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USING LARVAL TREMATODES THAT PARASITIZE SNAILS TO EVALUATE A SALTMARSH 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, USA. 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 a mean trematode prevalence (12%) and species richness (4.5 species) that were lower than control sites (28% trematode prevalence and 7 species). 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 species), while species richness at the control sites (7.8 species) 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.

Key words: *biological indicators;* Cerithidea californica; *estuarine restoration; parasites; restoration; salt marsh; 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, 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.

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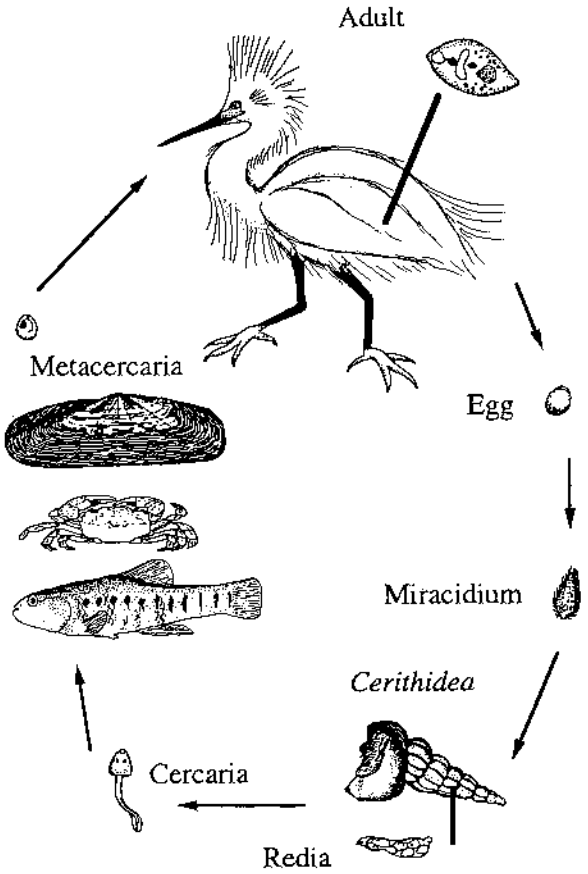

FIG. 1. Life cycle of trematodes using *Cerithidea californica* as a first intermediate host. The exact type of second intermediate host required (e.g., fishes, crustaceans, or molluscs) depends upon the species of trematode. Additionally, some trematode species will encyst on ingestible hard substrates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

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, e.g., 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 (Fig. 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 (Fig. 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; R. Hechinger and K. D. Lafferty, *unpublished manuscript*), 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 (Robson and Williams 1970, Pohley 1976). 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.

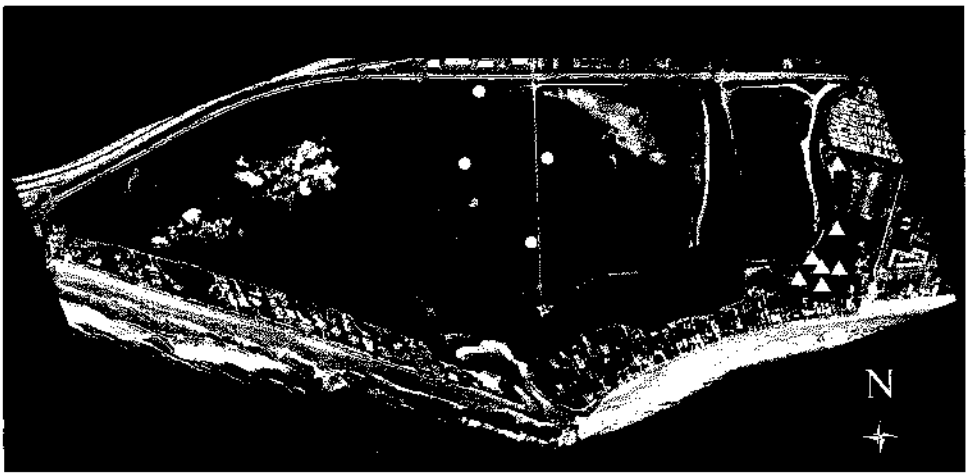

PLATE 1. Aerial photograph of Carpinteria Salt Marsh (34°24′00″ N, 119°31′30″ W) in southern California. White circles depict control sites within the University of California's Carpinteria Salt Marsh Reserve. White triangles depict restored sites within the Ash Avenue restoration. Photo credit: Pacific Western Aerial Photos.

