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Trematode Parasites as Estuarine Indicators: Opportunities, Applications, and Comparisons with Conventional Community Approaches

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Introduction

Biomarkers, Bioindicators, and Management

Wetland managers face a difficult task: how best to use resources to acquire information about wetland condition necessary to make appropriate management decisions. It can be difficult to choose from the array of potential approaches, ranging from directly monitoring whole biotic communities to using proxy biomarkers and bioindicators (Adams and Ryon, 1994). Indicators employing population and community measures vary considerably in the scope of the information conveyed, as well as in the costs associated with using them. This leaves us with the two basic and pertinent questions when evaluating an indicator: (1) Does it provide information useful for management? (2) Is it cost-effective relative to other options?

In this chapter, we review and elaborate on the application potential for using larval trematode parasites in snail hosts as biodiversity indicators. As we will demonstrate, larval trematodes in host snails are potentially very sensitive, ecologically relevant, and cost-effective bioindicators that reflect free-living community abundance, diversity, and trophic links. Our goals in this chapter are (1) to review the logical framework and supporting evidence of larval trematode parasites as bioindicators; (2) to list what types of these parasites and hosts are available around the world for use as indicators; (3) to articulate how larval trematode parasites could be included in a monitoring program; and (4) to explore the costs and benefits of using these parasites compared to traditional measures.

Larval Trematodes as Bioindicators

Parasites have great potential as population and community level bioindicators, as they may be highly sensitive to many types of impacts (either directly or indirectly through effects on host populations) (Marcogliese and Cone, 1997; Overstreet, 1997; Lafferty and Holt, 2003). However, parasite responses to environmental impacts are complex and do not yield a simple set of predictions (Lafferty, 1997). Whether a particular impact will increase or decrease parasites depends on whether the impact depresses host resistance (good for parasites), depresses host populations (bad for parasites), or affects parasites more than hosts (bad for parasites) (Lafferty and Holt, 2003). Generally, parasites requiring multiple hosts to complete their life cycles are negatively influenced by disturbance and impacts that could interfere with the efficiency of transmission from one host to the next.

One of the most promising parasite indicators is larval trematodes in snail hosts. These parasitic flatworms are typically hermaphroditic and sexually reproduce in vertebrate final (or "definitive") hosts (Figure 19.1). Trematode eggs pass in the excreta of the final host, and a ciliated swimming stage called a miracidium infects a specific mollusk host, usually a snail. If the appropriate snail species is present, the miracidium penetrates the host and asexually reproduces in this molluscan first intermediate host. Each infection typically persists for the life of the snail unless the trematode is displaced by a superior competitor (Lie et al., 1965; Heyneman and Umathevy, 1968; Lim and Heyneman, 1972). Some snail species (e.g., *Cerithidea californica*) serve as first intermediate host to several different species of trematode (Martin, 1972; Yamaguti, 1975; Kuris and Lafferty, 1994). Tailed swimming stages called

