**R/OPCENV-01**
**Parasites as Indicators of Coastal Wetland Health**
**3/1/2008–2/28/2012**

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**Project Leader**
Ryan F. Hechinger
Associate Research Biologist
UC Santa Barbara
Marine Science Institute
Santa Barbara, CA 93106-6510
T: 805-893-3998 F: 805-893-8062
Hechinger@lifesci.ucsb.edu

Kevin Lafferty
Research Ecologist
USGS
Western Ecological Research Ctr.
Marine Science Institute, UCSB
Santa Barbara, CA 93106-6510
T: 805-893-8778 F: 805-893-8062
lafferty@lifesci.ucsb.edu

**Project Hypothesis:**

- Trematodes can provide a powerful and cost-effective ecological indicator tool to assess and monitor biodiversity in estuarine habitats.

**Project Goals and Objectives:**

- To further evaluate the diversity relationships between trematodes and wetland biodiversity.
- To acquire baseline information on trematode communities for most of the appropriate estuaries in CA.
- To acquire baseline information on fish and invertebrate communities for 13 estuaries.
- To assess the temporal aspect of trematodes as indicators (how far back in time do they indicate?).
- To further clarify trematode life cycles to further resolve the trematode indicator tool.
- Communicate with managers and educators about the indicator tool.

**Briefly describe project methodology:**

Analyze previously collected data from 3 estuaries to examine diversity relationships between trematodes and invertebrates and fishes. Collect new data from 12 CA estuaries and 1 Baja California estuary on benthic invertebrate and fish abundance and diversity. Analyze data to evaluate biodiversity associations. Perform some trematode descriptive work to clarify life cycles. Perform growth studies of CA horn snails to inform the temporal nature of the trematode indicator tool. Compile previous long-term monitoring data to assess temporal nature of trematode indicator tool at sites with new trematode data.

**Describe progress and accomplishments towards meeting goals and objectives:**

The project suffered when the CA budget crises resulted in a pulling of our funds at a critical point in the project. Despite this, the resurrection of the third year funds permitted us to resume and do much catching up. We performed the analyses examining whether trematodes can serve as indicators of surrounding biodiversity at the habitat-level—finding that trematodes did positively indicate the abundance of several important estuarine animal groups (e.g., fishes and crabs). We also performed the major multi-wetland field survey of invertebrates and fishes—spending over 60 consecutive days in the field, sampling the abundance of fishes and invertebrates for thirteen different estuaries. We also collected the data required for the temporal studies of trematodes and snails at the San Diego estuary sites where we have access to long-term monitoring data of benthos and fishes. We also performed extensive studies on the growth of the snail that hosts the trematodes, informing the use of the tool. We've increased the utility of the trematode bioindicator tool by progressing with the life cycle studies, clarifying what hosts are used for two of our trematodes. We've progressed in our outreach goals, by discussing our project with numerous wetland managers up and down the coast, training one science instructor at the Lawrence Hall of Berkeley and one with the L.A. County Office of Education.

Also noteworthy is that an EPA scientist conducted and published a systematic evaluation of 36 indicators of estuarine condition (Weilhoefer CL (2010) A review of indicators of estuarine tidal wetland condition. Ecological Indicators 11:514-525). He found that trematode parasites scored as one of the highest diversity indicators. This can help promote the utility of using trematodes as indicators.

Importantly, our work is on-going. We have leveraged our Sea Grant/OPC project to acquire a large 5-year, $2.5M NSF-NIH grant to continue our work examining the role of parasites in estuaries. This will allow us to further enhance the development of trematodes as ecological indicators, and will greatly strengthen the output from this project. In fact this new funded project focuses on the estuaries that we started studying as part of this Sea Grant. Sea Grant and OPC will continue to reap benefits from this project for years.

**Project modifications:**

The biggest modification was the cessation of work forced during the project cancellation during the State's budget crises.

**Project Outcomes:**

Monitoring and evaluating biodiversity in estuaries is a key goal with respect to restoration, mitigation, and conservation. Informed management is critical because estuaries are perhaps the most endangered and impacted habitat in California (Zedler, 1982). Estuaries also serve as habitat for many listed species.

