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Running Head: Trematodes and Salt-marsh Restoration

Using larval trematodes that parasitize snails to evaluate a salt-marsh restoration project

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ABSTRACT

We conducted a Before-After-Control-Impact (BACI) study using larval digeneans infecting the California horn snail, *Cerithidea californica*, to evaluate the success of an ecological restoration project at Carpinteria Salt Marsh in California. Digenean trematodes are parasites with complex life cycles requiring birds and other vertebrates as final hosts. We tested two hypotheses for prevalence and species richness of larval trematodes in *C. californica*: (1) prior to the restoration, sites to be restored would have lower trematode prevalence and species richness relative to unimpacted control sites, and (2) that these differences would diminish after restoration. The sites to be restored were initially degraded for trematode species. They had an average trematode prevalence (12%) and species richness (4.5 spp) that were lower than control sites (28% prevalence and 7 spp). Despite the differences in prevalence, the proportional representation of each trematode species in the total community was similar between sites to be restored and control sites. Over the six years following restoration, trematode prevalence nearly quadrupled at restored sites (43%) while the prevalence at control sites (26%) remained unchanged. In addition, species richness at restored sites doubled (9 spp), while species richness at the control sites (7.8 spp) did not change. Immediately after restoration, the relative abundance of trematode species using fishes as second intermediate hosts declined while those using molluscs as second intermediate hosts increased. Trematode communities at restored and control sites gradually returned to being similar. We interpret the increase in trematode prevalence and species richness at restored sites to be a direct consequence of changes in bird use of the restored habitat. This study demonstrates a new comparative technique for assessing wetlands, and while it does not supplant biotic surveys, it informs such taxonomic lists. Most
importantly, it provides a synthetic quantification of the linkages among species in wetland food webs.
KEYWORDS

Biological indicators, *Cerithidea californica*, estuarine restoration, parasites, restoration, salt-mash, trematode
INTRODUCTION

Estuaries perform several functions, including provision of habitat, nutrient cycling, flood conveyance, sediment control, ground water recharge and discharge, shoreline protection and water quality improvement. In addition, estuaries have value for society in terms of providing sites for recreation, education, fisheries, research and appreciation of our natural heritage (Ferren et al. 1995). In the early part of the 20th century, governments around the world actively worked to eliminate estuarine habitats and convert them to more “productive” uses. In southern California alone, 90% of the original coastal wetland habitat has been lost to filling or dredging (National Oceanic and Atmospheric Administration 1990, Schoenherr 1992). Fragmentation, water quality degradation, introduction of invasive plants and animals, predation by feral animals, unregulated public access, and other forms of environmental perturbation have adversely impacted remaining estuarine habitat (Flack and Benton 1998, Zampella and Bunnell 1998). Provision of habitat for rare and endangered species is probably the most important remaining function of estuaries because many such plants and animals are restricted to what little habitat remains (Zedler et al. 1990).

Recognition of the importance of estuarine habitat has engendered efforts to restore, enhance and create this habitat (Broome et al. 1988). Unfortunately, studies on the effectiveness of estuarine restoration have been sobering (Zedler 1984, Zedler 1993, Zedler et al. 1992, Kentula 1996). Wetland managers have the dual task of restoring parcels of wetland habitat while concomitantly working to minimize current and future impacts on remaining wetlands. To achieve these tasks, managers and ecologists require specific criteria to evaluate restorations, including the ability to assess the extent to which a particular wetland is impacted or degraded, as well as baseline information to be able to detect changes in the future.
Assessing wetland habitats requires a diversity of techniques and expertise (Pacific Estuarine Research Laboratory 1990). Most environmental assessments involve quantifying physical measurements (such as the concentration of a pollutant (Karr 1994)), species of special interest, sentinel species and broader measures of the community such as the species richness of various guilds (Metcalfe 1989). Such qualitatively different data sets are extremely challenging to integrate for comparisons over space (both among and within estuaries) and time.

Parasitologists have recognized that environmental conditions affect parasites (reviewed in Lafferty 1997) and several have used parasites as indicators of degradation (Möller 1987, Khan and Thulin 1991, Poulin 1992, MacKenzie et al. 1995, Valtonen et al. 1997). Digenean trematodes are a particularly promising type of indicator parasite. Digenean parasitic flatworms require two or more hosts to complete their complex life cycles (Figure 1). Adult digeneans reproduce sexually, as cross-fertilizing hermaphrodites, in vertebrate definitive hosts. Adult worms produce eggs, usually voided in the definitive hosts' feces. A ciliated miracidium stage infects first intermediate host molluscs (usually snails), and this stage undergoes repeated asexual reproduction inside the snail host. Infected snails release cercariae that swim for several hours in search of second intermediate hosts. Upon contact with an appropriate second intermediate host, cercariae shed their tails and encyst as metacercariae. Metacercariae remain encysted in (or on) second intermediate hosts and are transmitted when a definitive host eats an infected second intermediate host. The second intermediate host required (e.g. fish, mollusc, crustacean) varies by species of trematode (Figure 1 and Appendix A). Some trematodes are relative generalists, for example, using several fish species as hosts (Martin 1972), while others are more host-specific, sometimes using just a single species (Martin 1950).
Although bird abundance and diversity are the most important factors structuring the trematode community in first intermediate host snails (Hoff 1941, Matthews et al. 1985, Bustnes and Galaktionov 1999, Smith 2001, Hechinger and Lafferty in prep.), the presence of second intermediate hosts such as fishes, clams and crabs also plays a critical role. When a life cycle is completed within a wetland, larval trematode infections in first intermediate host snails necessarily reflect the presence of particular second intermediate hosts, as well as predation of these hosts by definitive hosts. Consequently, a logical premise is that a diverse and abundant trematode community in first intermediate host snails is reflective of a diverse and abundant community of free-living host species in the marsh.