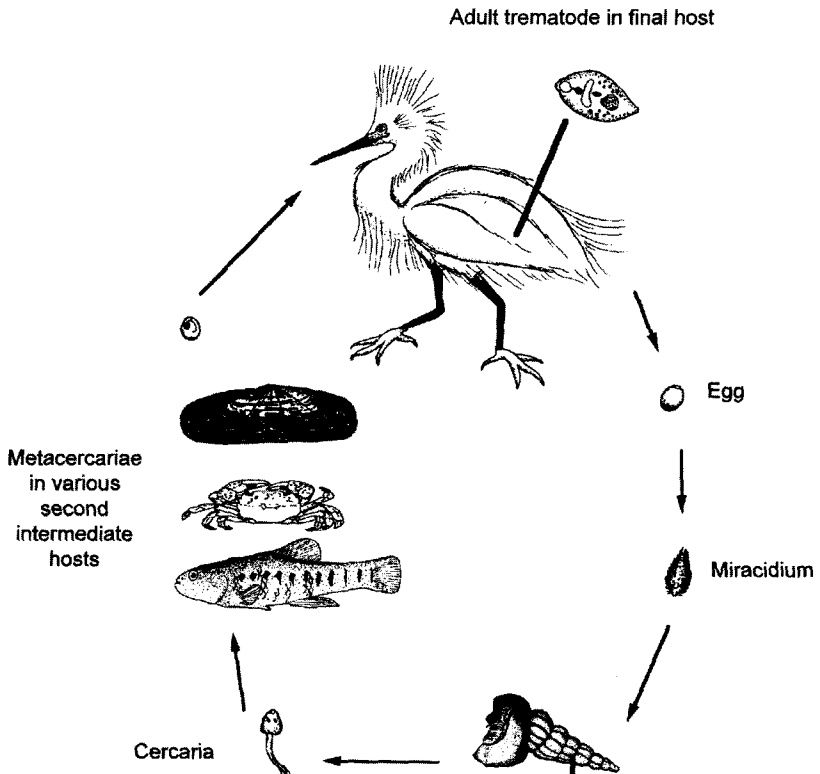

FIGURE 19.1 Generalized life cycle of trematodes using *Cerithidea californica* as a first intermediate host. (Modified from Huspeni and Lafferty, 2004). The exact type of second intermediate host used (e.g., fishes, crustaceans, or mollusks) depends on the species of trematode. *Note:* Not all reported second intermediate hosts for trematodes infecting *C. californica* are depicted in this figure.

cercariae are released from the first intermediate host mollusk and these cercariae may swim for several hours in search of an appropriate second intermediate host (e.g., fish, other mollusks, polychaetes, and crustaceans). Upon contact with an appropriate host, cercariae lose their tails and encyst in or on the second intermediate host. The type of, and specificity for, each second intermediate host depends on the trematode species. Some trematodes are highly host specific for the second intermediate host. For example, the trematode *Euhaplorchis californiensis* encysts only on the brain of the California killifish, *Fundulus parvipinnus* (Martin, 1950). Other trematode species are only specific to higher-level taxa (e.g., some can encyst in several species of fish, others in several species of crabs) and some simply encyst on hard substrates like crab exoskeletons and snail opercula. The trematode life cycle is completed when a final host preys on an infected second intermediate host. Because transmission to the vertebrate final host is by predation, we refer to this process as "trophic transmission" (Lafferty, 1999). This aspect of trematode life cycles permits inferences about which trophic links are functioning in a habitat (Marcogliese, 2001). Parasites in first intermediate host snails, therefore, are *positive* indicators of host communities and functioning trophic links. This runs counter to general impressions of parasites (i.e., that parasites indicate unhealthy conditions) and must be acknowledged and appreciated at the outset.

Hypotheses, Predictions, and Supporting Evidence

The complex life cycles of digenean trematodes (Figure 19.1) permit us to formulate host- and habitat-related hypotheses, and we discuss four of these below. While we acknowledge that in terms of scientific methodology hypotheses may only be falsified and not proved, we assert that the evidence from studies directly contradicts the alternative (i.e., falsifiable) hypotheses in each case presented below, and we therefore present the observed evidence as "support" for the hypotheses outlined below.

> **Hypothesis 1:** *The diversity and abundance of larval trematodes in mollusk first intermediate host populations directly reflects the diversity and abundance of final hosts.*

A diverse and abundant trematode community in first intermediate host snails is impossible without a diverse and abundant final host community. This is because final hosts are the sources of the trematode stages infectious to snails (Figure 19.1), and final hosts vary in what adult trematode communities they harbor. Therefore, more final hosts, and more species of final hosts, will result in more trematodes and more species of trematodes in first intermediate host snail populations.

Support for this hypothesis comes from several lines of evidence. The earliest observation of a correlation between larval trematode infections in first intermediate host snails and the presence of final hosts was made by Hoff (1941) who observed that snails infected with a trematode more frequently occurred near an aggregation of final host gulls. Subsequent to Hoff's study, many workers have noted that trematode infections in snails are higher at locations close to areas of heavier bird use (Robson and Williams, 1970; Matthews et al., 1985; Bustnes and Galaktionov, 1999). Smith (2001) was the first to explicitly relate bird density to trematode prevalence in snails. Working in Florida mangroves, she investigated the causal chain of events that link bird abundance to trematode prevalence in snails. She found significant correlations between bird abundance and number of roosting perches; between number of roosting perches and abundance of bird droppings; and between the abundance of bird droppings and prevalence of trematodes in caged sentinel snails. Finally, we have recently performed studies in a California salt marsh that directly link bird communities to larval trematode communities in snails (Hechinger and Lafferty, unpubl.), and we found positive significant associations between (1) bird abundance and trematode abundance and (2) larval trematode abundance and bird species richness.

> **Hypothesis 2:** *The diversity and abundance of larval trematode infections in first intermediate host snails indirectly reflects the diversity and abundance of prey species that may serve as second intermediate hosts.*

If final hosts disproportionately spend time at sites with abundant food (i.e., fishes and invertebrates), final hosts should transmit more trematodes to snails at sites with more abundant prey. Also, since different final host species prey on different food types, we expect that more final host species will use sites that have

greater species richness of fishes and invertebrates. Additionally, local completion of trematode life cycles requires the presence of second intermediate hosts because the trematodes need those hosts to infect final hosts.