Direct biodiversity assessment in estuaries typically focuses on three animal assemblages: birds, fishes, and benthic invertebrates. However, adequately assessing birds, fishes, and invertebrates requires extensive field and laboratory work (e.g., see methods in PERL, 1990). The costs of assessing this biodiversity generally preclude the desired amount of replication in space and time. Low replication reduces assessment accuracy. The high costs also mean that monitoring and assessment often is rarely undertaken.

The guilds of trematode parasites that live in snail populations provide an unconventional solution for monitoring estuaries. These easily sampled parasites indicate the diversity and abundance of the free-living components of estuarine ecosystems (Huspeni et al., 2005; Hechinger et al., 2007). Most of our research has focused on a guild of 20 trematode species that complete their life cycles in Pacific estuaries of California and Mexico (Fig. 1; Table 1). The trematodes parasitize the California horn snail (*Cerithidea californica*) as their first intermediate host. Clonally produced offspring leave the snails to encyst in second intermediate hosts. Different species of trematodes infect different types of second intermediate hosts, including fishes, clams, polychaetes, and crabs. The parasites are trophically transmitted to final host birds when birds eat second intermediate hosts. The trematodes mature in birds, and their eggs pass from the birds to infect snails. Although the 20 trematode species use a wide diversity of hosts, they all must converge in the easily sampled populations of the CA horn snail.

The trematodes' complex life cycles ensure that the diversity of trematodes in snail populations reflects surrounding biodiversity.

<u>This project demonstrated further that trematodes in snails can indicate birds.</u> We previously demonstrated positive correlations between the diversity and abundance of birds and trematodes infecting snails across several sites in a California estuary (Fig. 2). This is because birds, as hosts for adult trematodes, are the sources of trematode stages that infect snails (Fig. 1). We found that trematodes were particularly suited for assessing bird diversity at the small spatial scales often used to determine point source impacts (Hechinger and Lafferty, 2005). We have now also found that trematodes can indicate the presence of single bird species; a subset of trematode species indicated the presence of nesting territories of the endangered California clapper rail in the salt marsh at Naval Base Ventura County (Fig. 3). Further, estuaries with a greater abundance of rails had a greater abundance of rail-using trematodes in the snails. Hence,

in a sense, snails act as data-loggers, recording bird presence in the form of trematode infection to provide general and specific biodiversity assessments of bird communities.

This project also furthered our efforts examining whether trematodes can indicate the diversity of surrounding benthic invertebrates in estuaries (Fig. 4). These biodiversity correlations should occur for two reasons. First, fishes and benthic invertebrates attract birds (Colwell and Landrum, 1993), which consequently transmit a greater diversity and abundance of trematodes to snails. Secondly, the trematode species differ in what fishes and benthic invertebrates they use as second intermediate hosts. Therefore, a diverse and abundant trematode community in snails *requires* the presence of a bird community that feeds on diverse and abundant fishes and benthic invertebrates. The obligate connection of trematodes to surrounding biodiversity and food-web complexity is a major advantage of using them as biodiversity indicators compared to many other surrogates, which do not require but may potentially covary with surrounding biodiversity.

The specificity of trematodes to their second intermediate hosts (Table 1) greatly increases the power of trematodes as biodiversity indicators. Not only does the overall trematode diversity reflect the overall diversity of birds, fishes, and invertebrates, but this project showed that trematodes can indicate the abundance of particular components of surrounding fish and invertebrate diversity. For instance, we can classify the trematode species in snails into guilds reflecting their second intermediate host use. The right panels in Fig. 4 show that the abundance of particular trematode guilds in habitats can indicate the abundance of particular assemblages (e.g., fishes and crabs), and consequently, indicate the types of birds that forage on those assemblages. Host use of birds is primarily determined by their diet, which explains why we were able to accurately predict which trematode species would indicate the Clapper Rail (Fig. 3).

Sea Grant seed money for this project permitted us to demonstrate that the snails and their trematodes are widespread, being present in virtually every tidal southern CA estuary (Fig. 5). Additionally, we showed that the biodiversity correlations (such as those reflected in Figure 6) are strongest for the most common snail size-classes. This further increases the value of the snails and their trematodes as indicator tools.

