*Ecology*, 89(4), 2008, pp. 1068–1078


# USING PARASITES TO INFORM ECOLOGICAL HISTORY: COMPARISONS AMONG THREE CONGENERIC MARINE SNAILS

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*Abstract*. Species introduced to novel regions often leave behind many parasite species. Signatures of parasite release could thus be used to resolve cryptogenic (uncertain) origins such as that of *Littorina littorea*, a European marine snail whose history in North America has been debated for over 100 years. Through extensive field and literature surveys, we examined species richness of parasitic trematodes infecting this snail and two co-occurring congeners, *L. saxatilis* and *L. obtusata*, both considered native throughout the North Atlantic. Of the three snails, only *L. littorea* possessed significantly fewer trematode species in North America, and all North American trematodes infecting the three *Littorina* spp. were a nested subset of Europe. Surprisingly, several of *L. littorea*'s missing trematodes in North America infected the other *Littorina* congeners. Most likely, long separation of these trematodes from their former host resulted in divergence of the parasites' recognition of *L. littorea*. Overall, these patterns of parasitism suggest a recent invasion from Europe to North America for *L. littorea* and an older, natural expansion from Europe to North America for *L. saxatilis* and *L. obtusata*.

*Key words: biogeography; Chao2;* Cryptocotyle lingua; *enemy release; Europe; intertidal snails; introduced species;* Littorina littorea; Littorina obtusata; Littorina saxatilis; *nonindigenous species; North America; trematodes.*

## Introduction

As global human transportation continues to homogenize the world's biota, historical records and baseline biological surveys can help determine which species are truly native to a region. However, these records can be incomplete, resulting in uncertainties regarding some species' status as native or nonnative in a region (i.e., cryptogenic; Carlton 1996). Several scenarios may result in such doubt over a species' origin, including observations of a species with a disjunct biogeographical distribution, an odd ecological role within the community, or closely associating/co-occurring species that are known to have been introduced (Chapman and Carlton 1991, Ruiz et al. 2000). Here, we demonstrate that parasites may be useful as tools to aid in the resolution of the ecological histories of such cryptogenic species.

According to the hypothesis of enemy release, introduced species often leave behind predators and parasites in their native habitats (Torchin and Mitchell 2004). Because only a small number of individuals are typically exported in an invasion event, an introduced host will likely carry with it just a subset of its native parasite fauna, resulting in a reduction in parasite species richness in introduced populations compared to native populations (e.g., Dove 2000, Torchin et al. 2002, 2005, Tsutsui et al. 2003, Prenter et al. 2004). Thus, parasites may inform invasion histories through comparisons of patterns in their abundance and diversity in hosts from native and nonnative ranges.

In an extensive review of parasitism in nonnative vs. native hosts across many taxa, Torchin et al. (2003) found that nonnative populations possessed approximately half the parasite species richness and prevalence of infection of native host populations. Though many of these studies were terrestrial or freshwater, a few marine studies have also strongly supported the predictions of enemy release. For example, in northeastern North America, nonnative populations of the European green crab (*Carcinus maenas*) were found to possess roughly half the number of parasites compared to native European populations. Furthermore, nonnative green crabs were larger and exhibited a greater biomass than native conspecifics, consistent with predictions of both physiological and population-level benefits that nonnative hosts gain by escaping parasites (Torchin et al. 2001). Additionally, on the west coast of the United States, a nonnative snail, *Batillaria attramentia*, is infected by only a single parasitic trematode species, while at least eight trematodes infect it in its native range (Torchin et al. 2005).

Although invaders typically exhibit reduced parasite richness in an introduced population compared to their native range, this differential may decrease over time due to the probability of subsequent invasions of infected hosts or arrival of parasites through natural vectors or other hosts (Prenter et al. 2004). This difference in parasite composition between native and introduced

Manuscript received 18 May 2007; revised and accepted 17 August 2007. Corresponding Editor: K. D. Lafferty.

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regions depends on the time since the invasion, the amount of propagules transported between the regions, and the specificity of the host–parasite relationship (Torchin and Mitchell 2004). Larval trematode parasites of snails are highly specialized and are typically obligate to specific snail species (or a group of closely related snail species); thus they are a useful guild of parasites to explore enemy release signatures. This is because introduced hosts rarely acquire new trematode species infecting distantly related native hosts, which would dilute parasite release signatures.

Because of extensive, consistent support for decreased parasite richness in introduced populations (Torchin et al. 2003), we propose using patterns of enemy release in reverse, i.e., to use parasite signatures to inform the ecological origin of a given host. Specifically, we tested the predictions for parasite release among three North Atlantic marine congeneric snails that are believed to have very different invasion/colonization histories in their established populations. All three snail species serve as first intermediate hosts to host-specific digenean trematode (flatworm) parasites. While the enemy release hypothesis has been used to explain heightened invasion success and ecological impact, to our knowledge, this study represents the first endeavor to use its predictions to distinguish older, natural range expansions from a recent, and purportedly human-mediated, introduction.

## Study System

### *Littorine natural histories in the North Atlantic*

*Littorina saxatilis* (rough periwinkle) and *L. obtusata* (smooth periwinkle) are gastropod mollusks found in similar ranges and habitats throughout the North Atlantic, including western Europe and northeast North America, as well as Greenland and Iceland (Reid 1996). Both snails are considered native throughout the North Atlantic; their origins in the western Atlantic are generally believed to have been the result of a natural invasion from Europe many thousands of years ago (Ganong 1886, Ingolfsson 1992, Reid 1996, Wares and Cunningham 2001), as is suspected for many northwest Atlantic hard-bottom species (Vermeij 1991, Ingolfsson 1992, Wares and Cunningham 2001). *Littorina obtusata*, in particular, is believed to have colonized North America from Europe shortly after the last glacial maximum, which occurred ~20 000 years ago (Wares and Cunningham 2001). *Littorina saxatilis* and *L. obtusata* are both direct developers: *L. saxatilis* broods its young, while *L. obtusata* lays its egg casings on nearby rock and algae.