Support for the second hypothesis has only recently become available. Huspeni et al. (unpubl.) sampled larval trematodes, fishes, and benthic infauna at 27 sites in three California estuaries (Morro Bay, Carpinteria, and Pt. Mugu). They observed significant positive correlations between larval trematodes infecting snails and the benthic and fish communities at a site. Specifically, larval trematode prevalence in *C. californica* and the density of benthic infauna were positively associated, and trematode species richness was strongly correlated with infauna and fish species richness at a site.

Hypothesis 3: *The abundance of trematodes in first intermediate hosts reflects water quality.*

Miracidia and cercariae, as free-swimming trematode stages (see Figure 19.1), have direct contact with ambient environmental conditions. They are also sensitive to heavy metals and other toxins. Poor water quality, therefore, should impede trematode transmission. Evidence for this interpretation is provided by numerous studies showing the negative effects of toxics on both the molluscan hosts and the free-living stages of trematodes (Pietrock and Marcogliese, 2003). Free-living stages of trematodes are susceptible to environmental stressors such as increased temperature, extreme pH, and salinity shifts, as well as pollutants, particularly organics and heavy metals (Pietrock and Marcogliese, 2003). For example, trace metals kill free-living trematode cercariae and miracidia (Siddall and des Clers, 1994). Additionally, infected snails may be more susceptible to pollution than uninfected snails, and this can reduce the prevalence of infection in the snail population (Guth et al., 1977; Stadnichenko et al., 1995). Lefcort et al. (2002) also reported lower larval trematode diversity in first intermediate host snails at sites contaminated with heavy metals relative to reference sites.

Hypothesis 4: *Larval trematodes in first intermediate hosts are negatively influenced by environmental impacts and are less abundant and less diverse in impacted habitats.*

Because they have complex life cycles and require multiple hosts, perturbations affecting hosts or transmission at any point of the life cycle will lead to a reduction of trematodes measured in first intermediate host populations. Many workers have noted the logic and anecdotal evidence supporting this prediction (Kuris and Lafferty, 1994; MacKenzie et al., 1995; Lafferty, 1997). Cort et al. (1960) were the first to suspect that environmental impacts could result in drops of larval trematode prevalence in first intermediate host snails. They sampled trematode infections in a first intermediate host snails at several sites in a Michigan lake (Cort et al., 1937). More than 20 years later, Cort et al. (1960) resampled one of the sites and found a precipitous drop in trematode prevalence and suggested the cause was habitat loss and degradation of remaining habitat for final host bird populations. More recently, Keas and Blankespoor (1997) resampled three of Cort et al.'s 1937 sites and found that they had also dropped in trematode prevalence in snails. Huspeni and Lafferty (2004) used a salt marsh restoration project as an opportunity to explicitly test this hypothesis by performing a before-after-control-impact study on the larval trematodes in the California horn snail. Prior to restoration, impacted sites had significantly fewer larval trematodes (lower prevalence and species richness) in snails than control sites located in intact salt marsh (Figure 19.2A and B). After being restored, the impacted sites experienced a significant increase in trematode prevalence and species richness (Huspeni and Lafferty, 2004).

Methods: Employing Trematodes in Assessments: Worldwide Opportunities, Application Methodology, and Comparisons with Other Techniques

Opportunities exist for using larval trematodes in different habitats and regions of the world. We identify promising snails from a variety of aquatic habitats around the globe. To accomplish this, the literature was examined for snail first intermediate hosts reported to be parasitized by rich communities of larval trematodes. Because our principal focus for this chapter is estuarine habitats, we largely restricted our evaluation to estuarine snails. However, because trematodes are ubiquitous components of most aquatic and wetland