We also performed a study of the growth rates of the snails, which informs our ability use these "data loggers" to indicate estuary biodiversity over different time periods (Fig. 7).

We acquired long-term benthic invertebrate and fish monitoring data from 9 study sites in two San Diego area estuaries (Los Peñasquitos Lagoon, and Tijuana Estuary), from Dr. Joy Zedler and Janelle West. We also quantified trematode parasitism at the same sites. Analyses are on-going, but we will be able to further test the utility of the trematode tool using these data, particularly addressing the question about how the indicator reflects longer-term or shorter-term estuarine condition.

We also further demonstrated the widespread applicability of this indicator tool. We collaborated with colleagues to show that trematodes can reflect ecosystem recovery after a hurricane in the Yucatan, Mexico (Aguirre-Macedo et al., 2011). We also showed that trematodes have promise as ecological indicators in rocky intertidal systems in Chile and even in an African great rift lake (Hechinger et al., 2008).

We developed a broader theoretical context for our work, examining the how tight, in general, is the relationship between parasite diversity and free-living animal diversity. Past models have suggested that there could be linear or concave-down relationships between free-living species diversity and parasite diversity. Lafferty (in press) explored several models for the relationship between parasite richness and biodiversity loss. Life cycle complexity, low generality of parasites, and sensitivity of hosts reduced the robustness of parasite species to the loss of free-living species diversity. Food-web complexity and the ordering of extinctions altered these relationships in unpredictable ways. Each disassembly of a food web resulted in a unique relationship between parasite richness and the richness of free-living species because the extinction trajectory of parasites was sensitive to the order of extinctions of free-living species. However, the average of many disassemblies tended to approximate an analytical model. Parasites of specialist hosts and hosts higher on food chains were more likely to go extinct in food web models. Furthermore, correlated extinctions between hosts and parasites (for instance, if parasites share a host with a specialist predator) led to steeper declines in parasite richness with biodiversity loss. In empirical food webs with random removals of free-living species, the relationship between free-living species richness and parasite richness was, on average, quasi-linear, suggesting biodiversity loss reduces parasite diversity more than previously thought. This linear relationship greatly simplifies the predictions for using parasites as indicators.

Further supporting their value as biodiversity indicators, the EPA recently conducted a systematic evaluation of 36 indicators of estuarine condition, concluding that trematodes were the most promising biodiversity indicator (Weilhoefer, 2011). We will re-do their assessment when our data analyses are completed. Such an exercise should greatly strengthen the apparent value of this tool for managers.

We also made headway concerning outreach. We spoke with dozens of wetland managers up and down the coast. These managers were generally excited about the prospects of our research, and promoted our research to develop it, for instance, by permitting our work in their system. More substantially, several have written letters of support for our efforts in proposals (including for the proposal that resulted in this project being funded, and others): e.g., Michael Rouse (Marine Corps Base, Camp Pendleton), Daniel Shide (Naval Base Ventura Co.), Erin

Jones (Army Corps of Engineers), Tiffany Shepard (Naval Base Coronado), Jeff Crooks (TJ National Estuarine Research Reserve), Andy Brooks (Carpinteria Salt Marsh Reserve), and Eric Stein (SCCWRP).

We also performed at least one presentation that was indicator specific. In Dec 2010, PI Hechinger gave a presentation entitled "*Can trematode parasites save the day as ecological indicators of wetland condition?*" at the Ballona Wetlands Science and Research Symposium. The symposium was held at Loyola Marymount University. There were about 200 people in the audience, filled with members of the public, policy people, and managers, from throughout Southern California. The talk was very well received, and many people expressed excitement about the trematode biodiversity tool. A pdf of the symposium proceedings, the abstract & bio they had me submit, and of my talk is theoretically available online. Karina Johnston, the person who is monitoring biodiversity in the Ballona Wetlands, expressed interest in adopting the trematode tool at some point in the near future.

We reached out to the scientific community by including our indicator work in our general scientific presentations. This was most thoroughly accomplished by PI Lafferty, who presented at least 38 times to audiences throughout the US and other areas in the world. Relating to the success of our outreach, we had colleagues reach out to us to collaborate with them on constructing a project to use the trematode indicator tool to assess Gulf Coast estuaries and the impact from the BP oil spill.