*Littorina littorea* (common periwinkle) is also found in the North Atlantic rocky intertidal zone; however, both its biogeography and larval dispersal of young are different from congeners *L. saxatilis* and *L. obtusata*. *Littorina littorea* is presently found in western Europe and northeastern North America but is absent from Iceland and Greenland (Reid 1996), and it has pelagically dispersed larvae. Additionally, both *L. saxatilis* and *L. obtusata* are classified into the subgenus *Neritrema*, while *L. littorea* is classified into the subgenus *Littorina*; thus phylogenetically, *L. saxatilis* and *L. obtusata* are more closely related to one another than to *L. littorea* (Reid et al. 1996). *Littorina littorea* is a known native of Europe based upon extensive paleontological evidence (Reid 1996), but the history of its presence in North America remains less clear. What is known is that in the 1850s, *L. littorea* spread rapidly and sequentially southwards from Halifax, Nova Scotia, into the United States, reaching Delaware Bay only 30 years later (Steneck and Carlton 2001). What remains uncertain is whether Canadian populations were native and confined to Canada until the mid-1800s or were anthropogenically introduced from Europe. This ambiguity has been debated for more than 150 years, with evidence supporting both hypotheses (e.g., Ganong 1886, Clarke 1961, Berger 1977, Wares et al. 2002), but there has been no definitive resolution as of yet (Chapman et al. 2007, Wares and Blakeslee 2007).

### *Trematode parasites and trematode richness expectations in littorine snails*

Trematode parasites infect multiple hosts within their complex life cycles and primarily use gastropods as their first intermediate host. In their snail hosts, larval trematodes typically castrate (Kuris 1990) but do not kill their hosts, and infections are maintained throughout the duration of a snail's life. Trematodes asexually reproduce in their snail hosts, producing a free-swimming cercarial larval stage that is continually shed from the snail (though some species, e.g., "pygmaeus" group microphallids, do not have a free-swimming cercarial stage but instead use the snail as both a first and second intermediate host). The released cercariae then typically locate and encyst within a second intermediate host, which can include many species of fish, crabs, bivalves, or other mollusks (an exception is *Cercaria lebouri*, which encysts upon hard surfaces such as bivalve or crustacean shells and not within an organism). Second intermediate hosts must then be ingested by a definitive host, typically shorebirds (e.g., gulls, sea ducks, and other marine birds) or fish, in which the trematode sexually reproduces (Lauckner 1980).

We predicted *L. saxatilis* and *L. obtusata* would show some reduction (likely nonsignificant) in trematode species richness in North America, representing a subset of the snails' European-source trematode richness because we expected that older, natural invasions should have allowed sufficient time for the hosts to acquire trematode richness through subsequent host and trematode invasions. In contrast, if *L. littorea* is a recent invader from Europe, we expected a greater (likely significant) reduction in North American trematode species richness relative to its two congeners, based on the predictions for enemy release. Otherwise, we expected similar richness patterns to those exhibited by

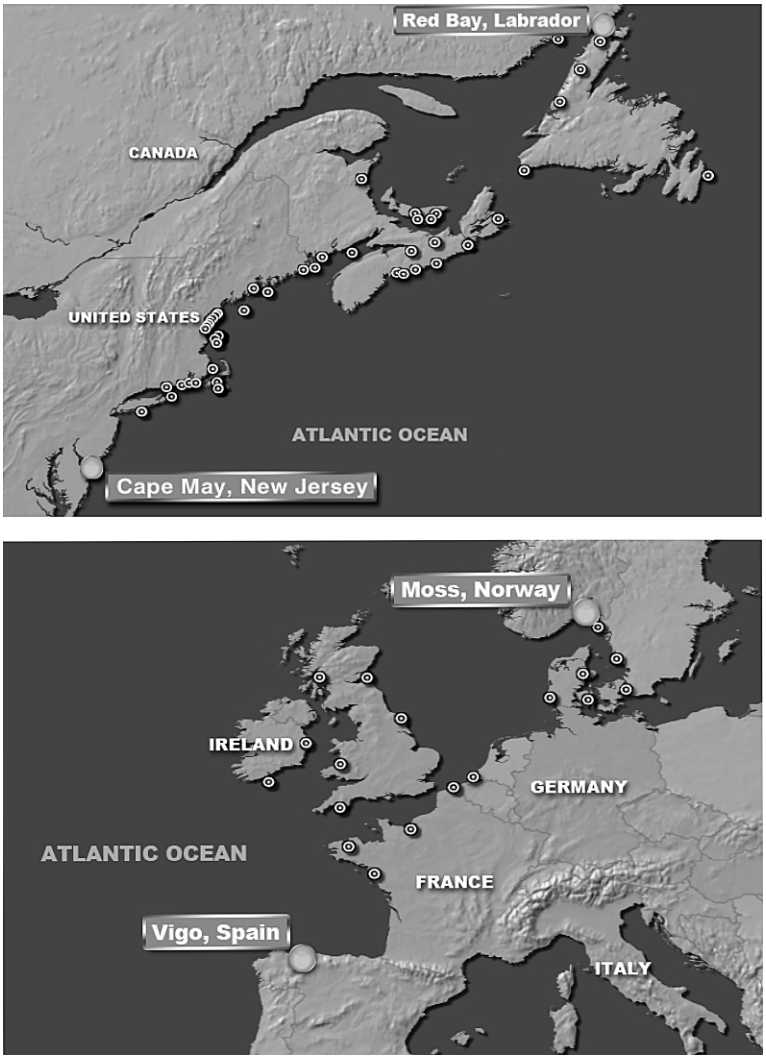


FIG. 1. North American and European collection sites for *Littorina littorea*, *L. saxatilis*, and *L. obtusata*. Altogether we collected *L. littorea*, *L. saxatilis*, and *L. obtusata* snails from 62 North American sites, ranging from Red Bay, Labrador, to Cape May, New Jersey, and we collected *L. littorea* snails from 20 European sites, ranging from Moss, Norway, to Vigo, Spain (see Appendix B for detailed information on these collection sites).

the two long-established congeners, *L. saxatilis* and *L. obtusata*.