Several studies have examined the relationship between generalized human "disturbance" (usually concerning the effects of humans or development on bird abundance) and larval digeneans in gastropods (reviewed in Kuris and Lafferty 1994, Lafferty 1997). The most common approach has been to compare the prevalence (percentage of hosts infected) or intensity (number of parasites of a species per host) of parasitism among hosts captured at a small number of control and impact sites (Moser and Cowen 1991), or at a single site before and after an impact (Marcogliese et al. 1990, Keas and Blankespoor 1997). Other authors have also speculated that the prevalence of digeneans declines with habitat degradation (Pohley 1976, Robson and Williams 1970). Cort et al. (1960) were the first to make such a comparison. They found that larval digenean diversity and species richness had declined in a Michigan lake over 20 years. They also noted increased human disturbance and reduced final host bird populations over that time. Keas and Blankespoor (1997) recently resampled these sites and observed continued declines in prevalence.
We used trematode communities to assess the success of an estuarine restoration. To our knowledge, this technique has not been used before in this manner. In addition to providing a measure of success for a wetland restoration, our study enables an experimental evaluation of the hypotheses that (1) environmental change affects parasites and (2) parasites can be used to monitor environmental change.

We used the suite of larval trematodes infecting the California horn snail, *Cerithidea californica*, to evaluate the success of the Ash Avenue Restoration at Carpinteria Salt Marsh (N 34 24' 00", W 119 31' 30") in southern California. *Cerithidea californica* and its suite of larval trematodes are ideally suited for estuarine assessments. *Cerithidea californica* is easy to collect and keep alive, and its trematodes are well described (Appendix A). Where *C. californica* occurs, it is typically abundant, often attaining densities of over 600 snails/m² (present study). *Cerithidea californica* can live several years (Sousa and Gleason 1989), and some individuals can live at least 12 years (Kuris, unpublished data). *Cerithidea californica* serves as first intermediate host for many species of larval trematodes (Martin 1972) (Figure 1 and Appendix A), and this assemblage of trematodes has been the subject of numerous studies at Carpinteria Salt Marsh (Kuris 1990, Lafferty 1993a, 1993b, Lafferty et al. 1994, Kuris and Lafferty 1994, Lafferty and Morris 1996, Stevens 1996, Huspeni 2000).

METHODS
Historically, the Ash Avenue parcel at Carpinteria Salt Marsh was tidally connected to the rest of Carpinteria Salt Marsh. It was gradually filled during the 1950’s. A restoration to improve tidal flow, construct new channels, and plant native estuarine flora in the Ash Avenue parcel was initiated in August, 1997. The original goal of this restoration, as for other ecological
restorations, was to return degraded habitat as close as possible to its original state (as measured by the reference sites in Carpinteria Salt Marsh Reserve). We employed a Before-After-Control-Impact (BACI) design (Stewart-Oaten and Murdoch 1986, Underwood 1994) using larval trematode communities to evaluate this restoration.

Prior to the restoration, in July 1997, we sampled *C. californica* for larval trematodes at four sites in remnant habitat within the six-hectare Ash Avenue parcel (hereafter referred to as “restored” sites), and at four control sites within the adjacent relatively undisturbed 49-hectare Carpinteria Salt Marsh Reserve (hereafter referred to as “control” sites). Sites were all banks of tidal creeks where *C. californica* occurs most abundantly. We chose sites within the pre-restored parcel so that they represented areas that would be affected by the restoration, and that had sufficient *C. californica* populations available for sampling prior to the restoration. Control sites were chosen randomly from channel habitat in Carpinteria Salt Marsh Reserve. Restoration construction in August, 1997 interrupted tidal flow to the restored areas to allow the initial grading and construction of new channels. Restoration construction was completed in October 1997 and the restored habitat was reconnected to tidal flow. All sites were re-sampled annually each July from 1998 - 2003. In July of 1999, we also sampled three sites representing newly created habitat within the restored Ash Avenue parcel. Prior to July 1999, created habitat within the restoration did not have sufficient populations of *C. californica* to sample for trematodes. We sampled these additional created sites with all others sites each July for 2000 - 2003.