We made headway concerning outreach to K-12. Steve McDonough who runs many of the L.A. County Office of Education hands-on marine science programs has committed to incorporating the trematodes and indicator concept into his curricula. Further, we trained Humberto Bracho, a public education specialist at the Lawrence Hall of Science, UC Berkeley. He then trained 25 high school students in the implementation and concepts of using trematodes as indicators. These students (of underrepresented backgrounds) were participating in Mills College Educational Talent Search (METS) Summer Program and they took the San Francisco Bay Biodiversity course presented by East Bay Academy of Young Scientist (EBAYS)/Lawrence Hall of Science. The success of this component of the course has led Lawrence Hall of Science to keep it in ongoing summer programs. Further, Mr. Bracho has presented to and trained his co-workers in the use of this tool, trained school teachers (e.g., 15 Oakland elementary school teachers), and established collaborations with other local educators and managers (e.g., at East Bay Parks) to implement the tool in the future.

Finally, we note that this project is on-going. We have numerous publications in the pipe-line concerning analyses of data collected during this project. We will also further increase our outreach to policy makers and managers, directly after we finish the final quantitative analyses. Supporting our efforts, we have been awarded a large 5-year, $2.5M NSF-NIH grant to continue our work examining the role of parasites in estuaries. This will allow us to further enhance the development of trematodes as ecological indicators, and will greatly strengthen the output from this Sea Grant/OPC project. In fact the newly funded project focuses on the same estuaries that we started studying as part of this Sea Grant/OPC project. Sea Grant and the OPC will reap benefits from this work for years to come.

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Aguirre-Macedo, M. L., V. M. Vidal-Martinez, and K. D. Lafferty. 2011. Trematode communities in snails can indicate impact and recovery from hurricanes in a tropical coastal lagoon. International Journal for Parasitology 41: 1403-1408.

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Hechinger, R. F., K. D. Lafferty, and A. M. Kuris. 2008. Trematodes indicate biodiversity in the Chilean intertidal zone and Lake Tanganyika. Journal of Parasitology 94: 966–968.

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Zedler, J. B. 1982. The ecology of southern California coastal salt marshes: a community profile. Pages ix + 110, U.S. Fish and Wildlife Service.

**Impact of project (e.g., work resulted in change in state policy):**

- At this point, we have primarily been "priming the pump" for using trematodes as indicators. However, managers and policy makers are positive about the idea. Several managers have expressed interest in adopting the tool. We expect that within a year or two that our on-going work will provide the requisite information and momentum to generate official adopting of trematodes as indicators.

**Publications:**

Aguirre-Macedo, M. L., V. M. Vidal-Martinez, and K. D. Lafferty. 2011. Trematode communities in snails can indicate impact and recovery from hurricanes in a tropical coastal lagoon. International Journal for Parasitology 41: 1403-1408.

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Hechinger, R. F., K. D. Lafferty, and A. M. Kuris. 2008. Trematodes indicate biodiversity in the Chilean intertidal zone and Lake Tanganyika. Journal of Parasitology 94: 966–968.

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Hechinger, R. F., K. D. Lafferty, J. P. McLaughlin, B. L. Fredensborg, T. C. Huspeni, J. Lorda, P. K. Sandhu, J. C. Shaw, M. E. Torchin, K. L. Whitney, and A. M. Kuris. 2011. Food webs including parasites, biomass, body sizes, and life stages, for three California/Baja California estuaries. Ecology 92: 791–791 [data paper].

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**Education:**

List all graduate students working on this project: Julio Lorda

How many student volunteers were involved with the project? 8

**Cooperating Organizations:**

Federal: US Geological Survey, US Marine Corps, US Navy, US Army Corps of Engineers

Local and state: L.A. Dept. of Parks and Rec (Cabrillo Aquarium)

Academic: UC Santa Barbara, UC San Diego, UC Berkeley

**International implications:**

Our work directly bears on Mexico, as Baja California is in the same biogeographic province as southern California and much of our work occurs in Baja.