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 (34°24′00″ N, 119°31′30″ W) in southern California, USA. *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. We often found densities of over 600 snails/m^2 (*data not shown*). *Cerithidea californica* can live several years (Sousa and Gleason 1989), and some individuals can live at least 12 years (A. M. Kuris, *unpublished data*). *Cerithidea californica* serves as first intermediate host for many species of larval trematodes (Martin 1972) (Fig. 1 and Appendix A), and this assemblage of trematodes has been the subject of numerous studies at Carpinteria Salt Marsh (Kuris 1990, Lafferty 1993*a*, *b*, Kuris and Lafferty 1994, Lafferty et al. 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 (see Plate 1). It was gradually filled during the 1950s. 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 it 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 prerestored 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 resampled annually each July from 1998 to 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 from 2000 to 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

three meters apart. Each transect was made using 500 cm^2 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 (percentage of snails infected) increases with snail size (i.e., age) (Kuris 1990, Sousa 1990, Lafferty 1993*a*, 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 or three years of age (Lafferty 1993*a*). 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 (percentage of 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 resampling 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, δ, between the mean values (trematode prevalence, trematode species richness) in the control (c) and restored (r) sites ($\delta = (c - r)/c$). Then, for 1000 iterations, we randomized the locations of the samples in space and calculated δ again. We recorded the number of times the randomized δ equaled or exceeded the known δ 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 δ 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).

TABLE 1. Wetland restoration effects on yearly mean trematode species richness and prevalence in first intermediate hosts, with values at restored sites shown.

Time since restoration (years)	Prevalence		Species richness	
	Proportion	P	Proportion	P
Restored vs. control sites				
0	**0.46**	0.02	**0.64**	0.021
1	0.49	0.06	**0.57**	0.044
2	0.82	0.27	0.70	0.094
3	0.78	0.18	**0.72**	0.030
4	0.78	0.14	0.80	0.091
5	1.06	0.69	0.88	0.354
6	1.62	0.84	1.16	0.995
Restored vs. prerestoration sites				
0	1.00	†	1.00	†
1	1.06	0.436	0.88	0.68
2	**1.77**	0.038	1.09	0.34
3	1.68	0.064	1.12	0.33
4	**1.67**	0.022	1.25	0.092
5	**2.21**	0.002	1.36	0.113
6	**3.50**	0.002	**1.81**	0.004

Notes: The calculation of P values for significant differences is described in the *Methods*. Means significant at $P \leq 0.05$ are noted in boldface.

† Not applicable.

Using a similar comparison, we also tested whether conditions significantly improved at the restored sites relative to prerestoration 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 mean 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 just described. Again, for presentation purposes, we provide this difference as a ratio in our summary tables, i.e., $(r_6/c_6)/(r_0/c_0)$ (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 prerestoration sites for each year. We also used a resampling 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 δ 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.,

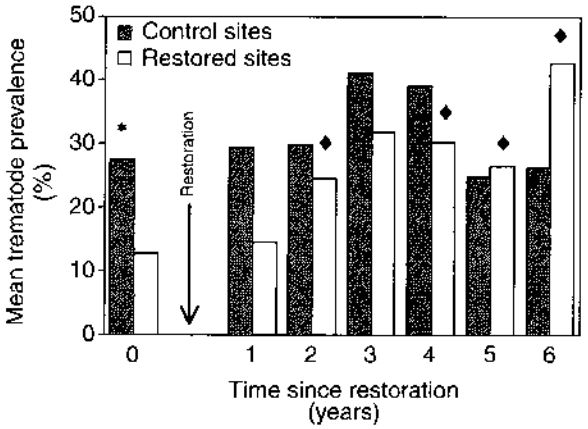

FIG. 2. Mean trematode prevalence at control and restored sites. Time 0 represents 1997 samples collected before the restoration. Asterisks (*) above the shaded bars represent years when control site means were significantly larger than restored site means for that year. Diamonds (♦) above open bars represent years when restored sites showed significant relative increases in mean prevalence compared to restored sites before the restoration (year 0).

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 vs. restored sites for each year.

RESULTS

Prior to the restoration, mean trematode prevalence was significantly lower at the sites to be restored (12%) relative to the control sites (27%, $P = 0.02$, Fig. 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 prerestoration values ($P = 0.038$, $P = 0.022$, $P = 0.002$, and $P = 0.002$, respectively; Fig. 2 and Table 1). By six years after restoration, mean trematode prevalence at restored sites well exceeded mean prevalence at control sites (Fig. 2).

Before the restoration, the mean species richness of trematodes at sites to be restored was significantly lower (4.5 species) than at control sites (7 species, $P = 0.021$, Fig. 3 and Table 1). In years 1 and 3 following restoration mean trematode species richness at restored sites remained significantly low relative to controls ($P = 0.044$ and $P = 0.030$, respectively). By year 6, mean trematode species richness at restored sites had significantly increased to twice the prerestored 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 Fig. 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 postrestoration trematode communities were as similar to control communities as they were prior to the restoration (Table 2).