We assessed the density of *C. californica* at each sampled site using three belt transects oriented perpendicular to the banks of the tidal channel and spaced 3 meters apart. Each transect was made using 500 cm² quadrats placed end to end from the vegetation edge to the center of the channel. We measured snails from the central transect to the nearest mm and assigned snails to 5
mm size classes to estimate the size frequency distribution at each site. Because trematode prevalence (% snails infected) increases with snail size (i.e. age) (Kuris 1990, Sousa 1990, Lafferty 1993a, Lafferty et al. 1994), we restricted our examination of snails for larval trematodes to snails of sizes 20-24.9 mm so that variation in snail size among sites would not influence our measure of trematode prevalence. This size class represented the middle range of the total size distribution of *C. californica* at our sites. Snails of this size are typically two to three years of age (Lafferty 1993a). From each site, we collected a total of 100 snails of the 20-24.9 mm size class. We examined these snails by dissection for larval trematode infection and identified larval trematodes using Martin’s (1972) key to larval trematodes infecting *C. californica*.

We tested two hypotheses regarding prevalence (% snails infected) and species richness of larval trematodes in *C. californica*. We hypothesized that prior to the restoration, the sites to be restored would have lower trematode prevalence and species richness relative to the unimpacted control sites, and that these differences would diminish over time. We similarly hypothesized that restoration would lead to relative increases in trematode prevalence and species richness.

To test each of these hypotheses, we employed a re-sampling algorithm that allowed us to determine the probability that the magnitude of the differences resulting from our comparisons could have occurred by chance (Sokal and Rohlf 1995). To assess degradation over time, we calculated the annual magnitude of the proportional difference, $\delta$, between the mean values (trematode prevalence, trematode species richness) in the control (c) and restored (r) sites (or, $\delta = (c-r)/c$). Then, for 1000 iterations, we randomized the locations of the samples in space and calculated $\delta$ again. We recorded the number of times the randomized $\delta$ equaled or exceeded the
known $\delta$ and used the proportion (out of 1000) to calculate a one-tailed $p$-value. This represented the probability that, for a particular year, we would have observed as great a difference between the control sites and the restored sites if the samples had been distributed randomly with respect to treatment. This approach has the advantage that it is free of most of the assumptions of parametric tests (Good 2000).

While $\delta$ has good statistical properties, it does not intuitively illustrate degradation. To provide a more easily understandable measure of treatment similarity, we present restored sites for a particular year as a proportion of the control sites in that year (e.g., $r_0/c_0$, $r_1/c_1$, $r_2/c_2$, $r_3/c_3$, $r_4/c_4$, $r_5/c_5$, $r_6/c_6$ where the subscript refers to the year following the restoration)(Table 1).

Using a similar comparison, we also tested whether conditions significantly improved at the restored sites relative to pre-restoration conditions. In this case, the initial values prior to the restoration ($r_0$) were compared to each of the subsequent years at the restored sites ($r_{1-6}$). We controlled for temporal variation by dividing the values for restored sites by the average values at the control sites for each year. For example, to compare values in the sixth year following the restoration, $\delta = (r_6/c_6) - (r_0/c_0)$ was evaluated for statistical significance as described above. Again, for presentation purposes, we provide this difference as a ratio in our summary tables ($(r_6/c_6)/(r_0/c_0))$ (Table 1).

To examine larval trematode community similarity between control and restored sites over time, we calculated Morisita-Horn similarity indices using the computer program EstimateS (Version 6.01b, Colwell 1997). Similarity indices were calculated to compare control and restored sites each year and also to compare restored sites with pre-restoration sites for each year. We also used a re-sampling approach to assess whether any of the Morisita-Horn indices indicated significant differences in community composition between control and restored sites.
over time. Because the Morisita-Horn index is already a standardized difference between samples, we used this for our $\delta$ value. We randomized infected snails between restored and control sites and counted the proportion of times the Morisita-Horn index was less than or equal to the observed value.

We also compared trematode communities in control and restored sites by grouping trematode species by general second intermediate host requirements (e.g. fishes, crabs, molluscs and ingestible hard substrates). In these groupings, each trematode species belonged to only a single second intermediate host group. For comparison, for each year, we plotted the proportion of total trematode community (all sites within a treatment pooled) made up by trematodes using each of these four host groups. We calculated the 95% confidence intervals on the proportion of each trematode group to compare control versus restored sites for each year.

RESULTS

Prior to the restoration, average trematode prevalence was significantly lower at the sites to be restored (12%) relative to the control sites (27%) ($p = 0.02$, Figure 2). Subsequent to the restoration, trematode prevalence was no longer significantly lower at restored sites. Prevalence increased over time, and at 2, 4, 5 and 6 years after restoration, restored sites had significantly higher prevalence relative to pre-restoration values ($p = 0.038$, $p = 0.022$, $p = 0.002$ and $p = 0.002$ respectively, Figure 2 and Table 1). By six years after restoration, average trematode prevalence at restored sites well exceeded average prevalence at control sites (Figure 2).

Before the restoration, the average species richness of trematodes at sites to be restored was significantly lower (4.5 spp) than at control sites (7 spp.) ($p = 0.021$, Figure 3 and Table 1). In years 1 and 3 following restoration average trematode species richness at restored sites
remained significantly low relative to controls ($p = 0.044$ and $p = 0.030$ respectively). By year 6, average trematode species richness at restored sites had significantly increased to twice the pre-restored species richness ($p = 0.004$, Table 1).