Our work also bears on countries around the world, as there are promising trematode indicator systems on or in every continent. We highlighted this in a book chapter (Huspeni et al., 2005), by listing promising systems. Further, we collaborated with colleagues to show that trematodes can reflect ecosystem recovery after a hurricane in the Yucatan, Mexico (Aguirre-Macedo et al., 2011). We also showed that trematodes have promise as ecological indicators in rocky intertidal systems in Chile and even in an African great rift lake (Hechinger et al., 2008).

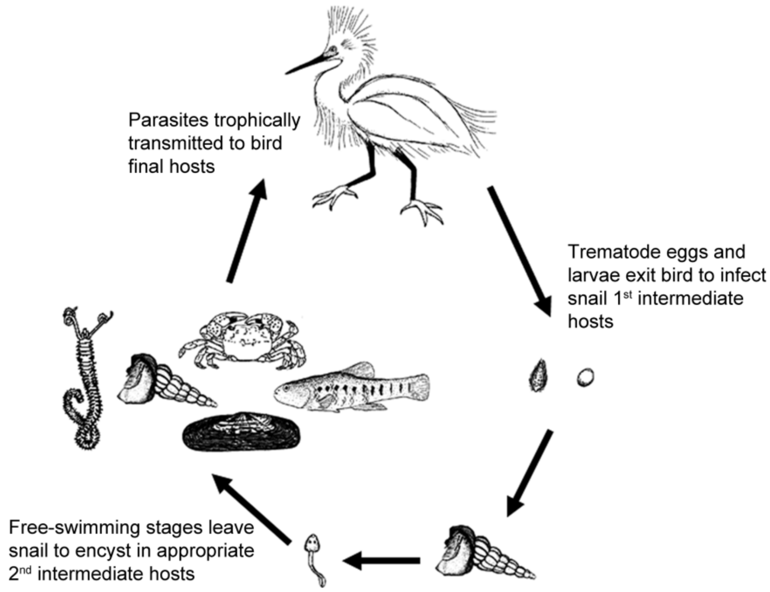


***Figure 1.*** *Representation of the trematode life cycles in estuaries.*

**Table 1. Trematode species that parasitize California horn snails (*Cerithidea californica*) as first intermediate host and the second intermediate hosts they use.**

| Species | Second intermediate hosts used[1] |
|---|---|
| *Austrobilharzia* sp. | none |
| *Mesostephanus appendiculatus* | fishes |
| Small cyathocotylid | fishes |
| *Catatropis johnstoni* | snails |
| *Acanthoparyphium spinulosum*[2] | clams, snails, polychaetes |
| *Himasthla rhigedana* | crabs, horn snails |
| *Himasthla* sp. B[3] | small snails, polychaetes |
| *Cloacitrema michiganensis* | clams, ghost shrimp |
| *Parorchis acanthus* | clams, ghost shrimp |
| *Probolocoryphe uca* | crabs |
| Small microphallid | amphipods |
| *Euhaplorchis californiensis* | killifish |
| *Phocitremoides ovale* | fishes |
| *Pygidiopsoides spindalis* | fishes |
| *Stictodora hancocki* | fishes |
| Large xiphidiocercaria A[4] | polychaetes |
| Large xiphidiocercaria B[4] | molluscs, polychaetes |
| *Renicola buchanani* | fishes |
| *Renicola cerithidicola* | fishes |

[1]Information on 2nd intermediate host use is based primarily on our familiarity with the system (but see Martin (1972), Huspeni and Lafferty (2004), Lafferty et al. (2006), Hechinger et al. (2011)).
[2]Based on preliminary data (Hechinger and Smith, unpublished data), and a note in Martin (1972), *A. spinulosum* may be two cryptic *Acanthoparyphium* species with differing second intermediate host specificities.
[3]*Himasthla* sp. B equals *Echinoparyphium* sp. of Martin (1972).
[4]These are two *Renicola* spp. (Hechinger, in prep).