## METHODS

### *Literature review*

To look for evidence of parasite release in *Littorina* spp., we performed an extensive literature review of trematode species richness in European and North American populations of *L. littorea*, *L. saxatilis*, and *L. obtusata*. We accepted studies that provided either trematode species richness (the total number of trematode species at a site), prevalence of infection (the proportion of snails infected by trematodes at a site), or both. We searched for every available study with these data. In all, we were able to use a total of 59 different European studies and 11 North American ones (Appendix A). Based on this review, we determined that while all three snail species have been well studied in Europe, they have been comparatively under-sampled in North America and studies have not encompassed the snails' full geographic ranges in North America compared to Europe.

### *Snail collections and dissections*

Observed species richness increases with sampling area and effort. Thus, any comparisons made using the much better sampled European literature data vs. the North American literature data would likely suffer from sampling bias. To remedy the apparent undersampling in North America, we collected ~100 *Littorina* sp. snails of each *Littorina* species from numerous sites throughout their North American ranges (*L. littorea*, $n = 49$

sites; *L. saxatilis*, $n = 19$ sites; *L. obtusata*, $n = 24$ sites; Fig. 1, Appendix B). We focused collections on *L. littorea* because it is more abundant and found at more sites than the other two littorines and because this was the species of the three we suspected would exhibit the signature of parasite release; thus, we wanted to ensure that sampling had been exhaustive to reveal all trematode species including potentially rare ones. Furthermore, we extensively sampled in Canada, especially north and east of Pictou, Nova Scotia (where *L. littorea* was first noted in North America), because the alternative hypothesis to an introduction of *L. littorea* to North America is that it was present in Canada historically. If true, these Canadian populations would be older and could harbor a richer parasite fauna that may not have completely advanced with the snail as it invaded the United States.

Although the European literature was quite extensive, we also collected *L. littorea* in Europe for corroboration with the literature, especially since many of the studies took place several decades ago. Also, we wanted to expand on the geographic range of samples reported from Europe, which, prior to our investigation, had centered on sites in the British Isles and the North Sea (Appendix A). Furthermore, as another potential signature of parasite release, we used these data to compare prevalence of infection for a standardized size class of snails between the two regions for *L. littorea* (length, North America, 18.78 ± 4.41 mm; Europe, 18.82 ± 4.42 mm [mean ± SD]) to determine whether this species showed lower prevalence of infection in North America compared to Europe. Standardization is important for prevalence comparisons because *L. littorea* size correlates with its age and thus the length of exposure to contract trematodes from its environment (Byers et al. 2008). Therefore, we collected and dissected ~100 *L. littorea* snails per site from 20 different European sites ranging from Moss, Norway, to Vigo, Spain (Fig. 1; Appendix B), recording trematode species richness and prevalence for each site. We did not perform field surveys in Europe for the other two *Littorina* species because the data in the literature was extensive, encompassing the majority of their ranges, and thus did not need further enhancement.

At each site, we haphazardly collected adult snails from the intertidal zone during low tide over the summer months of the years 2002–2005. Because both the snails and their trematode infections are long-lived, richness patterns at our sites were unlikely to change appreciably over the time period of our investigation, a fact we quantitatively confirmed for *L. littorea* at seven North American sites sampled in two study years. After we collected snails, we measured them from the apex to the anterior tip of the aperture and then dissected them under a stereomicroscope, and the gonadal and digestive tissues were examined for presence of trematode infection. Trematode species were identified under a compound microscope using multiple published keys and descriptions of trematodes infecting *Littorina* sp. (e.g., James 1968*a*, *b*, Werding 1969, Stunkard 1983).

### *Statistical analyses and species richness estimators*

To resolve whether our North American sampling was complete and to assess the total expected species richness and thus compare both populations using a standard metric, we employed EstimateS 8.0 (Colwell 2006) to construct species accumulation and species richness estimator curves from our field and literature data. EstimateS uses Monte Carlo resampling (through randomization of sample order over a number of replicates [e.g., 500]) to determine the mean accumulation of species ($S_{obs}$) as samples are added over the full data set (Gotelli and Colwell 2001), while also providing standard deviations and 95% confidence intervals for each data point (Colwell 2006). Although our data was sample-based, we rescaled our species accumulation curves to accumulated individuals in order to compare species richness across our data sets in a standardized manner (Gotelli and Colwell 2001).

Sample-based rarefaction curves may not capture the total species richness within a population for a particular sampling effort, especially if these curves have not reached a stable asymptote. Thus, nonparametric estimators, such as Chao2, can be useful in predicting the eventual asymptote in species richness for a particular population (Gotelli and Colwell 2001) and do so by including the effects of rare species on the total species richness (Witman et al. 2004, Chao 2005). Chao2 has been found to be one of the most robust estimators (see Colwell [2006] for Chao2 equation) when compared to empirical data from a variety of systems for revealing the missing species in a population and thus predicting the total expected species richness for the system (e.g., Walther and Morand 1998, Foggo et al. 2003). In fact, Walther and Morand (1998) advocated the use of Chao2 specifically for parasite species richness. In addition, Chao2 has been shown to remain precise even under changes in sampling effort (Walther and Morand 1998), and, because we included sites from both literature and field data of varying sample sizes, use of the Chao2 estimator was highly appropriate for our study.

Because a clearly asymptoting accumulation curve indicates complete capture of the total species richness in a population (Gotelli and Colwell 2001), estimator curves and species accumulation curves converging on the same asymptote reflect adequate sampling (Walther and Morand 1998). Therefore, we used this technique (with Chao2 as our estimator) to determine whether we had adequately sampled trematodes in North America since the snail hosts had been severely undersampled in the literature. Although the Chao2 method standardizes for variable sample sizes and thus accurately predicts the maximum expected species richness in each population, we performed an additional technique to standardize for sampling effort at the site level to determine whether average site-level richness corroborated results of the

TABLE 1. Overall prevalence (%) of each trematode species infecting *Littorina littorea*, *L. saxatilis*, and *L. obtusata* in Europe and North America.