During the course of this study, 15 species of trematode were observed infecting *C. californica* at control and restored sites. Fourteen of these 15 species occurred at both restored and control sites, and the least common species (*Renicola cerithidicola*) was observed in only five total infections at control sites (three in year 3 and two in year 6). The most common trematode at control sites was *Euhaplorchis californiensis*, averaging 16.0% prevalence over the study period. The next most abundant trematodes at control sites over the course of the study were *Himasthla rhigedana* (4.5%) and *Renicola buchanani* (2.3%). The most common trematode at restored sites was also *E. californiensis* (9.4%), followed by *Himasthla* sp. B (7.3%) and *Probolocoryphe uca* (3.3%). Life history information, including second intermediate and final hosts reported for each observed trematode species is provided in Appendix A. Data on the identity and frequency of occurrence of each trematode species (and double infection combinations) at restored and control sites for each year are summarized in Appendix B.

Morisita-Horn indices calculated on trematode species pooled across control sites and across restored sites showed interesting trends (Table 2). Before the restoration, relative species abundances at control sites were very similar to those at sites to be restored, despite overall prevalence being twice as high relative to the sites to be restored (Table 2 and Figure 2). Communities did not vary much over time at the control sites. Community similarity between restored and control sites generally increased after the initial change until post-restoration trematode communities were as similar to control communities as they were prior to the restoration (Table 2).
Before restoration, the relative proportion of trematode groups was similar between the control sites and sites to be restored, except that trematodes using molluscs as second intermediate hosts made up a higher proportion of the community in the sites to be restored (Figure 4). One year after restoration, the proportional representation of all groups was different between control and restored sites. In particular, restored sites were even more dominated by trematodes using molluscs as second intermediate hosts, and had reduced relative proportions of trematodes using fishes as second intermediate hosts (Figure 4). This was due to substantial increases in the absolute prevalence of *Himasthla* sp. B at restored sites (Appendix B). Restored sites experienced a decrease in the prevalence of *Euhaplorchis californiensis* immediately after the restoration (6.75% in year 0 versus 2.25% in year 1). Between years 2 and 6 after the restoration, the prevalence of *E. californiensis* increased substantially at restored sites, ending at 19.4% in year 6. *Euhaplorchis californiensis* uses the California killifish, *Fundulus parvipinnis*, as a second intermediate host. By year 6, restored sites were very similar to controls with respect to the proportions of each community requiring particular second intermediate host types (Figure 4).

**DISCUSSION**

The restoration had a significant positive effect on the abundance of larval trematodes at restored sites. There was substantial variation among the restored sites, especially in the first two years. In particular, the sites in the center of the restoration increased in trematode prevalence much more rapidly than two sites located on the periphery of the restoration. We suspect that the initial variation among sites within the restoration resulted from variation in the quality of restoration across the restored habitat. By year 5, trematode prevalences at the
peripheral sites were similar to prevalences at other restored sites. The apparent pattern of a
spread in the recovery of the trematode community out to more distant areas over time could
have occurred because of snail movement or an expansion in bird distributions, or both.

While we do not have specific bird data over time for our control and restored sites, we
interpret the increase in trematode prevalence at restored sites to be a direct consequence of
changes in bird use of restored habitat. We assert this for several reasons. Brawley et al. (1998)
observed significant differences in bird abundance and habitat use between reference sites and a
restored salt marsh site. They observed that bird abundance was greatest at a restored site, and
linked some of the bird distribution patterns to differences in available cover and prey between
habitats (Brawley et al. 1998). Smith (2001) observed shorebird densities in mangroves and
demonstrated that trematode prevalence in first-intermediate host snails increases as a positive
function of bird density. Additionally, Bustnes and Galaktionov (1999) demonstrated that
prevalence of larval trematodes in first intermediate host snails was higher near fish processing
plants relative to open shoreline habitat because the processing plants attracted definitive host
birds. Hechinger and Lafferty (in prep) have compared larval trematode communities in *C.
californica* with video observations of shorebird visitation frequency at sites within Carpinteria
Salt Marsh. At monitored sites, they observed a significant positive correlation between larval
trematode species richness and the species richness of visiting shorebirds.

There were differences between the trematode community composition at control and
restored sites, suggesting that the act of restoration temporarily favored trematodes that used
molluscs as second intermediate hosts and impaired trematodes that used fishes as second
intermediate hosts. The restoration particularly improved habitat for *Himasthla* sp. B, which
uses small *C. californica* (< 10mm) and the opisthobranch snail, *Acteocina inculta*, as its second
intermediate host (Appendix A). We speculate that the increase in *Himasthla* sp. B in *C. californica* first intermediate hosts was the result of increased abundances of small *C. californica* and *A. inculta* second intermediate hosts at restored sites. We observed very high abundances of *A. inculta* and small *C. californica* in the restored sites. The restoration grading likely increased habitat for *A. inculta*, and birds feeding on these snails in the restored habitats concomitantly infected *C. californica* as first intermediate hosts.

The increased abundance of *Himasthla* sp. B is consistent with Craft’s (2000) observations that in created salt marshes the development of the benthic invertebrate community is dependent upon soil formation and accumulation of organic matter in the soil, both of which are often slow processes. Our results are also consistent with findings of several authors who observed distinct assemblages of benthic invertebrates (Scatolini and Zedler 1996, Craft 2000) and size classes of some fishes (Williams and Zedler 1999, Talley 2000) in newly created salt-marsh habitats relative to older established salt-marsh habitats.

