Trematodes as indicators of the diversity and abundance of benthic invertebrates, fishes, and birds

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Ecology, Evolution, & Marine Biology

by

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September 2007
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ACKNOWLEDGEMENTS

I’ve been fortunate to have been surrounded by incredible people during my grad career. Excellent colleagues and friends have filled and fill my lab and my department. My main prof (Armand Kuris), and my older academic brother/advisor (Kevin Lafferty) were both instrumental in providing advice, opportunities, and helping me develop my science. Their importance in so many ways and for so many things cannot be overstated. We’ve also shared rich experiences and accomplished really good things. The nice thing is, we aren’t stopping. The good times are still rollin’.

The other members of my committee (Steve Gaines and Bob Warner) have also been instrumental in the formulation of my scientific perspective. Bob was really good at coming from the outside and not necessarily buying into things, causing me to sharpen my thinking. I hope we’re able to mess around more with castration and puppets. Steve has been really good at providing a load of skills and coming up with constructive and very complementary ideas about things. I hope within the next few years, we find the time to deal with competing worms and propagating error or who knows what.

Many grad students, post-docs and other lab-mates enhanced and supported my time as a grad student. My non-Nubian brothers Todd Huspeni and Mark Torchin; Mark Rigby, Lise Schickle-Goddard, Kathleen Whitney, Val McKenzie, Jenny Shaw (great bonding at finishing time), Charlie Boch, Nadia Talhouk, Julio Lorda, and Alice
Nguyen—they’ve all been, simply put, the god-damn bomb. Just like with the folks above, we’ve done good things and will keep on doing them. Pura vida.

I’ve also been lucky to have worked with a bunch of excellent undergrads on various projects: Eleca Dunham, Sarah Heerhartz, Christa Gallego, Josh “Fuah Fuah” Smith, Alan Wood, David Eghbali, Laura Cruz, Danni Storke, Victor Frankel, Lysette Arellano, and Josie Hardy.

Many people in at EEMB and MSI also provided important support. There are way too many names to list, but we are all lucky to have such a good supportive group of people from the shops to the administration!

And, of course, I’ve got to give kudos to my family. Both of my parents and my two brothers have always supported me in the path I’ve chosen. I love them.
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Nejat B. Ezal Fellowship Fund, 2001, $2,500

PUBLICATIONS SUBMITTED OR BEYOND

1. Hechinger RF, KD Lafferty, and AM Kuris. submitted. Trematodes indicate biodiversity in the Chilean intertidal zone and Lake Tanganyika.

2. Hechinger RF, KD Lafferty, FT Mancini III, RR Warner, and AM Kuris. in revision. How large is the hand in the puppet? Ecological and evolutionary effects on body mass of 15 trematode parasitic castrators in their snail host.


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**TALKS AND POSTERS PRESENTED**


Hechinger RF. 2005. Birds bring parasites to snails, and then... Invited presentation. Symposium: Effects of parasites on bird individuals and populations. Annual Meeting, American Ornithological Union. Santa Barbara, CA, August 2005


ABSTRACT

Trematodes as indicators of the diversity and abundance of benthic invertebrates, fishes, and birds

by

Ryan F. Hechinger

It is difficult to assess ecosystem biodiversity, yet essential to do so. Trematodes are particularly promising bioindicators, being connected to surrounding biodiversity and easily sampled in their first intermediate host snails. This investigation’s primary goal was to evaluate the hypothesis that these parasites reflect free-living animal diversity and abundance.

We quantified bird assemblages at several sites within an estuary to find that bird diversity and abundance positively correlated with that of trematodes in horn snails, Cerithidea californica.

We quantified assemblages of fishes, benthic invertebrates, and trematodes in horn snails at 32 sites in three California estuaries, finding that trematode diversity and abundance may indicate that of large benthic invertebrates and the diversity of small benthic invertebrates. Trematodes did not appear to indicate fish assemblages, but this likely was because typical surveys inadequately quantify fishes at that scale.
At larger scales (habitats in three estuaries in California and Baja California), we documented that trematode prevalence in horn snails correlated with the densities of fishes, crabs, burrowing shrimp, and snails, but did not significantly correlate with bivalves and polychaetes. Trematode assemblages on the whole changed in space congruently with combined fish and benthic invertebrate host assemblages. These results were strongest for trematodes in the most wide-spread snail size classes.

To assess the bioindicator promise of trematodes in non-estuarine ecosystems, we analyzed data from two published studies, one in the Chilean rocky intertidal zone, and another from an African rift lake. In both cases, we found that trematode prevalence was positively related to some element of surrounding diversity.

To facilitate the use of a trematode assemblage with promise for use as a bioindicator, I provide descriptions and an annotated key to identify eight species of trematodes that infect the common East Asian mud snail, Batillaria attramentaria.

On the whole, these findings support the idea that trematodes in snails indicate several types of free-living assemblages, and additionally provide an inexpensive bioindicator tool for surveillance of broader community change. In a time when the importance of biodiversity monitoring is escalating, we should seriously consider the widespread use of trematodes as bioindicators.
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Chapter 1

Introduction: larval trematodes as bioindicators of ecosystem condition

“It's hard work...But it's necessary work.”

G.W.Bush

It is difficult and costly to assess the biodiversity of ecosystems. However, it is essential to do so. The focus of my thesis has been to further the development of a method for assessing wetland ecosystem condition. The method uses larval trematode parasites as bioindicators of the diversity and abundance of benthic invertebrates, fishes, and birds, as well as for the ecological functioning of these ecosystems. Although this method will not necessarily supplant existing approaches, it has the potential to be less expensive, more integrative, more accurate than other available methods, and provide an explicit perspective on the functional properties of wetlands.

Why is measuring biodiversity hard and problematic? In biodiversity assessments, we often want information about multiple taxa from several replicated sites. It is challenging to get good information on the major components of communities using standard techniques. Perhaps the most important is the high cost of adequately surveying benthic invertebrates, fishes, and birds. This high cost generally precludes the amount of replication necessary for accurate assessments.
Table 1 gives examples from various studies in southern California of the low replicate number typically used to characterize entire wetlands. A second problem with such surveys is that many taxa (particularly mobile fishes and birds) are notoriously difficult to adequately sample using standard “snap shot” techniques. Frequently, either inadequate sampling is adopted, or these assemblages are simply not quantified at all. Thirdly, sampling benthic invertebrates and fishes can be environmentally destructive—particularly a concern in small systems being restored. Fourthly, and perhaps most significantly, integrating data in a meaningful way from various taxon-specific sampling procedures is quite challenging. Thus, more integrative and cost-effective methods for characterizing wetland biodiversity and ecosystem function are desired.

Can parasitic worms serve as bioindicators of surrounding animal diversity and of the functioning of food webs? Parasites, like all organisms, interact with various aspects of the environment. However, unlike other organisms, many parasites with multiple-host life cycles are predictably and directly linked to several types of invertebrate and vertebrate hosts. Additionally, at some steps in their life cycles, most of these parasites use predator-prey interactions to get from host to host. Thus, these parasites require both the presence of each of their hosts and a functioning food web (Gardner and Campbell 1992, Marcogliese and Cone 1998, Huspeni et al. 2005, Hudson et al. 2006). Among such parasites, trematode flatworms are particularly promising bioindicators (Huspeni et al. 2005). Not only are trematodes connected to
surrounding biodiversity and food webs, they are, easily sampled in their first intermediate hosts (Huspeni et al. 2005). Quantifying the trematode community at a site takes less time than does directly quantifying the various free-living assemblages (Huspeni et al. 2005). Table 2 is modified from Huspeni et al. (2005) and compares the amount of worker hours necessary to quantify various wetland assemblages using several standard direct methods and by using trematodes. Trematodes clearly are relatively easy to sample. Notably, trematodes have the added value that they indicate several components of the surrounding community that are otherwise each separately quantified using traditional techniques.

Trematodes logically should serve as good bioindicators, but data are necessary to validate and refine their use as such. One of the steps necessary in developing any ecological indicator is to demonstrate the occurrence of ecological associations between the indicator and the things to be indicated (McGeoch 1998). Before this investigation, there was little direct evidence that trematodes could indicate surrounding biodiversity. There were several studies showing that trematode assemblages varied in time or space in ways that were anecdotally associated with surrounding free-living assemblages (e.g., Hoff 1941, Cort et al. 1960, Robson and Williams 1970, Matthews et al. 1985, Keas and Blankespoor 1997, Bustnes and Galaktionov 1999), or with a wetland restoration (Huspeni and Lafferty 2004). Smith (2001) provided the only study quantitatively examining whether trematodes in snails were directly related to the abundance of a surrounding free-living assemblage. She
found that trematode prevalence in snails was positively related to bird abundance. The bulk of my thesis furthers the assessment of the use of trematodes as bioindicators by evaluating the ecological hypotheses underlying its use. That is, I evaluated the concept that larval trematodes reflect surrounding diversity and abundance of free-living animals.

Birds are a very important assemblage in estuaries, including most of the top predators (Erwin 1996). Chapter 2 shows that the diversity and abundance of larval trematodes in California horn snails positively correlates with the diversity and abundance of birds across several sites within a single estuary. This pattern likely exists because the various trematode species use different bird species as final hosts and because birds bring more trematodes to snails in areas where birds are more common. Further supporting the generality that trematodes can indicate bird abundance, the earlier study of Smith (2001), and now additionally Fredensborg et al. (2006), found positive associations between the abundance of birds and the prevalence of trematode parasites in snails. It is particularly desirable to have a good indicator of bird abundance and diversity because accurately quantifying bird communities at small spatial scales and over long time scales is difficult and time-consuming (Hechinger and Lafferty 2005, Huspeni et al. 2005). As an indication of their value as biomonitoring tools, in this study, trematode communities were much less time consuming to assess than were birds; collecting and processing trematode
data averaged 4.6 person-hours per site, while collecting and processing bird data averaged 53.2 person-hours per site.

In Chapters 3 and 4 we examine whether trematode abundance and diversity also indicate the abundance and diversity of fishes and benthic invertebrates. Trematodes should reflect the presence and abundance of fishes and benthos for two reasons (Huspeni et al. 2005, Hechinger et al. 2007). First, second intermediate hosts (and related animals) are food for predatory final hosts. To the extent predator final hosts spend more time in areas with abundant food, they will transmit more trematodes to snails. Second, trematodes cannot infect final hosts unless the final hosts eat second intermediate hosts. Therefore, at some scale, trematodes in snails rely upon the presence of appropriate second intermediate hosts. Given that trematode species vary with respect to species of second intermediate hosts they use, a diverse and abundant trematode assemblage in snails not only reflects, but requires, the presence of diverse and abundant second intermediate hosts (Gardner and Campbell 1992, Huspeni et al. 2005). In Chapter 3, we find that trematode diversity and abundance have promise for indicating the diversity and abundance of large benthic invertebrates also the diversity of small benthic invertebrates. That study was performed at a fine sampling scale, using 32 sites spread across three California estuaries. We found no evidence that trematode abundance and diversity could indicate that of fish assemblages at that scale. We predicted that trematode abundance truly could indicate fish abundance, but that fish likely move around too
much for snapshot techniques used at small scales to adequately measure fish assemblages for those small scales. Thus, our inability to detect associations between trematodes and fishes was due to inadequate quantification of fishes. Consequently, we predicted that increasing the scale of sampling may reveal the predicted associations between trematode and fish abundance.

In Chapter 4, we increased the scale of sampling to examine associations between trematodes and free-living communities. Here, we examined associations between trematodes and fishes and benthos across habitats in three different estuaries in California and Baja California. We also built upon previous work in three additional ways. Firstly, we examined the effect of using trematode assemblages in different size class snails. Overall, we found that various measures of trematode assemblages in the most common and wide-spread size classes of snails had the strongest associations with parallel measures of free-living assemblages. This increases the value of trematodes as a bioindicator tool, easing sampling and being more applicable in more places than if the strongest associations were found in the rarer size class snails. Secondly, we tested hypotheses about whether trematode assemblages provide information about the taxonomic composition of surrounding free-living assemblages. We found that trematodes do appear to indicate the abundance of certain taxa, particularly fishes, decapods, and snails. Thirdly, we used multivariate analyses to document that trematode assemblages, as a whole, change over space in parallel with the combined fish and benthic invertebrate assemblage.
This suggests that in situations lacking adequate support to monitor the all of the various free-living assemblages, larval trematodes in snails can provide a relatively inexpensive bioindicator tool for surveillance of community change.

In Chapter 5, we provide evidence suggesting that trematodes can serve as indicators in ecosystems other than estuaries. I reanalyzed data from two published studies, one in the Chilean rocky intertidal zone, and another from an African rift lake. In both cases, we found that trematode prevalence was positively related to some element of surrounding diversity. This supports the idea that, in a time when the importance of biodiversity monitoring is escalating (e.g., Noss 1990, Dobson 2005), the widespread use of trematodes as bioindicators should be seriously considered.

An additional step in using trematodes as bioindicators is to enable people to use the tool. This requires two things. First, it is necessary to indicate which snail-trematode systems to use as bioindicators. In Huspeni et al. (2005), we provide a table identifying numerous snail-trematode systems in estuaries throughout the world with promise for use as indicators. Also helpful to facilitate the use of trematodes as bioindicators is the creation of keys to identify trematode species to be used as indicators. In Chapter 5, I provide species descriptions and an annotated key to identify eight species of trematodes in a promising snail-trematode indicator system that is widespread on the eastern coast of Asia.
What work remains to be done to assess and develop the use of larval trematodes as bioindicators? One important issue is an evaluation of how well trematodes integrate the longer-term condition of ecosystems. Infections in long-lived snails accumulate over time, and potentially provide a type of information not possibly provided by direct quantification of free-living assemblages. An additional factor in the development of trematodes as bioindicator tools must take place after we have analyzed the strength of ecological associations between trematodes and free-living communities. We need cost-benefit analyses to evaluate the use of the indicator versus direct quantification of free-living assemblages. Such analyses must explicitly consider the error associated in estimates of both trematodes and free-living assemblages. The data necessary to perform these analyses is only now becoming available (largely provided in the following chapters). It will be interesting to see how things turn out.
Table 1. Examples of numbers of replicate sites used in various studies to characterize an entire wetland.

<table>
<thead>
<tr>
<th>Wetland</th>
<th>No. sampling stations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malibu Lagoon</td>
<td>5</td>
<td>Manion &amp; Dillingham, eds (1989)</td>
</tr>
<tr>
<td>Ballona Lagoon</td>
<td>2</td>
<td>MEC report</td>
</tr>
<tr>
<td>Ballona Wetlands</td>
<td>7 14</td>
<td>Schreiber, ed (1982)</td>
</tr>
<tr>
<td>Anaheim Bay</td>
<td>1 0</td>
<td>Levin et al.(1998)</td>
</tr>
<tr>
<td>Bolsa Chica</td>
<td>1 0</td>
<td>Levin et al.(1998)</td>
</tr>
<tr>
<td>Upper Newport Bay</td>
<td>1 0</td>
<td>Levin et al.(1998)</td>
</tr>
<tr>
<td>Los Peñasquitos Lagoon</td>
<td>5 5</td>
<td>See Desmond et al.(2002).</td>
</tr>
<tr>
<td>Mission Bay</td>
<td>1 0</td>
<td>Levin et al.(1998)</td>
</tr>
<tr>
<td>Paradise Creek Marsh</td>
<td>1 0</td>
<td>Levin et al.(1998)</td>
</tr>
<tr>
<td>Sweetwater Marsh</td>
<td>6 6</td>
<td>See Desmond et al.(2002).</td>
</tr>
<tr>
<td>Tijuana Estuary</td>
<td>4 3</td>
<td>See Desmond et al.(2002).</td>
</tr>
</tbody>
</table>

Note: birds are not included here since they are generally not quantified at the small scale within wetlands. This is because the high vagility of birds precludes adequate sampling with at small scales using standard techniques.
Table 2. Comparison of some estuarine community sampling methodologies. Table is modified from one presented in Huspeni et al. (2005).

<table>
<thead>
<tr>
<th>Community of interest</th>
<th>Investigator identification skill*</th>
<th>Sampling method</th>
<th>Primary gear required</th>
<th>Degree of habitat damage</th>
<th>Number of visits required</th>
<th>Estimated per site person hours per visit$</th>
<th>Field</th>
<th>Lab</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishes</td>
<td>low</td>
<td>Seining</td>
<td>Seine, blocking nets</td>
<td>High</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trapping</td>
<td>Fish traps</td>
<td>Low</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Benthic infauna</td>
<td>low</td>
<td>Large cores</td>
<td>Corers, wide mesh sieves</td>
<td>High</td>
<td>1</td>
<td>6 to 8</td>
<td>0</td>
<td>6 to 8</td>
<td>6 to 8</td>
</tr>
<tr>
<td>(large)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benthic infauna</td>
<td>high</td>
<td>Small cores</td>
<td>Corers, small mesh sieves, microscopes</td>
<td>Low</td>
<td>1</td>
<td>0.5</td>
<td>10 to 30</td>
<td>11 to 31</td>
<td></td>
</tr>
<tr>
<td>(small)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birds</td>
<td>moderate</td>
<td>Single bird survey</td>
<td>Binoculars, spotting scope</td>
<td>Low</td>
<td>1</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monthly bird survey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bird video</td>
<td>Video equipment</td>
<td>Low</td>
<td>30$</td>
<td>0.5$</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Larval trematode survey</td>
<td>Calipers, mesh bags, hammer, microscope</td>
<td>Low</td>
<td>1</td>
<td>0.5</td>
<td>3</td>
<td>3.5</td>
<td></td>
</tr>
</tbody>
</table>

$Person hours equal the number of workers multiplied by number of hours required to sample a site per visit.

*The relative amount of training necessary to identify species, given reference guides.

$This is for sampling two seasons, with 15 visits each season.

$Field time for video method also includes equipment set-up and removal (divided by 30 visits).
Chapter 2

Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts

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ABSTRACT

An unappreciated facet of biodiversity is that rich communities and high abundance may foster parasitism. For parasites that sequentially use different host species throughout complex life cycles, parasite diversity and abundance in “downstream” hosts should logically increase with the diversity and abundance of “upstream” hosts (which carry the preceding stages of parasites). Surprisingly, this logical assumption has little empirical support, especially regarding metazoan parasites. Few studies have attempted direct tests of this idea and most have lacked the appropriate scale of investigation. In two different studies, we used time-lapse videography to quantify birds at fine spatial scales and then related bird communities to larval trematode communities in snail populations sampled at the same small spatial scales. Species richness, species heterogeneity, and abundance of final host birds were positively correlated with species richness, species heterogeneity, and abundance of trematodes in host snails. Such community-level interactions have rarely been demonstrated and have implications for community theory, epidemiological theory, and ecosystem management.
1. INTRODUCTION

A rafting trip down the Congo will expose you to more parasitic diseases than a float down the Colorado. Although many factors drive such regional differences, this is partly an example of how parasitism, the most popular lifestyle on Earth (Price 1980; Thompson 1994; Poulin & Morand 2004), should be fostered by high host diversity and abundance. This makes sense, considering that hosts serve as both habitat and dispersal agents for parasites. For example, when transmission is density-dependent, an abundance of hosts should lead to an abundance of parasites. Further, because parasites tend to be host specific, increased species heterogeneity of host communities can facilitate increased species heterogeneity of parasite communities. Species richness, perhaps the key measure of biodiversity, is a function of individual abundance and species heterogeneity, and a high richness of hosts should contribute to a high richness of parasites.

These predictions may be specifically applied to parasites with complex life cycles that sequentially use different host species. The diversity and abundance of infection in “downstream” host species should logically increase with the diversity and abundance of “upstream” host species (upstream hosts carry stages of parasites that subsequently infect downstream hosts (Combes 1991)). A good example of this may be seen with trematode flatworm parasites and their hosts. Because various adult trematodes use different vertebrates as final hosts and trematode offspring infect snails as 1st intermediate hosts, snails should be at higher risk of infection by more species of trematode where vertebrate hosts are more abundant and more diverse.
Though perfectly logical, these assumptions have little empirical support. Are linkages between hosts so diffuse that spatial patterns break down? Or, have studies lacked the power or the appropriate scale of investigation to reveal existing trends? If patterns are found, are they general for the link between host diversity and parasitism?

It is a commonly held tenet that final host distribution governs larval trematode recruitment to snails, particularly regarding systems where final hosts are birds (e.g., Hoff 1941; Cable 1956; Cort et al. 1960; Robson & Williams 1970; Sousa 1993; Keas & Blankespoor 1997; Bustnes & Galaktionov 1999; Marcogliese et al. 2001; Skirnisson et al. 2004). This is because highly motile upstream host birds obviously vary in spatial distribution and, consequently, so should infections of larval trematodes in downstream host snails. However, most authors have based these logical assertions on circumstantial information with no data analysis. This is probably because it is difficult to relate the results of bird surveys to data on trematode parasites in snails. Since birds are highly vagile, typical bird surveys are usually performed at large spatial scales, such as several square kilometers (e.g., Ramer et al. 1991). On the other hand, since snail hosts are relatively dense and move little, larval trematode communities are usually sampled at much smaller scales, such as one to tens of square meters. However, as noted by Robson and Williams (1970), fine-scale heterogeneity of birds may be important for the distribution of trematode infections in snails, but the bird community observed over several square kilometers may not reflect the bird community’s use of any single small site within
that area. Thus, sampling upstream hosts and parasites in downstream hosts at
different spatial scales may obscure associations between the two communities.

Here, we report results from two independent observational studies conducted over
two years in a California coastal wetland. We circumvented the problem of assessing
highly vagile birds by using time-lapse videography to document bird use
simultaneously at numerous sites at small spatial scales. We then examined the
relationship between communities of birds and communities of larval trematodes in
snail populations collected at the same spatial scales. This allowed effective tests
with results supporting the hypothesis that host diversity begets parasite diversity.

2. METHODS

(a) Host-parasite system

In Californian tidal wetlands, various species of final host birds transmit more than 18
species of larval trematode to their 1\textsuperscript{st} intermediate host, the California horn snail,
\textit{Cerithidea californica} (Haldeman 1840) (Martin 1972). Infections in these abundant
snails are long-lived (Kuris 1990; Sousa 1993) and the trematodes continuously
asexually produce swimming stages that infect various 2\textsuperscript{nd} intermediate hosts. The
trematodes infect and then mature in birds that prey upon infected 2\textsuperscript{nd} intermediate
hosts (for a list of known hosts, see Huspeni & Lafferty 2004). Adult trematodes
produce eggs and larvae (miracidia) that reach the outside environment with the bird
excreta. These eggs and larvae are the trematode stages infective to snails.
(b) **Field sites**

Field work took place in two types of habitat in Carpinteria Salt Marsh (34°24’N, 119°32’W), California, USA. This 93 ha coastal wetland has a plain of vegetated salt marsh that is broken up by numerous tidal channels and pans (unvegetated shallow depressions). Birds often forage in channels and pans, and these habitats commonly support populations of 1st intermediate host snails. We chose 6 channel sites that have been the subjects of continuing long-term ecological monitoring projects. Channel sites were interspersed throughout the entire wetland, were situated in different tidal channels, and ranged in width from 2.8-7.7 m. We selected 7 pan sites to ensure interspersion throughout the wetland. The pans varied in area from 34-195 m².

Certain environmental factors may affect rates of parasitism in snails and potentially need to be controlled for in analyses. Benthic communities vary with environmental factors such as sediment grain size, sediment organic content, and tidal flushing (e.g., Bloom *et al.* 1972; Zedler *et al.* 1992). For channel sites, we measured channel width as a proxy for tidal flushing (which also correlates with sediment grain size (Zedler *et al.* 1992)). For pan sites, we determined the sediments’ percent mass sand and percent mass organic content. We collected sediment samples by taking 10 regularly interspersed 2.5 cm wide by 6.5 cm deep cores, which were frozen until analysis. In the laboratory, percent sand was determined by washing
sediments through a 63 μm mesh sieve and percent organic content was quantified by combusting samples at 475 °C for 12 hours.

(c) Birds and time-lapse videography

Accurately assessing the bird communities at small spatial scales is difficult because birds are extremely patchy in space and time. We therefore used time-lapse videography to quantify birds, using one camouflaged video camera at each plot simultaneously. Each camera was oriented so that a 3 meter stretch of habitat filled the top of the field-of-view. We determined the actual area of the plot visible to the camera using field measurements inputted into ImageJ 1.27 software (2002 NIH). Plot areas ranged between 13.2-19.6 m² for channels and 12.2-34.4 m² for pans. To capture seasonal variation, we sampled each habitat type throughout one winter month and one spring month (channels in November 2001 and May 2002, and pans in January-February and April-May 2003). Video cameras sampled during all tidal levels by running 5 sec min⁻¹ every minute for up to 12 hours day⁻¹. Thus, channel sites were sampled for 346.9 ± 13.4 (± s.d.) hours and pans for 268.8 ± 21.8 hours. An analysis of this videography technique, including a comparison to typical bird surveys, will be published elsewhere (Hechinger and Lafferty, unpublished data).