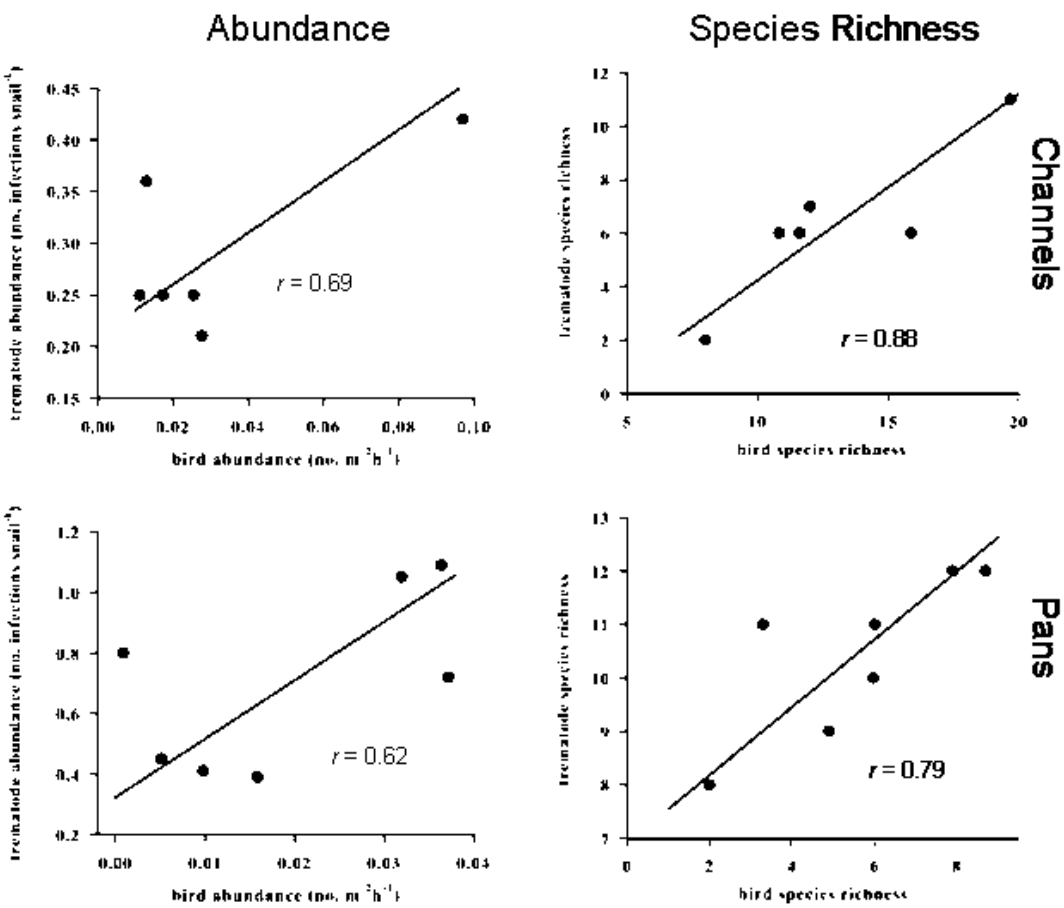


***Figure 2.*** *Positive correlations between abundance (left plots) and species richness (right plots) of trematodes in snail populations with the abundance and species richness of birds across sites in channels (top row; with 20-25 mm snails) and in pans (bottom row; with 25-30 mm snails).Combined P-value for abundance = 0.039; for species richness = 0.0017 (Hechinger and Lafferty, 2005).*

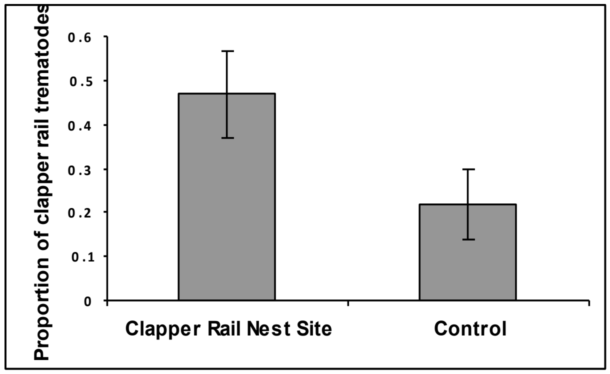


***Figure 3.*** *The effect of clapper rails on the proportion of clapper rail trematodes (# clapper rail trematodes/ # infected snails) in horn snails at Mugu Lagoon, Naval Base Ventura County ($t$ = 2.47, $P_{1\text{-tailed}}$ = 0.015, $N$ = 13). Values are means ± 1 SE (Whitney et al., 2007).*

