Title

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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.

Keywords

Cerithidea californica, Indicators, Biodiversity, Estuaries, Wetlands

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 Lafferty 2005; Huspeni et al. 2005).

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 1st 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, 2nd intermediate hosts. Different species of trematodes infect different types of 2nd 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 ≤ 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 ($F_{2,26} = 0.085$, $P = 0.92$, 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.

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Table 1 Trematode species sampled from 1st intermediate host California horn snails (*Cerithidea californica*), their total abundances, and 2nd intermediate host use

Species	Total abundance	Total pre-interactive abundance	Primary 2nd intermediate host used[a]
Euhaplorchis californiensis[b]	324	348	fish
Himasthla rhigedana	137	138	crabs
Small cyathocotylid	100	104	fishes
Probolocoryphe uca	78	87	crabs, amphipods[c]
Himasthla sp. B	49	52	snails, annelids
Renicola buchanani	45	51	fishes
Acanthoparyphium spinulosum	42	47	clams, snails, annelids[d]
Large xiphidiocercaria (renicolid)	39	46	annelids[e]
Catatropis johnstoni	36	46	snails
Austrobilharzia sp.	15	15	none
Parorchis acanthus	15	15	clams, shrimp
Phocitremoides ovale	12	14	fishes
Cloacitrema michiganensis	11	12	clams, shrimp
Pygidiopsoides spindalis	11	13	fishes
Mesostephanus appendiculatus	9	11	fishes
Renicola cerithidicola	3	4	fishes

[a] 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)).

[b] 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*.

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microphallid trematode species, one of which uses crabs and one that uses gammaridean amphipods (Hechinger and Smith, unpublished data).

[d] 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.

[e] Hechinger and Smith (unpublished data)