We recorded the presence and species identity of birds by inspecting the video tapes in the lab. The bird community at a site was thereby characterized using 20,813 ± 801 (± s.d.) video clips for channels and 16,128 ± 1306 video clips for pans.
(d) *Trematodes in snail populations*

We assessed the trematode communities in snails at the same 13 sites that we assessed the bird communities. We sampled snail populations from channels in February-March 2002 and from pans in July 2003, using three parallel belt transects composed of adjacent 10x50 cm quadrats. We measured, to the nearest 0.1 mm, the length of each snail found in the transects to generate size-frequency distributions. To assess the trematode community at each site, we randomly sampled 100 snails of the most common size class of each habitat type: 20-25 mm snails from channels and 25-30 mm snails from pans. We sampled a narrow size-range from each habitat because trematode species richness and infection abundance increase with snail size (Sousa 1983). We dissected the 1,300 snails in the lab to quantify trematode infections. We identified trematodes to species following Martin (1972) and Huspeni and Hechinger (*unpublished manuscript*).

(e) *Community measures*

(i) *Abundance*

For each site, we calculated bird abundance as the number of birds observed per meter squared per hour. We measured parasite abundance as the mean number of trematode infections per snail (terminology following Bush et al. (1997)). However, post-recruitment competitive loss can influence larval trematode communities in snails (Kuris 1990; Kuris & Lafferty 1994; Lafferty *et al.* 1994). Thus, the observed abundance of trematode infections will underestimate actual trematode recruitment to
snail populations. Therefore, to more accurately estimate recruitment of trematodes, we also calculated “pre-interactive” abundances of trematodes using the techniques outlined in Lafferty et al. (1994).

(ii) Heterogeneity

As a measure of local species heterogeneity of birds and trematodes, we used estimates of the “Fundamental Biodiversity Number” (Hubbell 2001). Hubbell (2001) derived the relationship $S = (1 + \theta \cdot \ln(1 + (J - 1)/\theta))$, where $S$ is species richness, $J$ is the number of individuals sampled, and $\theta$ is the Fundamental Biodiversity Number. Although not standardly used as a measure of local species heterogeneity, estimates of $\theta$ should serve well in this capacity, since, like Fisher’s $\alpha$ and Simpson’s index, it increases with the slope of species-individual accumulation curves.

To calculate $\theta$, we used the same general procedure for both bird and trematode communities. First, we generated randomized species accumulation curves (Gotelli & Colwell 2001) for each site by plotting the average number of species observed for various numbers of individual bird or trematodes observed (from 1 individual up to the maximum sampled for each site). The averages for number of species observed per number of individuals sampled were determined by permuting the data and resampling 100 times for each site. We then generated predicted species accumulation curves using Hubbell’s equation. We iteratively solved for the value of $\theta$ that resulted in the best fit of the curve to the data (the best fit was determined by
minimizing the sum of the squared deviations on log transformed abundance data).
One pan site was excluded from the heterogeneity analysis because it had so few
birds (5 individuals) that it was not possible to obtain a precise estimate for $\theta$.

(iii) Species Richness
Species richness may be measured using either observed values or some sort of
parametric or non-parametric estimator (Colwell & Coddington 1994; Magurran
2004). Species richness estimators are based on various assumptions. Even non-
parametric estimators assume the existence of relationships between the number of
observed rare species and the number of unobserved species. These assumptions may
not apply in the same way to bird communities and larval trematode communities in
snails. Thus, we decided to use observed species richness rather than a species
richness estimator. However, because observed species richness increases with
sampling effort, we standardized our effort when calculating the number of bird and
trematode species seen at a site. For birds, since there was variation in the amount of
time sites were video sampled, we used the average bird species richness observed in
1000 standard-sized random subsamples of the data for each site. The size of the
subsamples was standardized for each habitat type as the amount of time that
characterized the site with the smallest amount of sampling (19,983 minutes for
channels and 13,854 minutes for pans). For trematodes, observed species richness at
each site was standardized by counting the number of trematode species found in the
standard-sized sample of 100 snails.
(1) Analyses

We investigated all associations using product-moment correlation coefficients (r). We examined the relationships between bird abundance and trematode abundance in snails (using both observed and pre-interactive trematode abundances), between bird species heterogeneity and trematode species heterogeneity, and between bird species richness and trematode species richness in snails.

To determine whether the environmental factors, channel width and sediment character, were important variables affecting parasite abundance, we assessed whether there were relationships between trematode abundance and channel width, and between trematode abundance and percent sand and percent organics at pan sites. Also, to insure that our measurements of bird species richness were not confounded by varying sample plot size, we examined the effects of video plot size on observed species richness.

Although we ensured high interspersion of sites, spatial autocorrelation could still have resulted in non-independence of the data. Thus, we calculated the exact probability of a site being more similar to its nearest neighbor in the value of a variable than expected by chance (based upon complete enumeration of the similarities between all non-nearest neighbors for the appropriate habitat and measured variable).

We obtained one-tailed $p$-values for correlations by generating the null distribution of $r$ by randomly permuting the data 100,000 times (Edgington 1995), using the
Resampling Stats Excel Add-in 2.0 (2001 Resampling Stats, Inc.). When we could not specify the direction of a test \textit{a priori}, two-tailed \(p\)-values were similarly generated using |\(r|\). Since sampling timing, bird sampling effort, and snail size varied between channels and pans, we did not pool channel and pan data. Instead, we analyzed each habitat separately and, to assess the overall statistical support for the observed trends, we combined \(p\)-values (weighted by sample size) using the "z-transform" procedure (Strube & Miller 1986; Rice 1990).

3. RESULTS

Bird abundance and trematode abundance varied among sites within both channels and pans and, overall, were significantly positively correlated with each other (respectively, \(r = 0.69, n = 6, p = 0.15\) and \(r = 0.62, n = 7, p = 0.076\); combined \(p = 0.039\); figure 1). Similar results were seen when we used estimated pre-interactive trematode abundances (\(r = 0.72, n = 6, p = 0.15\) and \(r = 0.64, n = 7, p = 0.064\), respectively; combined \(p = 0.034\)). These results were not confounded by width of channels or variation in pan sediment character since there was no relationship between trematode abundance and channel width (\(|r| = 0.15, n = 6, p = 0.81\) or between trematode abundance and percent sand or percent organics (respectively, \(|r| = 0.036, n = 7, p = 0.97\) and \(|r| = 0.050, n = 7, p = 0.88\)). Also, spatial non-independence was not problematic since sites were not more similar to nearest neighbors than non-nearest neighbors in either habitat for bird or trematode abundance measures (all \(p > 0.05\), mean \(p = 0.44 \pm 0.32\) s.d.).
The species heterogeneity of bird and trematode communities also varied among sites in both studies. Bird species heterogeneity was strongly positively correlated with trematode species heterogeneity in both channels and pans (respectively, \( r = 0.95, n = 6, p = 0.012 \) and \( r = 0.81, n = 6, p = 0.029 \); combined \( p = 0.0017 \); figure 2). Spatial auto-correlation was not a problem, since sites were not more similar to nearest neighbors than non-nearest neighbors for bird or trematode heterogeneity in either habitat (all \( p > 0.11 \), mean \( p = 0.49 \pm 0.31 \) s.d.).

The species richness of bird and trematode communities varied among sites in both studies. Bird species richness was strongly positively correlated with trematode species richness in both channels and pans (respectively, \( r = 0.88, n = 6, p = 0.017 \) and \( r = 0.79, n = 7, p = 0.021 \); combined \( p = 0.0017 \); figure 3). Bird species richness was not confounded by variation in area of the plot sampled in channels or pans (respectively, \(|r| = 0.45, n = 6, p = 0.20 \) and \(|r| = 0.02, n = 7, p = 0.52 \); combined \( p = 0.31 \)). Spatial auto-correlation was not problematic, since there was no suggestion that sites were more similar to nearest neighbors than non-nearest neighbors in both habitats regarding observed species richness (all \( p > 0.16 \), mean \( p = 0.56 \pm 0.23 \) s.d.).

4. DISCUSSION

Findings from our two studies support the logical expectation that the spatial distribution of upstream hosts drives patterns of parasitism in downstream hosts. Host abundance influences parasite abundance, host heterogeneity facilitates parasite heterogeneity, and therefore host species richness begets parasite species richness.
This is the first time, to our knowledge, that the diversity of an upstream host community has been shown to be associated with the diversity of parasites in a downstream host population.

Only two previous studies have explicitly examined the relationship between bird communities and larval trematode communities in snails, yielding mixed results. Kube et al. (2002) characterized the bird use of an area by surveying birds over 5-10 km², while characterizing the snail trematode community in a restricted area, using “a few sweeps of a dip net”. The disparity between the scale of sampling for birds and snail hosts may explain why they found no association between bird abundance and trematodes in snails. Disparity in sampling scale may also explain why Latham and Poulin (2003), found only weak evidence for an association between final host birds and larval parasites in crabs (studying operationally similar acanthocephalan parasites). In the other previous study explicitly examining the relationship between birds and trematodes in snails, Smith (2001) sampled birds and larval trematodes in snails at the same spatial scale. She tackled the problem of quantifying vagile birds at small spatial scales by sampling replicated sites of varying number of naturally occurring bird perches. She found a positive association between bird abundance and trematode prevalence in snails. However, she did not investigate associations between bird diversity and trematode diversity.

We were able to uncover relationships between the diversity and abundance of upstream host birds and larval parasites in downstream host snails by using time-lapse videography to sample the two communities at the same spatial scale. The
associations between bird communities and parasite communities in snails were consistently in the same direction in both habitats sampled. The interesting outlier data point driving the positive correlation between channel bird abundance and trematode abundance in snails (figure 1a) occurs exactly where predicted. That is, the channel site that had by far the greatest bird abundance also had the greatest trematode abundance in snails. When such outliers occur, the best solution is to gather more data or do another study. This is what we did by studying pans, yielding results that further bolstered the hypothesis of a positive association between bird abundance and trematode abundance in snails (figure 1b).

Although we assessed the potential influence of some environmental conditions, some unmeasured factors may have affected spatial variation of parasitism in our systems. For example, temperature stress, ultraviolet radiation, and pollution may affect trematode survival and ability to infect snails (reviewed by Pietrock & Marcogliese 2003). It is possible these stresses varied between sites. However, our analysis demonstrated that nearest neighbor sites were not more similar to one another than expected by chance. Thus, we feel that it is unparsimonious to assume that birds, the known sources of trematode stages infectious to snails, were truly unrelated to trematode communities in snails and that some potential unknown environmental effect on parasitism spuriously covaried with bird abundance, heterogeneity and species richness.

Another possible explanation for residual variation in our results could be nocturnal final hosts, which were not adequately sampled with our videography.
technique. However, in our study system, there are primarily only two potential nocturnal final hosts: black-crowned night herons (*Nycticorax nycticorax*) and raccoons (*Procyon lotor*). Raccoons are almost certainly not important for our study because they defecate in only a few localized supra-tidal areas, none of which were at our study sites.

The flip-side of the relationships we studied should also occur. That is, birds as *downstream* hosts should be more frequently infected by more types of trematodes in areas where prey is more abundant and diverse. This is because many prey are *upstream* hosts for trematodes that are trophically transmitted to birds. Transmission to birds will often be more efficient in areas with greater prey (2*nd* intermediate host) density. Also, since trematode parasites differ in what host species they use, areas with more types of prey should support a greater diversity of trematodes. Indeed, there is some evidence that hosts with a more diverse diet are parasitized by more species of parasite (see review in Combes 2001). This may explain why communities of trematodes in gulls appear to be more similar in wetlands that share certain physical and biological characteristics (Simkova *et al.* 2003).

Further consideration of our findings illustrates that the distribution of an upstream host community can not only drive parasite community structure in downstream hosts, but can also be indirectly responsible for the distribution of varied and profound impacts of parasitism on several downstream host communities. It is generally recognized that species may significantly affect one another via indirect interactions, such as “apparent competition” (Holt 1977) and “parasite mediation”
(Price et al. 1986). Our work demonstrates that bird communities indirectly impact apparently unassociated snail populations by driving levels of trematode parasitism in snails. Trematode parasitism can strongly affect the ecological and evolutionary dynamics of snail populations since trematodes castrate, and can increase mortality in, snail 1st intermediate hosts (Kuris 1973; Sousa 1983; Lively 1987; Sousa & Gleason 1989; Lafferty 1993a; Lafferty 1993b). Further, birds may also indirectly affect fish and benthic invertebrates, because trematodes may also strongly impact these 2nd intermediate hosts, for example, by greatly increasing the rate of predation on infected individuals (e.g., Lafferty & Morris 1996).

Spatial variation in infection caused by the distribution of upstream hosts may also be extremely important for post-recruitment dynamics in parasite communities. For instance, spatial heterogeneity of trematode infection often increases competitive interactions between trematode species in snails (Kuris & Lafferty 1994). Lafferty et al. (1994) demonstrated that such intensification of interspecific competition is an important factor affecting parasite community structure in Carpinteria Salt Marsh. Our findings indicate that uneven use of the wetland ecosystem by birds drives the spatial heterogeneity in infection that causes this intensification of interspecific competition. Indeed, at our study sites, bird abundance and species richness both positively and significantly correlated with the proportion of trematodes lost to interspecific competition in snail populations (Hechinger and Lafferty, unpublished data).
Our results also strongly support exploring the use of trematode parasites as biomonitoring tools. Trematode communities in snails are common throughout the world (Yamaguti 1975; Kuris & Lafferty 1994; Poulin & Mouritsen 2003) and are easy to assess (Huspeni et al. 2005). If trematodes reflect the diversity and dynamics of surrounding free-living communities, they may be extremely useful indicators of ecological condition. Huspeni and Lafferty (2004) recently used trematode communities in California horn snails to evaluate the ecological effects of a wetland restoration project. They found that trematode abundance and species richness increased after the restoration and suggested that this occurred because more birds and more species of birds used the wetland following the restoration. Our findings support the basic reasoning behind this assertion. Further, we recently found correlations between the diversity and abundance of benthic invertebrates and trematodes in snail populations (Hechinger et al. unpublished manuscript). We expected this primarily because birds prey upon benthic invertebrates, and areas with greater diversity and abundance of benthos should attract a greater diversity and abundance of birds, which consequently bring a greater diversity and abundance of trematode stages infectious to snails (as documented in this report). As an indication of their value as biomonitoring tools, in our study trematode communities were much less time consuming to assess than were birds; collecting and processing trematode data averaged 4.6 person-hours per site, while collecting and processing bird data averaged 53.2 person-hours per site. We are currently performing a full quantitative
analysis of the cost-effectiveness of using trematodes as bioindicators compared to alternative methods of assessing ecosystem diversity.

At first sight, our finding that host diversity begets parasite diversity might seem to contradict results from research on a tick-transmitted disease. In the eastern United States, the risk of human exposure to Lyme disease decreases with increasing species richness of small mammals (e.g., see Ostfeld & Keesing 2000). Ixodid ticks vector Lyme disease (a spirochete bacterium) to humans only after first becoming infected by feeding on a competent reservoir host. Since the bacterium thrives in only a few host species, greater mammal richness increases the proportion of incompetent hosts that ticks feed on. This lowers the prevalence of the bacterium in ticks, which decreases the abundance of Lyme disease in humans. However, increasing the diversity of competent hosts (e.g., ground-nesting birds), apparently increases the abundance of Lyme disease (Ostfeld & Keesing 2000). Of course, increasing the diversity of any type of host will increase the diversity of parasites in general (although the abundance of a particular pathogen may decrease). In other words, there is no contradiction between what might superficially appear to be opposite findings.

5. CONCLUSION

Our work demonstrates consistent, positive, and significant associations between final host bird communities and trematode communities in intermediate host snail populations. Such a link is expected because upstream host birds are the source of
trematode eggs and larvae, which infect downstream host snails. Our work, along with Smith (2001), shows that abundant host bird communities drive recruitment of abundant larval trematode communities in host snails. We further demonstrate that species heterogeneity of bird communities leads to heterogeneity of parasite communities in snails. High abundance and heterogeneity increases species richness and this explains our findings that species rich bird communities are associated with species rich trematode communities. Thus, our work demonstrates, for the first time to our knowledge, that diverse upstream host communities can drive the development of diverse parasite communities in downstream host populations.

ACKNOWLEDGEMENTS

We thank A. Kuris, T. Huspeni, M. Rigby, K. Whitney, V. McKenzie, A. Mora, K. Phillips, N. Talhouk, J. Yee and several anonymous reviewers for helpful comments; A. Rice and D. Eghbali for assistance viewing videos; T. Huspeni for help with snail dissections; C. Boch for field and laboratory assistance; and W. Rice for statistical advice. We thank the University of California (UC) Natural Reserve System’s Carpinteria Salt Marsh Reserve, and the homeowners’ associations of Sandyland Cove and Sandpoint Road, for access to protected wetland habitat. This research received financial support from the UC Coastal Environmental Quality Initiative; the UC Toxic Substances and Research Program; the US EPA (EPA/R-82867601); and the NIH/NSF (DEB-0224565).
Figure 1. Positive correlations between abundance of trematodes in snail populations (mean number of individual infections per snail) and abundance of birds (# m$^{-2}$ h$^{-1}$) across sites in (a) channels, with 20-25mm snails and (b) pans, with 25-30mm snails ($r = 0.69$ and $r = 0.62$, respectively; combined $p = 0.039$).
Figure 2. Positive correlations between species heterogeneity (θ) of trematodes in snail populations and species heterogeneity of birds, across sites in (a) channels, with 20-25 mm snails, and (b) pans, with 25-30 mm snails ($r = 0.95$ and $r = 0.81$, respectively; combined $p = 0.0017$).
Figure 3. Positive correlations between species richness of trematodes in snail populations and species richness of birds, across sites in (a) channels, with 20-25mm snails, and (b) pans, with 25-30mm snails ($r = 0.88$ and $r = 0.79$, respectively; combined $p = 0.0017$).
Chapter 3

Can parasites indicate free-living diversity? Relationships between the species richness and abundance of larval trematodes with that of local benthos and fishes

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Abstract

Measuring biodiversity is difficult. This has spawned efforts to seek taxa whose species richness correlates with the species richness of other taxa. Such indicator taxa could then reduce the time and cost of assessing the biodiversity of the more extensive community. However, the search for species richness correlations has yielded mixed results. This may primarily be due to the lack of functional relationships between the taxa studied. Trematode parasites are highly promising bioindicators. Diverse assemblages of larval trematode parasites are easily sampled in intermediate host snails. Through their life cycles, these parasites are functionally coupled to the surrounding free-living diversity of vertebrate and invertebrate animals. Larval trematodes in snails have been demonstrated to positively correlate with bird diversity and abundance. Here, we explore whether trematodes also correlate with standard measures of fishes, large and small benthos, across 32 sites in three wetlands. We found associations between trematodes and benthic communities that were not consistent across wetlands. However, the associations were consistently positive for large benthic species richness and density. Additionally, some of the contrasting associations between trematodes and benthos may be explained by negative associations between large and small benthos. We found no associations with fish communities (likely due to the inadequacy of standard 'snapshot' sampling methodologies for highly mobile fishes). The results support further exploration of trematodes as bioindicators of diversity and abundance of animal communities.
Introduction

People love shortcuts. Scientists and habitat managers are not immune to this, particularly given the limited time and resources available to accomplish difficult tasks, such as assessments of biodiversity. In these assessments, we often want information about multiple taxa from several replicated sites. Yet, measuring the biodiversity of entire communities is extremely difficult or impossible. This difficulty has spawned searches for indicator taxa whose species richness is consistently correlated with the species richness of other taxa (e.g., Lawton et al. 1998; Vessby et al. 2002; Olsgard et al. 2003; Kati et al. 2004). Varied results from this search led Gunnarsson et al. (2004) to suggest focusing on groups of organisms that provide habitat or resources for other groups of organisms. Because these organisms are functionally coupled, correlations between the condition (e.g., diversity and abundance) of these groups would tend to be more consistent than those between taxa without such direct connections. But, the problem here is that the presence of the indicator group (the providers of habitat or resources) does not necessitate the presence of the groups to be indicated (the users of the habitat or resources). However, the converse situation does not suffer from this weakness. Organisms that obligatorily use other organisms as habitat or resources should be excellent indicators. The indicator groups directly depend upon the condition of the groups to be indicated. Many parasites have multiple-host life cycles that necessarily link them to several different taxa of surrounding animal communities. Hence, these sorts of parasites
may provide excellent indicators of other components of community structure and function (Gardner and Campbell 1992; Marcogliese and Cone 1998). Here, we explore how an easily sampled community of trematode parasites in snails is associated with more difficult to sample communities of fishes and benthic invertebrates. The discovery of relationships between these parasites and surrounding free-living community components would strongly support further exploration of these parasites as bioindicators.

Trematode flatworm parasites are a particularly promising indicator group (Kuris and Lafferty 1994; Huspeni and Lafferty 2004; Huspeni et al. 2005). This is because it is common for many trematode species to specialize on the same snail species as 1st intermediate host (where they can be efficiently sampled), but diverge as to what other hosts they use to complete their life cycles (where they are associated with many species at higher trophic levels) (Fig. 1). In these cases, a high species richness of parasites in snail hosts requires, and thus indicates, the presence of numerous other taxa (i.e., the hosts required for other parts of the life cycles).

Further, most trematodes are trophically transmitted to final hosts in their life cycles. Thus, not only do such parasites reflect the presence of surrounding taxa, they also directly indicate functioning trophic linkages (Gardner and Campbell 1992; Marcogliese and Cone 1998).
In many aquatic and marine systems, birds act as important hosts for adult
trematodes, and are the sources of trematode stages that infect snails (Fig. 1).
Hechinger and Lafferty (2005) demonstrated positive correlations between the
diversity of birds and diversity of trematodes infecting snail populations. This pattern
likely exists because various trematode species use different bird species as final
hosts. Additionally, Smith (2001), Hechinger and Lafferty (2005), and Fredensborg
et al. (2006) found positive associations between the abundance of birds and the
prevalence of trematode parasites in snails. This was expected because birds should
transmit more trematodes to snails in areas where birds are more common.
Trematodes make good indicators of birds because accurately quantifying bird
communities at small spatial scales and long time scales is difficult (Hechinger and

In this study, we investigate whether, like birds, local fish and benthic
invertebrate communities are positively associated with local trematode communities
in snails. This prediction has two general underpinnings (Huspeni et al. 2005). First,
fishes and benthic invertebrates attract birds (Colwell and Landrum 1993; Gawlik
2002), which are the sources of trematode stages that infect snails. Thus, sites with
greater species richness and abundance of fishes and benthos should have greater
richness and abundance of birds. Consequently, this brings a greater richness and
abundance of trematodes to infect snails. Secondly, as mentioned above, different
trematode species use different fishes and benthic invertebrates as 2nd intermediate
hosts. Therefore, a diverse and abundant trematode community in snails does not merely reflect, but at some scale requires, the presence of diverse and abundant fishes and benthic invertebrates. We also predicted, since we assessed benthic invertebrates and fishes using standard “snap-shot” techniques, that trematodes would most strongly associate with the more stationary component of the community—that is, the benthos—versus the vagile fishes. Determining if ecological relationships exist between trematodes and free-living communities is a critical step in assessing their value as bioindicators (see McGeoch 1998). Here, we find associations that demonstrate we should further investigate the use of larval trematodes as indicators of surrounding free-living species richness and abundance.

Materials and methods

Study system

We study a community of 20 trematode species that complete their life cycles (Fig. 1) in Pacific tidal wetlands of California and Mexico. These trematodes parasitize populations of the California horn snail (Cerithidea californica (Haldeman 1840)), as 1\textsuperscript{st} intermediate host (Martin 1972 (and references therein); Sousa 1983; Kuris 1990; Sousa 1990). Infections in snails are generally long-lived (Sousa 1983; Kuris 1990; Sousa 1990). The parasites continually asexually produce swimming stages (cercariae), which leave their host snail to encyst in, or on, 2\textsuperscript{nd} intermediate hosts. Different species of trematodes infect different types of 2\textsuperscript{nd} intermediate hosts, such as fishes, clams, polychaetes, and crabs. The parasites are trophically transmitted to
final host wetland birds when the birds eat 2nd intermediate hosts (except for one of the trematode species, which lacks a 2nd intermediate host and the cercariae infect birds directly). The trematodes mature in the birds, usually in the digestive tract. Trematode eggs or larvae pass with the birds’ excreta and subsequently infect snails. Thus, although the 20 species of these trematodes diverge regarding which hosts they require to complete their life cycles, they all converge in populations of the horn snail (see Huspeni and Lafferty (2004) and Lafferty et al. (2006), for information on which 2nd intermediate and bird final hosts are used by the various trematode species).