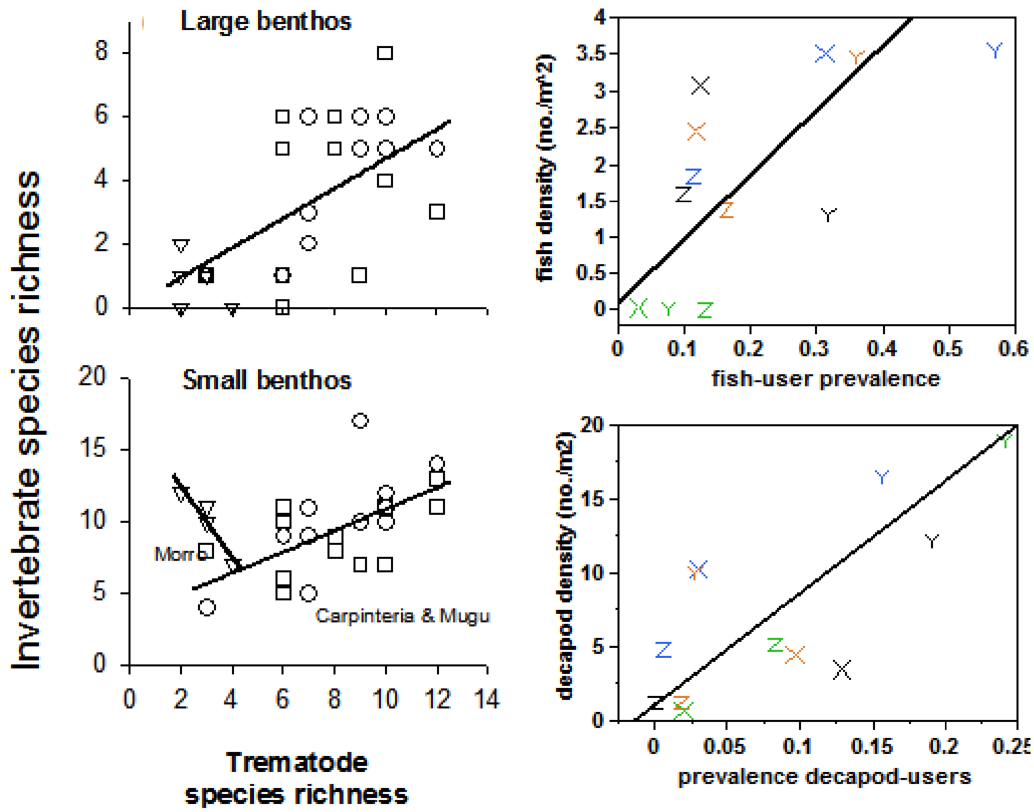


***Figure 4.*** *The two left panels show diversity correlations between trematodes and benthic invertebrates across sites in three California estuaries (Hechinger et al., 2007). Large benthos associations:* $R^2 = 0.43, P<0.000$*; small benthos:* $R^2 = 0.41$*, P = 0.002. The two right panels show the taxonomic resolution provided by the trematode indicator tool (Hechinger, 2007). Habitats with a greater abundance of fish-using trematodes in snails have more fishes (r = 0.67, P = 0.018). The same can be said for crab- and shrimp-using trematodes and crabs and shrimps (r = 0.75, P = 0.005) (crabs and shrimps are decapods).*