| | *L. littorea* | | | | *L. saxatilis* | | | *L. obtusata* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Europe | | North America | | Europe | North America | | Europe | North America | |
| Trematode species | Literature | Field | Literature | Field | Literature | Literature | Field | Literature | Literature | Field |
| *Cryptocotyle lingua* | 14.38 | 5.23 | 9.66 | 9.37 | 1.53 | 6.60 | 2.62 | 0.33 | 6.32 | 1.53 |
| *Cercaria parvicaudata* | ND | 2.23 | 0.62 | 1.13 | 0.04 | 1.82 | 0.53 | 0.15 | ND | 0.42 |
| *Renicola roscovita* | 4.75 | 2.04 | 0.09 | 0.27 | 0.47 | 0.68 | 1.33 | 0.13 | 1.09 | 1.18 |
| *Microphallus similis* | 0.01 | 0.15 | ND | 0.02 | 11.61 | 0.73 | 4.14 | 8.92 | 1.82 | 2.96 |
| "Pygmaeus" microphallid group | 1.06 | 0.23 | 0.02 | 0.02 | 7.69 | 23.38 | 0.49 | 7.39 | 8.21 | 0.28 |
| *Cercaria lebouri* | 0.23 | 0.15 | | | 0.06 | | 0.53 | 0.06 | 0.55 | 0.14 |
| *Himasthla elongata* | 5.73 | 0.92 | | | 0.01 | | 0.04 | 0.01 | | 0.10 |
| *Himasthla littorinae* | ND | 0.27 | | | ND | 1.35 | 0.04 | ND | 0.30 | 0.17 |
| *Podocotyle atomon* | 0.18 | 1.00 | | | 0.54 | 0.05 | 0.13 | 0.35 | 0.18 | 0.28 |
| *Cercaria emasculans* | 0.28 | 0.04 | | | 0.01 | | | | | |
| *Cercaria littorinae* | 0.04 | | | | | | | 0.01 | | |
| *Cercaria littorinae saxatilis I* | | | | | <0.01 | | 0.04 | | | |
| *Cercaria littorinae saxatilis II* | | | | | 0.01 | | 0.04 | | | |
| *Cercaria littorinae saxatilis III* | | | | | 0.01 | | | | | |
| *Cercaria littorinae saxatilis IV* | | | | | 0.01 | | 0.09 | 0.01 | | 0.10 |
| *Cercaria littorinae saxatilis VI* | | | | | <0.01 | | 0.04 | | | |
| *Cercaria littorinae saxatilis VII* | | | | | 0.02 | | | 0.03 | | 0.03 |
| *Maritrema arenaria* | | | | | 0.11 | | 0.27 | | | |
| *Parvatrema homeotecnum* | | | | | 0.09 | | | 0.09 | | 0.10 |
| *Cercaria littorinae obtusatae* | | | | | | | | 0.18 | | 0.07 |
| *Parapronocephalum symmetricum* | | | | | 0.11 | | | 0.02 | | |
| *Maritrema linguilla* | | | | | 0.32 | | | | | |
| *Cercaria brevicauda* | | | | | <0.01 | | | | | |
| *Notocotyloides petasatum* | | | | | | | | 0.01 | | |
| *Cercaria islandica I* | | | | | | | | 0.03 | | |

*Notes:* Data stem from our extensive literature and field surveys (see Appendices A and B). The percentage of infection of a trematode species among all snails investigated (i.e., prevalence) is listed for each survey. The abbreviation "ND" indicates no data and refers to studies recording presence but not prevalence of a trematode species. Taxonomic issues for some trematode species may affect our reported prevalences (especially for European *L. saxatilis* and *L. obtusata*, for which all data were extracted from the literature), and thus our data may not precisely reflect natural prevalence for these species (see Appendix C for detailed information). Furthermore, because of ambiguities regarding species identifications in the field and literature, we have combined four species of morphologically similar microphallid species into one category ("pygmaeus" microphallid group) in both Europe and North America (Appendix C).

Chao2 technique and the observed richness ($S_{obs}$) in each population. To do this, we performed Monte Carlo resampling (using EstimateS 8.0) on each site to randomly select 75 individuals (the minimum number of individuals that had been sampled at every site). Those few sites/studies with fewer than 75 individuals were excluded from this analysis. Following this selection, the adjusted site-level species richness value (mean ± SE) was recorded for each field site and literature study (Appendices A and B). These standardized values were then used in a single-factor ANOVA for each species to determine whether there were significant differences in mean site-level richness in North American vs. European populations.

## RESULTS

Our sampling dramatically increased the total number of snails and sites previously investigated in North America for trematodes of all three littorines (Tables 1 and 2, Appendix A). In total, for *Littorina littorea*, we

TABLE 2. Trematode investigation metadata for *Littorina littorea*, *L. saxatilis,* and *L. obtusata* in Europe and North America.

| | *L. littorea* | | | | *L. saxatilis* | | | *L. obtusata* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Europe | | North America | | Europe | North America | | Europe | North America | |
| Metadata | Literature | Field | Literature | Field | Literature | Literature | Field | Literature | Literature | Field |
| Total trematode species richness | 11 | 10 | 5 | 5 | 21 | 7 | 14 | 17 | 8 | 13 |
| Study sites | 16 | 20 | 7 | 49 | 16 | 2 | 19 | 13 | 1 | 24 |
| Total infected snails | 18 787 | 319 | 670 | 888 | 5029 | 666 | 233 | 2811 | 304 | 215 |
| Total snails investigated | 70 460 | 2600 | 6447 | 8210 | 22 196 | 1925 | 2248 | 15 867 | 1645 | 2875 |

*Notes:* The metadata are presented for each snail species (*L. littorea*, *L. saxatilis*, or *L. obtusata*) in each population (Europe or North America) and in each survey (literature or field) and include: the total number of trematode species recorded or observed in these surveys; the total number of sites from which the trematode data were recorded or observed in these surveys; the total number of infected snails recorded or observed in these surveys; and the total number of snails investigated in these surveys.