Field sampling

We sampled fish, benthic invertebrate, and snail trematode assemblages in three California coastal wetlands (Morro Bay (35.34°N, 120.83°W), Carpinteria Salt Marsh (34.40°N, 119.53°W), and Mugu Lagoon (34.10°N, 119.10°W)) in the summers of 2001 and 2002. We sampled 20 sites in 2001 (4 at Morro, 8 at Carpinteria, and 8 at Mugu) and 12 different sites in 2002 (4 at Morro, 3 at Carpinteria, and 5 at Mugu). Each site was a 20 meter stretch of tidal creek running through pickleweed (Salicornia virginica) salt marsh. Tidal creeks ranged 2.8-29.5 m in width (mean = 10.5 m, median = 7.9 m). The sites with horn snails were selected as part of other extensive ecological projects (e.g., see the website for the Pacific Estuarine Ecosystem Indicator Research Consortium at http://www.bml.ucdavis.edu/peeir/). In 2001, the sites were selected to ensure interspersion throughout each wetland. In 2002, in each wetland, sites were positioned along one or more tidal creeks to ensure
interspersion throughout the reach of each creek. Although sites were in different tidal creeks or separated by at least 150 m, spatial autocorrelation could still have resulted in non-independence of the data. To assess whether this was a problem for measures of trematode abundance and species richness, we calculated the exact probability of each site being more similar to its nearest neighbor than expected by chance (based upon the frequency of being equally or more similar to non-nearest neighbors). Only 3/32 sites (for trematode abundance) and 1/30 sites (for trematode species richness) had nearest neighbor similarities with \( P \leq 0.05 \). Since the probability of observing this many or fewer \( P \)-values \( \leq 0.05 \) by chance alone is respectively 0.73 and 0.34 (calculated using the binomial probability function (Sokal and Rohlf 1981)), we did not consider that spatial autocorrelation was a problem.

To assess the fish community at each site, we employed a standard technique widely used to monitor coastal wetlands in southern California (PERL 1990). During mid-tidal levels, we sectioned off a 20 m stretch of channel by rapidly deploying two blocking nets. We then sequentially made five passes between the blocking nets with a 10 m two-pole seine. All nets had a mesh size of 3.2 mm. All captured fish were identified to species (following Miller and Lea 1972) and counted on-site. For each site, we calculated the species richness and density of the total fish community.

At each site, we separately characterized both large and small benthic animals. We sampled the large benthos (e.g., crabs and bivalves) using large cores, and the
small benthos (e.g., amphipods and polychaetes) using small cores. Large cores were 78.5 cm$^2$ in area, 50 cm deep and were sieved on-site through a 3 mm mesh screen. We identified to species the specimens retained on the sieves (primarily using Smith and Carlton 1975; McLean 1978; Morris et al. 1980; Coan et al. 2000). We released most animals at the site of capture, but some were returned to the lab for identification and to provide voucher specimens. We excluded taxa from the large core analysis that were primary targets of small cores (mainly polychaetes). Small cores were 19.6 cm$^2$ in area and 5 cm deep. These were fixed in 10% formalin after collection and sieved later in the lab through a 0.5 mm mesh. The contents remaining on the sieve were then stained with Rose Bengal, sorted, and the specimens were identified to lowest possible taxonomic category primarily by using the above identification guides. To position replicate cores at each site, we adopted an elevationally-stratified sampling layout widely used to monitor coastal wetlands in southern California (PERL 1990). We sampled three elevations at each site: (1) high (just below the lower limit of the pickleweed), (2) low (the deepest part of the channel), and (3) mid (the elevational mid-point). At each elevation, we took three replicates of both core types (large and small), spaced 10 m apart (in 2002, we took five replicates of each core type at the low elevation, 5 m apart). For each site, for large and small benthos, we calculated species richness ("taxonomic richness" for small cores) and total density. To control for sampling effort when calculating species richness, we ignored the two additional large cores taken at the low elevation in 2002 (but the additional cores were still used to provide improved density
estimates). Some small cores were lost, before processing, for three Morro Bay sites.
Two of these sites were excluded from all analyses, and one (for which most cores
were not lost) was still used for small benthos density estimates.

To assess larval trematode communities, we haphazardly collected 100 20-25
mm *Cerithidea californica* snails from within 10 m of each site (from within 50 m, in
a few cases, due to low snail density). Snails were dissected in the laboratory and
their infection status determined. We identified all trematodes to species, following
Martin (1972) and Huspeni and Hechinger (unpublished manuscript). For each site,
we described the trematode community by calculating species richness and the
summed prevalence of all trematode species (x100). Since we encountered multiple-
species infections in some snails, the summed prevalence of trematode species better
describes levels of trematode recruitment than does simple prevalence of infected
snails. Also, trematode assemblages in snail populations often have high levels of
interspecific competition and competitive loss (due to dominant trematode species
recruiting to, and killing, infections of subordinate species in individual snails) (Kuris
and Lafferty 1994). Thus, observed trematode prevalence may significantly
underestimate actual recruitment (because many of the subordinates have been killed
(Kuris and Lafferty 1994; Lafferty et al. 1994)). Therefore, we also calculated
summed “pre-interactive” prevalence using the techniques outlined in Lafferty et al.
(1994). The formulas, using knowledge of the trematode dominance hierarchy (Kuris
1990; Huspeni 2000), simply apply the prevalence of each trematode species in
“competitor-free” snails to the portion of the snail population infected with dominant
trematode species. This provides an estimate of how many subordinate infections
have been killed. Pre-interactive prevalence for each trematode species is then,
simply, the number of killed infections, plus the number of observed infections,
divided by the total number of sampled snails. To determine whether pre-interactive
prevalence offered additional insight, we used these values in parallel analyses to
those using observed trematode prevalences (detailed below). We excluded two sites
from species richness analyses because we were unable to sample 100 1st intermediate
host snails due to low snail abundance.

Data analysis

Our primary goal was to determine whether there were any associations between the
trematode community in snails at a site and common measures of the free-living fish
and benthic invertebrate communities at that site. Thus, we separately analyzed
relationships between the species richness and prevalence of trematodes and the three
measured free-living assemblages (fishes, small benthos, and large benthos). We
used general linear models with trematode measures as predictor variables and the
free-living measures as response variables.

We were also interested in whether relationships were consistent between
wetlands. Thus, each initial full model included, as predictor variables, the trematode
measure (species richness or prevalence), wetland (Mugu Lagoon, Carpinteria Salt
Marsh, and Morro Bay), and the trematode X wetland interaction (i.e., the initial full model fitted separate regression lines, for each wetland, between trematodes and the free-living assemblages). Following Neter et al. (1996) and Quinn and Keough (2002), when interactions were included, we used centered trematode predictor variables to eliminate problems of collinearity. We sequentially deleted interaction terms and wetland when their contributions to the model were not significant (p > 0.10). When there was an indication that the relationship between trematodes and a free-living assemblage differed between wetlands (i.e., when the trematode X wetland interaction was significant), we asked whether the two most similar wetlands should be pooled. This was done primarily to determine whether trematodes consistently indicated the free-living assemblage in those two wetlands. To do this, we performed partial $F$-tests, comparing the full model (with all three wetlands and their interaction with trematodes) to the reduced model (pooling the two most similar wetlands and maintaining a wetland X trematode interaction term) (see Neter et al. 1996; Quinn and Keough 2002). Additionally, since we sampled each of the three wetlands over two years, we determined whether the effect of wetland was influenced by year. The effect of wetland on all of the fish or benthic invertebrate measures was consistent across years (i.e., there were no wetland X year interactions, (all p values >0.20)). Thus, we did not consider year further and focused on wetland as the potential cofactor with the trematode measures.
Some of the free-living benthic species do not generally serve as hosts for any of the horn snail trematodes (but all of the fish species are potential hosts). This is particularly true for the small benthos (e.g., nematodes and insects are not trematode hosts in our system). We would expect relationships between trematodes and free-living assemblages to be strongest for those taxa that are potential hosts for trematodes. Thus, we performed parallel analyses for the large and small benthic assemblages, one set using only potential host species and another using all encountered species. We determined which species or taxa were potential hosts using the best available knowledge (published data and our unpublished data on trematode host use (see Martin 1972; Lafferty et al. 2006 and references therein).

In some cases, we examined relationships between various measures of the free-living assemblages. This was done to gain insight into potential mechanisms explaining the relationships with trematodes. These associations were analyzed using GLMs, following the same general procedure that we described above.

We ensured assumptions were met, regarding homogeneity of variance and approximate normality of residuals, by inspecting plots of residuals versus model-predicted values, and normal quantile plots with Lilliefors' curves (see Neter et al. 1996; Quinn and Keough 2002). All \( P \)-values are conservatively two-tailed (even though our hypotheses were one-tailed). Also, we focus on nominal \( P \)-values (with a critical value of 0.05 for each test), but we also considered the effect of multiple
comparisons on the family-wide error rate for the final tests in each taxon. All significant $P$-values remained significant after controlling the family-wide error rate using the sharper sequentially rejective Bonferroni procedure (Hochberg 1988). We performed all analyses using the software platform JMP v.5.1.2 (2003 SAS Institute).

**Results**

We sampled 39,930 fish comprising 18 species, 894 individual large benthic animals of 20 species, and 60,345 individual small benthic animals comprising 92 taxa (see electronic supplementary material (ESM) Table S1). Further, we dissected 3,079 snails and encountered 926 individual trematode infections belonging to 16 species (Table 1). We estimated a total pre-interactive trematode prevalence of 1,003 individuals (Table 1).

In all cases, there were positive associations between benthic species richness and density measures using potential hosts and the measures including all species (Fig. S1). These correlations were extremely (and necessarily) strong for the large benthos (Figs. S1a and S1b), since there were only a few rare species of large benthos that are not potential hosts for trematodes (Table S1). The positive relationships were also very strong small benthos densities, but less so for species richness (Figs. S1c and S1d).
Fish species richness was not associated with trematode species richness (Fig. 2a, $R^2 = 0.069, F_{1,28} = 2.09, P = 0.16, n = 30$). Neither wetland nor its interaction with trematode richness were significant factors ($F_{2,26} = 0.085, P = 0.92, \text{ and } F_{2,24} = 0.21, P = 0.81$, respectively).

The species richness of potential host large benthos positively associated with trematode species richness across all three wetlands (Fig. 2c, $R^2 = 0.43, F_{1,28} = 21.2, P < 0.0001, n = 30$). Here, neither wetland nor its interaction with trematode richness were significant factors ($P = 0.60$ and $P = 0.45$, respectively). We obtained similar results when we included non-host large benthic invertebrate species (Fig. S2a).

Our final model for potential host small benthos species richness indicated that trematode species richness was positively related to potential host small benthos richness across two wetlands and negatively in the other wetland (Fig. 2e; effect of trematode richness and its interaction: partial $R^2 = 0.41, F_{2,23} = 8.13, P = 0.0021$; full model $R^2 = 0.41, P = 0.0060, n = 27$). The initial complete model suggested that there was an interaction between wetland and trematode species richness (interaction effect, $F_{2,21} = 3.76, P = 0.070$; full model $R^2 = 0.51, P = 0.0075, n = 27$). The regression line for Morro Bay had a negative slope, while those for Carpinteria Salt Marsh and Mugu Lagoon were positive. This model (allowing separate effects and regressions for all three wetlands) was not significantly better than the final model, pooling Carpinteria Salt Marsh and Mugu Lagoon ($F_{2,21} = 2.00, P = 0.16$). We
obtained similar results when we included non-host species in the analysis (ESM S2c).

There was a positive association between large and small benthos species richness (which we examined since they both were positively related to trematode richness). We found this positive relationship whether we analyzed host species only, or all species of large and small benthos (Fig. S3). In the analysis using all benthic species (Fig. S3b), there was a significant effect of wetland, with Morro Bay having more small benthic species than either Carpinteria Salt Marsh or Mugu Lagoon (~3-13 more species, 95% confidence intervals, Tukey HSD).

Fish density was not associated with trematode prevalence (Fig. 2b; partial $R^2 = 0.0033$, $F_{1,28} = 0.092$, $P = 0.76$, $n = 32$). Here, wetland was a significant factor affecting fish density ($F_{2,28} = 3.60$, $P = 0.041$), but the wetland X trematode prevalence interaction was not significant ($F_{2,26} = 0.89$, $P = 0.42$).

Our final model for density of potential host large benthos indicated that trematode prevalence was positively related to density of potential host large benthos, strongly in one wetland, and weakly over the other two wetlands (Fig. 2d; effect of trematode prevalence and its interactions, partial $R^2 = 0.54$, partial $F_{2,28} = 16.3$, $P < 0.0001$; full model $R^2 = 0.66$, $P < 0.0001$, $n = 32$). The initial complete model showed that the relationship between large benthos density and trematode prevalence
varied across wetlands (interaction effect: $F_{2,26} = 8.0, \ P = 0.0020$; full model $R^2 = 0.67, \ P < 0.0001, \ n = 32$). The regression slope for Carpinteria Salt Marsh was strongly positive, while the slopes for Morro Bay and Mugu Lagoon were less so (Morro Bay being almost flat). Further model comparison demonstrated that Morro Bay and Mugu Lagoon could be pooled. That is, keeping all three wetlands separate did not significantly provide further explanatory power than did pooling the two similar wetlands ($F_{2,26} = 0.55, \ P = 0.59$). Inclusion of non-host large benthos species yielded similar results (Fig. S2b).

Our final model for potential host small benthos density showed that trematode prevalence associated with host small benthos density, positively at one wetland, and negatively at the other two wetlands (Fig. 2f; effect of trematode prevalence and interactions, partial $R^2 = 0.30, F_{2,26} = 5.44, \ P = 0.011$; full model $R^2 = 0.33, \ P = 0.015, \ n = 30$). The initial complete model indicated there was significant variation across wetlands with respect to the relationship between trematode prevalence and the density of small benthos (interaction effect: $F_{2,24} = 5.32, \ P = 0.012$; full model $R^2 = 0.43, \ P = 0.015, \ n = 30$). Further analysis revealed that this model (keeping each wetland separate) was not significantly better than the model pooling the two most similar wetlands, Carpinteria Salt Marsh and Mugu Lagoon ($F_{2,24} = 2.08, \ P = 0.15$). Inclusion of non-host small benthos species yielded similar results (Fig. S2d).
Since there were inconsistent associations for trematode abundance with large and small benthos density, we examined the relationship between these two components of the benthos. There was a negative association between the density of large and small benthos across all three wetlands, whether we used potential host species, or all benthic species (Fig. S4).

Similar results were obtained when we used pre-interactive trematode prevalence to examine associations with free-living assemblage densities (see ESM S5).

**Discussion**

Previous work identified consistent positive relationships between the diversity and abundance of trematode communities in snails and final host bird communities (Smith 2001; Hechinger and Lafferty 2005; Fredensborg et al. 2006). Here, we find evidence suggesting the existence of associations between free-living benthic communities and the communities of trematode parasites in snails. However, our results also indicate that these relationships may not be consistent in all wetlands. Overall, the clearest findings were for species richness and for large benthos. Trematode species richness was positively associated with the species richness of large and small benthic invertebrates (although, for small benthos, only in the two wetlands for which we have the most data). Regarding abundance measures, we found no consistent relationships between trematode prevalence and benthos or fish.
densities. However, trematode prevalence was positively associated with the density of large benthic invertebrates in all wetlands.

Why did we find no associations between larval trematode communities and the measures of the fish community? We had expected this outcome because we used a standard “snap-shot” sampling of the vagile (and thus highly temporally variable) fish community. Seining with blocking nets is not likely to accurately characterize the fish community’s temporally integrated use of a site. An example of this may be seen in Fig. 2b: the four data points with the highest fish densities (which also have low trematode prevalences) are sites where we happened to capture large groups of mobile schooling fishes (*Fundulus parvipinnis* or *Atherinops affinis*), which may have simply been “passing through.” On the other hand, trematode infections in snails should integrate the temporal variation in the fish use of a site (because trematodes are long-lived in snails). This suggests that the lack of an association between fishes and trematodes might reflect the inadequacies of standard seining methods to quantify fishes, rather than the inadequacies of trematodes to act as indicators of fish communities. This question probably needs to be examined at the scale of a drainage system within wetlands, rather than at the small scale we evaluated here (see below), or fishes need to be sampled in a more time-integrative fashion.
Even when trematodes are significantly associated with the benthos, there is quite a bit of variation about the regression lines for these relationships (Fig 2). In particular, there are sites with relatively large numbers of trematode species, but with few species of large benthic invertebrates (Fig. 2b). Can insufficient sampling of benthos also explain much of the unexplained variation in their relationships with trematodes? We know from personal experience that standard coring can miss benthic species present at a site. Of course, predatory birds (which bring the trematodes to snails) would not be as likely to miss the presence of benthic prey.

The associations we observed between trematodes and the benthos were not strongly influenced by whether we included non-host species in the analyses. This was expected for large benthos, since non-host taxa are few and rare (necessitating the observed tight correlations between measures using or excluding non-host taxa (Figs. S1a and S1b). However, the similar results are particularly interesting for small benthos, since a relatively large number of taxa and individuals are non-hosts (e.g., nematodes and insects; Table S1). However, here too, the similar results are explained because measures of small benthic hosts strongly covary with measures of small benthos that include non-hosts (Figs. S1c and S1d), although the species richness relationship has more unexplained variation.

Associations among the free-living taxa could confound interpretations of the associations between free-living taxa and trematodes. For example, we suggest that
the unexpected negative association in two wetlands between trematode prevalence and small benthos density (Fig. 2f) can be explained by the negative correlation between the densities of large and small benthos in those wetlands (Fig. S4). Large benthic animals (e.g., brachyuran crabs) may directly lower the density of small benthos (e.g., polychaetes) by predation (e.g., Quammen 1984) and, logically, via asymmetrical competition for space. This negative association could also arise indirectly through the different components of the benthos responding differently to environmental conditions. For example, some small benthic animals (e.g., the polychaete, *Capitella capitata*) can dominate the benthos in conditions unfavorable for most animals (e.g., Nordby and Zedler 1991). The negative association between large and small benthos densities contrasts with the positive correlation between the species richness of the same communities in the same two wetlands (Fig. S3). This positive association for species richness may also be both causal and due to covariance with additional factors. For example, some large benthic animals modify the environment (e.g., via burrowing) and this increased heterogeneity may provide microhabitats for a greater diversity of small benthic animals. Also, conditions favorable to the development of a rich community of large benthos (e.g., high tidal flushing (Nordby and Zedler 1991)) may also be conducive to the development of a rich community of small benthos. Whatever the mechanism that influences the relationship between large and small benthos, the species richness of both components was positively associated with trematode species richness in snails (with the apparent exception of small benthos species richness at Morro Bay). We expect
this relationship to be proximally driven because more species of birds would be expected to visit sites with more species of benthic prey, and these birds will consequently bring more species of trematodes to infect snails at those sites.

We investigated relationships between communities at very local scales (e.g., 20 meter reaches of tidal creeks). But, we expect that associations between trematode communities in snails and surrounding animal communities will also occur at scales of entire wetlands or regions. Although our study was not designed to thoroughly assess patterns among wetlands, there is some evidence for this in our data. There were average differences among wetlands in species richness and abundance. For example, Morro Bay had the lowest values for trematode species richness and prevalence (Fig. 2). Morro Bay also had low values for large benthos species richness and density (Figs. 2c and 2d). Surveys of birds in these wetlands indicate that the tidal creeks at Morro Bay have lower densities of wetland birds than either Carpinteria Salt Marsh or Mugu Lagoon (Lafferty, unpublished data). This is consistent with previously demonstrated positive associations between abundance (Smith 2001; Hechinger and Lafferty 2005; Fredensborg et al. 2006) and species richness (Hechinger and Lafferty 2005) of birds and trematodes in snails at local scales. Thus, we speculate that fewer birds use tidal creeks at Morro Bay. This lack of habitat use results from the lower abundance of benthic invertebrates in these creeks. As a consequence of lower bird use, fewer trematodes are present in these snail populations. It is worth noting, however, that the Morro Bay sites did not have
relatively low values for species richness and density of small benthos or fishes. More extensive sampling of Morro Bay would be worthwhile to clarify any associations there.

The patterns we observed using trematode prevalence were not altered when we analyzed data that accounted for competitive loss of trematode infections. This is worth noting, since calculating “pre-interactive” prevalence requires knowledge or postulation of the dominance hierarchy among trematode species (Kuris 1990; Kuris and Lafferty 1994; Lafferty et al. 1994). Although determining pre-interactive prevalence is not difficult, it does add an additional step to the analysis of trematode communities. Regarding using trematodes as a tool, it would be simpler if it were possible for wetland assessors to only use observed prevalences. Although our results suggest that it may be possible to use observed prevalences, we infrequently found more than 50 infections per 100 snails. In situations where observed prevalences are higher, and thus trematode interspecific competition more intense, pre-interactive prevalences will be more important (Kuris and Lafferty 1994). Additionally, trematode competitive displacement imparts a ceiling on observed prevalence (by driving the number of infections toward one per snail). Pre-interactive prevalence removes this asymptote on observed abundance, because it provides as estimate of trematode recruitment that can exceed one per snail. This should increase its value as an indicator in areas where prevalence is high.
Since we found evidence for some positive associations, these results support continued exploration of larval trematode communities as bioindicators of other community components (particularly large benthic invertebrates). These parasites occur throughout the world (Yamaguti 1975; Kuris and Lafferty 1994; Poulin and Mouritsen 2003), and logically should provide comprehensive, temporally integrative, environmentally safe, and cost-effective information about community structure and trophic linkages (Huspeni et al. 2005). It is important to highlight that, unlike previously proposed indicators, trematodes may also reflect abundance of individuals within the various assemblages, not only species richness. Huspeni and Lafferty (2004) evaluated the ecological effects of a wetland restoration using trematode communities. They found that trematode species richness and prevalence increased after the restoration. Unfortunately, they did not survey the free-living communities (it was too costly). Nonetheless, it seems likely that the trematode community became enriched in the restored wetland because birds were attracted to a newly established community of fishes and benthic invertebrates. Similarly, two other recent studies highlight the promise of trematodes as indicators in other types of ecosystems. Loot et al. (2005) recently documented higher levels of trematode parasitism in Chilean rocky intertidal reserves compared to exploited areas. Also, McIntyre et al. (2005) found greater levels of trematodes in snails in non-disturbed compared to disturbed sites in an east African rift lake.
Can trematodes be used as indicators only in systems where trematode life cycles are well-studied? As pointed out by Huspeni et al. (2005), it is straightforward to identify, to taxonomic family, unknown larval trematodes from snail hosts, and that this knowledge is usually sufficient to identify both the general type of 2nd intermediate host (e.g., mollusc, copepod, fish, etc.) and the type of final host (e.g., fish, amphibian, reptile, bird or mammal). Thus, although detailed knowledge of 2nd intermediate host use certainly increases the resolution offered by trematodes as indicators, we suspect that trematodes may be usefully employed as ecological indicators, even in little-studied systems.

What steps should we take to further the development of trematode communities as indicators? First, we should more thoroughly explore the ecological relationships between larval trematodes and surrounding communities of free-living organisms. For instance, are the various trematode populations in snails directly influenced by the abundance of their particular 2nd intermediate hosts? Are crab-using trematodes in snails more common in areas with greater abundances of crabs? Second, it is also necessary to explore associations at the scale of whole habitats and entire wetlands, particularly because this is the scale upon which management frequently operates. Finally, after any further ecological relationships between larval trematode and free-living communities have been established, evaluation of larval trematode communities as bioindicators must quantitatively analyze the most efficient way to combine the use of trematodes and traditional methods of assessing
biodiversity. Such cost-benefit analyses must account for the strength of the relationship between trematodes and measures of free-living communities, as well as the effort and cost required to obtain a sample of the target variable with comparable predictive accuracy.

To conclude and summarize, although previous work demonstrates that trematodes may serve as good indicators for bird communities, it is still not clear to what extent they may serve as indicators of benthos and fishes. Although we found some positive associations between the species richness and abundance of trematodes in snail populations and surrounding benthic communities, the results were inconsistent. Our results indicate trematodes in snails can potentially be developed as indicators of large benthic invertebrates. Future work should more extensively explore within wetland associations, examine larger-scale patterns, and then carefully quantify the costs and benefits of various sampling techniques. Diverse communities of trematodes are common throughout the world in both fresh water and marine habitats (Kuris and Lafferty 1994; Poulin and Mouritsen 2003; Huspeni et al. 2005). Because it is important, yet costly, to monitor biodiversity in these habitats, we should seriously explore the relatively inexpensive use of trematodes as bioindicators of species diversity, abundance and trophic function in these ecosystems.