Larval trematodes in first intermediate hosts engage in fierce interspecific competitive interactions (Lie 1973, reviewed in Kuris and Lafferty 1994). These competitive interactions frequently result in the exclusion of subordinate species (Kuris 1990, Sousa 1990), and could have affected our results. For example, a diverse bird community could transmit several trematode species to a snail population, but subordinate trematode species from these birds might not be detected in sample of the snail population if competitively dominant trematode species are abundant. For this reason, Lafferty et al. (1994) provided a method to estimate the expected prevalence of trematodes that would have occurred in a sample of snails in the absence of competition. We acknowledge that for our analysis, such expected prevalences are the best measure of what trematode species recruit from birds to snails. Although we calculated both
expected and actual observed prevalences, we present only the observed prevalences because these are more intuitive for the reader and, as described below, not substantially different from the expected prevalences in our study. Firstly, our measure of diversity, species richness, was not affected by our choice of observed or estimated prevalences. Estimated values of overall prevalence were, however, 7% higher than observed values. Although this difference varied among samples, on average, it was identical between restored and control sites, indicating that the overall differences seen in our treatments were unaffected by the method we used to assess prevalence. We did observe one minor change in the statistical significance of our results. Using observed values, years 2, 4, 5 and 6 showed a significant increase in prevalence at the restored sites. Using expected values, years 3, 4, 5 and 6 showed significant increases in prevalence. This difference neither changes the general pattern we observed, nor our interpretation of the results. While we also used estimated values of overall prevalence in comparing larval trematode communities at control and restored sites in the repeated measures analysis, use of these values had no effect on the analysis.

Our study underscores the importance of having adequate control sites to compare with restored sites when measuring ecological functioning at the restoration sites. Without adequate control sites, it would have been possible to conclude, incorrectly, that increases in trematode prevalence at restored sites in years 3 and 4 post-restoration were due solely to restoration effects (see Figure 2). Because control sites also exhibited increases in prevalence during these years, it is likely that a factor (or factors) independent of the restoration accounted for some of these increases (unless there was a wider spillover effect of the restoration which operated at the scale of the entire marsh).
This study demonstrates a new comparative technique for assessing wetlands. While this approach does not supplant the faunal and floral surveys which are still needed for assessments specific to particular taxa, it does inform the taxonomic lists. Unlike other approaches, this methodology provides a unique synthetic quantification of the linkages among species in wetland food webs. Our results suggest some trophic interactions (e.g., a Short-billed Dowitcher eats *A. inculta*) are initially more likely to occur at restored sites relative to other types of interactions (e.g., a heron eats *Fundulus parvipinnis*) at control sites. We hypothesize this is correlated with differences in the presence and abundances of available prey items that serve as second intermediate hosts at these sites, and we are currently investigating this question. While parasites have been used here to elucidate particular trophic interactions, it is also worth noting that it has been demonstrated that parasites can actually *increase* the rate of predation by increasing susceptibility of infected second intermediate hosts to predation (Lafferty & Morris 1996, Kuris 1997).

Trematodes are ubiquitous components of wetland communities, both as adult worms in definitive hosts and as larval stages in molluscs and second intermediate hosts (Dawes 1946, Skrjabin 1979). Multiple species of trematode have been described from molluscan first intermediate hosts in freshwater and estuarine habitats (Yamaguti 1971, 1975). Frequently, several trematode species having a single first intermediate host species will use many different types of second intermediate hosts (as seen here with trematodes infecting *C. californica*). For another example, the widespread freshwater snail *Lymnaea stagnalis* is infected with at least 18 different species of larval trematodes (Loy and Haas 2001). While some of these trematodes are not completely described, they can be readily apportioned into four second intermediate host groups: fishes, amphibians, molluscs and annelids. Additionally, some of these 18 species use
mammals as definitive hosts, while the remaining species use birds (Loy and Haas 2001), permitting separate inferences of bird and mammal activity at a site.

Estuarine systems also have many trematodes available for environmental comparisons. The estuarine snail, *Ilyanassa obsoleta*, on the Atlantic coast of North America has a well-studied suite of at least nine species of larval trematode (Curtis 1995, 1997). Trematodes infecting other species of *Cerithidea* will also provide excellent subjects for this type of environmental assessment. As a genus, *Cerithidea* has a worldwide tropical and subtropical distribution (Houbrick 1984). Many of these species of *Cerithidea* are commonly parasitized by multiple species of larval trematodes (Harada and Suguri 1989, Al-Kandari et al. 2000, Mani and Rao 1993). Within North America alone, in addition to *C. californica* on the California and northern Baja California, Mexico coasts, *C. mazatlanica* inhabits Pacific estuaries in Mexico (Lafferty 1993a, Huspeni 2000), *C. pliculosa* is present in the Gulf of Mexico (Wardle 1974, McNeff 1978), *C. scalariformis* is seasonally abundant on the Gulf and Atlantic estuaries of Florida (Holliman 1961, Smith 2001), and *C. costata* is present in the Caribbean (Cable 1956). Each of these *Cerithidea* species acts as a host to suites of larval trematodes related to those in *C. californica* (Huspeni 2000), and offer further opportunities for environmental assessments like the study reported here.
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LITERATURE CITED


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Table 1. Yearly average trematode prevalence and species richness at restored sites as a proportion of control sites and pre-restoration values.