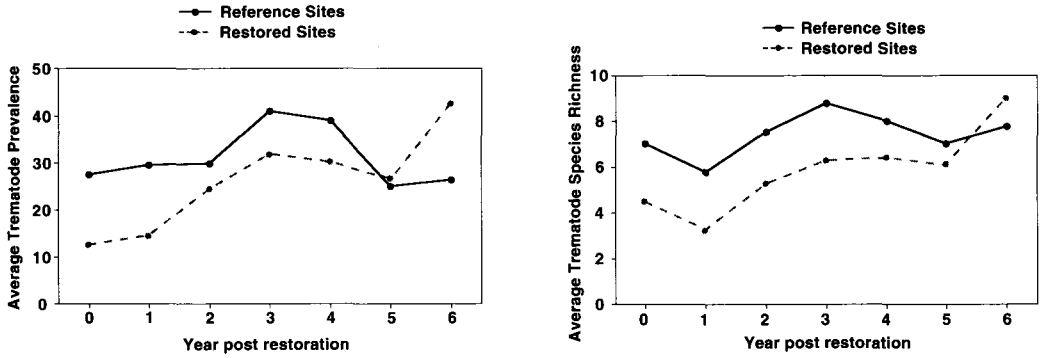

FIGURE 19.2 (A) Average trematode prevalence at control and restored sites in Carpinteria salt marsh. Time 0 represents samples collected before the restoration. Times 1 to 6 represent number of years since the restoration was completed. Year 0 prevalence at control sites was significantly greater than at sites to be restored ($p < 0.05$). Restored sites were significantly higher in trematode prevalence in years 2, 4, 5, and 6 relative to prevalence at those sites in year 0. (B) Average trematode species richness at control and restored sites in Carpinteria salt marsh. Time 0 represents samples collected before the restoration. Times 1 to 6 represent number of years since the restoration was completed. Year 0 species richness at control sites was significantly greater than at sites to be restored ($p < 0.05$). Restored sites were significantly higher in trematode species richness in year 6 relative to species richness at those sites in year 0. (Data reproduced from Huspeni and Lafferty, 2004.)

habitats (Dawes, 1946; Yamaguti, 1971; Yamaguti, 1975), some candidate snail hosts from other aquatic systems are also reported.

To date, the specific steps required to include larval trematodes in a monitoring or management program have not been fully articulated. This chapter outlines steps for using larval trematodes as bioindicators of the abundance, richness, and trophic links operating in surrounding communities. We describe how to sample and identify larval trematodes, and construct a methodology to analyze these data to infer information about the abundance, richness, and trophic interactions of surrounding benthic, fish, mammal, and bird communities.

Finally, the costs (i.e., effort) and benefits (i.e., informational content) of various traditional community assessment techniques are compared to using larval trematodes to evaluate ecosystem function. The extent to which the various techniques provide information about spatial and temporal variation in community organization is especially examined.

Results

Larval Trematodes: Worldwide Opportunities for Application

Although trematodes are ubiquitous components of nearly all aquatic and marine habitats, those most tractable for estuarine assessments infect snails from intertidal or shallow subtidal zones. Our review of the literature found several estuarine snails and families that might be suitable for use in monitoring programs using larval trematodes (Table 19.1). Species in the potamidid genus *Cerithidea* have a worldwide tropical and subtropical distribution in estuaries, and are frequently abundant and long-lived (Houbrick, 1984). *Cerithidea* species also host rich larval trematode communities comprising either the same trematode species, or trematode species closely related to those in *C. californica* (Huspeni, 2000). For example, in North America, *C. californica,* which serves as first intermediate host to 18 trematode species, has already been examined, and occurs in many critical habitats in southern California and northern Baja California (Martin, 1972). *Cerithidea mazatlanica* occurs from southern Baja California through the Gulf of California south to Ecuador (Keen, 1971), and shares *C. californica's* 18 trematode species (Huspeni, 2000). *Cerithidea pliculosa* hosts at least 12 trematode species (Wardle, 1974) and is found in estuaries in the Gulf of Mexico, while *C. costata* hosts a similar 12 species and is found in the Gulf of Mexico and throughout the Caribbean (Cable, 1956). Outside North America, few other *Cerithidea* species have been examined for trematodes, but *C. cingulata* in the Persian Gulf, India, and Japan hosts at least 15 trematode species (Mani and Rao, 1993; Abdul-Salam and Sreelatha, 1998) (Table 19.1).

TABLE 19.1

Estuarine Snail Species Reported as Infected with Larval Trematodes[a]