Acknowledgments
We gratefully acknowledge our colleagues of the Pacific Estuarine Ecosystem Indicator Consortium. We thank Shelly Anghera, Stephanie Mutz, Monique Myers for field work and much of the laboratory processing of the small benthos; Eleca Dunham for lab and field assistance; and Leslie Harris for identifying polychaetes. We also appreciate the helpful insight of three anonymous reviewers and the editors. Financial support for this project came from the US Environmental Protection Agency’s Science to Achieve Results (STAR) Estuarine and Great Lakes (EaGLE) program through funding to the Pacific Estuarine Ecosystem Indicator Research (PEEIR) Consortium, US EPA Agreement #R-882867601 and the National Science Foundation through the NIH/NSF Ecology of Infectious Disease Program (DEB-0224565). It has not been subjected to any EPA review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. RFH received funding from the UC Toxic Substances and Research Program. Our work complied with U.S. Federal and California State law.
Table 1  Trematode species sampled from 1st intermediate host California horn snails (*Cerithidea californica*), their total abundances, and 2nd intermediate host use

<table>
<thead>
<tr>
<th>Species</th>
<th>Total abundance</th>
<th>Total pre-interactive abundance</th>
<th>Primary 2nd intermediate host used&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Euhaplorchis californiensis</em></td>
<td>324</td>
<td>348</td>
<td>fish</td>
</tr>
<tr>
<td><em>Himasthla rhigedana</em></td>
<td>137</td>
<td>138</td>
<td>crabs</td>
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<tr>
<td><em>Small cyathocotylid</em></td>
<td>100</td>
<td>104</td>
<td>fishes</td>
</tr>
<tr>
<td><em>Probolocorype uca</em></td>
<td>78</td>
<td>87</td>
<td>crabs, amphipods&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Himasthla sp. B</em></td>
<td>49</td>
<td>52</td>
<td>snails, annelids</td>
</tr>
<tr>
<td><em>Renicola buchanani</em></td>
<td>45</td>
<td>51</td>
<td>fishes</td>
</tr>
<tr>
<td><em>Acanthoparyphium spinulosum</em></td>
<td>42</td>
<td>47</td>
<td>clams, snails, annelids&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Large xiphidiocercaria (renicolid)</td>
<td>39</td>
<td>46</td>
<td>annelids&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Catatropis johnstonii</em></td>
<td>36</td>
<td>46</td>
<td>snails</td>
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<tr>
<td><em>Austrobilharzia</em> sp.</td>
<td>15</td>
<td>15</td>
<td>none</td>
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<tr>
<td><em>Parorchis acanthus</em></td>
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<td>15</td>
<td>clams, shrimp</td>
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<td><em>Phocitremoides ovale</em></td>
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<td>fishes</td>
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<td><em>Cloactirema michiganensis</em></td>
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<td>12</td>
<td>clams, shrimp</td>
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<tr>
<td><em>Pygidiosoides spindalis</em></td>
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<td>13</td>
<td>fishes</td>
</tr>
<tr>
<td><em>Mesostephanus appendiculatus</em></td>
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<td>11</td>
<td>fishes</td>
</tr>
<tr>
<td><em>Renicola cerithicola</em></td>
<td>3</td>
<td>4</td>
<td>fishes</td>
</tr>
</tbody>
</table>

<sup>a</sup>Information on 2nd intermediate host use is based primarily on our familiarity with the system (but see Martin (1972) and references therein, Huspeni and Lafferty (2004), and Lafferty et al. (2006)).

<sup>b</sup>Approximately 7% of these are *Stictodora hancocki*, which was unrecognized in 2001. For consistency across years, we pooled the 23 S. hancocki encountered in 2002 with *Euhaplorchis californiensis*.

<sup>c</sup>We have recently discovered that our ‘*Probolocorype uca*’ were actually two microphallid trematode species, one of which uses crabs and one that uses gammaridean amphipods (Hechinger and Smith, unpublished data).

<sup>d</sup>Based on preliminary data (Hechinger and Smith, unpublished data), and a note in Martin (1972), *Acanthoparyphium spinulosum* may be two cryptic *Acanthoparyphium* species with differing second intermediate host specificities.

<sup>e</sup>Hechinger and Smith (unpublished data)
Figure 1 A generalized representation of the trematode life cycles in our system
Figure 2  Trematode species richness and abundance associations with potential host (a,b) fishes, (c,d) small benthos and (e,f) large benthos in three coastal wetlands, Morro Bay (△), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). Trend lines are shown for associations where trematodes were significantly associated with the free-living community component. The dashed lines in (b) indicate the significant effect of wetland (P = 0.041). The names of the appropriate wetlands are placed near the lines when separate regressions for different wetlands best described associations. R^2 and P-values for the effect of trematodes (including their interactions for (d), (e), & (f)) are, for (a) R^2 = 0.069, P = 0.16; (b) R^2 = 0.0033, P = 0.76; (c) R^2 = 0.43, P < 0.0001; (d) R^2 = 0.54, P < 0.0001; (e) R^2 = 0.41, P = 0.0021; and (f) R^2 = 0.30, P = 0.011
## Electronic Supplementary Material

**Table S1** List of free-living species and their total abundances observed with our three sampling methods

<table>
<thead>
<tr>
<th>Fishes (seines, blocking nets)</th>
<th>Large Benthos (lg. cores, 3 mm mesh sieves)</th>
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<tbody>
<tr>
<td><strong>Species</strong></td>
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<td><strong>Clevelandia ios</strong></td>
<td>Crustacea</td>
</tr>
<tr>
<td><strong>Gillichthys mirabilis</strong></td>
<td>Crustacea</td>
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<td><strong>Ilypnum gilberti</strong></td>
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</tr>
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<td><strong>Hypopsipeta guttalata</strong></td>
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<td><strong>Cymatogaster aggregata</strong></td>
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</tr>
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<td><strong>Engraulis mordax</strong></td>
<td>Mollusca</td>
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<tr>
<td><strong>Quietula y-cauda</strong></td>
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</tr>
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<td><strong>Pleuronectoidea</strong></td>
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</tr>
<tr>
<td><strong>Sygnathus sp.</strong></td>
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</tr>
<tr>
<td><strong>Gasterosteus aculeatus</strong></td>
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</table>

---

\*a\* Denotes species not previously described.
### Table S1 (continued)  List of free-living species and their total abundances observed with our three sampling methods

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Species</th>
<th>Total Abundance</th>
<th>Taxon</th>
<th>Species</th>
<th>Total Abundance</th>
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</table>

<sup>a</sup>We excluded these "non-host" taxa from analyses using only "potential hosts."

<sup>b</sup>The assignment of genus for this individual is questionable.
Figure S1  Strong associations between benthos measures that use potential host species with measures using all species for large benthos (a,b) and small benthos (c,d) in three coastal wetlands, Morro Bay (Δ), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). For (a) $R^2 = 0.99$, $P < 0.0001$, $n = 32$; (b) $R^2 = 1.00$, $P < 0.0001$, $n = 32$; (c) $R^2 = 0.88$, $P < 0.0001$, $n = 29$, and the separate slopes and intercepts for lines represent the effect of wetland ($P = 0.016$) and an interaction between wetland and total small benthos richness ($P = 0.10$); and (d) $R^2 = 0.98$, $P < 0.0001$, $n = 30$, and wetlands significantly differed only in intercepts ($P = 0.0016$).
Figure S2  Relationships between benthos and trematodes (using all encountered benthic species in analyses) for large benthos (a,b) and small benthos (c,d) in three coastal wetlands, Morro Bay (△), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). Results are very similar to analyses using only potential host species of benthos (Fig. 2). The names of the appropriate wetlands are placed near the lines when separate regressions for different wetlands best described associations. $R^2$ and $P$-values are for the effect of trematodes (including their interactions for (b), (c), & (d)). For (a) $R^2 = 0.40, P =0.0002, n =30$; (b) $R^2 =0.54, P <0.0001, n =32$; (c) $R^2 =0.36, P =0.0055, n =27$; and (d) $R^2 =0.28, P =0.013, n =30$
Figure S3  Positive association between large and small benthos species richness measures using host species of benthos (a), or all species of benthos (b) in three coastal wetlands, Morro Bay (△), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). The names of the appropriate wetlands are placed near the lines when separate regressions for different wetlands best described associations. For (a) $R^2 = 0.28$, $P = 0.0033$, $n = 29$, and wetland was not significant ($P = 0.12$); (b) $R^2 = 0.37$, $P < 0.0008$, $n = 29$ and wetland was a significant effect (partial $R^2 = 0.45$, $P = 0.0005$)

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Figure S4  Negative association between small and large benthos density measures, for potential host species (a), and all species of benthos (b) in three coastal wetlands, Morro Bay (Δ), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). For (a) $R^2 = 0.20$, $P = 0.013$, $n = 30$, and wetland was not a significant effect ($P = 0.59$); (b) $R^2 = 0.23$, $P < 0.0073$, $n = 30$, and wetland also was not significant ($P = 0.30$)
**Electronic Supplementary Material S5:** Results of analyses using preinteractive trematode prevalence

We obtained very similar results when we used pre-interactive trematode prevalence to examine associations with free-living assemblage densities. Specifically, as with observed trematode prevalence, fish density was not associated with trematode pre-interactive prevalence (partial $R^2 = 0.0023$, $F_{1,28} = 0.065$, $P = 0.81$, $n = 32$), and wetland was a significant factor affecting fish density ($F_{2,28} = 3.60$, $P = 0.037$). Also as with observed prevalence, trematode pre-interactive prevalence appeared to positively relate to large benthos density, weakly over two wetlands, and more strongly at one (for hosts only: effect of trematode prevalence and its interactions, partial $R^2 = 0.58$, $F_{2,28} = 19.2$, $P < 0.0001$; full model $R^2 = 0.68$, $P < 0.0001$, $n = 32$; including non-hosts: effect of trematode prevalence and its interactions, partial $R^2 = 0.57$, $F_{2,28} = 18.9$, $P < 0.0001$; full model $R^2 = 0.65$, $P < 0.0001$, $n = 32$). Finally, as with observed trematode prevalence, pre-interactive prevalence also appeared to be associated with small benthos density. This was a positive association at one wetland, but was negative at the other two wetlands (for hosts only: effect of trematode pre-interactive prevalence and interactions; partial $R^2 = 0.29$, $F_{2,26} = 5.21$, $P = 0.012$; full model $R^2 = 0.32$, $P = 0.017$, $n = 30$; including non-hosts: partial $R^2 = 0.27$, $F_{2,26} = 4.83$, $P = 0.017$; full model $R^2 = 0.34$, $P = 0.011$, $n = 30$).
Chapter 4

Trematode parasites reflect several aspects of fish and benthic invertebrate assemblages across estuarine habitats

1. Introduction

Can trematode parasites accurately and efficiently indicate the condition of estuaries? Monitoring and evaluating the status of our estuaries is important for restoration, mitigation, and conservation (PERL 1990, Bortone 2005). There are many biotic and abiotic measures of estuary ecosystems, but one of the most challenging to quantify is animal biodiversity and the functioning of food webs. Assemblages of trematode parasites in snail populations are promising bioindicators of animal biodiversity and the functioning of food webs. Indeed, there is mounting evidence that the diversity and abundance of trematode assemblages in snail populations does reflect the diversity and abundance of surrounding free-living assemblages (Smith 2001, Huspeni and Lafferty 2004, Hechinger and Lafferty 2005, Huspeni et al. 2005, Fredensborg et al. 2006, Hechinger et al. 2007, Hechinger et al. submitted).

However, several questions remain concerning trematodes as indicators. Here, we address some of these issues, finding that trematodes indicate several aspects of free-living assemblages at larger scales than has been previously examined, and that trematodes can provide more taxonomic information than has previously been considered.

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Diverse trematode assemblages in snails should serve as efficient indicators of many of the other harder to sample elements of surrounding free-living biodiversity. This is because diverse trematode assemblages in snails are easy to sample and depend on the biodiversity surrounding them because they generally must use several types of host to complete their life cycles. Typically, larval trematodes occur in first intermediate host snails where they continually produce clonal stages that leave the snails. These stages (cercariae) seek out and encyst in or on second intermediate hosts. The type of second intermediate host is some sort of invertebrate or vertebrate, and this depends on the trematode species. Adult trematodes live in vertebrate final hosts, which usually become infected by eating second intermediate hosts. Sexually produced offspring leave the final host with its excreta and subsequently infect snail first intermediate hosts. The species in a diverse community of trematodes diverge in many parts of their life cycles. However, they all use a limited set of snail first intermediate host species. The most promising trematode indicators are those diverse trematodes assemblages that use the same single snail species as first intermediate host (where they are easily sampled), but widely differ in other parts of their life cycles (connecting them to many elements of the surrounding free-living community) (Huspeni et al. 2005).

Unfortunately, poor-quality data on free-living species assemblages would make it difficult to clearly demonstrate the value of trematodes as indicators of free-
living animal diversity and abundance. There are usually large errors and low sample sizes associated with data on free-living abundance and diversity. Also, snap-shot assessments of vagile free-living assemblages do not adequately characterize those assemblages’ use of sites. Another uncertainty about snap-shot estimates of free-living communities is that they may not represent the longer-term condition of a site (particularly for smaller and shorter-lived species) while trematode assemblages in snails integrate longer time periods (see below). These factors would tend to obscure what might be strong associations between the diversity and abundance of trematodes with that of free-living assemblages.

The abundance and diversity of trematodes in snails should most clearly associate with the abundance and diversity of final hosts. This is because worms in final hosts provide the stages that infect snails. Supporting this idea, Hechinger and Lafferty (2005) found clear associations between the diversity of birds and the diversity of trematodes in snails. Further, areas with greater abundances of bird, mammal, or fish final hosts have greater abundances of trematodes in snail populations (Smith 2001, Hechinger and Lafferty 2005, Lafferty and Dunham 2005, Fredensborg et al. 2006, Whitney et al. 2007, Hechinger et al. submitted). As a result, trematode diversity predicts final host diversity and trematode abundance predicts final host abundance.
Often, we desire information on more elements of a community than only final host assemblages. Several arguments suggest trematodes should reflect the presence and abundance of their second intermediate hosts and related animals (Huspeni et al. 2005, Hechinger et al. 2007). First, second intermediate hosts (and related animals) are food for predatory final hosts. To the extent predator final hosts spend more time in areas with abundant food, they will transmit more trematodes to snails. Second, trematodes cannot infect final hosts unless the final hosts eat second intermediate hosts. Therefore, at some scale, trematodes in snails rely upon the presence of appropriate second intermediate hosts. To the extent that trematode species vary with respect to species of second intermediate hosts they use, a diverse and abundant trematode assemblage in snails not only reflects, but requires, the presence of diverse and abundant second intermediate hosts (Gardner and Campbell 1992, Huspeni et al. 2005, Hechinger et al. 2007). Support for this idea is provided by Simkova et al. (2003), who found that the trematode communities in final host gulls varied in similarity across estuaries in ways related to the manner that fishes and invertebrates varied in similarity. Further, Hechinger et al. (2007) found positive diversity and abundance correlations between trematodes and large benthic invertebrates; positive diversity and negative abundance associations with small benthic invertebrates; and no associations with fishes. To address several unresolved questions, we expand and build on their work by using the diverse group of trematodes parasitizing the California horn snail (Cerithidea californica). An important point here is that trematodes in snails should reflect the presence of all
members of the free-living community that serve as prey or attract final hosts (which bring the infections to the snails). However, trematodes should most strongly indicate the abundance and diversity of those members of the free-living community who also serve as hosts, and thus foster the completion of trematode life cycles.

A remaining issue concerning using trematodes as bioindicators has to do with sampling scale. All previous work examining associations between trematodes in snails and free-living assemblages have sampled snails at the small scale (Smith 2001, Hechinger and Lafferty 2005, Fredensborg et al. 2006, Hechinger et al. 2007). That is, replicates have been sites (usually tens to a few hundred square meters) within one type of habitat within an estuary. Do trematodes reflect biodiversity at larger scales? Do areas ranging from one to hundreds of hectares that have a greater diversity and abundance of free-living animals also have a greater diversity and abundance of trematodes?

We predicted that two general patterns would emerge by increasing the sampling unit to entire habitats (e.g., comparing communities in from vegetated marsh with communities from mudflats). First, we expected that associations between trematodes and free-living assemblages that have been documented at fine scales would simply scale up to the habitat level, perhaps even being stronger given the potential for increased variation in communities among habitats than exists in communities among sites within a habitat. For example, Hechinger et al. (2007)
found positive diversity and abundance associations between trematodes and large benthic invertebrates (e.g., crabs and clams) across small scale sites within the same habitat type (tidal channels) across three estuaries. We expected that positive associations between trematodes and large benthic invertebrates would also occur after using habitats as replicates. That is, habitats with a greater diversity and abundance of trematodes would have a greater diversity and abundance of benthic invertebrates.

Increasing sampling scale may reveal associations not detectable at fine scales. For example, as Hechinger et al. (2007) pointed out, fishes probably move around too much for snapshot techniques used at small scales to adequately measure fish assemblages at those small scales. Hechinger et al. (2007) expected that the lack of associations between the diversity and abundance of trematodes and fishes sampled at fine scales (across sites within the same habitat type in three estuaries) resulted from the inability of single, localized seine hauls to represent the long-term use of fishes at a particular site. Because distributed replicate snapshots would likely provide a more accurate depiction of the fish assemblage over larger areas, we predicted that entire habitats with a greater abundance of trematodes in snails would have greater abundance of fishes.

Another remaining question pertains to whether trematodes can provide information concerning the taxonomic composition of free-living assemblages.
Earlier work has largely focused on relating the various assemblages as wholes (or was limited to examining associations for a single final host species—see below). Yet, different trematode species use different types of second intermediate hosts. Thus, different subsets of the trematode assemblage (i.e., different trematode guilds, classified by second intermediate host use) should be directly associated with different components of the surrounding free-living assemblage. Logic is not the only support for this idea. Lafferty and Dunham (2005) and Whitney et al. (2007) both presented evidence that single members of the final host assemblage are associated with particular trematode species. Additionally, earlier work used abundance and diversity metrics where all species are treated equally. For instance, using standard diversity metrics or aggregate density measures, there is no way to distinguish a site with ten species of polychaete from a site with five species of polychaetes and five species of crustaceans. However, differing host use becomes increasingly common the more distantly related are the trematode species (Yamaguti 1975, Schell 1985). Thus, trematode assemblages composed of more taxonomically disparate species should reflect more taxonomically disparate free-living assemblages compared to trematode assemblages with the same species diversity, but lower taxonomic diversity. The use of a diversity metric that captures the degree of relatedness among individuals might detect taxonomic diversity associations between trematode assemblages and free-living assemblages.
Our ability to use trematode diversity to assess host diversity depends on the level of host specificity of trematodes for second intermediate hosts. For instance, most of the trematodes that use fishes as second intermediate hosts are generalists, each trematode species using virtually all the fish species (JC Shaw, unpublished data). Therefore, increased fish diversity should not favor completion of the life cycles of more species of fish-using trematodes. This drove our prediction that we would not uncover positive diversity associations between fishes and trematodes in snails, despite expecting positive abundance associations.

Associations between snail trematode and free-living assemblages have been examined only with the use of univariate metrics. However, multivariate methods can frequently more powerfully detect community change in space or time than can univariate metrics (Warwick and Clarke 1991, Clarke and Warwick 2001). In such analyses, rather than a single data point describing each sample, many data points describe a sample (for instance, the sample’s similarity to all other samples). Thus, employing multivariate methods of analysis would extend previous work examining associations between trematodes in snails and free-living assemblages, and potentially increase our ability to determine how trematodes may serve as bioindicators.

A final question we address is whether relationships between trematodes and surrounding free-living assemblages depend on the snail size class examined for
trematodes. Trematode prevalence typically varies among snail size classes (Kuris and Lafferty 1994). Typically, prevalence increases with snail size, because larger (older) size classes of snails tend to accumulate infections over time. Consequently, trematode assemblages in larger size classes of snails integrate longer periods of time than do trematode assemblages in smaller snails. This could have two non-mutually exclusive effects on the indicator value of different size classes of snails. First, larger snail size classes should have greater indicator value for understanding the longer-term condition of the surrounding ecosystem. Longer-term condition itself may be of more interest than is current condition. Additionally, larger snail size classes have had time to accumulate trematode infections that represent surrounding diversity. This increases the range in values obtained for our indicator (namely, trematode prevalences), potentially allowing greater discrimination among sites. Contrastingly, infections in small size class snails reflect more recent events than do infections in larger size class snails. To the extent that surrounding diversity has varied over time, relationships between trematodes in snails and the surrounding free-living fauna may be stronger in smaller snail size classes. Given these size-dependent indicator values, it would be useful and important to know how associations between trematodes in snails and free-living assemblages vary across snail size classes.

Here, we do five new things. First, we examine whether trematodes associate with fishes and benthic invertebrates at scales larger than previously examined (the scale of entire habitats within estuaries). Second, we examine whether particular...
components of the benthic invertebrate and fish assemblages are associated with particular guilds of trematodes, classified by what host groups the post-snail trematode stages use. Third, in addition to using standard diversity indices, we employ a diversity index that accounts for the degree of taxonomic relatedness among the sampled individuals. Fourth, in addition to standard univariate measures of diversity and abundance, we examine whether trematode assemblages associate with surrounding free-living assemblages using multivariate methods. In particular, we examine the extent to which similarity matrices for habitat units based on trematodes relate to those based on the combined assemblage of fishes and benthic invertebrates. Fifth, we examine whether our findings differ depending on the snail size class used to quantify trematode assemblages. We find that trematodes in snails are associated with several, but not all, aspects of the surrounding fish and benthic invertebrate host assemblages at the habitat scale and that snail size affects this strength of this association.

2. Methods

2.a. Field and lab work

We used data we acquired during an intensive quantification of free-living assemblages and their parasites in the intertidal zone of three Pacific Coast estuaries. The estuaries were the 61 ha Carpinteria Salt Marsh, California, USA (CSM), the 707 ha Estero de Punta Banda, Baja California, Mexico (EPB), and the 144 ha Bahia Falsa in Bahia San Quintín, Baja California (BSQ). We quantified assemblages of
benthic invertebrates, fishes, and trematodes in snails at 69 sites throughout the three estuaries. The 23 sites in each estuary were randomly laid out, stratified by the four major intertidal habitat types in these ecosystems: channels, flats, marsh, and pans (unvegetated areas surrounded by marsh). Because we randomly sampled each habitat, we were able to average across replicate sites within an estuary’s habitat type to generate mean values of our various abundance and diversity measures (see below) for the entire habitat. To delineate habitat areas and generate the random points, technicians used satellite imagery and ArcGIS 8.3 software (ESRI 1996).

Our field teams quantified the density of fishes at each site (excluding marsh sites). We used 3 mm mesh blocking nets to enclose an area. We then captured fish using five sweeps with two-pole seines (the last sweep performed with the blocking nets). The pole seines were also 3 mm mesh and were eight or 11 m long by 1.8 m high. At channel sites, we used the blocking nets to enclose a 10 meter stretch of channel. At flat sites, we used the blocking nets to enclose a similarly sized area. We seined the entire area of pan sites. We identified all individual fishes to species and counted them on-site. We calculated density by simply dividing the number of captured individuals by the area enclosed by the blocking nets. Some fish enter marsh habitat at high tide (West and Zedler 2000). However, there was no submerged marsh habitat when we sampled fish (during mid-tide levels). So, we assumed fish abundance in marsh habitats was zero for our analyses.
We also sampled the benthic invertebrates at each site within a plot with maximum dimensions of 10 x 10 m (dimensions sometimes limited by channel or pan size). We separately characterized large and small benthic animals. We randomly sampled large benthic invertebrates in three ways. First, we used ~20 quadrats (10 x 50 cm) to sample densities of large (≥ 10mm length) epibenthic invertebrates. Second, we sampled large infaunal invertebrates with 5-20 large (0.1 m diameter) cores sieved through a 5 mm mesh. Large benthic invertebrates were typically identified to species and counted on-site. Because benthic species are usually highly aggregated, we sometimes employed a stratified random sampling scheme (Thompson 2002) within a site for some large benthic animals to reduce variance in our estimates when we could readily identify habitat from non-habitat. We sampled crabs by using five random ‘cores’ (each comprised of three adjacent 0.24 m diameter cores) randomly placed within the crab burrow habitat. We used these large crab cores because the 0.1 m diameter large cores insufficiently captured these relatively mobile animals. We quantified the small benthic invertebrates using 5 or more pooled randomly placed small cores (0.05 m diameter). We took these cores back to the lab and placed them in refrigeration until we sieved them through a 1 mm mesh (usually within several hours after collection, sometimes within a day). We identified individual small benthic invertebrates to species or, more frequently, to “morphospecies” belonging to some higher taxonomic category.
We quantified larval trematode assemblages in randomly sampled California horn snails from each of the 54 sites at which they were present in the 12 estuary-habitat units we sampled. Prevalence (proportion of infected hosts) in horn snails tends to increase with snail size (Sousa 1983). Our research team dissected snails belonging to four 5-mm length size classes; 15.0-19.9 mm (15s), 20.0-24.9 mm (20s), 25.0-29.9 mm (25s), and 30.0-34.9 mm (30s). We focused on the two most abundant and widespread size classes (20s and 25s). Our target sample size for snails dissected in each size class was 100 for 20s and 25s, and 50 for 15s and 30s. In some situations of low snail density, to reach our random sample, we haphazardly collected additional snails spread throughout the site. Sometimes snails were absent or so rare we could not reach our target. Snails were returned to the lab, dissected, and their trematode infections enumerated. We identified all trematodes to species, following Martin (1972) and Huspeni and Hechinger (unpublished manuscript). Table 1 gives the names and second intermediate host use of these trematodes. Table 2 gives the number of sampled sites and numbers of snails dissected per habitat for each snail size class.