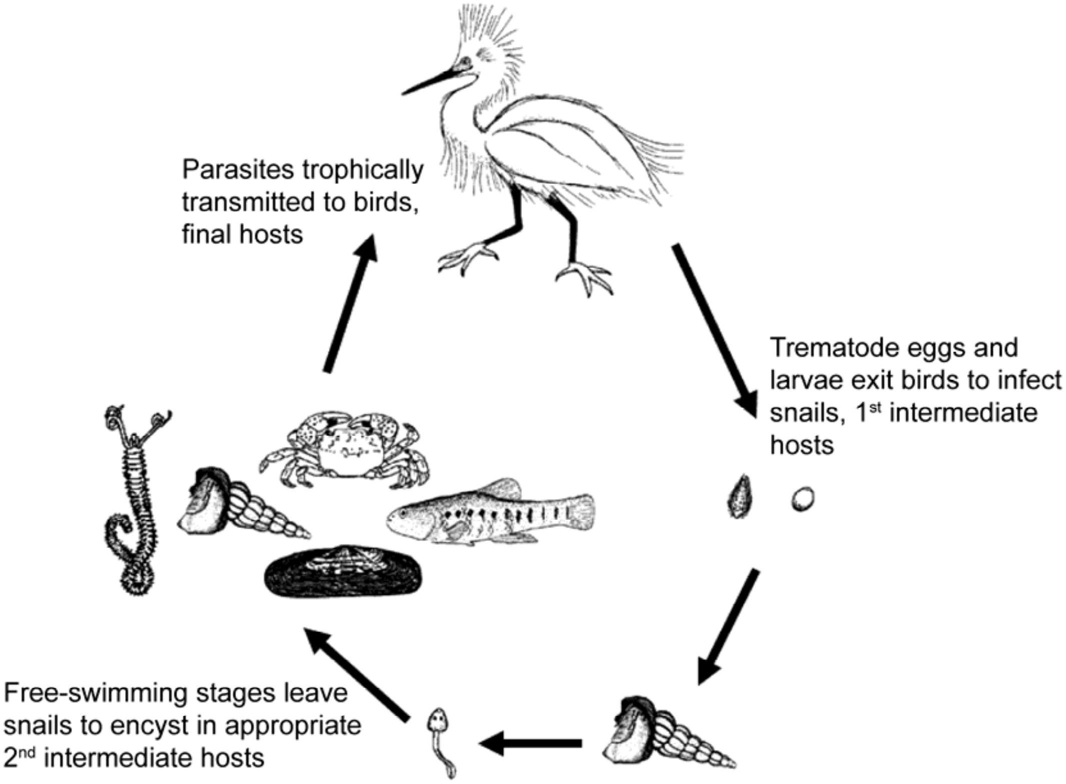

Figure 1 A generalized representation of the trematode life cycles in our system

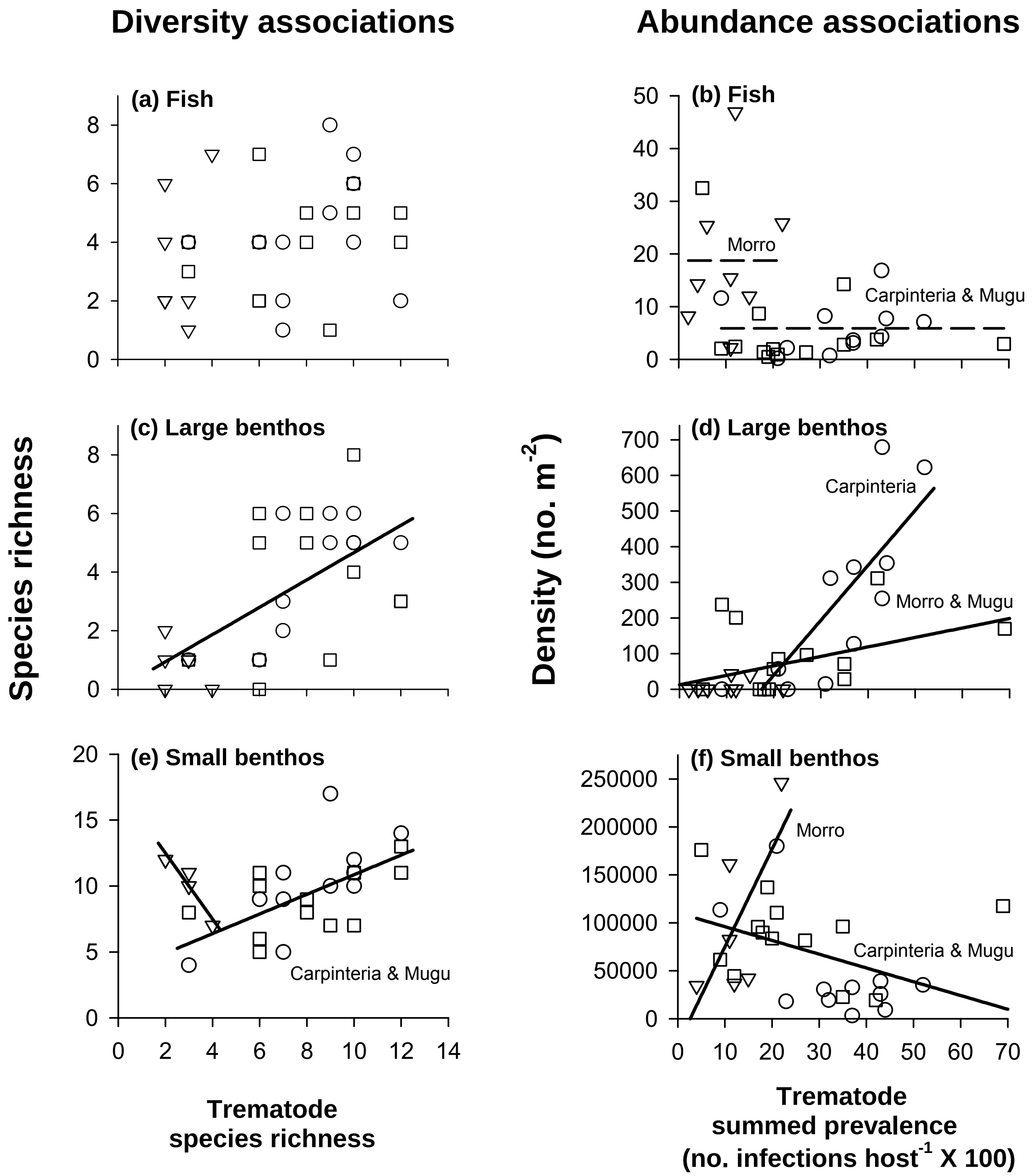

Figure 2 Trematode species richness and abundance associations with potential host (a,b) fishes, (c,d) small benthos and (e,f) large benthos in three coastal wetlands, Morro Bay (Δ), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). Trend lines are shown for associations where trematodes were significantly 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) $R^2 = 0.30$, $P = 0.011$