Snail Family and Species	Region	No. of Reported Trematode Species	Second Intermediate Host Types	Final Host Types	Ref.
Potamididae					
Cerithidea californica	W. North America	18	Crustaceans, fishes, and mollusks	Birds and mammals	25, 33
Cerithidea cingulata	S. Asia, Indopacific	15	Crustaceans, fishes, mollusks, and vegetation	Birds and fishes	1, 32
Cerithidea costata	S.W. North America, Caribbean, E. Central America	12	Crustaceans, fishes, and mollusks	Birds and mammals	7
Cerithidea mazatlanica	S.W. North America, N.W. South America	18	Crustaceans, fishes, and mollusks	Birds and mammals	23, 33[b]
Cerithidea pliculosa	Gulf of Mexico to Central America	12	Crustaceans, fishes, and mollusks	Birds and mammals	34, 49
Cerithidea rhizophorarum	Japan	3	Crustaceans and mollusks	Birds	18, 19
Cerithidea scalariformis	S.E. North America	12	Crustaceans, fishes, and mollusks	Birds and mammals	21, 44
Tympanotonus microptera	Japan	3	Hard substrates	Birds	26
Velacumantus australis	Australia	8	Crustaceans, fishes, and mollusks	Birds	3–5, 16, 47, 48
Cerithiidae					
Cerithium mediterraneum	Mediterranean	3	Fishes	Birds	40–42
Cerithium litteratum	Caribbean and Venezuela	3	Fishes	Birds and fishes	8, 35
Cerithium moniliferum	Australia	11	Crustaceans, fishes, and mollusks	Birds and fishes	9, 10
Cerithium muscarum	Gulf of Mexico	3	Fishes and mollusks	Birds	14, 37, 46
Cerithium rupestre	Mediterranean	3	Crustaceans and fishes	Birds	6, 39, 41

Cerithium scabridum	S.W. Asia, Suez Canal	12	Crustaceans, fishes, and mollusks	Birds and fishes	2
Cerithium stercusmuscarum	W. North America	5	Crustaceans, fishes, and mollusks	Birds, mammals, and fishes	15, 24
Cerithium vulgatum	Mediterranean and Black Sea	3	Fishes	Birds	36, 38, 50
Nassariidae					
Ilyanassa obsoleta	E. North America	9	Fishes, crustaceans, cnidarians, polychaetes, turbellarians, and mollusks	Birds and fishes	11, 12, 17, 45
Nassarius orissaensis	South Asia	3	Mollusks	Fishes	30, 31
Batillariidae					
Batillaria cumingi	East Asia	8	Crustaceans, fishes, and mollusks	Birds	19, 20, 43
Batillaria minima	Gulf of Mexico	4	Crustaceans and mollusks	Birds	7, 46
Hydrobiidae					
Hydrobia acuta	Mediterranean	15	Crustaceans, fishes, and mollusks	Birds and fishes	13
Hydrobia ulvae	N. and W. Europe	32	Crustaceans, fishes, and mollusks	Birds and fishes	13, 22
Hydrobia ventrosa	N. and W. Europe, Mediterranean, Black Sea	19	Crustaceans, fishes, and mollusks	Birds and fishes	13, 27–29

[a] Hosts with fewer than three reported trematode species are not listed.

[b] Huspeni (2000) demonstrated that the trematode species present in *Cerithidea californica* are also present in *C. mazatlanica*. Consequently, Martin's 1972 key to the larval trematodes infecting *Cerithidea californica* can be used for trematodes infecting *C. mazatlanica*.

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Other estuarine snails reported to host larval trematodes are also listed in Table 19.1. Snail species in the family Nassariidae and in the genera *Cerithium and Batillaria* that have been examined for larval trematodes typically host rich larval trematode faunas. Like *Cerithidea* species, *Cerithium, Batillaria,* and nassariid species are frequently abundant where they occur, but relatively few of the species in these taxa have been thoroughly examined for larval trematode parasites. *Ilyanassa obsoleta* on the East Coast (and introduced to the West Coast) of the United States is ideally suited for trematode assessments and, more broadly, other nassariid species are likely to host rich trematode faunas.

Table 19.1 is not intended to be an exhaustive list of hosts that should have rich communities of larval trematodes. The hosts listed are necessarily limited by both our scope of review (e.g., many snail species that had fewer than three species of larval trematodes reported were excluded), as well as basic scientific

exploration of hosts for larval trematodes. There is strong consistency between snail species in the same family regarding whether or not they host rich trematode communities (Ewers, 1964). Snail species are ecologically and taxonomically related to those reported (i.e., intertidal and shallow subtidal species in the same genus or family) will also likely host rich larval trematode communities.

While the principal focus in this chapter is to produce information on promising snails for potential use in larval trematode assessments in estuaries, a more general listing of potential snail hosts in other aquatic habitats is also provided. Specifically, we examined the literature for reported diverse larval trematode communities in snail hosts from shallow freshwater habitats (lakes, ponds, and streams) and intertidal and shallow subtidal marine habitats (rocky and sandy beach). In freshwater habitats, lymnaeids (e.g., Rees, 1932; Anteson, 1970; Brown et al., 1988), physids (e.g., Brown et al., 1988; Snyder and Esch, 1993; Sapp and Esch, 1994), planorbids (e.g., Goater et al., 1989; Fernandez and Esch, 1991a,b), and hydrobiids (e.g., Winterbourn, 1974; Krist et al., 2000) are frequently parasitized by diverse guilds of larval trematodes. The hydrobiids are a very large group of snails that are found in freshwater to marine habitats, and many hydrobiid species are undescribed. In temperate freshwater habitats, many snails often live only 1 year, limiting inferences to a very recent timescale (see below). Muricids (e.g., Ching, 1991) and littorinids (e.g., Matthews et al., 1985) are good candidates in rocky intertidal habitats, while olivellids and buccinids are likely candidates in sandy beach habitats.