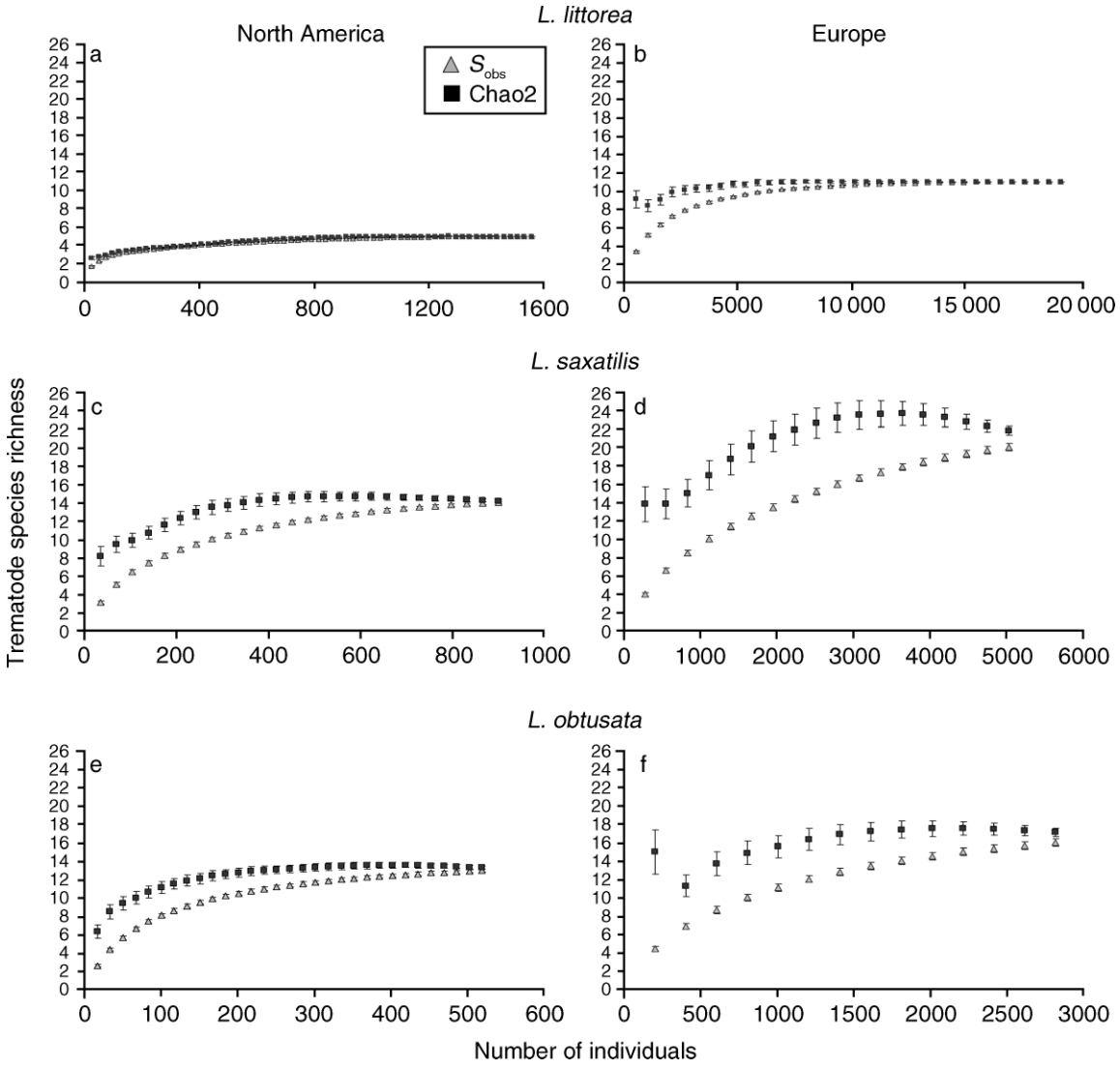


FIG. 2. Trematode species richness as a function of the number of infected *Littorina* sp. snails from both literature and field data. Richness of trematodes infecting *Littorina* sp. in both North America and Europe was estimated using species accumulation and species estimator curves (Colwell 2006). Each panel shows $S_{obs}$ and the Chao2 species richness estimate for (a, b) *L. littorea*, (c, d) *L. saxatilis*, and (e, f) *L. obtusata*. Error bars are standard error for 500 runs in EstimateS (Colwell 2006). For *L. littorea*, $S_{obs}$ and Chao2 reached an asymptote at a trematode species richness value of 5 in North America and a value of 11 in Europe. For *L. saxatilis*, the $S_{obs}$ and the Chao2 curves reached an asymptote at 14 trematode species in North America; in Europe, the $S_{obs}$ culminated at 20 species, and the Chao2 achieved a value of 21 species. For *L. obtusata*, $S_{obs}$ and Chao2 culminated at 13 species in North America; in Europe, $S_{obs}$ culminated at 16 species, and Chao2 achieved a value of 17 species.

found 11 trematode species in Europe vs. five in North America, a 55% reduction in trematode richness in North America. For *L. saxatilis*, there were a total of 21 European vs. 14 North American trematode species, a reduction of 33% in North America. Finally, for *L. obtusata*, the difference was 17 to 13 species in Europe vs. North America, a reduction of 24% in North America. For all three snail species, the trematode species richness of North America was a subset of the European trematode richness (Table 1).

Species accumulation ($S_{obs}$) and Chao2 species richness estimator analyses both reached an asymptote at a trematode species richness of five species for North American *L. littorea* (Fig. 2a) and at 11 species for European *L. littorea* (Fig. 2b), indicating that no further trematode species are expected in either population. Confidence intervals in North America and Europe for both $S_{obs}$ and Chao2 were zero or nearly zero. For *L. saxatilis*, the North American $S_{obs}$ and Chao2 curves reached an asymptote at a trematode species richness value of 14 species ($S_{obs}$, CI = 13–15; Chao2, CI = 14–18; Fig. 2c), while the European $S_{obs}$ achieved a value of 20 species and the Chao2 curve culminated at 21 species ($S_{obs}$, CI = 17–23; Chao2, CI = 20–31; Fig. 2d). For

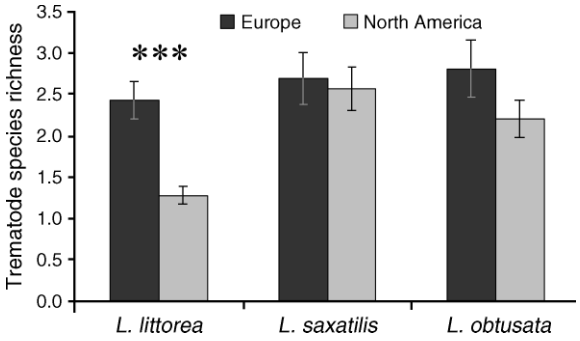


FIG. 3. Standardized site-level trematode species richness (adjusted mean ± SE) for *Littorina littorea*, *L. saxatilis*, and *L. obtusata* in Europe vs. North America. *Littorina littorea* shows a significantly greater (*** $P < 0.001$) site-level trematode richness in Europe (2.43 ± 0.23 trematode species) compared to North America (1.28 ± 0.11 trematode species). *Littorina saxatilis* and *L. obtusata* both show lower trematode richness in North America (*L. saxatilis*, 2.57 ± 0.26 trematode species; *L. obtusata*, 2.20 ± 0.23 trematode species) compared to Europe (*L. saxatilis*, 2.69 ± 0.31 trematode species; *L. obtusata*, 2.81 ± 0.35 trematode species), but these differences were not significant ($P = 0.82$ and $P = 0.25$, respectively).