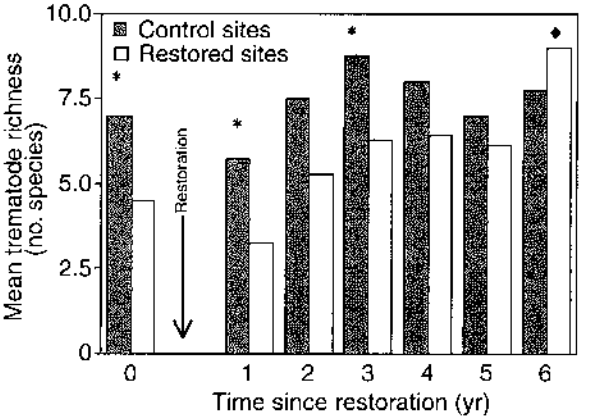

FIG. 3. Mean trematode species richness at control and restored sites. Time 0 represents 1997 samples collected before the restoration. Asterisks (*) above the shaded bars represent years when mean species richness was significantly greater at control sites compared to restored sites for that year. Diamonds (♦) above open bars represent years when restored sites showed significant relative increases in mean species richness compared to restored sites before the restoration (year 0).

TABLE 2. Trematode community similarity indices for restored sites vs. control and prerestoration sites.

Time since restoration (years)	Morisita-Horn community similarity indices			
	Restored vs. control sites		Restored vs. prerestoration sites	
	Index	*P*	Index	*P*
0	0.95	0.90	1.00	†
1	0.34	0.06	0.53	0.29
2	0.46	0.23	0.66	0.44
3	0.80	0.58	0.83	0.92
4	0.72	0.67	0.80	0.65
5	0.84	0.75	0.86	0.97
6	0.95	0.90	0.94	0.99

† Not applicable.

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 (Fig. 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 (Fig. 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 vs. 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 (Fig. 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 saltmarsh 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. R. Hechinger and K. D. Lafferty (*unpublished manuscript*) 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* (<10 mm) 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 saltmarsh habitats relative to older established saltmarsh habitats.

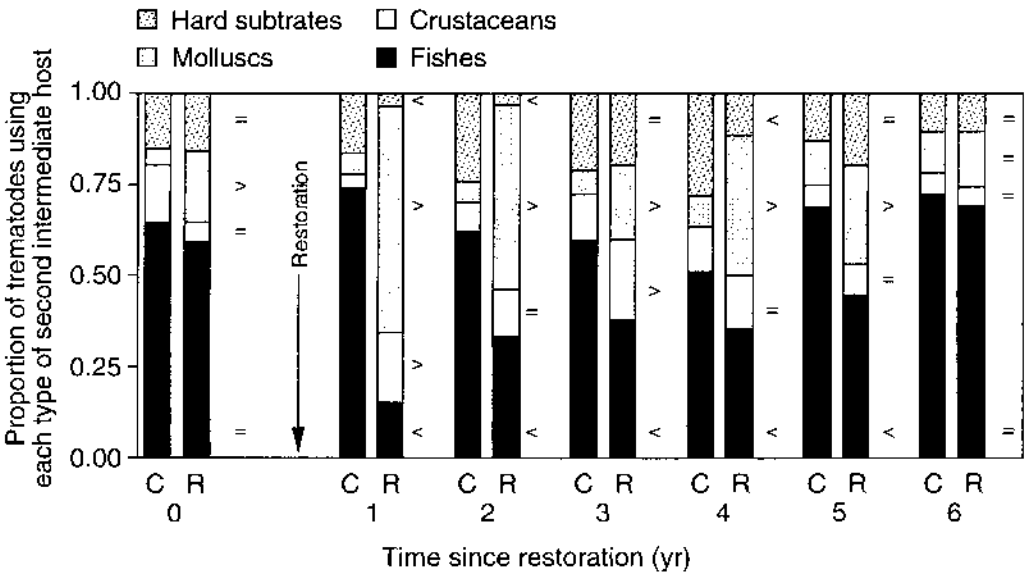

FIG. 4. The relative proportions of larval trematode communities by second intermediate host group at control and restored sites. Trematode species were grouped by the second intermediate host they require. The four host groups were fishes, crustaceans, molluscs, and hard substrates. Bars represent the proportion of the total trematode community that utilized 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 the abundance of a second intermediate host group in restored sites was significantly greater (>), less (<), or not different (=) than in the control sites for that year.

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 a 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 we describe in this paragraph, 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.

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 after restoration were due solely to restoration effects (see Fig. 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 eating *A. inculta*) are initially more likely to occur at restored sites relative to other types of interactions (e.g., a heron eating

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 and 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, Mani and Rao 1993, Al-Kandari et al. 2000). Within North America alone, in addition to *C. californica* on the US California and northern Mexican Baja California coasts, *C. mazatlanica* inhabits Pacific estuaries in Mexico (Lafferty 1993*a*, 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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APPENDIX A

Data on life cycles and hosts used by larval trematodes infecting *Cerithidea californica* are available in ESA's Electronic Data Archive: *Ecological Archives* A014-016-A1.

APPENDIX B

Data on larval trematodes infecting *Cerithidea californica* at control and restored sites by year are available in ESA's Electronic Data Archive: *Ecological Archives* A014-016-A2.