Steps Required for Conducting a Larval Trematode Assessment

1. Choose the Snail First Intermediate Host

Trematode communities will vary greatly among snail species and, therefore, comparisons among sites should hold the host snail species constant. Because trematodes are ubiquitous, many snail hosts are available. If present in a habitat, we believe the snail hosts and families listed in Table 19.1 and above are likely to be infected with diverse trematode guilds. Other considerations are important as well, and to be useful as a bioindicator of the diversity and abundance of final hosts and the broader free-living community, a snail host should be abundant and at densities that permit sampling for parasites (e.g., averaging at least one snail per square meter).

2. Control for Habitat

Within an estuary, larval trematode infections in snails may vary between habitat types (e.g., channels vs. mudflats). For example, significant differences were found in prevalence and community composition of larval trematode infections in *Cerithidea californica* across channels, vegetated marsh, mudflat, and pans at Estero de Punta Banda, Baja California (Huspeni et al., unpubl.). To control for this potential source of variation, we recommend limiting comparisons to specific habitat types (e.g., channels or mudflats), or sampling habitat types equally across sites.

3. Control for Snail Age

Each snail is analogous to a datalogger that records an infection event over time. Therefore, trematode prevalence and diversity in samples of first intermediate host snails will increase with the amount of time during which snails have been alive and exposed to infection. Because older snails are likely to be larger, it is important to control for snail size when comparing among samples. If snails vary significantly in size, an initial collection of snails across all sizes present should first be attempted. Focus should be given to larger size classes of snails because, in most systems, prevalence and species richness of larval trematodes increases with snail size (i.e., age) (Kuris, 1990; Sousa, 1993; Kuris and Lafferty, 1994; Lafferty et al., 1994). That is, older snails are more likely to be infected than younger snails. The magnitude of this effect can be demonstrated by plotting prevalence (i.e., percent snails infected) as a function of size in a system under consideration. If a positive relationship exists, it will be necessary to control for size in analyses, and this can be accomplished by restricting subsequent collections to a narrow size class.

Three factors should be considered when choosing the size class for sampling. First, sampling smaller-sized classes will emphasize more recent conditions, while sampling large-sized classes will provide a longer temporal integration. Variation in exposure time with snail size could permit assessment of changes in estuarine community abundance, richness, and trophic functioning over time by comparing changes in the trematode community across snail size classes. This has not been attempted to our knowledge, but could allow the identification of temporal changes in the absence of baseline data. Second, the selected size class should be abundant across sites to ensure a sufficient sample size. Third, the size class should have, on average, an intermediate prevalence of infection in order to increase statistical power when making comparisons (i.e., it is difficult to compare samples for differences when almost none or nearly all the snails are infected). For example, in *C. californica,* the largest adults measure 30 to 35 mm in size. We frequently use 20- to 25-mm snails in our comparisons, as this size class is common and has intermediate prevalences.

4. Choose the Sampling Effort and Scales

Snails move very little, so samples at a particular site will reflect conditions on the order of tens of meters. This makes it possible to assess fine-scale heterogeneity in habitat quality within an estuary. It is important to control for sampling effort and a constant number of snails should be assessed from each site. In our studies, 100 snails has proved to be a good minimum sample size to assess trematode prevalence and species richness at a site because this sample size is sufficient to produce a small variance when estimating prevalence of trematodes in a population of hosts.

Repeated sampling may provide information on year-to-year variation. Because some estuarine fauna have strong seasonal variation in abundance and distribution, it is important to control for sample season when comparing samples. Some snail hosts are only seasonally abundant and, therefore, need to be sampled at particular times of year. Assessments of trematode communities over time or that compare sites within or between wetlands require appropriate levels of replication for statistical tests to be valid (generally five or more sites per wetland). Tests for significant differences in prevalence can be done with simple comparisons of confidence intervals (calculated for proportions) for sites or sites pooled across habitats. Other robust comparisons for prevalence and species richness may be made using resampling statistical procedures (Lafferty et al., 1994; Edgington, 1995). Other significance tests are possible for comparing sites with standard community measures and are outlined in Krebs (1999).