Because trematodes logically must most directly associate with their hosts (see Introduction), to increase our ability to detect associations we focus on the members of the fish and benthic invertebrate community that can serve as second intermediate hosts. We excluded from analyses taxa of benthic invertebrates and fishes that do not serve as hosts for the California horn snail trematodes. This resulted
in excluding very little of the fish assemblage (only one non-host fish species out of 17 fish species), less than one quarter of the small benthic invertebrate species (29 non-host species out of 88 species), and also very little of the large benthic invertebrate assemblage (only two non-host large benthic invertebrate species out of 24 species). Thus, the bulk of the fish and benthic invertebrate free-living assemblages remained in our analyses.

California horn snails are first intermediate host for all the trematode species in our study and also serve as second intermediate host for several of the trematode species (Table 1 and Lafferty et al. (2006)). Because trematodes may negatively affect snail abundance (Lafferty 1993), and because host density can influence transmission of parasites (Anderson and May 1979, May and Anderson 1979), there may be a complicated relationship between the density of California horn snails and the prevalence of trematodes in those snails (see discussion). For this reason, we performed some parallel analyses excluding horn snails from the benthic invertebrate host assemblage to determine whether their presence affected the results. We exclude site-size classes where we did not dissect at least 10 snails.

2.b. Characterization of assemblages

2.b.i. Univariate analyses: abundance measures
We characterized the abundance of fishes and benthic invertebrates as density
(no./m²). To express the abundance of trematodes, we used prevalence (proportion of
hosts infected each of the 5-mm size classes). We used prevalence, rather than
density, as our measure of trematode abundance because prevalence is far easier to
calculate than is trematode density. Hence, prevalence has the greatest indicator
value. We calculated two forms of prevalence for each trematode species, observed
and “pre-interactive.” Trematode assemblages in snail populations often have high
levels of interspecific competition and competitive loss (due to dominant trematode
species recruiting to, and killing, infections of subordinate species in individual snails
(Kuris 1990, Kuris and Lafferty 1994)). Thus, observed prevalence may significantly
underestimate actual trematode recruitment (because many subordinates have been
killed and replaced by dominant species (Kuris and Lafferty 1994, Lafferty et al.
1994)). Yet, actual recruitment levels should be more closely related to free-living
assemblages than are observed recruitment levels in situations where there has been
competitive loss of many subordinate trematodes. Therefore, we also calculated
expected pre-interactive prevalences using the techniques outlined in Lafferty et al.
(1994). The formulas, using knowledge of the trematode dominance hierarchy (Kuris
1990, Huspeni 2000), simply apply the prevalence of each trematode species in
“competitor-free” snails to the portion of the snail population infected with dominant
trematode species. This provides an estimate of how many subordinate infections
have been killed (Lafferty et al. 1994). Because results were very similar whether we
used observed or expected prevalence, and because pre-interactive prevalences
logically should more closely indicate surrounding diversity, we present analyses using pre-interactive prevalences in the main body of the paper. We present parallel analyses using observed prevalences in the Appendix. At the appropriate places in the results, we indicate when differences in significance occurred between correlations using pre-interactive and observed prevalences.

2.b.ii. Univariante analyses: diversity measures

Researchers use a wide variety of diversity indices to characterize communities (Krebs 1999, Magurran 2004). We picked four types of diversity measure, each of which captures different elements of host community diversity.

First, we calculated species richness (the number of species in a sample) for each host assemblage. Because the number of individuals sampled varied across sites, we calculated rarified species richness using Hurlbert’s (1971) equation. We used the expected number of species observed if two individuals were sampled ES(2), since there were some sites with few encountered individuals. Sites with zero individuals in any particular assemblage have predicted species richness of zero for that assemblage.

The second measure of diversity that we used was Simpson’s diversity, 1/D. This index captures elements of both richness and evenness (see below). Simpson’s index of dominance, D, is the probability that two randomly sampled individuals
from a sample belong to the same species (Simpson 1949). Thus, \( I/D \) increases with species richness and abundance. This is one of the most highly recommended indices (Magurran 2004). It is stable and not nearly as sensitive to sample size as is the more popular Shannon-Weiner Index.

The third diversity measure we used was evenness. Evenness simply captures the degree to which species abundances are similar within a sample (Krebs 1999, Magurran 2004). We used Simpson’s evenness \( E(I/D) \), because it is based upon Simpson’s diversity, which we used as our diversity index. Simpson’s evenness is simply Simpson’s diversity divided by the observed number of species in a sample.

The fourth way that we quantified diversity was to capture the taxonomic diversity represented by host species in a sample. To do this, we calculated taxonomic diversity, \( \Delta \) (Clarke and Warwick 1998). This index is related to Simpson’s diversity, but the degree of relatedness among the species is considered. Larger weights are attached to species combinations that are farther apart in a taxonomic hierarchy than are attached to those species more closely related.

2.b.iii. Multivariate analyses

We generated similarity matrices expressing how habitat units were related to one another based upon host species abundances, using Bray-Curtis similarities. This similarity metric has been widely used in studies characterizing benthic and fish
assemblages (Clarke 1993, Clarke and Warwick 2001). This index is based on differences in individual species abundances across all pairs of samples. It ranges from 0 – 100. Higher scores represent samples that more closely match each other in species composition and species abundances. Zero similarity indicates that no species are shared between samples. These matrices contain information that is multivariate, because they contain multiple data points for each habitat unit (their similarity to all other habitat-units).

2.c. Data analyses

2.c.i. In general

Because our questions in this study pertain to how well trematodes indicate free-living assemblages at large habitat-level scales, the replicates for this study are estuary-habitat units. We calculated measures for habitats using replicate values of those measures. For instance, the value for the pan habitat at CSM is the average of five pan sites in that estuary. Given the four habitat types per each of the three estuaries, we generally have a maximum sample size of 12 estuary-habitat units. We performed all analysis in parallel for each of the relatively common snail size classes (15-20, 20-25, 25-30, and 30-35). All calculations and analyses were performed using the software platforms Primer 6 (Primer-E), JMP 7 (SAS), and Excel 2003 (Microsoft).
In this analyses, we focus on and present nominal p-values and do not adjust for multiple comparisons (such comparisons are easily made by parties interested in doing so, given the information provided here).

2.c.ii. Univariate analyses

We examined correlations between the various univariate measures of trematodes and the different components of the free-living community. Since there were four snail size classes and several types of community measures, there were a very large number of such analyses. Therefore, for clarity, we summarize the findings by presenting the correlation coefficients with 95% confidence limits. The confidence limits for r were calculated using Hotelling’s z* transform procedure (Sokal and Rohlf 1981). We present the scatter plots for the significant correlations (with two-tailed p-values ≤ 0.05). In the appendix, we present all scatter plots.

2.c.iii. Multivariate analyses

For the entire free-living fish-benthic invertebrate host community, we generated one similarity matrix using Bray-Curtis similarities based on untransformed densities and one matrix based on fourth root transformed densities (Tables A1a-d). The important difference between using raw and fourth root transformed data is that less abundant species play more major roles in the similarity values when using fourth root transformed densities (Clarke and Green 1988, Clarke and Warwick 2001). This is because fourth root transformation reduces the numerical disparity between very
common and less common species, precluding very common species from
dominating the similarity metric. To demonstrate the effect of fourth root
transforming species densities, Table A1 lists the most important benthic invertebrate
and fish species in driving the change in community structure across habitat units
between BSQ and CSM using either untransformed or fourth root transformed
densities. The most important species are those that have the highest percent
contributions to the total dissimilarities (1-similarity) between wetlands (stopping
when a cumulative 90% of the total dissimilarity has been explained). The effect of
fourth root transforming in increasing the importance of the less dense large benthic
invertebrates and fishes is clear by examining these species lists.

We also generated Bray-Curtis similarity matrices for trematode assemblages,
using untransformed prevalences of each species in each of the four snail size classes
(Tables A3a-d). There was no reason to perform the analogous fourth root transform
for trematode assemblages, because prevalences and summed prevalences have far
less of a value range than do the densities used to characterize the benthic
invertebrates and fishes.

We examined whether trematode assemblages shifted across habitat-units in
conjunction with the combined fish and benthic invertebrate host assemblage. We did
this by evaluating whether the similarity matrices based on each assemblage were
more similar to each other than expected by chance using a permutation-based test,
RELATE (Clarke and Warwick 2001). The RELATE test is very similar to a Mantel test (Manly 1997), but is based on the Spearman-rank correlation between the two compared matrices. We used 10,000 permutations of the matrices to generate one-tailed p-values, for the RELATE tests. To visualize the tested associations between similarity matrices, we created scatter plots of similarity coefficients belonging to the corresponding cells in tested matrix pairs.

3. Results

3.a. Density of free-living taxa versus trematode guilds that infect them

Overall, trematodes in 20 and 25 mm size classes more strongly correlated with densities of host taxa than did trematodes in 15 and 30 mm snail size classes.

The prevalence of fish-using trematodes in all snail size classes positively associated with fish host density (Figure 1). However, the positive correlation was significant only for the 20-25 mm snail size class (Figures 1 and 2).

Similarly, the prevalence of polychaete-using trematodes in all snail size classes also positively associated with polychaete host density. Yet, these correlations were not as strong as those between fish host density and fish-using trematode prevalence, never being statistically significant (Figure 3).
Surprisingly, habitats with greater prevalences of mollusk-using trematodes had lower host mollusk-densities. This association was significant for trematodes in the three largest snail size classes (Figures 4a and 5). Breaking mollusks into snails and bivalves indicated that the negative association was largely driven by a negative correlation with snails (Figure 4b, 6). Mollusk-using trematodes still negatively correlated with host snail density when California horn snails were excluded from the snail density calculation (Figures 4c and 6). Trematodes very weakly associated with host bivalve density, but these correlations were small and non-significant for all snail size classes (Figure 4d).

Consistent with the positive abundance associations between trematodes and host fishes, habitats with greater prevalences of decapod-using trematodes tended to have greater decapod densities (Figure 7a). This association was significant for the 20-25 and 25-30 mm snail size classes (Figures 7a and 8). The borderline significance for the 30-35 mm size class was significant when using observed prevalences (Figure A6a). Splitting decapods into crabs (brachyurans) and burrowing shrimp (thallasinideans) showed that the densities of both crabs and burrowing shrimp tended to positively correlate with the prevalences of crab- and shrimp-using trematodes respectively (Figures 7b, 7c, 9, and 10). The correlations for crabs closely mirrored those for decapods (Figures 7a, 7b, 8 and 9). Those for burrowing shrimp were positive for the three largest snail size classes, but only significant for 20s (Figures 7c and 10).
Results for parallel associations using observed prevalences are in the appendix (Figures A1-A4), as are scatter plots for all comparisons made using pre-interactive and observed prevalences (Figures A8-A28).

3. b. Univariate diversity measures

Overall, as predicted the generalist fish-using trematode guild did not exhibit significant or consistent diversity associations with the host fish assemblage (Figure 11), although there were consistently positive non-significant correlations for Simpson’s diversity (Figure 11b).

The guild of trematodes using benthic invertebrates exhibited no overall diversity trend consistent with small benthic host invertebrate hosts across habitat units (Figure 12). However, Simpson’s and taxonomic diversity of small benthic host invertebrates tended to negatively associate with that of benthic invertebrate-using trematodes (Figures 12b,d). But, this was only statistically significant for one snail size class for each metric (Figures 12b,d, 13 and 14), and the association for taxonomic diversity was only of borderline significance using observed prevalences (Figure A6d). Additionally, there was a suggestion of a positive association between these trematodes and small benthic host invertebrates for Simpson’s evenness, although this only approached significance in two size classes (Figure 12c).
Unlike small benthic invertebrate hosts, large benthic invertebrate hosts tended to exhibit more positive diversity correlations with benthic-invertebrate using trematodes (Figure 15). This was only consistent in direction for Simpson’s and taxonomic diversity (Figures 15b,d, 16 and 18). However, the correlations were significant for only one snail size class for each Simpson’s and taxonomic diversity. The evenness of benthic invertebrates-using trematodes also positively and significantly correlated with the evenness of large benthic invertebrates for the 25 and 30 mm snail size classes (Figures 15c and 18). However, the latter association was not significant when using observed prevalences (Figure A7c). Also, the positive association for rarified species richness of benthic invertebrates-using trematodes with large benthic invertebrates (Figure 15a) was significant when we used observed trematode prevalences (Figure A7a).

Results for all parallel associations using observed prevalences are in the Appendix, as are scatter plots for all comparisons made using pre-interactive and observed prevalences.

3.c. Multivariate analyses
Trematode assemblages in 20 and 25 mm snail size classes usually shifted in species composition and abundance across habitats in parallel with the species abundances of host fishes and benthic invertebrates (Figures 19 and 20). That is, the multivariate pattern of changes across habitat units in trematode species’ prevalences in 20 and 25...
mm snail size classes corresponded with the pattern of changes of host fish and benthic invertebrate species abundances (using either raw or fourth root transformed species densities, Figures 19 and 20). Rarer free-living species also played a role in the association of fishes and benthic invertebrates with trematodes (evident because significant associations were significant when using fourth root transformed densities, Figures 19 and 20). However, the trematode assemblage in 20 mm snails did not significantly relate with untransformed density of host fishes and benthic invertebrates after excluding horn snails from the benthic invertebrates (Figure 19c). Trematode assemblages in 15 and 30 mm size classed snails usually did not change congruently with the fish and benthic invertebrate host assemblage (Figures 19, 20a, 20b, 20g, and 20h).

4. Discussion

In addition to indicating communities of final hosts (in this system, primarily birds, Hechinger and Lafferty 2005, Whitney et al. 2007), our findings suggest trematodes in snails have value for indicating some second intermediate hosts (e.g., fishes and decapods) at relatively large spatial scales. For several of the univariate and multivariate measures, trematode assemblages in snails changed over space in parallel with the combined assemblage of second intermediate hosts. Thus, trematode assemblages may serve as indicators for the broad community of second intermediate hosts in general. Because trematodes infect most fishes and invertebrates in this system, these findings directly indicate trematodes in snails also effectively reflect the
entire community of fishes and invertebrates, which might be of interest for monitoring purposes. The ability of trematodes to indicate specific members of the second intermediate host community varied as did the strength of associations using differently sized snails. We found that trematodes from intermediate size classes of snails seemed to give the best indicators of host diversity, further helping refine the use of this tool.

4.a. Univariante density associations

A notable finding is that the prevalences of various trematode groups directly associated with the densities of their hosts. The prevalence of fish-using, crab-using, shrimp-using, and snail-using trematodes may thus serve to indicate the respective densities of fishes, crabs, shrimps, and snails. These free-living assemblages are important components of estuary communities (Mitsch and Gosselink 2000) that are difficult to quantify using standard techniques (Huspeni et al. 2005).

In contrast to previous work conducted at finer spatial scales (Hechinger et al. 2007), habitats with greater prevalences of fish-using trematodes in snails tended to harbor greater densities of fishes (although significantly so only for the 20-25 mm size class of snails). For a vagile—and therefore temporally variable—free-living assemblage such as fishes, a broad sampling scale is necessary to obtain a description of the assemblage suited for such an analysis. In this study, we increased the sampling scale in space (by averaging across several replicate snap shots throughout
habitats), allowing detection of associations between fishes and trematodes. This is not the first time adequate sampling of vagile host species has permitted the detection of an association between trematodes and hosts. Previous studies examined associations between trematodes and a different vagile free-living assemblage—birds (Smith 2001, Hechinger and Lafferty 2005, Fredensborg et al. 2006). By repeatedly sampling the same small sites, those studies obtained average measures of the bird assemblage that allowed detection of associations between trematode and bird abundance (Smith 2001, Hechinger and Lafferty 2005, Fredensborg et al. 2006) and diversity (Hechinger and Lafferty 2005). These examples underscore the power of trematodes as indicators. A snapshot sample of trematodes is easy to obtain and it reflects a much more difficult to obtain integrated view of the host community at longer temporal scales. In other words, the long period of time over which hosts pass infections to snails in the field result in trematode community that has accumulated over time with exposure to the long-term presence of the host community.

The strong association between the prevalence of decapod-using trematodes was and the density of decapod hosts suggests that trematodes could serve as specific indicators of this group. This relationship was mirrored when we split decapod density into the constituent crab and burrowing shrimp density, and broke the prevalence of decapod-using trematodes into prevalences of crab- and shrimp-users. These positive associations are consistent with previous findings using small-scale replication that trematode prevalence positively associated with large benthic

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invertebrates density (where crabs were a major component of the large benthos, (Hechinger et al. 2007)). From a practical standpoint, trematodes may also make efficient indicators of this group. We found the digging necessary to obtain accurate densities of crabs and shrimps to be both demanding in time and energy as well as destructive to the habitat (unpublished observations).

Trematode prevalence may also positively indicate polychaetes but this was not clear with our methods. However, the correlations for polychaete-using trematodes and polychaete density were consistently positive, despite being non-significant. This suggests a potential lack of power to detect significance, which is not unexpected given the fact that we had, at most, 12 habitat units to compare. Perhaps future study at finer scales with more replication will reveal significant associations between polychaete-using trematode prevalence and polychaete density. Also potentially obscuring associations between the prevalence of polychaete-using trematodes and polychaete density is that several of the polychaete-using trematodes also use mollusks as second intermediate hosts (table 1). Compared to quantifying trematode prevalence, quantifying polychaete density is more difficult in both the field and the lab (Fauchoald 1977, Huspeni et al. 2005). Hence, it would be an important indicator value for trematodes if trematode prevalence does turn out to indicate polychaete density.
Contrary to the general positive associations between trematode prevalence with their second intermediate hosts, mollusk-using trematodes exhibited negative associations with snail density and no associations with bivalve density. We had expected the negative relationship with the snail assemblage would be driven by the documented negative association between trematode prevalence and the density of first intermediate host California horn snails (Lafferty 1993). However, very interestingly, this relationship occurred when California horn snails were excluded from the calculations of snail density. Perhaps non-host snails are a source of mortality for trematode stages infectious to horn snails, as has been experimentally demonstrated for several freshwater snail-trematode systems (e.g., Chernin 1968, Sapp and Loker 2000). The snails in our system are among the easiest benthic invertebrates to quantify (being relatively large and living on the surface. Therefore, if snails were the only assemblage of interest for monitoring, they should likely be directly quantified versus indirectly quantified using trematodes. However, trematodes have added indicator value if trematode prevalence indicates snail density in addition to indicating other harder-to-quantify components of the free-living community.

4.b. Univariante diversity associations

Because six of the seven fish-using trematode species do not specialize on any particular fish species, it is not surprising that trematodes did not exhibit any consistent or significant diversity trends with the diversity of fish hosts. The weak
positive associations between trematodes and fishes for Simpson’s diversity and evenness might have resulted from the positive association between diversity and abundance of fishes across the studied habitats (unpublished data).

Contrary to our predictions, trematodes did not exhibit consistent positive diversity associations with surrounding benthic invertebrates. This was unexpected because previous work at finer scales found that trematodes positively reflected both small and large benthic invertebrate species richness (Hechinger et al. 2007). The size of the invertebrate taxa seemed to affect direction of the association between trematodes and invertebrate hosts. For several measures of diversity, large benthic invertebrates tended to positively correlate, while small benthic invertebrates tended to negatively correlate with trematodes. Given that the density of various large benthic invertebrates (e.g., crabs, shrimp, and snails) correlated with trematode prevalence, perhaps there is a direct association between large benthic invertebrate host diversity and trematode diversity in snails, and the negative association between trematodes and small invertebrate hosts is indirect. The diversity of large and small benthic invertebrate hosts is weakly, but negatively correlated across the estuary-habitat units we studied (unpublished data). In other words, if large benthic invertebrates drive levels of trematode infection in snails, and the diversity of large benthic invertebrates is negatively associated with the diversity of small benthic invertebrates, the negative associations between small benthic invertebrate host diversity and trematode diversity are the results of a spurious, indirect association.
4.d. General considerations

The trematode similarity matrices more strongly and positively related to those of fishes and benthic invertebrates when California horn snails were included in the free-living community. If trematodes reflect surrounding diversity then they would indicate the abundance of California horn snails, which are one of the most important (based upon numbers and biomass) members of the benthic invertebrates in these systems (Zedler 1982, Kuris et al. submitted). Also, of course, California horn snails are important for the trematodes, perhaps mostly for their role as the first intermediate host (horn snails are also are eaten by birds (Lafferty et al. 2006) and are important second intermediate hosts for several of the trematode species). The trematodes in this assemblage can also affect the density of horn snails (Lafferty 1993). Thus, given the ecological importance of horn snails in general, and the direct importance of horn snails and trematode for each other, it should be no surprise that the trematode assemblage most strongly associated with the free-living assemblage when horn snails were included in that assemblage.

Associations between trematodes and free-living assemblages were generally strongest for trematodes in the most common size classes of snails (20-25 and 25-30 mm snails). This occurred for the correlational analyses as well as in the multivariate analyses. This is convenient from the standpoint of using trematodes as bioindicator tools because these size classes are the most common and widespread (table 2).
The strength of associations did not depend on whether we used pre-
interactive or observed trematode prevalences, despite the fact that community
measures frequently differ when calculated using pre-interactive or observed
prevalences, particularly in larger size classes where competition is more important
(unpublished data, and see Figures A10-A30). However, a lack of strong differences
in associations for the two types of prevalences is consistent with previous studies
examining associations between trematodes and free-living assemblages (Hechinger
and Lafferty 2005, Hechinger et al. 2007, Whitney et al. 2007). If it turns out that pre-
interactive prevalences are not necessary in using trematodes as indicators, as
Hechinger et al. (2007) pointed out, this eases the use of trematodes because using
observed prevalences is simpler than calculating pre-interactive prevalences.

4.e. Summary and conclusions

Previous work has demonstrated that the abundance and diversity of final hosts
relates to the abundance and diversity of larval trematodes in snails (Smith 2001,
Hechinger and Lafferty 2005, Lafferty and Dunham 2005, Fredensborg et al. 2006,
results suggesting trematode prevalence and diversity relate at fine scales to the
abundance and diversity of benthic invertebrate second intermediate hosts. Our
results provide evidence that the prevalence of trematodes in the most easily sampled
size classes of snails may provide information on the density of host fishes, crabs,
burrowing shrimp, and snails. Additionally, we found that the entire trematode assemblage in snails changes over space in parallel with the combined assemblage of fishes and benthic invertebrate second intermediate hosts. This suggests that in situations lacking adequate support to monitor the various free-living assemblages, larval trematodes in snails may provide a relatively inexpensive bioindicator tool to be used for surveillance for community change as was done in Huspeni and Lafferty (2004) and Morely and Lewis (2006). Sometimes, the trematode community will also reveal information about specific groups or organisms as well.
Table 1. Trematode species that parasitize California horn snails (*Cerithidea californica*) as first intermediate host and the second intermediate hosts they use.