**Prevalence**

Restored sites as a proportion of:

<table>
<thead>
<tr>
<th>Year post-restoration</th>
<th>Control sites $(p)$</th>
<th>Pre-restoration sites $(p)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.46* (0.02)</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>0.49 (0.06)</td>
<td>1.06 (0.436)</td>
</tr>
<tr>
<td>2</td>
<td>0.82 (0.27)</td>
<td>1.77* (0.038)</td>
</tr>
<tr>
<td>3</td>
<td>0.78 (0.18)</td>
<td>1.68 (0.064)</td>
</tr>
<tr>
<td>4</td>
<td>0.78 (0.14)</td>
<td>1.67* (0.022)</td>
</tr>
<tr>
<td>5</td>
<td>1.06 (0.69)</td>
<td>2.21* (0.002)</td>
</tr>
<tr>
<td>6</td>
<td>1.62 (0.84)</td>
<td>3.50* (0.002)</td>
</tr>
</tbody>
</table>

**Richness**

Restored sites as a proportion of:

<table>
<thead>
<tr>
<th>Year post-restoration</th>
<th>Control sites $(p)$</th>
<th>Pre-restoration sites $(p)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.64* (0.021)</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>0.57* (0.044)</td>
<td>0.88 (0.68)</td>
</tr>
<tr>
<td>2</td>
<td>0.70 (0.094)</td>
<td>1.09 (0.34)</td>
</tr>
<tr>
<td>3</td>
<td>0.72* (0.030)</td>
<td>1.12 (0.33)</td>
</tr>
<tr>
<td>4</td>
<td>0.80 (0.091)</td>
<td>1.25 (0.092)</td>
</tr>
<tr>
<td>5</td>
<td>0.88 (0.354)</td>
<td>1.36 (0.113)</td>
</tr>
<tr>
<td>6</td>
<td>1.16 (0.995)</td>
<td>1.81* (0.004)</td>
</tr>
</tbody>
</table>
The left column represents time after restoration. Center column values represent the proportion of restored site values relative to control sites for each year. Right column values represent the proportion of restored sites relative to pre-restoration values for each year. The calculation of $p$ values for significant differences is described in the text and $p$ values are given in parentheses next to proportional values. Significant $p$ values are depicted with an asterisk.
Table 2. Trematode community similarity indices for control and restored sites.

**Morisita-Horn community similarity indices:**

Similarity of restored sites relative to:

<table>
<thead>
<tr>
<th>Year post-restoration</th>
<th>Control sites ( (p) )</th>
<th>Pre-restoration sites ( (p) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.95 ( (0.90) )</td>
<td>1.00 ( ------- )</td>
</tr>
<tr>
<td>1</td>
<td>0.34 ( (0.06) )</td>
<td>0.53 ( (0.29) )</td>
</tr>
<tr>
<td>2</td>
<td>0.46 ( (0.23) )</td>
<td>0.66 ( (0.44) )</td>
</tr>
<tr>
<td>3</td>
<td>0.80 ( (0.58) )</td>
<td>0.83 ( (0.92) )</td>
</tr>
<tr>
<td>4</td>
<td>0.72 ( (0.67) )</td>
<td>0.80 ( (0.65) )</td>
</tr>
<tr>
<td>5</td>
<td>0.84 ( (0.75) )</td>
<td>0.86 ( (0.97) )</td>
</tr>
<tr>
<td>6</td>
<td>0.95 ( (0.90) )</td>
<td>0.94 ( (0.99) )</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. Life cycle of trematodes using Cerithidea californica as a first intermediate host.  The exact type of second intermediate host required (e.g. fishes, crustaceans or molluscs) depends upon the species of trematode.  Additionally, some trematode species will encyst on ingestible hard substrates (e.g. crab exoskeletons, snail opercula, bivalve shells).  (See Appendix A for more specific host information.)

Figure 2. Average trematode prevalence at control and restored sites.
Time 0 represents 1997 samples collected before the restoration.  Asterisks (*) above the shaded bars represent years when control site averages were significantly large relative to the restored sites for that year.  Diamonds (♦) above the white bars represent years where restored sites showed significant relative increases in average prevalence compared to restored sites before the restoration (year 0).

Figure 3. Average trematode species richness at control and restored sites.
Time 0 represents 1997 samples collected before the restoration.  Asterisks (*) above the shaded bars represent years when average species richness was significantly greater at control sites compared to restored sites for that year.  Diamonds (♦) above the white bars represent years where restored sites showed significant relative increases in average species richness compared to restored sites before the restoration (year 0).
Figure 4. The relative proportions of larval trematode communities by second intermediate host group at control and restored sites.