5. Assess Snails for Larval Trematode Infections

Prior to dissecting, snails should be rinsed to remove mud and organic matter, and the length (or width, if appropriate) of each snail should be recorded. Begin a dissection by placing a snail in a shallow container and gently cracking its shell. A hammer or vise is useful for large snails, and pressing down with the bottom of a glass vial is sufficient for smaller ones. Pieces of shell can be removed with fine forceps and the internal organs exposed. Fortunately for the dissector, larval trematode stages typically make up from 30 to 50% of the tissue weight of infected snails (Kuris and Lafferty, 1994). Examine the gonad and digestive gland carefully, as these are common sites of trematode infection. The kidney, heart, pericardial region, and mantle regions are also possible sites of larval trematode infection. In some cases, multiple species may be observed infecting a single snail, particularly when overall percent of infected snails is high. In these cases, it is important to identify and record each species.

6. Identification of Trematode Species

Most basic parasitology texts (e.g., Roberts and Janovy, 2000) provide descriptions of the general types of larval stages of trematodes. Such texts also provide a good overall introduction to digenean trematodes. Schell's classic *How to Know the Trematodes* (1970) is also a great resource for descriptions of the intramolluscan stages. Using this or other keys, it is straightforward to identify the cercaria stage to a digenean family. A determination of trematode infection to this level in the vast majority of cases is sufficient to identify the typical second intermediate host (e.g., mollusk, copepod, fish, etc.) and final host (e.g., fish, amphibian, reptile, bird, or mammal). In the cases of well-studied snail first intermediate hosts (Table 19.1),

many trematode life cycles have been completely described, with second intermediate and final hosts identified. In other cases, a worker may simply work with operational taxonomic units (e.g., Bucephalid 1, Heterophyid 1, etc.). At initial screening stages, the focus should be on carefully identifying infections to the lowest operational taxonomic unit.

7. Data Analysis

Several summary measures are useful in comparing larval trematode infections in snails across sites.

a. Prevalence and Abundance

Prevalence is a measure of the percent of hosts with a larval trematode infection. For example, if 100 snails were assessed and 25 trematode infections were observed, then the trematode prevalence at this site would be 0.25 or 25%. At sites with a significant frequency of multiply infected snails (i.e., double or triple infections), a modified prevalence (abundance) may be calculated by dividing the total number of infections observed (e.g., with double infections in a single snail counting as two) and dividing this number by the total number of snails examined. At high prevalence sites, abundance may exceed 1.0 or 100%. It is worth remembering that asexual reproduction of trematode stages takes place within infected snails. Therefore, the appropriate unit of measure is not the number of individual clonal stages in a snail, but the number of independent infections that have occurred. This is estimated by the number of different species infecting an individual snail.

If two trematode species infect the same snail, one often predictably outcompetes the other (Kuris and Lafferty, 1994). Because of this, in cases where infection rates are high, the infection rate of subordinate species may be underestimated. In other words, using the "snail as a datalogger" analogy, infections with dominant species can "overwrite" records of previous subordinate infections. These overwritten data are meaningful because they correspond to the presence of a final host that carried a subordinate trematode species. Fortunately, it is possible to estimate the frequency at which each subordinate species has been replaced by a dominant because analytical techniques have been developed to estimate the pre-interactive prevalence of subordinate species (Lafferty et al., 1994). Doing so requires knowledge of the trematode dominance hierarchy, which can be postulated for most trematode communities using simple rules (Kuris, 1990; Kuris and Lafferty, 1994). Pre-interactive prevalence is the preferred indicator of transmission from final hosts to snails and should be calculated where possible.

b. Species Richness and Other Community Measures

Species richness is simply the number of trematode species (or operational taxonomic units) observed at a site. It is extremely important to control for sampling effort when comparing species richness (i.e., examining an equal number of hosts per site, or using analytical resampling approaches), because more trematode species are likely to be found as more hosts are examined. Other diversity measures (e.g., Simpson's index or the Shannon–Wiener index) can be calculated as well, and instructions and recommendations for these are given in Krebs (1999). Community similarity indices may also be useful for evaluations that compare impacted or restored sites with reference sites.

c. Second Intermediate Host Use

To reveal specific trophic links operating in a habitat, larval trematode communities can be compared on the basis of second intermediate hosts required. To accomplish this, it is necessary to partition the community of larval trematodes according to second intermediate host use. For example, 100 snails assessed at a site may yield 40 infections (e.g., prevalence = 40%), of which 30 are heterophyids (using fishes), five are microphallids (using crustaceans), and five are echinostomes (using mollusks). The proportional breakdown of the larval trematode community permits inferences about the diversity of final hosts and their likely prey choice at a site. Sites and habitats can then be compared on the basis of second intermediate host use (see Huspeni and Lafferty, 2004).

d. Final Host Use

In some cases, infections in first intermediate host snails may be partitioned into groups based on final host use. For example, *I. obsoleta* hosts nine different larval trematode species: four that use birds as final hosts, four that use fishes as final hosts, and one that uses turtles as final hosts (Table 19.1). Partitioning the larval trematode community in snails on the basis of final hosts used permits inferences to be made regarding final host communities present at each site.