<table>
<thead>
<tr>
<th>Species</th>
<th>second intermediate hosts used¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austrobilharzia sp.</td>
<td>none</td>
</tr>
<tr>
<td>Mesostephanus appendiculatus</td>
<td>fishes</td>
</tr>
<tr>
<td>Small cyathocotylid</td>
<td>fishes</td>
</tr>
<tr>
<td>Catatrops johnstoni</td>
<td>snails</td>
</tr>
<tr>
<td>Acanthoparyphium spinulosum²</td>
<td>clams, snails, polychaetes</td>
</tr>
<tr>
<td>Himasthla rhigedana</td>
<td>crabs, horn snails</td>
</tr>
<tr>
<td>Himasthla sp. B³</td>
<td>snails, polychaetes</td>
</tr>
<tr>
<td>Cloacitrema michiganensis</td>
<td>clams, ghost shrimp</td>
</tr>
<tr>
<td>Parorchis acanthus</td>
<td>clams, ghost shrimp</td>
</tr>
<tr>
<td>Probolocoryphe uca</td>
<td>crabs</td>
</tr>
<tr>
<td>Small microphallid</td>
<td>amphipods</td>
</tr>
<tr>
<td>Euhaplorchis californiensis</td>
<td>killfish</td>
</tr>
<tr>
<td>Phocitremoides ovale</td>
<td>fishes</td>
</tr>
<tr>
<td>Pygidiopsoides spindalis</td>
<td>fishes</td>
</tr>
<tr>
<td>Stictodora hancocki</td>
<td>fishes</td>
</tr>
<tr>
<td>Large xiphidiocercaria⁴</td>
<td>polychaetes</td>
</tr>
<tr>
<td>Renicola buchanani</td>
<td>fishes</td>
</tr>
<tr>
<td>Renicola cerithidicola</td>
<td>fishes</td>
</tr>
</tbody>
</table>

¹Information on 2nd intermediate host use is based primarily on our familiarity with the system (but see Martin (1972), Huspeni and Lafferty (2004), and Lafferty et al. (2006)).

²Based on preliminary data (Hechinger and Smith, unpublished data), and a note in Martin (1972). *A. spinulosum* may be two cryptic *Acanthoparyphium* species with differing second intermediate host specificities.

³Himasthla sp. B equals *Echinoparyphium* sp. of Martin (1972).

⁴Hechinger and Smith (unpublished data).
Table 2. The number of sampled sites, mean number snails dissected per site, and sum snails dissected to quantify trematode communities, by 5 mm size class for each estuary-habitat unit.

<table>
<thead>
<tr>
<th>estuary</th>
<th>habitat</th>
<th>15 no. sites / mean no. snails per site / sum snails</th>
<th>20 no. sites / mean no. snails per site / sum snails</th>
<th>25 no. sites / mean no. snails per site / sum snails</th>
<th>30 no. sites / mean no. snails per site / sum snails</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSQ</td>
<td>flat</td>
<td>2 / 51 / 102</td>
<td>2 / 100 / 200</td>
<td>1 / 105 / 105</td>
<td>1 / 45 / 45</td>
</tr>
<tr>
<td>BSQ</td>
<td>marsh</td>
<td>1 / 33 / 33</td>
<td>4 / 78 / 310</td>
<td>5 / 68 / 338</td>
<td>3 / 50 / 149</td>
</tr>
<tr>
<td>EPB</td>
<td>channel</td>
<td>7 / 51 / 357</td>
<td>7 / 100 / 703</td>
<td>6 / 71 / 426</td>
<td>1 / 10 / 10</td>
</tr>
<tr>
<td>EPB</td>
<td>flat</td>
<td>6 / 51 / 304</td>
<td>6 / 100 / 602</td>
<td>6 / 78 / 467</td>
<td>3 / 32 / 95</td>
</tr>
<tr>
<td>EPB</td>
<td>marsh</td>
<td>5 / 47 / 236</td>
<td>5 / 100 / 502</td>
<td>4 / 49 / 195</td>
<td>1 / 12 / 12</td>
</tr>
<tr>
<td>EPB</td>
<td>pan</td>
<td>2 / 100 / 200</td>
<td>2 / 113 / 225</td>
<td>0 / 0 / 0</td>
<td>0 / 0 / 0</td>
</tr>
<tr>
<td>CSM</td>
<td>channel</td>
<td>3 / 50 / 149</td>
<td>7 / 99 / 696</td>
<td>7 / 100 / 702</td>
<td>3 / 82 / 247</td>
</tr>
<tr>
<td>CSM</td>
<td>flat</td>
<td>1 / 54 / 54</td>
<td>5 / 95 / 476</td>
<td>5 / 101 / 507</td>
<td>4 / 101 / 402</td>
</tr>
<tr>
<td>CSM</td>
<td>marsh</td>
<td>0 / 0 / 0</td>
<td>1 / 110 / 110</td>
<td>1 / 100 / 100</td>
<td>1 / 15 / 15</td>
</tr>
<tr>
<td>CSM</td>
<td>pan</td>
<td>1 / 54 / 54</td>
<td>2 / 100 / 200</td>
<td>3 / 70 / 210</td>
<td>3 / 75 / 224</td>
</tr>
</tbody>
</table>

sum sites / sum snails: 36 / 1913 52 / 5215 49 / 4155 27 / 1417

note: BSQ = Bahia San Quintín, CSM = Carpinteria Salt Marsh, EPB = Estero de Punta Banda
Figure 1. Results of correlations between fish density and summed pre-interactive prevalence (in snails of four size classes) of the trematode guild using fishes as second intermediate hosts.
Figure 2. Summed pre-interactive prevalence of fish-using trematodes and fish density positively correlated across habitat-units for the 20-25 mm snail size class (see Figure 1). X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans. The line is the reduced major axis, reflecting the bivariate nature of the data (Sokal and Rohlf 1981).
Figure 3. Results of correlations between density of polychaetes and summed pre-interactive prevalence in snails of four size classes of the trematode guild using polychaetes as second intermediate hosts.
Figure 4. Results of correlations between density of (a) mollusks, (b) snails, (c) snails excluding California horn snails, and (d) bivalves with summed pre-interactive prevalence in snails (of four size classes) of the trematode guild using the taxa as second intermediate hosts.
Figure 5. Mollusk density (aggregate snails and bivalves) negatively correlated to summed pre-interactive prevalence of mollusk-using trematodes across habitat-units for (a) 20-25 mm, (b) 25-30 mm, and (c) 30-35 mm snail size classes (see Figure 4a). X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans. All lines are the reduced major axes (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 6.

with horn snails

without horn snails

Snail density (no./m²)

mollusk-using trematode prevalence
Figure 6. Snail density, with (left) or without (right) California horn snails, positively correlated to summed pre-active prevalence of mollusk-using trematodes across habitat-units for (a,b) 20-25 mm, (c,d) 25-30 mm, and (e,f) 30-35 mm snail size classes (see Figures 4b and 4c). $X =$CSM, $Y =$EPB, $Z =$BSQ; blue =$channels, green =$marsh, black =$flats, orange =$pans. The lines are the reduced major axes (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 7. Results of correlations between density of (a) decapods, (b) crabs and (c) burrowing shrimp with summed pre-interaction prevalence in snails (of four size classes) of the trematode guild using decapods as second intermediate hosts.
Figure 8. Decapod density (crabs + burrowing shrimp) positively correlated to the summed pre-interactive prevalence of decapod-using trematodes across habitat-units for (a) 20-25 mm and (b) 25-30 mm snail size classes (see Figure 7a). $X = \text{CSM}$, $Y = \text{EPB}$, $Z = \text{BSQ}$; blue = channels, green = marsh, black = flats, orange = pans. The lines are the reduced major axes (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 9. Crab density positively correlated to the summed pre-interactive prevalence of crab-using trematodes across habitat-units for (a) 20-25 mm and (b) 25-30 mm snail size classes (see Figure 7b). X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans. The lines are the reduced major axes (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 10. Burrowing shrimp density positively correlated with summed pre-interactive prevalence of shrimp-using trematodes across habitat-units for the 20-25 mm snail size class (see Figure 7c). X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans. The line is the reduced major axis (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 11.
Figure 11. Results of correlations between four measures of diversity of fishes with those measures of diversity (in four size classes of snails) of the guild of trematodes that use fishes as second intermediate hosts. (a) is species richness, rarified to two individuals, ES(2); (b) is Simpson’s diversity, 1/D; (c) is Simpson’s evenness, E(1/D); and (d) is taxonomic diversity, delta. Indices for trematode assemblages were based on pre-interactive prevalences of trematode species. The lines are the reduced major axes (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 12. Results of correlations between four measures of diversity of small benthic invertebrates with those measures of diversity (in four size classes of snails) of the guild of trematodes that use benthic invertebrates as second intermediate hosts. (a) is species richness, rarified to two individuals, ES(2); (b) is Simpson’s diversity, 1/D; (c) is Simpson’s evenness, E(1/D); and (d) is taxonomic diversity, delta. Indices for trematode assemblages were based on pre-interactive prevalences of trematode species.
Figure 13. Small benthic invertebrate diversity (1/D) negatively correlates with diversity of benthic invertebrate-using trematodes across habitat-units for the 20-25 mm snail size class (see Figure 12b). X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans. Indices for trematode assemblages were based on pre-interactive prevalences of trematode species. The line is the reduced major axis (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 14. Small benthic invertebrate taxonomic diversity (delta) negatively correlates with taxonomic diversity of benthic invertebrate-using trematodes across habitat-units for the 25-30 mm snail size class (see Figure 12d). \( X = \text{CSM}, Y = \text{EPB}, Z = \text{BSQ} \); blue = channels, green = marsh, black = flats, orange = pans. Indices for trematode assemblages were based on pre-interactive prevalences of trematode species. The line is the reduced major axis (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 15. Results of correlations between four measures of diversity of large benthic invertebrates with those measures of diversity (in four size classes of snails) of the guild of trematodes that use benthic invertebrates as second intermediate hosts. (a) is species richness, rarified to two individuals, ES(2); (b) is Simpson's diversity, 1/D; (c) is Simpson's evenness, E(1/D); and (d) is taxonomic diversity, delta. Indices for trematode assemblages were based on pre-interactive prevalences of trematode species.
Figure 16. Large benthic invertebrate diversity (1/D) positively correlates with diversity of benthic invertebrate-using trematodes across habitat-units for the 15-20 mm snail size class (see Figure 15b). Diversity index for trematodes was based on pre-interactive abundances. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans. The line is the reduced major axis (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 17. Large benthic invertebrate evenness (E(1/D)) positively correlates with evenness of benthic invertebrate-using trematodes across habitat-units for the (a) 25-30 mm and the (b) 30-35 mm snail size classes (see Figure 15c). Diversity index for trematodes was based on pre-interactive abundances. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans. The lines are the reduced major axes (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 18. Large benthic invertebrate taxonomic diversity (delta) positively correlates with the taxonomic diversity of benthic invertebrate-using trematodes across habitat-units for the 25-30 mm snail size class (see Figure 15d). Diversity index for trematodes was based on pre-interactive abundances. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans. The line is the reduced major axis (Sokal and Rohlf 1981), reflecting the bivariate nature of the data.
Figure 19.

(a) 0.0054 0.0021 0.26 **
(b) 0.30 0.0087 0.0025 0.12 **
(c) 0.072 0.12 0.0069 0.38 **
(d) 0.31 0.031 0.0014 **

4th root transformed densities

From RELATIVE

Snail 5 mm size class

with C. cali.

without C. cali.
Figure 19. Results from RELATE analyses examining whether trematode assemblages shift congruently across estuary-habitat units with the assemblage of fishes and benthic invertebrates. Similarity matrices for the combined fish and benthic invertebrate host assemblage either included California horn snails (top) or excluded California horn snails (bottom), and were based on raw (left) or fourth root transformed (right) species densities. The y-axes are the $\rho$ statistics indicating the strength of the association between two independently derived similarity matrices for habitat units: one based on trematode assemblages (using pre-interactive prevalences) and one based on the combined fish and benthic invertebrate host assemblage. We examined relationships using trematode assemblages found in four different snail size classes (x-axes). Asterisks above symbols indicate p-values for $\rho$ statistics: one asterisk indicates $p \leq .05$, two indicate $p \leq .01$. Actual p-values are above the asterisks.
Figure 20.

Raw densities

$4^{th}$ root densities

Fish & benthos assemblage similarity

Trematode assemblage similarity

15s

20s

25s

30s

132
Figure 20. Associations between corresponding elements in independently calculated trematode and fish & benthic invertebrate host assemblage similarity matrices among estuary-habitat units. These scatter plots help visualize the results in Figures 19a,b of the RELATE tests for association of similarity matrices based upon trematode assemblages with those based on fish & benthic invertebrate host assemblages. Plots are shown for comparisons between assemblages of trematodes (using pre-interactive prevalences) in each of the four snail size classes and for Bray-Curtis community similarities calculated for using both raw (left) and fourth root transformed (right) fish and benthic invertebrate host species densities (see text for details).
Figure A1. Results of correlations between density of fishes and summed observed prevalence in snails of four size classes of the trematode guild using fishes as 2nd intermediate hosts.
Figure A2. Results of correlations between density of polychaetes and summed observed prevalence in snails of four size classes of the trematode guild using polychaetes as 2nd intermediate hosts.
Figure A3. 

- a. density snails v prevalence mollusc-users 
  - correlation coefficient

- b. density bivalves v prevalence mollusc-users 
  - 95% CI

- c. density molluscs v prevalence 
  - 95% CI

- d. density snails (exc. C. cal.) v prevalence mollusc-users 
  - correlation coefficient

136
Figure A3. Results of correlations between density of (a) mollusks, (b) snails, (c) snails excluding California horn snails, and (d) bivalves with summed observed prevalence in snails (of four size classes) of the trematode guild using the taxa as second intermediate hosts.
Figure A4.

a. **density decapods v prevalence decapod-users**

b. **density crabs v prevalence crab-users**

c. **density shrimp v prevalence shrimp-users**
Figure A4. Results of correlations between density of (a) decapods, (b) crabs and (c) burrowing shrimp with summed observed prevalence in snails (of four size classes) of the trematode guild using decapods as second intermediate hosts.
Figure A5. Results of correlations between four measures of diversity of fishes with those measures of diversity (in four size classes of snails) of the guild of trematodes that use fishes as second intermediate hosts. (a) is species richness, rarified to two individuals, ES(2); (b) is Simpson’s diversity, 1/D; (c) is Simpson’s evenness, E(1/D); and (d) is taxonomic diversity, delta. Indices for trematode communities were based on observed prevalences of trematode species.
Figure A6. Results of correlations between four measures of diversity of small benthos with those measures of diversity (in four size classes of snails) of the guild of trematodes that use benthos as second intermediate hosts. (a) is species richness, rarified to two individuals, ES(2); (b) is Simpson’s diversity, 1/D; (c) is Simpson’s evenness, E(1/D); and (d) is taxonomic diversity, delta. Indices for trematode communities were based on observed prevalences of trematode species.
Figure A7. Results of correlations between four measures of diversity of large benthos with those measures of diversity (in four size classes of snails) of the guild of trematodes that use benthos as second intermediate hosts. (a) is species richness, rarified to two individuals, ES(2); (b) is Simpson’s diversity, 1/D; (c) is Simpson’s evenness, E(1/D); and (d) is taxonomic diversity, delta. Indices for trematode communities were based on observed prevalences of trematode species.
Figure A8. Scatter plots of fish density vs. fish-using trematode prevalence in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 1. Associations using observed prevalences (bottom row) correspond to results in Figure A1. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A9. Scatter plots of polychaete density vs. polychaete-using trematode prevalence in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 3. Associations using observed prevalences (bottom row) correspond to results in Figure A3. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A10. Scatter plots of mollusk density vs. mollusk-using trematode prevalence in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 4a. Associations using observed prevalences (bottom row) correspond to results in Figure A3a. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A11. Scatter plots of snail density vs. mollusk-using trematode prevalence in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 4b. Associations using observed prevalences (bottom row) correspond to results in Figure A3b. $X = \text{CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh,}$
$\text{black = flats, orange = pans.}$
Figure A12. Scatter plots of snail (excluding California horn snail) density vs. mollusk-using trematode prevalence in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 4c. Associations using observed prevalences (bottom row) correspond to results in Figure A3c. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A13. Scatter plots of bivalve density vs. mollusk-using trematode prevalence in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 4d. Associations using observed prevalences (bottom row) correspond to results in Figure A3d. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A14. Scatter plots of decapod density vs. decapod-using trematode prevalence in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 7a. Associations using observed prevalences (bottom row) correspond to results in Figure A4a. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A15. Scatter plots of crab density vs. crab-using trematode prevalence in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 7b. Associations using observed prevalences (bottom row) correspond to results in Figure A4b. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A16. Scatter plots of shrimp density vs. shrimp-using trematode prevalence in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 7c. Associations using observed prevalences (bottom row) correspond to results in Figure A4c. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A17. Scatter plots of fish rarified species richness (ES(2)) vs. fish-using trematode rarified species richness ES(2) in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 11a. Associations using observed prevalences (bottom row) correspond to results in Figure A5a. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A19. Scatter plots of fish rarified diversity (1/D) vs. fish-using trematode rarified species richness diversity (1/D) in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 11b. Associations using observed prevalences (bottom row) correspond to results in Figure A5b. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A19. Scatter plots of fish evenness (E(1/D)) vs. fish-using trematode evenness (E(1/D)) in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 11c. Associations using observed prevalences (bottom row) correspond to results in Figure A5c. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A20. Scatter plots of fish taxonomic diversity (delta) vs. fish-using trematode taxonomic diversity (delta) in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 11d. Associations using observed prevalences (bottom row) correspond to results in Figure A5d. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A21. Scatter plots of small benthos rarified species richness (ES(2) vs. small benthos-using trematode rarified species richness ES(2) in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 12a. Associations using observed prevalences (bottom row) correspond to results in Figure A6a. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A22. Scatter plots of small benthos diversity (1/D) vs. small benthos-using trematode rarified species richness ES(2) in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 12b. Associations using observed prevalences (bottom row) correspond to results in Figure A6b. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A23. Scatter plots of small benthos evenness, $E(1/D)$, vs. small benthos-using trematode evenness, $E(1/D)$, in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 12c. Associations using observed prevalences (bottom row) correspond to results in Figure A6c. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A24. Scatter plots of small benthos taxonomic diversity (delta) vs. small benthos-using trematode taxonomic diversity (delta) in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 12d. Associations using observed prevalences (bottom row) correspond to results in Figure A6d. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A25. Scatter plots of large benthos rarified species richness, ES(2), vs. large benthos-using trematode rarified species richness ES(2) in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 15a. Associations using observed prevalences (bottom row) correspond to results in Figure A7a. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A26. Scatter plots of large benthos diversity (1/D) vs. large benthos-using trematode rarified diversity (1/D) in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 15b. Associations using observed prevalences (bottom row) correspond to results in Figure A7b. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A27. Scatter plots of large benthos evenness, E(1/D), vs. large benthos-using trematode evenness, E(1/D), in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure 15c. Associations using observed prevalences (bottom row) correspond to results in Figure A7c. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Figure A28. Scatter plots of large benthos taxonomic diversity (delta) vs. large benthos-using trematode taxonomic diversity in four size classes of snail (by column). Associations using pre-interactive prevalences (top row) correspond to the results in Figure A7d. Associations using observed prevalences (bottom row) correspond to results in Figure A7d. X = CSM, Y = EPB, Z = BSQ; blue = channels, green = marsh, black = flats, orange = pans.
Table A1a. Similarity matrix for habitat units using Bray-Curtis similarities on raw density data of species of fishes and benthic invertebrates.

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Table A1c. Similarity matrix for habitat units using Bray-Curtis similarities on raw density data of species of fishes and benthic invertebrates (excluding CA horn snails).

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Table A1d. Similarity matrix for habitat units using Bray-Curtis similarities on fourth root transformed density data of species of fishes and benthic invertebrates (excluding CA horn snails).

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Table A2. Top species contributors to the Bray-Curtis dissimilarities between the estuaries BSQ and CSM (using habitats as units) based on the raw and fourth root transformed densities of species of fishes and benthic invertebrates. Lists include those species contributing to the top 90% of dissimilarities and demonstrates the effect of fourth root transformation of increasing the role played by fishes and large benthos.

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Table A3a. Similarity matrix for habitat units using Bray-Curtis similarities on observed prevalence data of trematode species in 15-20 mm size classed snails.

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Table A3b. Similarity matrix for habitat units using Bray-Curtis similarities on observed prevalence data of trematode species in 20-25 mm size classed snails.

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Table A3c. Similarity matrix for habitat units using Bray-Curtis similarities on observed prevalence data of trematode species in 25-30 mm size classed snails.

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</table>
Table A3d. Similarity matrix for habitat units using Bray-Curtis similarities on observed prevalence data of trematode species in 30-35 mm size classed snails.

|       | BSQchan | BSQflat | BSQpan  | CSMchan | CSMflat | CSMpan  | CSMmarsh | CSMpan  | EPBchan | EPBflat | EPBpan  | EPBmarsh | EPBpan |
|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|--------|
| BSQchan| 49.503  |         |         |         |         |         |         |         |         |         |         |         |         |        |
| BSQflat|         | 49.769  | 58.321  |         |         |         |         |         |         |         |         |         |         |        |
| BSQpan | 57.706  | 43.33   | 47.436  |         |         |         |         |         |         |         |         |         |         |        |
| CSMchan| 41.637  | 25.273  | 37.641  | 51.645  |         |         |         |         |         |         |         |         |         |        |
| CSMflat| 28.572  | 17.648  | 25.555  | 43.332  | 59.955  |         |         |         |         |         |         |         |         |        |
| CSMmarsh| 49.014 | 33.365  | 33.482  | 36.476  | 39.882  | 29.849  |         |         |         |         |         |         |         |        |
| CSMpan | 28.429  | 18.954  | 26.424  | 46.028  | 71.944  | 82.675  | 25.647  |         |         |         |         |         |         |        |
| EPBchan| 5.9043  | 8.7018  | 21.856  | 11.03   | 9.8086  | 1.8153  | 8.724   | 2.0017  |         |         |         |         |         |        |
| EPBflat| 28.748  | 19.546  | 29.341  | 55.333  | 55.984  | 46.131  | 26.462  | 55.897  | 29.084  |         |         |         |         |        |
| EPBmarsh| 23.19  | 17.532  | 23.141  | 23.923  | 43.68   | 22.822  | 30.317  | 31.858  | 17.56   | 41.371  |         |         |         |        |
Chapter 5

Trematodes indicate biodiversity in the Chilean intertidal zone and Lake Tanganyika

Ryan F. Hechinger, Kevin D. Lafferty and Armand M. Kuris
Abstract

Trematode communities in populations of estuarine snails may reflect surrounding animal diversity, abundance, and food web function. We know less about the potential for trematodes to serve as bioindicators in other habitats. Here, we reanalyze data from two published studies concerning trematodes, one in the Chilean rocky intertidal zone and the other from the East African rift lake, Lake Tanganyika. Our analyses indicate that trematodes in both habitats are directly and positively related to surrounding hosts and biotic interactions. This further supports the potential for using trematodes in first intermediate hosts as bioindicators.

Can parasitic worms serve as indicators of surrounding animal diversity, abundance, and food web function? Parasites, like all organisms, interact with various aspects of the environment. However, unlike other organisms, many parasites with multiple-host life cycles are predictably and directly linked to several types of invertebrate and vertebrate hosts. Additionally, at some steps in their life cycles, most of these parasites use predator-prey interactions to get from host to host. Thus, these parasites require both the presence of each of their hosts and a functioning food web (Gardner and Campbell, 1992; Marcogliese and Cone, 1998; Huspeni et al., 2005; Hudson et al., 2006). Among such parasites, trematode flatworms are particularly promising ecological indicators (Huspeni et al., 2005). Not only are trematodes connected to surrounding biodiversity and food webs, they are, importantly, easily
sampled in their first intermediate hosts (Huspeni et al., 2005). For instance, trematode communities can correlate with surrounding bird communities (Smith, 2001; Hechinger and Lafferty, 2005; Fredensborg et al., 2006). This has led to work developing the use of larval trematodes as indicators in estuarine habitats, directly assessing the association of trematode communities with surrounding free-living fauna (Huspeni and Lafferty, 2004; Hechinger and Lafferty, 2005; Lafferty and Dunham, 2005; Lafferty et al., 2005; Hechinger et al., 2007; Whitney et al., in press).

We know less about the potential for trematodes as indicators from non-estuarine habitats. However, trematodes are widespread and their ability to act as indicators should not be restricted to estuaries. Here, we briefly consider two studies of trematodes and their hosts, one from the rocky intertidal in Chile (Loot et al., 2005) and the other from a rift lake in East Africa (McIntyre et al., 2005). We analyze data from these studies to evaluate the promise of developing trematodes as indicators in these types of ecosystems.