Trematode species were grouped by the second intermediate host they require. The four host groups were fishes, crustaceans, molluscs and hard substrates. Bars represent proportion of total trematode community that utilize each type of second intermediate host at control (C) and restored (R) sites. Values were based on pooled total infections for control and restored sites. Symbols for each group in each year represent whether 95% confidence intervals indicated that a second intermediate host group in restored sites was significantly greater (\(>\)), less (\(<\)), or not different (\(=\)) than in the control sites for that year.
Figure 1.

![Diagram of the life cycle]

- **Adult**
- **Egg**
- **Metacercaria**
- **Miracidium**
- **Cercaria**
- **Cerithidea**
- **Redia**
Figure 2.

- ♦️♦️♦️♦️ Average trematode prevalence
- ♦️♦️♦️♦️ Average prevalence at control sites
- ♦️♦️♦️♦️ Average prevalence at restored sites

<table>
<thead>
<tr>
<th>Year Post Restoration</th>
<th>Average trematode prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
</tr>
</tbody>
</table>

Restoration
Figure 3.

Average trematode species richness

- □ Average species richness at control sites
- ☐ Average species richness at restored sites

Year post restoration

0 1 2 3 4 5 6

Average species richness

0 2.5 5 7.5 10

Restoration

* * * ♦ ♦ ♦ ♦
Figure 4.

Propportion of trematode community using each type of second intermediate host with restoration.
APPENDIX A. Life cycles and hosts used by larval trematodes infecting *Cerithidea californica*.

Columns represent species of trematode described or reported from *C. californica*, second intermediate hosts, and reported definitive hosts for these trematode species. References are included at the bottom of this appendix.

APPENDIX B. Larval trematodes infecting *Cerithidea californica* at control and restored sites by year.

C and R represent pooled infections from control and restored sites respectively. Values given in the first portion of the Appendix B represent total infections and include the double infections listed in the lower portion. The lower portion of Appendix B provides species combinations and numbers of observed double infections.
## APPENDIX A.  Life cycles and hosts used by larval trematodes infecting *Cerithidea californica*

<table>
<thead>
<tr>
<th>Trematode Species Infecting <em>Cerithidea californica</em></th>
<th>Type of Second Intermediate Host</th>
<th>Commonly Observed Second Intermediate Host Species</th>
<th>Reported Definitive Host Species</th>
</tr>
</thead>
</table>
|                                                     |                                  | **Bivalves:**
|                                                     |                                  | **Nereid polychaetes** [3, 5]                    | Western Sandpiper [7]           |
|                                                     |                                  | **Crustaceans:**
|                                                     |                                  |                                                  | Dunlin [7]                      |
|                                                     |                                  |                                                  | Western Sandpiper [7]           |
|                                                     |                                  |                                                  | Rice Rat (*Oryzomys palustris*) [9]|
|                                                     |                                  | **Bivalves:**
|                                                     |                                  |                                                  | Willet [10]                     |
|                                                     |                                  |                                                  | Short-billed Dowitcher [7]      |
|                                                     |                                  |                                                  | Dunlin [7]                      |
|                                                     |                                  |                                                  | Western Sandpiper [7]           |
|                                                     |                                  |                                                  | Least Sandpiper [7]             |
|                                                     |                                  |                                                  | California Gull [7]             |
|                                                     |                                  | *Cerithidea californica* (< 10mm) [12]           | Spotted Sandpiper               |
|                                                     |                                  | **Crustaceans:**
|                                                     |                                  | *Pachygrapsus crassipes* [4]                    | Spotted Sandpiper               |
|                                                     |                                  | *Uca spp.* [3]                                  |                                |
|                                                     |                                  | **Neotrypaea californiensis** [3]                |                                |
**Mesostephanus appendiculatus** [18]  | Fishes  | *Fundulus parvipinnis* [18]  
|  |  | *Gillichthys mirabilis* [19]  
|  |  | *Clevelandia ios* [19]  
|  |  | Unknown  

**Parorchis** spp. [14, 20, 21]  | Hard substrates  | **Gastropods:**  
|  |  | *Cerithidea californica* [12]  
|  |  | **Bivalves:**  
|  |  | *Tagelus* spp. [3]  
|  |  | **Crustaceans:**  
|  |  | *Hemigrapsus oregonensis* [4]  
|  |  | Marbled Godwit [7]  
|  |  | Long-billed Curlew [17]  
|  |  | Willet [7]  
|  |  | Short-billed Dowitcher [7]  
|  |  | Herring Gull [21]  
|  |  | Dunlin [7]  
|  |  | Western Sandpiper [7]  
|  |  | Least Sandpiper [7]  

**Phocitremoides ovale** [22]  | Fishes  | *Fundulus parvipinnis* [22]  
|  |  | *Atherinops affinis* [22]  
|  |  | Unknown  

**Probolocoryphe uca** [5, 23]  | Crustaceans  | *Hemigrapsus oregonensis* [4]  
|  |  | *Pachygrapsus oregonensis* [3]  
|  |  | *Uca* spp. [3, 23]  
|  |  | Willet [4]  

**Pygidiopsoides spindalis** [24]  | Fishes  | *Fundulus parvipinnis* [24]  
|  |  | *Gillichthys mirabilis* [25]  
|  |  | *Clevelandia ios* [26]  
|  |  | Unknown  