North American *L. obtusata*, the $S_{obs}$ curve achieved a value of 12 species, while the Chao2 curve culminated at ~12 species ($S_{obs}$, CI = 9–15; Chao2, CI = 12–20; Fig. 2e). For European *L. obtusata,* the $S_{obs}$ curve culminated at a trematode species richness of 16 species, while the Chao2 curve achieved a value of 17 species ($S_{obs}$, CI = 13–19; Chao2, CI = 16–25; Fig. 2f). The difference in the total trematode richness in European *L. saxatilis* and *L. obtusata* recorded in Table 2 (21 and 17 trematode species for *L. saxatilis* and *L. obtusata*, respectively) and the final $S_{obs}$ values shown in Fig. 2d, f (20 and 16 trematode species for *L. saxatilis* and *L. obtusata*, respectively) are the result of missing *H. littorinae* prevalence data, which is necessary for inclusion in the $S_{obs}$ analysis (Table 1).

After standardizing for sampling effort at each site (Appendices A and B) and statistically comparing the adjusted site-level trematode richness in each region, we found European *L. littorea* to possess a significantly greater site-level trematode species richness compared to North America ($F_{1,86} = 28.27$; $P < 0.001$). The site-level richness was nearly 50% lower for North America (1.28 ± 0.11 species; mean ± SE) compared to Europe (2.43 ± 0.23 species; Fig. 3), mirroring the overall reduction in trematode richness using the Chao2 approach above. Both *L. saxatilis* and *L. obtusata* showed lower average trematode richness in North America compared to Europe. For *L. saxatilis*, the decline in North American (2.57 ± 0.26 species) richness compared to Europe (2.69 ± 0.31 species) was only about 5%, while for *L. obtusata*, the decline in North America (2.20 ± 0.23 species) was ~22% compared to Europe (2.81 ± 0.35 species). The reduction was nonsignificant for both species (*L. saxatilis*, $F_{1,32} = 0.05$, $P = 0.82$; *L. obtusata*, $F_{1,34} = 1.37$, $P = 0.25$; Fig. 3). Power, however, was low for the *L. saxatilis* and *L. obtusata* analyses, due principally to the low effect size differences between the European and North American site-level richness. When we performed power analyses for these two snails using their sample sizes and variability, using an effect size equivalent to that seen in *L. littorea*, we found high power for both *L. saxatilis* (0.90) and *L. obtusata* (0.89).

Finally, we compared site-level trematode prevalence of infection in European vs. North American *L. littorea* with a single-factor ANOVA analysis. Differences in prevalence between the two populations were not significant (Europe, 10.2% ± 2.0%; North America, 9.7% ± 1.5% [mean ± SE]; $F_{1,76} = 0.02$, $P = 0.88$), nor were they significantly different when we compared the prevalence of just the five trematode species common between North America and Europe (Europe, 8.9% ± 1.9%; North America, 9.7% ± 1.5%; $F_{1,74} = 0.08$, $P = 0.78$).

## DISCUSSION

Both our extensive literature review and supplemental field sampling identified total trematode species richness that was 55% lower for *Littorina littorea* in North America vs. Europe (Table 2). Mean site-level richness was also significantly lower in North America compared to Europe (Fig. 3) and the decline (47%) was nearly equivalent to the decline based on the total species richness (55%). Moreover, the Chao2 results confirmed that this difference in trematode species richness was not the result of undersampling in North America but was the accurate total species richness for this region. Furthermore, all Chao2 curves for *L. littorea* reached an asymptote at the same value as the observed trematode richness (Fig. 2a, b). In contrast, for *L. saxatilis* and *L. obtusata*, smaller, nonsignificant reductions in trematode species richness in North America vs. Europe were demonstrated (33% and 24%, respectively; Table 2). Mean site-level richness for *L. saxatilis* and *L. obtusata* also showed much lower declines in North America vs. Europe compared to *L. littorea* (Fig. 3). The decline based on mean site-level richness in North American *L. obtusata* (22%) was essentially equivalent to that witnessed in the declines based on the total richness (25%) in each region, while for *L. saxatilis*, the decline based on average site-level richness was much

lower (5%) than that witnessed in the declines based on the total richness (33%) in each region. *Littorina saxatilis*, although on the whole about one-third lower in total trematode richness in North America compared to Europe, exhibited comparable site-level richness between the regions. The similarity in site-level richness is likely explained by the fact that one species in particular, *Microphallus similis*, was found in *L. saxatilis* at the majority of North American sites. Because this trematode species was present at most sites, it boosted the North American site-level richness without further adding to the overall regional richness.

In both *L. saxatilis* and *L. obtusata*, the maximum expected trematode richness in each population calculated by the Chao2 estimator equaled or nearly equaled the observed species richness in each region (Fig. 2c–f). This result demonstrates that our field and literature sampling captured all trematode species expected in North America and nearly all in Europe (e.g., for European *L. saxatilis*, the Chao2 suggested that one more species could be found with further sampling). Thus, we can be confident that the much lower differences in total richness and mean site-level richness observed in *L. saxatilis* and *L. obtusata* compared to *L. littorea* were accurate and not a result of undersampling. On the whole, our analyses demonstrate a substantial distinction between *L. littorea* and its two congeners, in that the differences in total and site-level richness between the regions for *L. saxatilis* and *L. obtusata* is about half that of the difference for *L. littorea*.