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

Fishes (seines, blocking nets)

Species	Total Abundance
Fundulus parvipinnis	16069
Atherinops affinis	15198
Clevelandia ios	5875
Gillichthys mirabilis	1356
Leptocottus armatus	1037
Ilypnus gilberti	168
Hypsopsetta guttalata	114
Cymatogaster aggregata	31
Engraulis mordax	26
Quietula y-cauda	17
Pleuronectoidea	11
Sygnathus sp.	11
Gasterosteus aculeatus	6
Paralichthys californicus	4
Anchoa compressa	2
Acanthogobius flavimanus	2
Lepidogobius lepidus	2
Eucyclogobius newberryi	1

Large Benthos (lg. cores, 3 mm mesh sieves)

Taxon	Species	Total Abundance
Anthozoa	Zoantharia[a]	2
Crustacea	*Hemigrapsus oregonensis*	12
	Neotrypaea californiensis	5
	Pachygrapsus crassipes	4
	Upogebia pugettensis	1
Mollusca	*Cerithidea californica*	398
	Macoma nasuta	151
	Protothaca staminea	136
	Tagelus spp.	98
	Cryptomya californica	62
	bivalves, unid. spp.	9
	Nutricola tantilla	4
	Chione undatella	2
	Tellina carpenteri	2
	Cumingia sp.[b]	1
	Laevicardium sp.	1
	Leporimetis obesa	1
	Lyonsia californica	1
	Nuttallia nuttallii	1
	polyplacophoran[a]	1

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

Small Benthos (sm. cores, 0.5 mm mesh sieves)

Taxon	Species	Total Abundance
Annelida	Oligochaetes	19989
	Streblospio benedicti	10196
	Capitellidae	7699
	Spionidae	1834
	Exogone lourei	570
	Eteone	313
	Paranais litoralis	29
	Polychaete, unid. sp.	24
	Glycera spp.	23
	Polychaeta, 8 unid. spp	22
	Apoprionospio pygmaea	17
	Aphelochaeta sp.	12
	Mediomastus sp.	5
	Armandia brevis	3
	Glyceridae	3
	Goniadidae	3
	Nephtys ferruginea	3
	Hemipodus borealis	2
	Phyllodocidae	2
	Polydora ligni	2
	Prionospio heterobranchia	2
	Protocirrineris sp.	2
	Scoloplos sp.	2
	Brania sp.	1
	Scoloplos acmeceps	1
Insecta[a]	Chironomidae	107
	Dolichopodidae	70
	Diptera pupa	25
	Psychodidae	25
	Insecta, 6 unid. spp.	17
	Coleoptera	11
	Diptera, 2 unid. spp.	11
	Diptera (larvae)	3
	Cicindela gabbi	2
	Gerris argentatus	2
	Cicindelidae	2
	Culicidae	1
Crustacea	*Corophium* sp.	10055
	Leptochelia dubia[a]	1048
	Ostracoda[a]	607
	Gammaridae	467
	Copepoda[a]	450
	Caprella brevirostris[a]	29
	Paracerceis sp.	24
	Cumacea[a]	20
	"Shrimp"[a]	10
	Isopoda	9
	Pandalus sp. [a]	5
	Hemigrapsus oregonensis	3
	Grapsidae	2
	Crustacea	2
	Pachygrapsus crassipes	1
	Peramphithoe sp.	1
	Tanaidacea[a]	1
Mollusca	*Acteocina* spp.	1927
	Bivalvia (juveniles)	138
	Cerithidea californica	92
	Assiminea californica[a]	74
	Leporimetis obesa	51
	Macoma nasuta	38
	Protothaca staminea	33
	Gastropoda	2
	Tagelus spp.	4
	Olea hansineensis[a]	3
	Musculista senhousia	1
	Tellina carpenteri	1
Misc. Taxa[a]	Nematoda	2974
	Turbellaria	894
	Nemertea	189
	Phoronida	136
	Zoantharia	7
	Holothuria	5
	Acari (mite)	3
	Clevelandia ios	1
	Gillichthys mirabilis	1
	Hydrozoa	1
	Sipuncula	1

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[a] We excluded these “non-host” taxa from analyses using only “potential hosts.”