**Renicola buchanani** [27, 28]  | Fishes  | *Fundulus parvipinnis* [28]  
|  |  | *Gillichthys mirabilis* [19]  
|  |  | Short-billed Dowitcher [7]  
|  |  | Dunlin [7]  
|  |  | Least Sandpiper [7]  
|  |  | California Gull [5]  

**Renicola cerithidicola** [28]  | Fishes  | *Fundulus parvipinnis* [28]  
|  |  | *Gillichthys mirabilis* [26]  
|  |  | Short-billed Dowitcher [7]  
|  |  | Dunlin [7]  
|  |  | California Gull [5]  
|  |  | Least Sandpiper [7]  

|  |  | *Gillichthys mirabilis* [26]  
|  |  | *Clevelandia ios* [26]  
|  |  | *Atherinops affinis* [26]  
|  |  | Unknown  

*Euhaplorchis californiensis* has only been found in significant intensities the brain of *Fundulus parvipinnis*, despite extensive searches in *Gillichthys mirabilis*, *Clevelandia ios*, and *Atherinops affinis*.

The species referred to here as *Himasthla* species B was referred to as *Echinoparyphium* sp. in Martin s 1972 key and elsewhere in the ecological literature. Careful examination of collar spine morphology and life cycle characteristics indicates this species belongs in the genus *Himasthla*, and is referred to here as *Himasthla* sp. B.
Martin (1972) refers to this species as a microphallid. Microphallids typically use crustaceans as second intermediate hosts. However, the morphology of the cercaria of this species indicates that it is a type of renicolid that uses molluscs as second intermediate hosts.

REFERENCES

2  T. Huspeni and R. Hechinger, *unpublished data*
3  R. Hechinger, *unpublished data*
12  T. Huspeni, *unpublished data*
15  K. Whitney, *unpublished data*
19 J. Shaw and R. Hechinger, unpublished data
26 R. Hechinger and J. Shaw, unpublished data
APPENDIX B. Larval trematodes infecting *Cerithidea californica* at control and restored sites by year

<table>
<thead>
<tr>
<th>Trematode species</th>
<th>Total trematode infections by year of restoration :</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>R</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td><em>Acanthoparyphium spinulosum</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Catatropis johnstoni</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Cloacitrema michiganensis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Euhaplorchis californiensis</em></td>
<td>63</td>
</tr>
<tr>
<td><em>Himasthla</em> sp. B</td>
<td>2</td>
</tr>
<tr>
<td><em>Himasthla rhigedana</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Large xiphidiocercaria</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Mesostephanus appendiculatus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Parorchis acanthus</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Phocitremoides ovale</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Probolocoryphe uca</em></td>
<td>15</td>
</tr>
<tr>
<td><em>Pygidiopsoides spindalis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Renicola buchanani</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Renicola cerithidicola</em></td>
<td>0</td>
</tr>
<tr>
<td>Small cyathocotylid</td>
<td>0</td>
</tr>
<tr>
<td>Double infections by year post restoration:</td>
<td>0</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td><strong>E. californiensis/ R. buchanani</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>H. rhigedana/ E. californiensis</strong></td>
<td></td>
</tr>
<tr>
<td><strong>E. californiensis/ P. uca</strong></td>
<td>1</td>
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<tr>
<td><strong>E. californiensis/ Small cyathocotylid</strong></td>
<td>2</td>
</tr>
<tr>
<td><strong>H. species B/ P. uca</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>C. johnstoni/ P. uca</strong></td>
<td></td>
</tr>
<tr>
<td><strong>A. spinulosum/ P. uca</strong></td>
<td>2</td>
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<tr>
<td><strong>H. rhigedana/ Large xiphidiocercaria</strong></td>
<td></td>
</tr>
<tr>
<td><strong>H. species B./ E. californiensis</strong></td>
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<tr>
<td><strong>Large xiphidiocercaria/ P. uca</strong></td>
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</tr>
<tr>
<td><strong>R. buchanani/ P. uca</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>A. spinulosum/ P. uca</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>C. johnstoni/ R. buchanani</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>E. californiensis/ Large xiphidiocercaria</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>E. californiensis/ M. appendiculatus</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>E. californiensis/ R. cerithidicola</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>H. rhigedana/ R. buchanani</strong></td>
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<td><strong>R. buchanani/ Small cyathocotylid</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>P. ovale/ R. buchanani</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>E. californiensis/ C. johnstoni</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>R. buchanani/ Large xiphidiocercaria</strong></td>
<td>1</td>
</tr>
</tbody>
</table>

**Total number of snails examined:**
400 400 400 400 400 700 400 700 400 700 400 700 400 700

**Total number of uninfected snails:**
290 351 287 343 288 531 249 492 248 495 309 520 302 433

**Total number of infections:**
110 51 118 58 119 171 164 224 156 212 100 186 105 298
C and R represent pooled infections from control and restored sites respectively. Numbers listed above the C and R symbols are year post-restoration. Values given in the upper table are total infections and include the double infections listed in the lower table.

Numbers in this lower table represent the frequency and species composition of double infections observed for each year at control and restored sites. Summary values for total snails, total infected snails, and total infections are given for each year at control and restored sites.