In European snails, taxonomic inflation of trematode species may exist as the result of a longer history of trematode exploration, which has produced accounts of ambiguous and difficult-to-distinguish congeners. We partially controlled for this by lumping four of the more indistinguishable congener species, which have often been referred to in the literature (e.g., Galaktionov and Skirnisson 2000) as the "pygmaeus" group of microphallids (refer to Appendix C for details). Despite the potential for inflated richness in the native region, trematode species richness still was not significantly depressed in *L. obtusata* or in *L. saxatilis* in North America relative to Europe. In contrast, *Littorina littorea* had almost no taxonomically challenging species to inflate its European trematode richness, yet it was the only snail to demonstrate a significant decrease in its North American trematode species richness. The reduction in trematode richness in *L. littorea* cannot be due to different environmental conditions that have resulted in across-the-board reductions of littorine trematodes in North America because *L. obtusata* and *L. saxatilis* show no significant decline in trematode richness and are infected by the same species of trematodes that are missing in *L. littorea* North American populations. This suggests that the significantly lower trematode richness in North American vs. European *L. littorea* requires additional and alternative explanation. Thus, our results strongly support a distinctly different ecological history for *L. littorea* compared to its two congeners.

We suggest that the nonsignificant reduction in trematode richness in North American compared to European *L. saxatilis* and *L. obtusata* (Fig. 3) supports an older invasion for these two snails because a longer time interval should allow for subsequent invasions to enhance the number of parasite species in the invaded population (Prenter et al. 2004). Furthermore, all trematode species infecting the snails in North America were a subset of the European trematode population (Table 1). Our data is therefore consistent with other evidence that has suggested that *L. saxatilis* and *L. obtusata* naturally invaded North America from Europe, probably as recolonization events following the last glacial maximum (e.g., Ganong 1886, Ingolfsson 1992, Reid 1996), which could have been as long as 20 000 years ago (Wares and Cunningham 2001). This natural crossing from Europe to North America was likely through dispersal to shallow water and intertidal habitats of islands in the North Atlantic, where both *L. saxatilis* and *L. obtusata* (but not *L. littorea*) are presently found, including populations in the Faeroe Islands, Iceland, and Greenland (Ganong 1886, Reid 1996). Such a scenario has been suggested for much of the western North Atlantic hard-bottom fauna, as evidenced by the Iceland fauna, which is almost entirely a subset of European fauna, and northeastern North America, which is a further reduced subset of the European and Icelandic fauna (Ingolfsson 1992). From our European literature data set, it in fact appears that the Iceland populations match Europe more closely in trematode diversity than northeastern North America (Sannia and James 1977, Galaktionov and Skirnisson 2000, Skirnisson and Galaktionov 2002), further corroborating historical, natural movement of the two littorines from the British Isles to Iceland and suggesting a filtering out of trematode species with increasing distance from the source.

*Littorina littorea*, on the other hand, is not found to exist on any of the aforementioned North Atlantic islands that were likely stepping stones for the natural invasions of *L. obtusata* and *L. saxatilis* (Johannesson 1988, Reid 1996). Although *L. littorea* has pelagic larvae (unlike its direct developing congeners), such broadcast spawning species often have trouble retaining and establishing populations in small, isolated areas, especially islands (Johannesson 1988, Byers and Pringle 2006). Furthermore, Kraeuter (1976) suggests that current patterns from the British Isles across the North Atlantic make a direct crossing by *L. littorea* larvae "impossible" and that any drifting adults would likely end up south of most of *L. littorea*'s present-day North American range and far south of its first reported sightings in southern Canada. Thus, the patterns in trematode richness we observed for this snail coupled with its natural history suggest a recent introduction to North America from Europe.

One of the striking results of our data set is that almost all of the trematode species that infect *L. littorea* in Europe are found in North America infecting *L. saxatilis* and *L. obtusata* (Table 1), yet four of these species do not infect *L. littorea* in North America (*Cercaria lebouri*, *Himasthla elongata*, *H. littorinae*, and *Podocotyle atomon*). The lack of occurrence of these four trematodes in North American *L. littorea* is surprising given that they all use *L. littorea* as a host in Europe and two of the four (*H. elongata* and *Cercaria lebouri*) are believed to use *L. littorea* as their preferred primary host (James 1968*b*, Williams and Ellis 1976, Matthews et al. 1985). The absence of these four trematode species in North American *L. littorea* is not the result of a sampling issue because the sample size from our North American *L. littorea* field surveys is four times higher than the other littorines and our species accumulation curves suggest complete capture of all North American trematodes for *L. littorea* (Fig. 2a). The absence is also not due to lack of infection opportunities or ecological proximity because *L. littorea* was present at all sites where we observed these four species infecting *L. saxatilis* and *L. obtusata* in North America. Moreover, two species in particular, *H. elongata* and *H. littorinae*, have miracidia that directly penetrate their snail hosts and do not require ingestion for infection to occur (Stunkard 1966, Matthews et al. 1985). In addition, Matthews et al. (1985), in a study performed in Ireland, suggested that *H. elongata*'s free-swimming miracidia actively host-selected for *L. littorea* and not for the other two littorines. For all these reasons, it is highly likely that the four former *L. littorea* parasites have had many opportunities to infect *L. littorea* in North America and their absence must be due to some physiological constraint between these trematodes and North American *L. littorea* snails, a pattern consistent with a genetic divergence between these four trematode species and their former host.