[b] The assignment of genus for this individual is questionable.

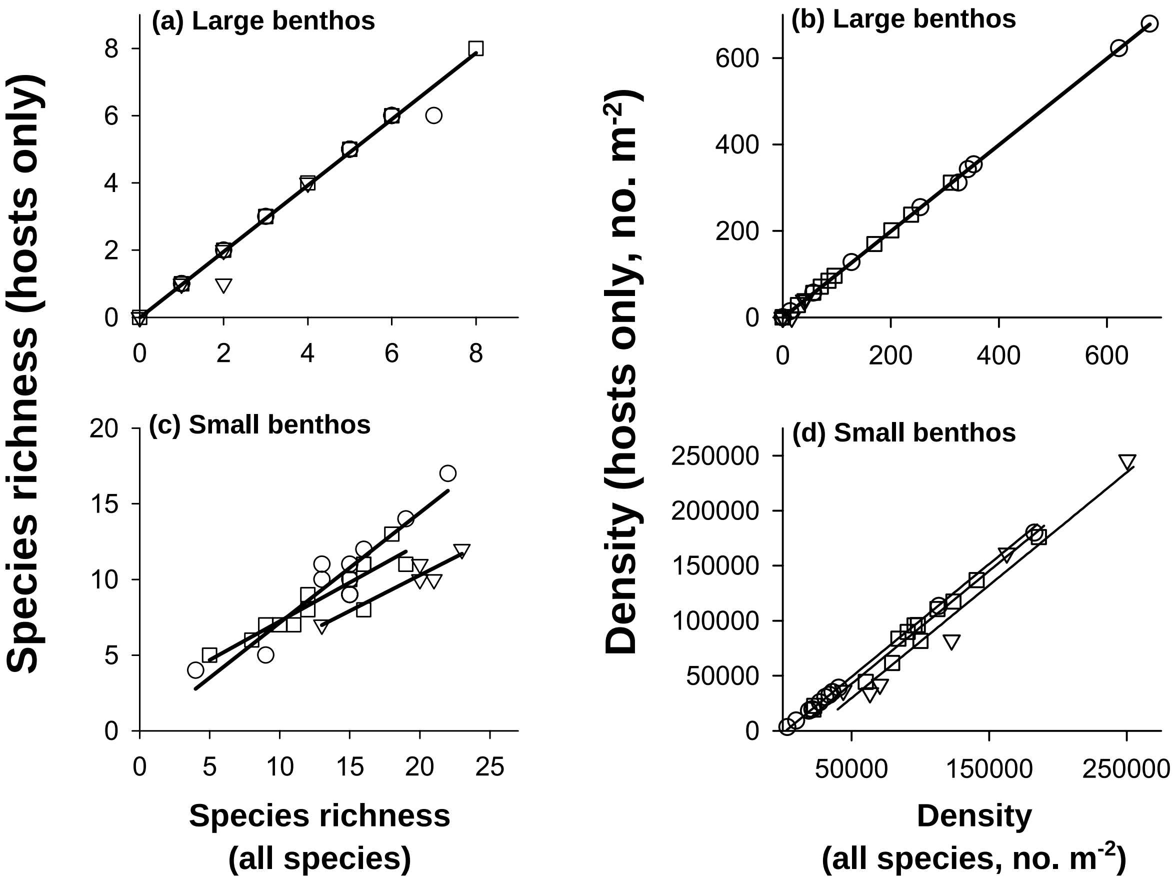

Figure S1 Strong associations between benthos measures that use potential host species with measures using all species for large benthos (a,b) and small benthos (c,d) in three coastal wetlands, Morro Bay (Δ), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). For (a) $R^2 = 0.99$, $P < 0.0001$, n = 32); (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 ($P = 0.0016$)