In Chile, Loot et al. (2005) studied parasitism in two rocky intertidal reserves and two areas fully open to exploitation. The trematode, Proctoecces lintoni, was more common in reserves than in exploited areas, in first intermediate host mussels and second intermediate host limpets. Loot et al. (2005) suspected that the reserves had higher levels of parasitism because the reserves harbored greater densities of final host fishes (which should increase the transmission of parasites to mussels). Indeed,
our reanalysis of their data shows that reserves also harbored both greater densities of infected fish and greater densities of adult trematodes (whose offspring infect mussels) (Fig. 1). This further supports the idea (Hudson et al., 2006) that a 'healthy' ecosystem is one that is rich in parasites.

Could measures of trematodes provide an assessment of the success of the rocky intertidal reserves in protecting biodiversity? Generally, for an indicator to be useful, it must be both easier to sample than the character of interest and tightly associated with it (McGeoch, 1998). In Loot et al.'s (2005) study system, fish are more difficult to sample than mussels, thus using mussel parasites as indicators is appealing. Do levels of trematode parasitism in first intermediate host mussels indicate the abundance of surrounding fish? Although there are only four data points from Loot et al.'s study, fish density is tightly related with trematode parasitism in mussels, providing proof of concept that trematodes may serve as indicators for the effectiveness of the reserve for protecting fished species (Fig. 2a).

A study by McIntyre et al. (2005) on effects of disturbance on a snail assemblage in an East African rift lake (Lake Tanganyika) provides similar insights into the use of trematodes as indicators. Here, trematodes are less prevalent in snails at disturbed sites. Do the disturbed sites harbor a lower abundance or diversity of surrounding animal life? Although McIntyre et al. (2005) did not present data on the rest of the community at their study sites, they provided data that serves as a proxy
for crab abundance: they used snail shell damage as an indicator of the intensity of interactions with predatory crabs. These crabs may serve as prey for vertebrate final hosts and also likely act as second intermediate hosts for some of the unidentified trematodes. Thus, areas with more crabs may have more trematodes in snails. Do areas with greater levels of crab damage have higher trematode prevalence in snails? Here too, we have only four data points, but plotting crab attack rate versus the prevalence of trematode infection in snails indicates that these variables are tightly and positively correlated (Fig. 2b). A link between crab abundance and trematodes could result from increased life cycle completion of trematodes that use crabs as second intermediate hosts. The relationship could also occur if an abundance of crabs attracts mammal final hosts (otters prey upon crabs in this system (P.B. McIntyre pers. comm.)). In the latter case, trematodes in snails would also be good indicators for the abundance of otters.

Despite the low sample sizes, each of these studies provides at least marginally significant results that lie exactly in the direction predicted by the hypothesis that trematodes in first intermediate hosts indicate surrounding free-living diversity and food web function. However, we can go further and statistically assess the overall support these two analyses give the hypothesis by combining the independent $p$-values using the “Z-transform” procedure (Strube and Miller, 1986; Rice, 1990). This “mini-meta-analysis” yields a $p$-value of 0.012, demonstrating the
consensus of the two analyses—that trematodes do positively indicate surrounding diversity and ecological function.

Our reanalysis of data from the studies of Loot et al. (2005) and McIntyre et al. (2005) simply highlights the potential of trematodes to serve as indicators of biodiversity and food web function in widely differing ecosystems. We found significant results despite small sample sizes, indicating the associations are very strong. Promising trematode-host indicators are common throughout the world in many types of aquatic and marine habitats (Huspeni et al. 2005). Most importantly, in many cases, a speciose guild of trematodes uses the same species of first intermediate host snail. Due to the divergent life cycles of different trematode species (i.e., they use different second intermediate and final hosts), such guilds capture much of the complexity of the surrounding community. These parasites are very easily sampled compared to surveying entire communities of invertebrates, fishes, birds, and mammals. This indicates that trematodes in first intermediate hosts can provide cost-effective and useful information about the surrounding ecosystem. In a time when the importance of biodiversity monitoring is escalating (e.g., Noss, 1990; Dobson, 2005), the widespread use of trematodes as bioindicators should be seriously considered.

Acknowledgements

We are grateful to P. B. McIntyre, E. Michel, and G. Loot for sharing information and providing very useful comments on this manuscript. N. Talhouk also provided helpful
comments. This manuscript benefited from financial support from the US NIH/NSF Ecology of Infectious Disease Program (DEB-0224565) and from the US EPA's STAR EaGLE program (through funding to the Pacific Estuarine Ecosystem Indicator Research Consortium (R-882867601)). It has not been subjected to any EPA review and therefore no official endorsement should be inferred.
Figure 1.

**a.**

Overall heterogeneity

$$F_U = 10.7$$

$$p = 0.0007$$

Protected different than exploited?

$$t_{35} = 3.6$$

$$p = 0.0010$$

**Study sites**

<table>
<thead>
<tr>
<th>No. of trematodes (in fish) / 100 m of shore / 30 min</th>
<th>ECIM</th>
<th>EQ</th>
<th>LCS</th>
<th>LCN</th>
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<tr>
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<tr>
<td>exploited</td>
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</tbody>
</table>

**b.**

Overall heterogeneity

$$F_U = 3.8$$

$$p = 0.018$$

Protected different than exploited?

$$t_{35} = 2.5$$

$$p = 0.019$$

**Study sites**

<table>
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<tr>
<th>No. of trematodes (in fish) / 100 m of shore / 30 min</th>
<th>ECIM</th>
<th>EQ</th>
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FIGURE 1

(a) Density of infected final host fish (± se), and (b) density of trematodes living in final host fishes (± se) at protected and exploited study sites in the rocky intertidal. These data are derived from data given in Loot et al. (2005). We calculated the density of infected fish by multiplying the density of all fish by the prevalence of infection. Trematode density is the product of the density of fish and the mean number of trematodes per fish individual (mean abundance). We performed statistics on our derived measures by using standard errors obtained by applying general rules of error propagation (Taylor, 1982) to the standard errors and degrees of freedom provided in Loot et al. (2005). The $F_C$-statistic is from an ANOVA allowing unequal variances (Rice and Gaines, 1989; Rice and Gaines, 1993) examining whether there is overall heterogeneity across sites, and the $t$-test (allowing unequal variances (Norman and Streiner, 2000)) directly assesses whether protected sites differ from exploited sites.
Figure 2.

(a) Mean no. fish / 100 m of shore / 30 min vs. prevalence of trematode in mussels.

\[ R^2 = 0.78 \]
\[ p = 0.059 \]

(b) Crab attack level (scar frequency) vs. prevalence of trematodes in snails.

\[ R^2 = 0.80 \]
\[ p = 0.054 \]
FIGURE 2

Scatter plots suggesting that trematodes can serve as indicators of (a) fish abundance and (b) crab abundance (for which crab attack rates on snails may serve as a proxy). Data for (a) is replotted from Loot et al. (2005) and data for (b) are from McIntyre et al. (2005) and are mean scar frequency (density-weighted) across elevations and prevalence of parasitism in adult-sized snails. The $p$-values are one-tailed. Taken together, both studies support the general hypothesis that trematodes indicate surrounding diversity, with a consensus $p$-value of 0.012. Fitted lines are ordinary least squares regression lines.
Chapter 6

Annotated key to the trematode species infecting *Batillaria attramentaria*

(Prosobranchia: Batillariidae) as first intermediate host

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DOI: 10.1016/j.parint.2007.06.004
Abstract

In eastern Asia and western North America at least nine morphologically distinguishable species of digenean trematode infect the mud snail, *Batillaria atramentaria* (Sowerby 1855) (=*B. cumingi* (Crosse 1862)), as first intermediate host. Further, molecular and morphological evidence indicates that several of these trematode species comprise complexes of cryptic species. I present an identification key to these nine trematode morphospecies (including four newly reported species). Additionally, I provide an annotated list, which includes further diagnostic information on the larval stages (cercariae, parthenitae, and metacercariae), information on second intermediate host use, links to the previous relevant reports of trematodes infecting *B. atramentaria* and notes on other aspects of the species’ biology.
Introduction

In eastern Asia, at least nine morphologically recognized species of digenean trematode infect the mud snail, *Batillaria attramentaria* (see Ito 1957, Shimura and Ito 1980, Rybakov 1987, Harada 1989, Harada and Suguri 1989 and this report). Additionally, one of these trematode species is common in *B. attramentaria* populations introduced to the west coast of North America (Torchin et al. 2005, Miura et al. 2006b). There exists no comprehensive listing of the trematodes of *B. attramentaria*, nor a key to their identification. This absence is an impediment to research on this ensemble (sensu (Fauth et al. 1996)) of larval trematodes.

The snail, *B. attramentaria* (Sowerby 1855) (=*B. cumingi* (Crosse 1862)) ranges widely along the east coast of Asia (Hasegawa 2000). Additionally, this snail has been introduced to the west coast of North America (Byers 1999). The snails are common in the intertidal on soft and hard bottoms of estuaries and protected outer coast sites (e.g., see Adachi and Wada 1997).

I recently (11 June – 30 June 2003) took part in an ecological study of *B. attramentaria* and its trematode parasites (Torchin et al., unpublished work). During this survey, I examined trematodes from 17 populations of *B. attramentaria* from Osaka, Wakayama, Chiba, and Miyagi prefectures, Honshu, Japan. I recognized, based on morphology, eight species of digenean trematodes infecting *B. attramentaria* as their first intermediate host. To my knowledge, two of these species
have been described from this snail in both Japan (Ito 1957, Shimura and Ito 1980, Harada 1989, Harada and Suguri 1989) and eastern Russia (Rybakov 1987) and two have been previously reported only from eastern Russia (Rybakov 1987). A ninth trematode species has been described infecting *B. atramentaria* in eastern Russia (Rybakov 1987), but this species has never been reported from Japan. Thus, I encountered undescribed larval stages of four species of trematodes that infect *B. atramentaria*. However, they are relatively easily placed in appropriate taxonomic families (following Cable 1956, Holliman 1961), sometimes tentatively to genus, and provisionally recognized as “morphospecies.”

Here, I provide a key to, and an annotated listing of, the species of digenean trematodes known to infect *B. atramentaria*. I include species that have been clearly described from *B. atramentaria* (from both Japan and Russia) and the four newly reported species that I encountered during the aforementioned ecological survey. Trematodes are frequently highly specific for their first intermediate host snails (Dawes 1946, Ewers 1964, Ginetsinskaya 1968, Yamaguti 1975, Gibson and Bray 1994). In the absence of data demonstrating otherwise, I believe it is prudent to assume trematodes are different species if they infect different snail species, particularly sympatric snails that belong to different genera. Further support for hesitating to assume conspecific identity of trematodes in different snail species is the continual discovery of cryptic trematode species existing even within the *same* host species (Donald et al. 2004, Lucas et al. 2005), including some that use *B.*
attramentaria (Miura et al. 2005). Consequently, I do not include in the key two species previously reported infecting B. attramentaria. This is because these species were described from a different genus of snail and we are lacking descriptions of specimens from B. attramentaria. However, in the annotations for related species, I alert workers to the possible existence of these additional species and detail morphological characters that might distinguish them from the species in the key. This key should be considered provisional, as the existence of cryptic species has been demonstrated by molecular genetic work and is suggested by morphological evidence. Additionally, although I provide descriptive details and working names for the newly reported species, I am not attaching formal species names or designating type specimens—further work is necessary for complete descriptions. I hope this key facilitates ecological work and highlights needed taxonomic research on this ensemble of larval trematodes.

**Materials and Methods**

The key primarily uses characters of the cercariae and should be used in conjunction with the figures and species annotations. The line drawings primarily complement the key, and I only included details that appeared to be consistently and readily discernable in live specimens. The annotations have more descriptive detail, as well as information on parthenitae (sporocysts or rediae) and metacercariae. I additionally provide notes on the types of second intermediate host potentially used by the various trematode species. All of these trematodes probably use birds as final hosts (based

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upon known life cycles of taxonomically related trematodes). I also provide potential links to previous records of trematodes in *B. attramentaria*, as well as notes of additional biological interest.

Except as otherwise indicated, all drawings are based on my observations of live specimens from dissected snails. For each species I encountered, I obtained measurements using an ocular micrometer on haphazardly-picked cercariae and parthenitae, which originated from a single dissected host, were heat-killed, fixed in 10% formalin, stained with Semichon's acetocarmine, and mounted wet in glycerin. If cercarial bodies or tails fixed dorso-ventrally flexed (noted below when this was consistent for a species), I took length measurements in several straight sections along the lateral view. For bilaterally paired features (e.g., eye spots, lateral fin lengths) I alternatingly measured either the left or the right structures for each new individual. For cercarial counts in parthenitae, I attempted to include all embryos larger than 5 μm. Unless otherwise noted, all measurements are in μm ± 1 s.d. Also, I use the term "flame bulb" instead of "flame cell," believing it to more clearly reflect the structure of a prostonephridium. To facilitate access, vouchers have been deposited at the U.S. National Parasite Collection.
Results and Discussion

*Key to the digenean trematodes known to use* Batillaria attramentaria as first intermediate host

1 a. Cercariae with eye spots.................................................................2
   b. Cercariae without eye spots..........................................................3

2 a. Tail of cercaria with two lateral fins (proximally) and one dorsal-ventral fin (distally)..............*Cercaria batillariae* Shimura and Ito 1980
   b. Tail of cercaria forked...............................................................Schistosomatid sp. I

3 a. Tail of cercaria forked...............................................................Cyathocotylid sp. I
   b. Tail of cercaria not forked........................................................4

4 a. Tail of cercaria with parenchymous cells and an invaginated posterior tip...........................................(philophthalmids) 5
   b. Tail of cercaria without such characters........................................6

5 a. Mature cercaria translucent (immature stages more opaque, with well-defined cyst glands), with hundreds of obvious thin ducts (from cyst glands) in tegument of ventral surface (potentially misinterpreted as

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spines); metacercariae may form flask-shaped cysts in dissection dish...........................................................................................................Philophthalmid sp. I

b. Mature and immature cercaria opaque, with many dark, poorly-defined cyst glands throughout body; cercaria without obvious tegumental ducts or spines; metacercariae can form circular cysts in dissection dish...........................................................................................................Philophthalmid sp. II

6 a. Cercaria with distinctive pinnately-branched excretory system; with no oral stylet; develop in rediae.................................................................
..................................................................................................................(Acanthoparyphium) 7

b. Cercaria without a pinnately-branched excretory system; with oral stylet; develop in sporocysts................................................................. 8

7 a. Cercaria without pronounced cephalic collar; cercarial body longer than ~290 μm................................................................. Acanthoparyphium sp. I

b. Cercaria with pronounced cephalic collar; cercarial body shorter than 200 μm.................................................................

........Acanthoparyphium macracanthum Rybakov and Lukomskaya 1988

8 a. Cercaria with prominent oral stylet (~21 μm long); body translucent, with highly visible penetration ducts and glands; V-shaped excretory
bladder extending less than one fifth into the body; ventral sucker not present..........................*Cercaria hosoumininae* Shimura and Ito 1980

b. Cercaria with small indistinct oral stylet (~9 μm long); most of body filled with opaque cyst glands; penetration glands and ducts not clearly visible; cercaria with distinctive Y-shaped excretory bladder extending about half way up the body; ventral sucker present..........................*Renicola* sp. I

**Species annotations**

*Acanthoparyphium* sp. I (*Echinostomatidae*)—Fig. 1, Fig. 2

Cercariae develop in active rediae in gonadal region of snail visceral mass. Cercariae positively phototactic, swim by cupping body at ventral sucker, lash tail and wiggle side to side, pivoting along long axis.

This species has not previously been reported (but see below). I examined specimens from Chiba Prefecture (Obitsu River) and Miyagi Prefecture (Torinoumi), the description being based on an infection from the latter locality (voucher deposited, USNPC No. 099682.00).

*Diagnosis*: Cercaria non-oculate, pharyngeate, with oral and ventral suckers. Cercaria body oblong to oval in dorsal view, dorsal-ventrally flattened, length 289-350 (318 ± 16, n = 13), max width 130-161 (144 ± 8, n = 12) at or slightly anterior to ventral sucker. Collar not very pronounced, with 23 spines of similar
size in single row, interrupted ventrally. Length of terminal collar spine 6.2-8.8
(8.1 ± 0.9, n = 10), width at base 1.5-2.5 (2.2 ± 0.4, n = 10). Tegument otherwise
without spines, but with papillae discernable laterally. Tail simple, circular in
cross-section, attached subterminally, length 267-363 (322 ± 32, n = 12), width
29-41 (36 ± 4, n = 12) just posterior to base. Cystogenous glands obscure much of
internal structure. Oral sucker ± circular, length 41-51 (47 ± 3, n = 13), width 43-
46 (44 ± 1, n = 13), with undetermined number of glands, with apparently two
pairs of ducts (one pair/side) opening anteriorly. An additional two pairs of ducts
(one pair/side) observed from just posterior to pharynx extending anteriorly and
opening at sides of oral sucker, their origin (presumably at penetration glands) not
observed and obscured by cystogenous glands. Ventral sucker ± circular, about
2/3 body length from anterior end, length 55-73 (65 ± 5, n = 13), width 56-67 (61
± 3, n = 13), may appear wider than long in live specimens. Prepharynx evident,
shorter than pharynx. Pharynx length 24-32 (27 ± 2, n = 12), width 12-18 (15 ± 2,
n = 12). Esophagus slender, bifurcating just anterior to ventral sucker. Digestive
ceca curve around ventral sucker, continue posteriorly to middle of excretory
bladder, almost to posterior of body. Excretory bladder thin-walled, more-or-less
square, with short anterior-medial connection receiving main collecting ducts.
Main collecting ducts extend anteriorly from connection with bladder, laterally
around ventral sucker, anteriorly to oral sucker, with several medially and
laterally projecting diverticula between ventral sucker and pharynx. Rest of body
excretory ducts and complete flame bulb formula not observed. Caudal excretory
duct extends into tail, bifurcating and opening laterally. Mass of primordia (probably genital) evident upon acetocarmine staining, along midline, just posterior to ventral sucker, length 13-18 (15 ± 2, n = 7), width 15-20 (17 ± 2, n = 7). Redia with orange-pigment clusters in tegument. Redia length 501-1065 (799 ± 165, n = 14), width 150-258 (214 ± 29, n = 14). Redia with collar, relative position of collar apex along body 0.10-0.30 (.17 ± .05, n = 12). Redia with posterior pair of appendages, relative position along body 0.66-0.91 (0.77 ± 0.07, n = 11). Redia pharynx length 63-105 (78 ± 14, n = 13), width 47-79 (60 ± 9, n = 12). Rediae with 8-27 (18 ± 6, n = 14) cercariae in various stages of development.

Species of Acanthopyryphium use mollusks (e.g., Bearup 1960, Kim et al. 2004, Martorelli et al. 2006) and polychaetes (Hechinger and Smith, unpublished data) as second intermediate hosts. In an unpublished study, Armand Kuris and I found metacercariae of an Acanthopyryphium species in the feet of clams (Venerupis (=Ruditapes) philippinarum) only at the locality where we found first intermediate host infections of Acanthopyryphium sp. I in B. atramentaria (Hechinger and Kuris, unpublished data).

Harada and Suguri (1989) reported an Acanthopyryphium-like cercaria from B. atramentaria in Japan, and identified that cercaria as Cercaria yamagutii Ito 1957. However, Ito (Ito 1957) described and reported C. yamagutii from the three snail species, Cerithidea rhizophorarum, Cerithidea largillieri, and Tympanotonus
microptera. As mentioned in the introduction, it seems preferable to assume that
trematodes using different first intermediate host species, particularly different
genera, are different species unless evidence shows otherwise. Barring further study, I
consider the C. yamagutii reported from B. atramentaria by Harada and Suguri
(1989) to be the same as Acanthoparyphium sp. I, although several of the characters
(e.g., size of the body, suckers and collar spines) that I measured for
Acanthoparyphium sp. I are smaller than those Harada and Suguri reported for C.
yamagutii.

_Acanthoparyphium macracanthum_ Rybakov 1987 (Echinostomatidae)—Fig. 1

Cercariae develop in rediae, which reside in the gonad and digestive gland of
snail (Rybakov 1987).

Rybakov (1987) described this species infecting _B. atramentaria_ from Peter
the Great Bay, eastern Russia. I never encountered this species, and all descriptive
information is from Rybakov (1987) (M.C. and E. Rigby kindly provided the
translation and I have slightly modified their language).

_Diagnosis_ (range (average), no n was provided): cercaria body length 152-169
(162), width 61-68 (63), tail length 139-155 (147), tail width 17-20 (19), oral
sucker length 21-24 (22), oral sucker width 24-27 (26), pharynx length 11-14
(13), pharynx width 5-8 (7), ventral sucker length 29-34 (32), width 27-32 (30).
Cercariae belong to the morphological group Echinostomata. Spiny collar has 23 spines. Tegument on ventral side from spiny collar to ventral sucker has 15 to 18 rows of spines, and dorsal side from spiny collar to level of ventral sucker has wide horizontal creases. Rest of body without armor. Digestive system well developed, cecal branches reach posteriorly to anterior end of urinary bladder. Only two pairs of penetration glands were found. Additionally, in dorsal part of oral sucker, three unique glandular cells were observed. Cystogenous glands are located very close together under tegument over entire body of cercaria.

Excretory system is as usual for echinostomatids, flame bulb formula, perhaps, is 2[(3+3+3+3+3) + (3+3+3+3+3)] = 60.

Rybakov and Lukomskaya (1988) described the life cycle of this trematode in Peter the Great Bay, finding that the trematode used three bivalve species (including Ruditapes philippinarum) as second intermediate hosts, and that they were able to use chickens as experimental final hosts. They also described A. macracanthum as a new species in that publication, but it seems that Rybakov (1987) was a valid description and thus has priority as author of the species name.

*Cercaria batillariae* Shimura and Ito 1980 (Heterophyidae)—Fig. 1

Cercariae develop in active rediae, residing primarily in the gonadal region of the snail.
Shimura and Ito (1980) described this species from *B. atramentaria* from Kanagawa and Chiba Prefectures, Japan. I examined infections from nine localities on the Pacific coast of Honshu, from Wakayama Prefecture to Miyagi Prefecture. The following description is based on an infection from Waka River (voucher deposited, USNPC No. 099683.00).

*Diagnosis:* Cercaria biocolate, pleurolophocercous (lateral and dorso-ventral fins), pharyngeate, with oral and ventral suckers. Cercaria body oval to oblong in dorsal view, dorsal-ventrally flattened, body length 142-149 (146 ± 2, n = 12), max width 56-62 (60 ± 2, n = 11) at midbody. Tegment with small spines, arranged in transverse rows, particularly evident over oral sucker. Tail attached in pronounced subterminal socket, tail length 255-295 (282 ± 13, n = 8), width 20-24 (22 ± 2, n = 8) just posterior to base. Tail with two lateral fins and one continuous dorso-ventral fin, extending around the tail tip. Lateral fin length 110-120 (113 ± 3, n = 8), width 10-17 (13 ± 3, n = 4). Dorsal portion of dorso-ventral fin length 159-196 (184 ± 12, n = 8), dorsal origin 6-34 (19 ± 11, n = 6) anterior to insertion of lateral fins, ventral insertion of dorso-ventral fin ~7-11 (9 ± 2, n = 3) posterior to insertion of laterals. Oral sucker very well developed, rounded, length 25-27 (26 ± 1, n = 12), width 20-27 (24 ± 2, n = 11). At least two transverse rows of oral spines present, but number of rows or spines not accurately observed. Ventral sucker not developed. Prepharynx length 22-28 (25 ± 2, n = 11), not very prominent. Pharynx length 6-9 (8 ± 1, n = 11), width 9-11 (10 ± 1, n = 11). Eye

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spots black, cuboidal to slightly rectangular, length 6.1-7.5 (6.7 ± 0.6, n = 11),
width 5.5-7.4 (6.5 ± 0.6, n = 11). Esophagus and ceca not observed. Excretory
bladder thick-walled, v-shaped to cordate, appears to empty directly into the
subterminal notch, just below tail. Flame bulb formula not observed. Seven pairs
of penetration glands (each ~13 diameter), clustered just past midbody, partly
surrounding anterior edge of primordial mass (which appears clear, before
staining). This mass of primordia (probably genital) evident upon acetocarmine
staining, along midline, just posterior to cluster of penetration glands, anterior to
excretory bladder, diamond-shaped, length 15-20 (18 ± 1, n = 10) along longest
side. Redia sausage-shaped, with no appendages, length 756-877 (809 ± 35, n =
10), width 108-149 (133 ± 12, n = 10). Redia pharynx length 21-27 (24 ± 2, n =
10), width 22-25 (24 ± 1, n = 10). Gut of redia not discernable due to cercariae.
Redia packed with ~20-31 (26 ± 3, n = 10) cercariae in various stages of
development (cercariae are generally more developed in anterior of redia).