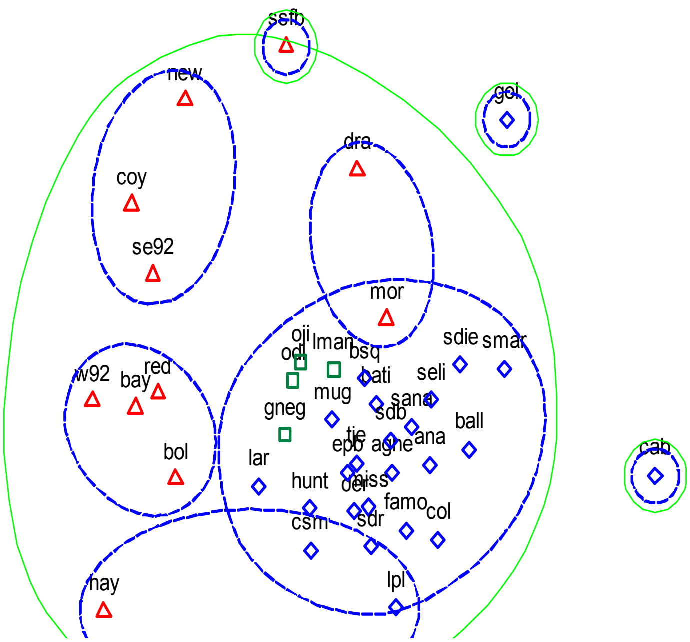


***Figure 5: Multidimensional scaling plot representing background data on trematode communities in California horn snail populations from 41 estuaries.*** *Estuaries closer to one another are more similar in trematode community composition (Bray-Curtis Similarity). Triangles = central CA estuaries; diamonds = southern CA and northern Baja CA estuaries; squares = southern Baja CA estuaries. Green lines encircle estuaries with 20% similarity in cluster analysis, blue those with 40% similarity. Hechinger et al. (in prep.).*

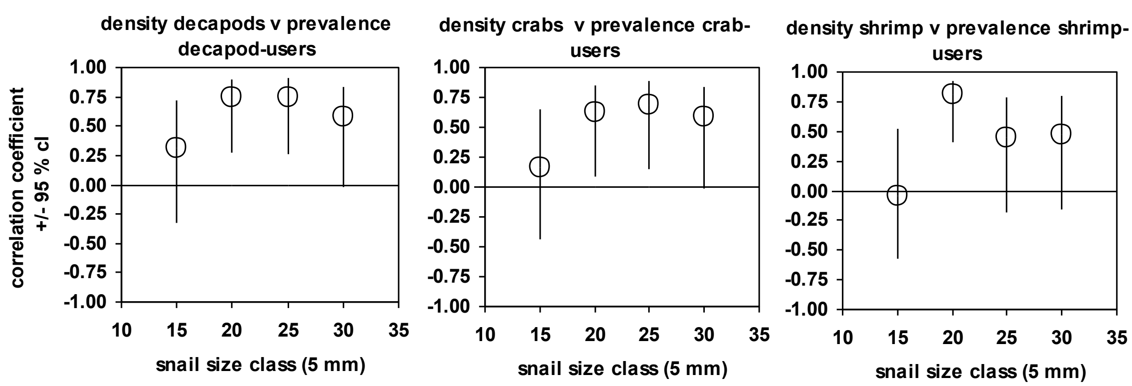


***Figure 6: Biodiversity correlations are greatest for most common snail size classes, further increasing indicator value.*** *Results of correlations between density of (a) decapods, (b) crabs and (c) burrowing shrimp with summed pre-interactive prevalence in snails (of four size classes) of the trematode guild using decapods as second intermediate hosts. Results are similar for other groups (Hechinger et al., in prep.).*

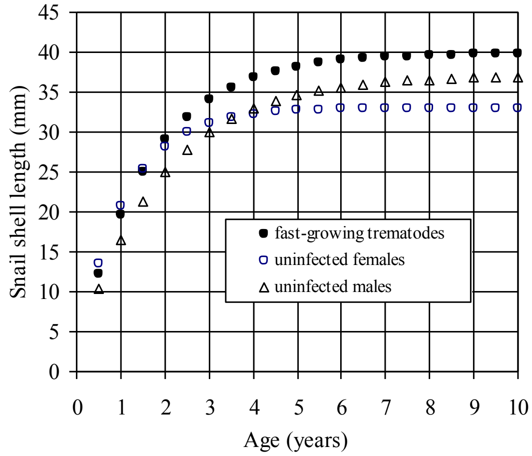


***Figure 7:. Von Bertalanffy growth curve for California horn snails showing different growth rates of uninfected snails by gender and trematode species that cause snails to grow fast.*** *Analysis of data from Hechinger (2010), which come from 17 sites in three southern California estuaries. These data, and much more extensive growth data from up and down the coast (unpublished), will allow us to examine how sampling snails of different sizes indicates biodiversity at different time periods in the recent past, and the importance for factoring in variation in growth.*