The most parsimonious explanation for this pattern is that upon a recent introduction of *L. littorea* to North America, these four former *L. littorea* parasites (being present in historical North American populations of *L. saxatilis* and *L. obtusata*) no longer recognized *L. littorea* as a suitable host due to the divergence that had occurred over their long separation. *Littorina saxatilis* and *L. obtusata* are believed to have naturally invaded North America following the last glacial maximum, ~20 000 years ago (e.g., Ganong 1886, Ingolfsson 1992, Reid 1996, Wares and Cunningham 2001), and they likely carried some of these former *L. littorea* trematode species with them upon invasion (*L. littorea*'s trematodes are a nested subset of the other two littorine species; see Table 1). Definitive seabird hosts, such as *Larus argentatus* (Herring Gull) and *Larus marinus* (Black-backed Gull; Stunkard 1966), may have also brought trematode species to North America; however, trans-Atlantic flights by these gull species are believed to be uncommon (J. Ellis and T. Good, *personal communications*). Because these former *L. littorea* trematodes have low prevalence in European *L. saxatilis* and *L. obtusata* (e.g., from a few Iceland sites, the prevalence of *Cercaria lebouri* was 0.2% and 0.3% for *L. saxatilis* and *L. obtusata*, respectively, and it was 3.5% and 4% for *P. atomon* in *L. saxatilis* and *L. obtusata*, respectively [Galaktionov and Skirnisson 2000, Skirnisson and Galaktionov 2002]), trematode colonizations in North America would have likely included extremely small founding populations. Small populations are highly susceptible to genetic drift, in which genotypes allowing for physiological compatibility between parasites and their primary host could have been lost, leading to a divergence between these trematode populations on either side of the Atlantic and thus a situation in which these four trematode species can no longer infect their former host.

A loss of infectivity of hosts for certain parasite genotypes has been empirically and even experimentally demonstrated. For example, Little et al. (2006) experimentally showed that after several generations a particular genotype of a bacterial parasite, *Pasteuria ramosa*, lost the ability to infect a host genotype of its crustacean host, *Daphnia magna*, while other *P. ramosa* genotypes did not. Similarly, Richards (1977) found that certain strains of the trematode *Schistosoma mansoni* were less infective to the freshwater snail *Biomphalaria glabrata* than other strains and that changes in its infectivity may have been the result of shifts in gene frequencies. Finally, trematode species previously thought to represent one species have been found to be genetically distinct cryptic taxa. For example, Huspeni (2000) showed that the trematode "species" *Parorchis acanthus* actually represents four genetically distinct species and that, for one of these distinct species, there were also two divergent clades representing genetic differences within this species complex. Thus, due to isolating events, morphologically similar members of a species may actually become genetically distinct cryptic taxa (Huspeni 2000). A loss of infectivity due to trematode genotype shifts or losses is a likely explanation for the absence of these four trematode species in North American *L. littorea*. Ultimately, given a small, natural, and historical inoculation of the former *L. littorea* trematodes to North America, the separation of *L. littorea* from its parasites necessary for divergence in the loss of infection capability was most likely driven by an absence of *L. littorea* in North America over historical time.

The nested subset of *L. littorea* trematodes also helps eliminate alternative explanations for the absence of several of its European trematode species in North America. First, the absence cannot be due to the lack of appropriate second intermediate and definitive hosts in the trematodes' complex life cycle. Not only are appropriate second intermediate and definitive hosts present in North America (e.g., Pohley 1976, Stunkard 1983), but their ecological functioning as hosts also is

assured by the successful completion and persistence of all of *L. littorea*'s trematodes using the other two *Littorina* sp. snails. Second, although glaciation is believed to have been more severe in the western than the eastern Atlantic (Ingolfsson 1992), any explanation that invokes a pre-Ice Age North American history for *L. littorea* would have to explain how glaciers wiped out trematode species just from *L. littorea* that were not subsequently restored with the North American colonization of *L. obtusata* and *L. saxatilis* and their shared trematode species shortly after the last glacial maximum.

Finally, we found prevalence of trematode infection in *L. littorea* to be similar in North America and Europe. Although prevalence has been shown to be significantly lower in founder vs. source populations in other systems, Torchin et al. (2001) also showed that when only species common between populations were compared, the prevalence between the populations was not different. Presumably this was because parasite species carried with their hosts were able to achieve equally high prevalence in the introduced range as in their source population. Because the five species common between our two populations of *L. littorea* account for 81% of the occurrence of all trematode species in Europe, it is perhaps not surprising that we did not find higher prevalence of infection in Europe vs. North America.

In conclusion, the results of our trematode species richness analyses corroborate prior historical, molecular, and ecological evidence supporting an older, natural invasion of North America for both *L. saxatilis* and *L. obtusata* and meets expectations of enemy release for North American *L. littorea*, thus supporting a recent invasion for this snail. An interesting facet of parasite release uncovered here is that although *L. littorea* has escaped some trematodes in North America, it has not escaped those parasites physically, but physiologically due to an incompatibility that has apparently developed over the long separation between these trematodes and their former host. Our work represents the first endeavor to use parasites to inform invasion histories. Because parasite release is an easily recognizable signature, it may prove useful in helping to resolve the cryptogenic status of species in many systems.

## Acknowledgments

We thank I. Altman, T. Backeljau, M. Blakeslee, J. Carlton, R. Coleman, L. Collins, E. Dewey, M. Donovan, A. Figueroa, A. Fowler, A. Freeman, J. Grahame, B. Griffen, M. Janamma, A. Houghton, R. Houghton, T. Huspeni, K. Johannesson, A. Kintner, J. Lee, M. Lesser, T. Maguire, J. Meyer, L. Page, R. Ramsay, A. Rosenberg, C. Suckling, S. Teck, V. Taibe, and D. Zdankoweicz for assistance in the field and laboratory, snail collections, insights, and/or manuscript comments. We also thank three anonymous reviewers for their comments and suggestions. Support was provided by NSF (OCE-0503932), NH Sea Grant, USDA Hatch, and the UNH Vice President for Research Discretionary Fund to J. Byers. A. Blakeslee was supported by the Sloan Foundation History of Marine Animal Populations (HMAP) and small grants from the American Malacological Society, Sigma Xi, Lerner Gray, UNH Center for Marine Biology, UNH Zoology Department, and UNH Graduate School. We also thank the Shoals REU program. This paper is scientific contribution no. 2348 from the New Hampshire Agriculture Experiment Station.

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## APPENDIX A

Literature review for *Littorina littorea*, *L. saxatilis*, and *L. obtusata*, documenting sample sizes and trematode species richness by site (*Ecological Archives* E089-064-A1).

## APPENDIX B

Field collection sites in North America and Europe for *Littorina littorea*, *L. saxatilis*, and *L. obtusata*, documenting sample sizes and trematode species richness by site (*Ecological Archives* E089-064-A2).

## APPENDIX C

Trematode taxonomy operational procedures for Table 1 prevalence data (*Ecological Archives* E089-064-A3).