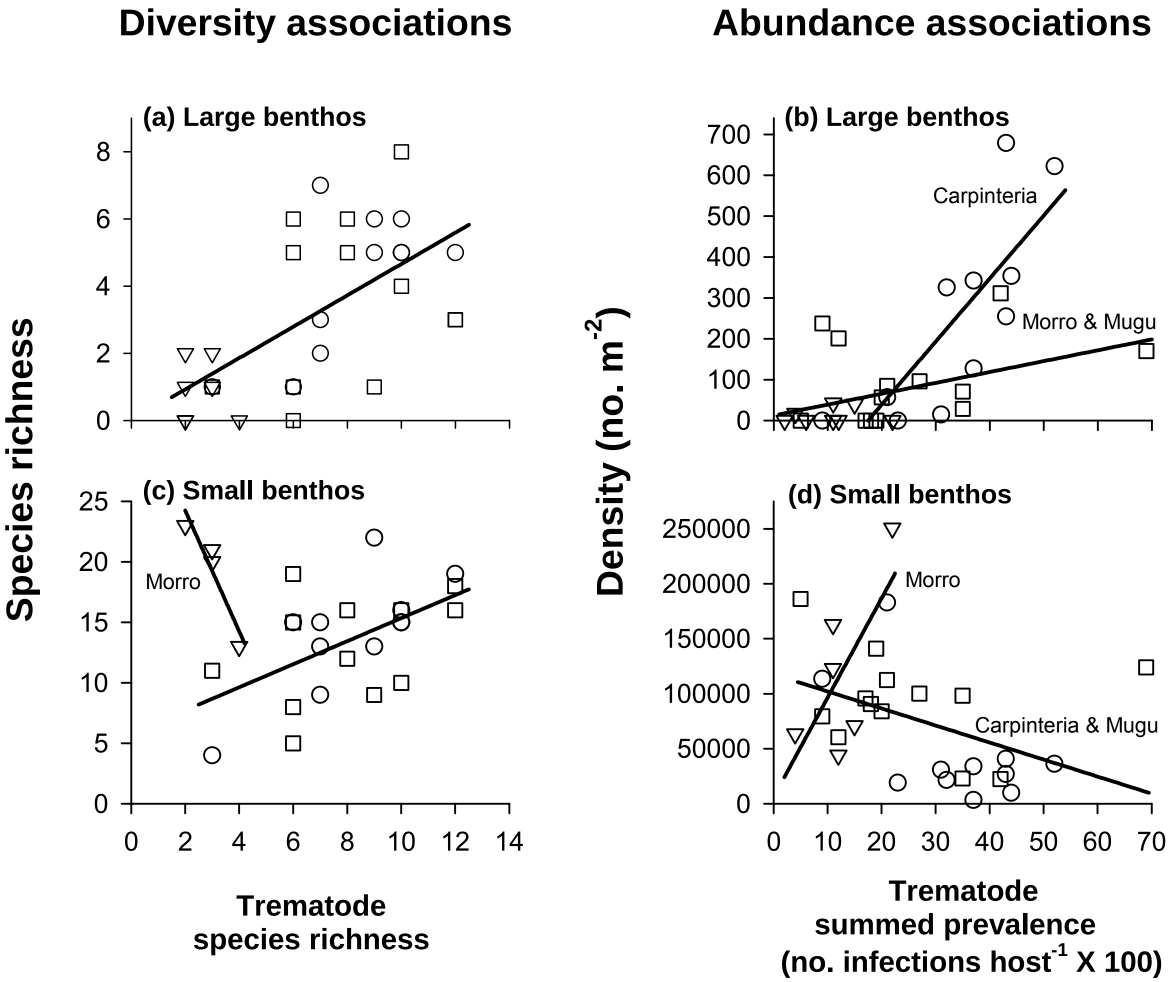

Figure S2 Relationships between benthos and trematodes (using all encountered benthic species in analyses) for large benthos (a,b) and small benthos (c,d) in three coastal wetlands, Morro Bay (Δ), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). Results are very similar to analyses using only potential 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