Shimura and Ito (1980), and I (unpublished data) have shown experimentally
that C. batillariae infects fish as second intermediate hosts. I also obtained adults by
infecting laboratory mice with metacercariae, but have not succeeded in retrieving
adults with the female components of the reproductive system matured (unpublished
work).
*C. batillariae* has been the subject of several ecological and population genetics studies. *C. batillariae* is the single morphospecies of trematode that has invaded the West Coast of North America with its snail host, *B. atramentaria* (Torchin et al. 2005). Miura et al. (2005) recently used molecular genetic methods to demonstrate that *C. batillariae* likely is a complex of eight cryptic species in Japan. Subsequently, Miura et al. (2006b) determined that at least three of the eight cryptic species are present in the introduced range on the west coast of North America, with only two being common. Additionally, Miura et al. (2006a) showed that *C. batillariae* strongly affects its host snail’s growth and distribution in the intertidal zone.

The above measurements and my description largely fit within the range of those provided in Shimura and Ito’s description (Shimura and Ito 1980). But there are exceptions. First, the measurements that Shimura and Ito provided for the prepharynx length (53-66, ave. 60) are more than twice as large as my measurements. However, I believe Shimura and Ito’s prepharynx measurements may have been reported in error, for they do not correspond with their figure of the cercarial body (from which I estimate the prepharynx to be ~33 μm—very close to my measurements). The second major difference between our descriptions pertains to the dorso-ventral tail fin. Shimura and Ito report that the dorsal fin originates posterior to the posterior end of the laterals and the ventral inserts anterior to the posterior end of the laterals. The dorso-ventral fins on the specimens that I have examined carefully (dozens from Japan and the United States), have the opposite arrangement (as described above).
This difference is either due to a mistake in the original description, or it is real and due to either intraspecific variation or variation across cryptic species. The other exceptions are that my measurements are smaller in tail and lateral fin length and the eyespots of Shimura and Ito’s specimens appear to be more rectangular, versus tending to be cuboidal as were the specimens upon which I based the above description (although I have examined many other specimens with more rectangular eyes). These differences may be an artifact of my dealing only with cercariae from dissected snails, as such cercariae are potentially not fully developed. Alternatively, the differences may be due to intraspecific variation, or these differences may reflect variation across the cryptic species of *C. batillariae* uncovered by Miura et al (2005).

Rybakov (1987) reported and provided a description of *C. batillariae* Shimura and Ito 1980 from *B. atramentaria* in Peter the Great Bay, eastern Russia. The cercariae he described are about 30 μm longer in body length, and over 100 μm longer in tail length. It is possible that the difference for body length is because Rybakov made measurements on live specimens. Additionally, the dorsal tail fin originates very far anterior (near the base of the tail). Either these differences are due to intraspecific variation, or, perhaps more likely, Rybakov described a different cryptic species of *C. batillariae*.

Harada and Suguri (1989) reported a “Cercaria sp. 5” from *B. atramentaria*. Their measurements appear to slightly differ from those I provide here, but this is
hard to assess since they provided averages with no indication of the dispersion of the data. The oral spine arrangement they reported for Cercaria sp. 5 (6-10, 6-10, 4) differs from that reported by Shimura and Ito (1980) in the original description of *C. batillariae* (7-10, 8-9, 5-6). Cercaria sp. 5 is probably a member of the cryptic species complex of *C. batillariae* of Miura et. al (2005).

*Cercaria hosoumininae* Shimura and Ito 1980 (*Microphallidae*)—Fig. 1

Cercariae develop in inactive sporocysts residing primarily in gonadal region of snail host.

Shimura and Ito (1980) described this species infecting *B. attramentaria* from Kanagawa and Chiba Prefectures. I examined infections from Wakayama Prefecture (Uchino River, Yukashi Lagoon, and Hashiguiiwa) and Miyagi Prefecture (Mangoku River and Nagazura River). The following is based on an infection from Uchino River (voucher deposited, USNPC No. 099684.00).

*Diagnosis:* Cercariae non-oculate, apharyngeate, monostomate (no ventral sucker), with simple tail and oral stylet. Cercaria body oval to oblong to elongate in dorsal view, dorsal-ventrally flattened, usually fixed dorsal-ventrally flexed. Body length (taken in lateral view, to not underestimate because body flexed) 132-155 (140 ± 7, n = 13), max width 37-49 (43 ± 4, n = 13) at or slightly anterior to midbody. Tail attached slightly subterminally, circular in cross-section, with
fine tegumental crenulations, length 84-112 (104 ± 8, n = 9), width 10-12 (11 ± 1, n = 9). Oral sucker not very strongly developed, length 32-34 (33 ± 1, n = 13), width difficult to discern and not measured. Oral sucker with well developed stylet. Stylet sclerotized more than half its anterior length, narrow, slightly higher (dorso-ventrally) than wide (left to right), base non-sclerotized and rounded, sometimes forming a bulb. Stylet length 17-23 (20 ± 2, n = 10), width 2.6-5.5 (4.5 ± 0.8, n = 12). Three pairs of pronounced penetration glands sequentially arranged anterior to posterior, restricted roughly to third quarter of body; anterior two pairs filled with coarse granules, posterior pair with fine granules. Penetration gland ducts pronounced, extend anteriorly to anterior of oral sucker where they narrow and turn sharply medially. Penetration gland ducts from anterior pair positioned singly and medial to posterior two pairs, which are positioned more laterally, and are bundled together. Excretory bladder v-shaped. Rest of excretory system not observed. Mass of primordia (putatively ventral sucker primordium) evident upon acetocarmine staining, along midline, filling region posterior to penetration glands and anterior to excretory bladder. Sporocyst simple, thin-walled (~2-6 μm thick), round to oval, length 198-316 (251 ± 34, n = 10), width 159-221 (191 ± 20, n = 10), densely packed with estimated number of cercariae 13-27 (20 ± 4, n = 10) mostly in late stage of development, with few germ balls.

This species probably uses crustaceans as second intermediate hosts, as do most microphallids (see Yamaguti 1975).
The description I provide agrees with that provided by Shimura and Ito (1980) in their description of *C. hosoumininae* except that the measurements of the cercariae I examined (including those from at least two other sites, data not given here) are apparently smaller in body, tail and stylet length. This may be an artifact of my dealing only with cercariae from dissected snails, as such cercariae are potentially not fully developed, or represent intraspecific variation. Alternatively, the infections I encountered may belong to a different species of microphallid. Indeed, molecular evidence indicates that there are cryptic species of *C. hosoumininae* (O.Miura, pers. comm.). Further study is called for to resolve this issue.

Rybakov (1987) reported and described *C. hosoumininae* Shimura and Ito 1980 in *B. attramentaria* from Peter the Great Bay, eastern Russia. His measurements agree with Shimura and Ito’s description.

Harada and Suguri (1989) reported many infections of the microphallid, *Cercaria lanceolata* Holliman 1961, infecting *Cerithidea rhizophorarum* snails and two infections in *B. attramentaria*. Holliman described *C. lanceolata* from *Cerithidea scalariformis* in the Gulf of Mexico (Holliman 1961). Harada and Suguri assigned the microphallids they encountered to *C. lanceolata* on the basis of the flame bulb pattern of $2[(2+2)+(2+2)]=16$ (while the formula for *C. hosoumininae* is $2[(1+1)+(1+1)]=8$) and the shared genus of snail host (although that does not explain
applying the name to the infections in *B. atramentaria*). In the Gulf of Mexico, *C. lanceolata* was subsequently determined to a species of *Probolocoryphe* (Heard 1976). Given the arguments discussed above regarding host specificity, it seems very possible that a species of *Probolocoryphe* infects *C. rhizophorarum*. But, it does not seem likely that the same species would also infect *B. atramentaria*. However, since Harada and Suguri reported two infections of *C. lanceolata* in *B. atramentaria*, workers studying trematodes in *B. atramentaria* should watch for a second microphallid species with 16 flame bulbs. I would refer to such a species as “Microphallid sp. II,” rather than *Probolocoryphe lanceolata*, until further work on adults or late-stage metacercariae is undertaken. If such a second microphallid species infects *B. atramentaria*, I suspect it will differ from *C. hosoumininae* not only in flame bulb formula, but also in other morphological characters, including stylet morphology.

**Cyathocotylid sp. I**—Fig. 1, Fig. 3

Cercariae develop in very active sporocysts in the gonadal region of the snail visceral mass.

This species has not previously been reported. I examined infections from specimens from Wakayama Prefecture (Yukashi Lagoon and Hashiguiiwa) and Miyagi Prefecture (Nagazura River). The description below is based on an infection from Nagazura River (voucher deposited, USNPC No. 099685.00).
Diagnosis: Cercariae non-oculate, pharyngeate, monostomate (no ventral sucker), longifurcate. Cercaria body oval to pyriform in dorsal view, dorsal-ventrally flattened, length 158-204 (176 ± 19, n = 8), max width 96-123 (110 ± 11, n = 10) at or slightly posterior to midbody. Tail attached to dorsal side of posterior body, width constricted just before attachment. Tail stem laterally flattened, length (to posterior-most part of tail) 412-489 (447 ± 30, n = 11), width at attachment to furcae 40-51 (46 ± 3, n = 11). Tail furcae laterally flattened, length 247-355 (303 ± 29, n = 11), width at base 27-32 (29 ± 1, n = 11), each provided with continuous dorsal-ventral fin-fold. Anterior organ/oral sucker length 31-47 (40 ± 6, n = 8), width 27-39 (32 ± 5, n = 9), with undetermined number of penetration glands. Excretory system collecting ducts follow general pattern typical of cyathocotylid cercariae. Single, blind ducts extend anterio-laterally from juncture of lateral and cross-commissural ducts. Fifteen pairs of protonephridia (flame bulbs, or cells) observed in cercarial body, but capillaries, and thus, flame bulb formula, not observed. Flame bulbs not observed in tail stem. Mass of primordia (probably genital primordia, perhaps with acetabular primordia at anterior) evident upon acetocarmine staining, along midline, in region between the two medial collecting ducts just posterior to point where ducts fuse, length 18-25 (22 ± 2, n = 9), width 12-27 (20 ± 5, n = 9). Sporocysts with transverse annulations, long and thin (can be over 4 mm long), highly intertwined and difficult to separate intact.
This species most likely infects fish as second intermediate hosts, as do most cyathocotylids (Yamaguti 1975, Combes et al. 1980, Schell 1985).

**Philophthalmid species I ("clear philophthalmid")**—Fig. 1, Fig. 4

Cercariae develop in active rediae, primarily in gonadal region of snail visceral mass. Cercariae often swim to top, attach to surface tension, and get an air bubble in their ventral sucker. Cercariae infrequently encyst in dish and form flask-shaped metacercariae (Fig. 1).

This species has not previously been reported. I examined specimens from Chiba Prefecture (Obitsu River) and Miyagi Prefecture (Torinoumi and Ogatsu Bay). The description below is based on an infection from Ogatsu Bay (voucher deposited, USNPC No. 099686.00). I provide measurements, although I had very little fixed material for study.

*Diagnosis:* Cercaria non-oculate, pharyngeate, with oral and ventral suckers. Cercaria body oval to oblong to slightly spatulate in dorsal view, dorsal-ventrally flattened, length 575-653 (602 ± 44, \( n = 3 \)), max width 144-155 (149 ± 6, \( n = 3 \)) slightly anterior to ventral sucker. Tegument apparently without spines, but with prominent thin ducts from cystogenous glands (potentially misinterpreted as spines), particularly over lateral and posterior half of ventrum. Tail simple (except for terminal gland), circular in cross-section, with parenchyma cells, attached.
slightly subterminally, length 404-417 (411 ± 9, n = 2), width 40-48 (44 ± 4, n = 3) just posterior to base. Tail terminal gland length 116-162 (133 ± 25, n = 3). Cystogenous glands obscure much of internal structure. Oral sucker round, length 59-74 (65 ± 8, n = 3), width 52-56 (55 ± 2, n = 3). Ventral sucker round to slightly oval (may occur more oval in fresh specimens), at beginning of second half of body, length 71-86 (78 ± 8, n = 3), width 66-79 (71 ± 7, n = 3). Prepharynx evident, length 15-32 (25 ± 9, n = 3). Pharynx length 32-41 (36 ± 5, n = 3), width 22-25 (23 ± 2, n = 3). Esophagus bifurcates about half way distance from anterior of cercaria body to ventral sucker, length 39-42 (41 ± 2, n = 2). Digestive ceca diverge laterally, continue posteriorly almost to end of body to around anterior of excretory bladder. Excretory bladder thin-walled, more-or-less square, with short anterior-medial connection receiving main collecting ducts. Main collecting ducts extend anteriorly from connection with bladder, laterally around ventral sucker, or cross over ventral sucker’s sides, continue anteriorly to recurve just before oral sucker around level of pharynx. Complete flame bulb formula not observed. Mass of primordia evident upon acetocarmine staining, along midline, about half way between ventral sucker and excretory bladder, sometimes extends anterior as thin strip toward cecal bifurcation. Redia translucent, length 652-1250 (1042 ± 140, n = 16), width 123-218 (184 ± 22, n = 16), with one or two appendages, positioned at ~9/10 redial length, not always prominent. Redia pharynx length 42-55 (49 ± 4, n = 15), pharynx width 44-57 (48 ± 4, n = 15). Redia gut length 245-358 (286 ±
36, n = 9). Cercariae-producing rediae with number of cercariae 3-17 (13 ± 4, n = 16), in all stages of development.

This trematode may be a species of *Philophthalmus* as this genus is known to form flask-shaped cysts (e.g., Thakur and Cheng 1968, Fried and Grigo 1976, Abdul-Salam et al. 2004) and infect other species of *Batillaria* (e.g., Fried and Grigo 1976).

**Philophthalmid species II ("opaque philophthalmid")—Fig. 1**

Cercariae develop in large active rediae, which primarily reside in the gonadal region of the snail visceral mass. Cercariae sometimes encyst in dish and form round to oval metacercariae (Fig. 2).

This species has not previously been reported in Japan, but probably has been reported in eastern Russia (see below). I examined infections from Miyagi Prefecture (Ogatsu Bay and Nagazura River), and the following is based on an infection from the former locality (voucher deposited, USNPC No. 099687.00).

**Diagnosis:** Cercaria non-oculate, pharyngeate, with oral and ventral suckers.

Cercaria body oval to oblong to slightly spatulate in dorsal view, dorsal-ventrally flattened, length 352-502 (431 ± 51, n = 8), max width 167-218 (200 ± 16, n = 8) slightly anterior to ventral sucker. Tegument without spines. Tail simple (except for terminal gland), circular in cross-section, with parenchyma cells, attached
slightly subterminally, fixed in varying states of contraction, length 145-412 (233 ± 90, n = 8), width 39-74 (56 ± 14, n = 5) just posterior to base. Tail terminal gland 51-86 (65 ± 10, n = 8). Oral sucker round, length 60-69 (66 ± 4, n = 8), width 59-74 (69 ± 6, n = 8). Ventral sucker round, at beginning of posterior half of body, length 71-98 (87 ± 8, n = 8), width 79-105 (96 ± 9, n = 8). Cystogenous glands ~10 diameter, obscure almost all internal structure, few organs being evident even in highly flattened unfixed specimens. Prepharynx present, apparently shorter than pharynx. Esophagus bifurcates about half way between anterior of cercaria body to ventral sucker. Digestive ceca diverge laterally, continue posteriorly at least to ventral sucker, but posterior limits not observed. Excretory bladder thin, sacate, with short medio-anterior duct receiving main collecting ducts. Main collecting ducts extend anteriorly from connection with bladder, path not observed in middle of body, recurve at level of pharynx. Caudal tubule bifurcates a short distance into tail. Redia translucent, length 1002-1225 (1117 ± 92, n = 4), width 277-350 (304 ± 34, n = 4), with one or two appendages, positioned ~9/10 radial length, not always prominent. Redia pharynx length 54-68 (58 ± 7, n = 4), width 49-55 (51 ± 3, n = 4). Redia gut usually obscured by embryos, length 245-466 (356 ± 156, n = 2). Cercariae-producing rediae with number of cercariae 10-14 (12 ± 2, n = 4), in all stages of development.

In a molecular genetic study, Miura et al. (2005) showed that Philophthalmid species II is a complex of three cryptic species.
Rybakov (1987) reported and described a philophthalmid species (that he
tentatively termed "Cercaria Parorchis sp.") from B. atramentaria from Peter the
Great Bay, eastern Russia. I provisionally consider Cercaria Parorchis sp. to be the
same as Philophthalmid species II, noting that the former is larger in most
dimensions. These differences either reflect differences in laboratory technique
(Rybakov made measurements on live specimens, and he noted that he had very little
material to study), indicate intraspecific variation, or occur because the specimen
reported by Rybakov represents one of the cryptic species uncovered by Miura et al.
(2005). I do not use Rybakov's name because I believe there is no evidence to assign
this philophthalmid to Parorchis, versus other philophthalmid genera (e.g.,
Cloacitrema). Indeed, there is evidence against this trematode being a species of
Parorchis, since it is lacking the strong body spination and the spined collar that
characterizes Parorchis spp. (Cable 1956). Thus, I use a temporary morphospecies
name that reflects only the trematode's probable familial affiliation.

Harada (1989) and Harada and Suguri (1989) reported one infection of a
philophthalmid, Cercaria shikokuensis, in B. atramentaria. However, Harada (1989)
described C. shikokuensis from the snail Cerithidea rhizophorarum. As discussed in
the introduction, based on general principles and without strong evidence to the
contrary, it seems questionable to assume C. shikokuensis (described from a species
of Cerithidea) would also infect B. atramentaria. Nevertheless, workers should
certainly watch for a *C. shikokuensis*-like cercaria infecting *B. attramentaria*. The most obvious distinguishing trait would be the presence of prominent body spines in *C. shikokuensis* (and, apparently, *C. shikokuensis* has a longer body, smaller ventral sucker, and a longer esophagus than does Philophthalmid sp. II). If such a cercaria is found, I would refer to it as “Philophthalmid sp. III,” rather than *C. shikokuensis*, barring further study. Perhaps Harada (1989) and Harada and Suguri (1989) recognized one of the three cryptic species of Philophthalmid Cercaria II reported by Miura et al. (2005).

*Renicola* sp. I (= *Cercaria Renicola* sp. of Rybakov (Rybakov 1987))—Fig. 1

Cercariae develop in inactive sporocysts residing in the gonad and sometimes digestive gland regions of the snail.

This species has previously been reported in Japan (Miura and Chiba 2007, see below) and eastern Russia (Rybakov 1987, see below). I examined infections from Miyagi Prefecture (Matsushima and Mangoku River), and the below is based on an infection from Matsushima (voucher deposited, USNPC No. 099688.00).

*Diagnosis:* Cercariae non-oculate, pharyngeate, distomate (oral and ventral suckers), with simple tail and oral stylet. Cercaria body oval to elongate in dorsal view, dorsal-ventrally flattened. Body length 148-233 (174 ± 27, n = 9), width 61-74 (68 ± 4, n = 9) at midbody. Tail attached slightly terminally, circular in cross-
section, length 103-123 (114 ± 8, n = 9), width 16-22 (18 ± 2, n = 9). Oral sucker well developed, length 27-35 (30 ± 3, n = 8), width 25-31 (28 ± 2, n = 9). Oral sucker with bullet-shaped stylet anteriorly (and dorsal to mouth), very hard to see in non-compressed fixed specimens, stylet length 8.5-8.6 (8.6 ± 0.1, n = 3), width 2.6-2.9 (2.7 ± 0.2, n = 3). Ventral sucker oval, positioned at mid-body, length 25-33 (28 ± 3, n = 9), width 27-33 (29 ± 2, n = 9). Tegument covered with minute spines, the full distribution of which was not observed. Cystogenous glands fill most of body and obscure internal structures. Pharynx round, just posterior to oral sucker, barely discernable, not measured. Esophagus difficult to see, slender, branching about half way to ventral sucker into two slender cecae, the posterior extent not observed. Excretory bladder prominent and Y-shaped, with lateral arms not extending to anterior of ventral sucker. Rest of excretory system not observed. Sporocyst simple, thick-walled (at least 5-7 thick), round to oval, length 198-472 (313 ± 73, n = 15), width 112-206 (157 ± 24, n = 15), densely packed with estimated number of cercariae 9-16 (11 ± 2, n = 11) in all stages of development.

Renicolid cercariae with stylets are known to encyst in mollusks (Stunkard 1964) and polychaete worms (Hechinger and Smith, unpublished data).

Rybakov (1987) reported and described this species, from eastern Russia, with the temporary name of “Cercaria Renicola sp.” His measurements slightly differed from those I present, but these differences could easily reflect differences in
laboratory technique (he made measurements on live specimens) or intraspecific variation. I do not use Rybakov’s temporary name to maintain consistent naming throughout this manuscript.

Miura and Chiba (2007) provide information (using the name “renicolid cercaria I” from a early version of this manuscript) on the distribution of Renicola sp. I along an elevational gradient and among host sizes; as well as on the frequency of double infections with C. batillariae.

**Schistosomatid sp. I**—Fig. 1, Fig. 5

Cercariae develop in inactive whitish sporocysts spread throughout the gonad and digestive gland of the snail.

This species has apparently not previously been reported from B. attramentaria. I encountered only one infection at one locality in Miyagi Prefecture (Mangoku River) (voucher deposited, USNPC No. 099688.00).

*Diagnosis*: Cercariae oculate, aphyryngeate, distomate (with oral and ventral sucker), brevifurcate. Unless otherwise indicated, n = 12. Cercaria body pyriform in dorsal view, dorsal-ventrally flattened, length 197-252 (215 ± 18), width 74-91 (82 ± 5) at or slightly posterior to mid-body. Tail attached terminally. Tail stem length (from insertion to anterior base of furcae) 211-238 (226 ± 8),

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width at bulge just posterior to insertion 32-42 (38 ± 2), width at furcal attachment 7-21 (15 ± 5). Tail furca with no fins, length 98-130 (118 ± 8), width at base 10-21 (14 ± 3). Anterior organ length 69-93 (80 ± 9), width 54-61 (57 ± 2, n = 11), with several types of glands. Mouth slightly subterminal. Ventral sucker located just posterior to midbody, length 20-27 (24 ± 2, n = 8), width 24-34 (29 ± 3, n = 8). Eyespots black, circular, diameter 10-12 (11.5 ± 0.9). Daughter sporocyst sausage-shaped, with concavity (~30 diameter) observed on one end, potentially marking outside of birth pore. Daughter sporocyst length 412-1311 (765 ± 255, n = 14), width 127-247 (179 ± 36, n = 14), number of cercariae 4-20 (14 ± 4, n = 13), in all stages of development.

These cercariae most likely directly infect bird final hosts as do many other schistosomatids in marine snails (Yamaguti 1975, Blair et al. 2001).

Acknowledgements

For hosting and logistics in Japan, I am grateful to S. Chiba, T. Koga, A. Kogima, A. Wada, and O. Miura. I thank my compadres, A. Kuris, M. Torchin, and E. Dunham, with whom I worked on the ecological survey. A. Kuris, K. Lafferty, J. Shaw, and two anonymous reviewers provided helpful comments on the ms. For translation of Russian, I am very grateful to M. and E. Rigby. I thank O. Miura for translation of Japanese, comments, and sharing information concerning the biology of these trematodes. I also thank J. McLean at the Los Angeles County Natural History
Museum for helping sort out the nomenclature of *B. atramentaria*. This manuscript benefited from financial support of a grant from the National Science Foundation through the NIH/NSF Ecology of Infectious Disease Program (DEB-0224565) to A. Kuris and K. Lafferty.
Figure 1.
Figure 1. General appearance of the cercariae and parthenitae of digenean trematode species known to infect Batillaria attramentaria as first intermediate host. Metacercariae are included for the philophthalmids. The drawing of Acanthoparyphium macracanthum is modified from (Rybakov and Lukomskaya 1988). All scale bars are 100μm.
Figure 2. Photograph of cercaria of *Acanthoparyphium* sp. I. Specimen was alive and under some cover-slip pressure. Scale bar = 100μm.
Figure 3. Photograph of cercaria of Cyathocotylid sp. I. Specimen was formalin-fixed and acetocarmine stained. Scale bar = 100μm.
Figure 4. Photograph of cercaria of Philopthalmid sp. I. Specimen was alive and under heavy cover-slip pressure. Scale bar = 100μm.
Figure 5. Photograph of cercaria of Schistosomatid sp. I. Specimen was formalin-fixed and acetocarmine stained. Scale bar = 100μm.
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