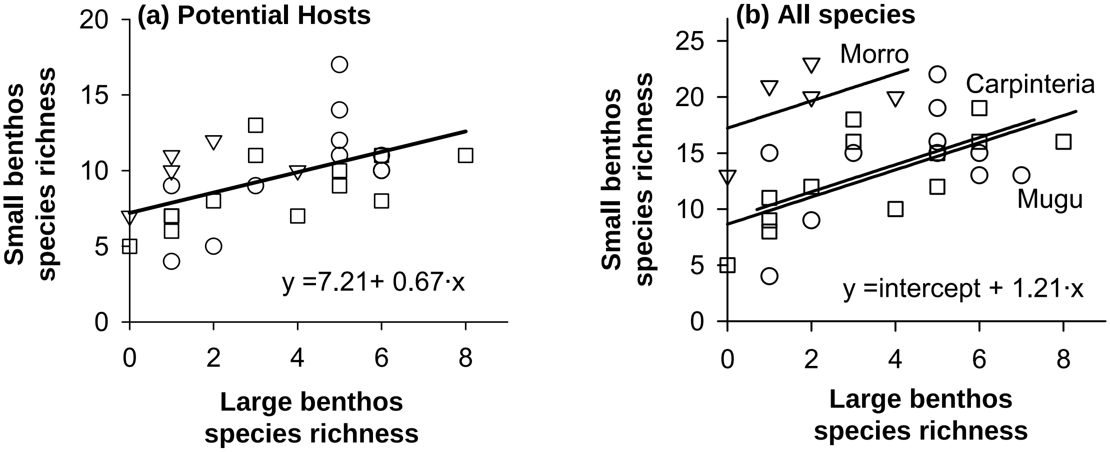

Figure S3 Positive association between large and small benthos species richness measures using host species of benthos (a), or all species of benthos (b) in three coastal wetlands, Morro Bay (Δ), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). The names of the appropriate wetlands are placed near the 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)

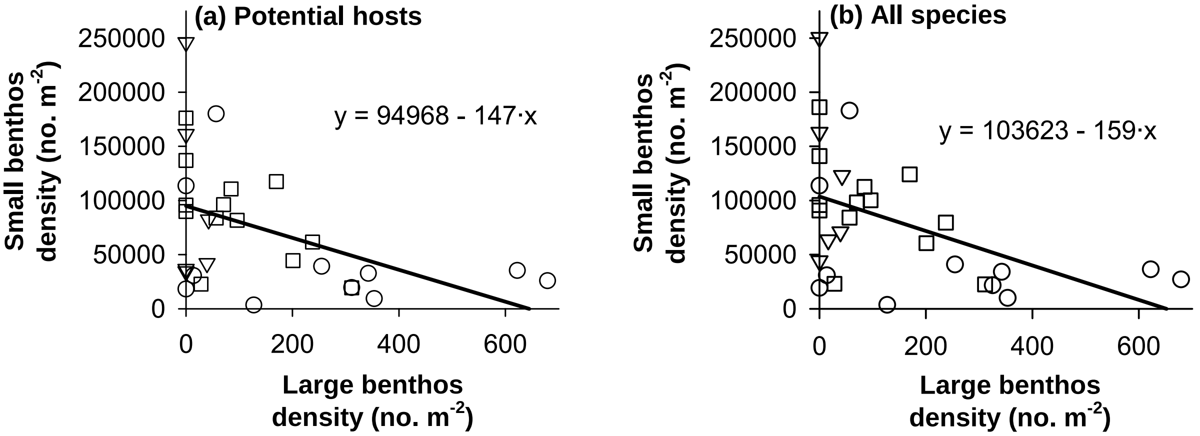

Figure S4 Negative association between small and large benthos density measures, for potential host species (a), and all species of benthos (b) in three coastal wetlands, Morro Bay (Δ), Carpinteria Salt Marsh (○), and Mugu Lagoon (□). For (a) R^2 = 0.20, P = 0.013, n = 30, and wetland was not a significant 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).