Comparison of Larval Trematodes with Other Community Assessment Approaches

Table 19.2 shows a comparison of commonly employed estuarine sampling techniques for community assessments. Estuarine communities frequently assessed by managers include fishes, large and small benthic invertebrates, birds, and plants (Table 19.2). These communities differ markedly in the way they may vary temporally at a site. For example, because of their vagility, fish and bird communities at a site often vary significantly over short time intervals. Large benthic infauna (e.g., those captured on a 3-mm mesh) are less temporally variable; but small benthic infauna (e.g., those captured on a 0.5-mm mesh) often vary seasonally. Plant communities are often the least temporally variable in estuarine systems.

Not surprisingly, conventional sampling techniques vary in their ability to capture temporal variation in the target community. They also differ in the environmental damage that results from sampling. For example, a single seining effort with blocking nets provides a very limited temporal snapshot of the fish community, requires at least several person-hours to accomplish, and is destructive to muddy habitats. Fish traps can be deployed *in situ* for several days and are, therefore, more temporally integrative than seining. However, many fish species do not enter traps, traps vary in efficiency for the fish species they do capture, and traps cannot provide an estimate of absolute density of fishes at a site. While not environmentally damaging, single bird surveys are also limited in their ability to capture the temporal variation in bird communities at a site. More temporally integrated views of the bird community can be achieved through repeated surveys or videotaped observations, but each of these requires substantial effort. Benthic coring and processing for large infauna is laborious in the field and destructive to the habitat. Sampling for the smaller benthic community requires less field labor, but can be incredibly laborious in the laboratory, depending on the taxonomic level of identification desired.

By comparison, assessments of larval trematodes in first intermediate host snails provide significant information about local communities with relatively little field laboratory effort, and minimal environmental disturbance, and provide temporally integrated views of bird, fish, and benthic communities. Furthermore, trematode surveys of snail hosts can provide a temporally integrated view of the final host use of the habitat over the life of the snail host. With the exception of larval trematode surveys, all other methods provide information largely limited to the community of focus. For example, bird surveys, either using standard bird count surveys or videography, provide information largely limited to the bird community (although observations of foraging birds will indirectly indicate benthic invertebrate or fish communities). The sampling techniques that have a high information yield typically require significant field and/or laboratory effort, are usually destructive to the habitat, and sample a narrow range of species.

Discussion

Assessing larval trematode infections in snail hosts can provide a quantitative, comparative, comprehensive, temporally integrative, environmentally safe, and cost-effective approach for inferring community structure and trophic linkages in an estuary. Larval trematodes in first intermediate host snails provide indirect information about vertebrate and invertebrate communities as well as trophic links between second intermediate and final hosts.

It is important to reemphasize here that unlike many parasites (e.g., ciliates on the gills of a fish), larval trematodes in first intermediate host snails should be viewed as positive indicators of broader host communities. If this predicted connection seems tenuous, we submit the following. Several ecological indicators have

TABLE 19.2

Comparison of Estuarine Community Sampling Methodologies

Sampling Method	Community of Interest	Temporal Variation in Community	Ability of Method to Capture Temporal Variation	Primary Gear Required to Sample a Site (field and lab)	Degree of Habitat Destruction Caused by Assessment Method	No. of Visits Required to Sample a Site	Estimated per Site Person-Hours[a]		
							Field	Lab	Total
Seining	Fishes	High	Low	Seine, blocking nets	High	1	4	0	4
Trapping	Fishes	High	Moderate	Fish traps	Low	2	1	0	1
Large cores	Benthic infauna (large)	Low	High	Corers, wide mesh sieves	High	1	6–8	0	6–8
Small cores	Benthic infauna (small)	Moderate	Moderate	Corers, small mesh sieves, microscopes	Low	1	0.5	10–30	11–31
Single bird survey	Birds	High	Low	Binoculars, spotting scope	Low	1	0.5	0	0.5
Monthly bird survey	Birds	High	Moderate		Low	12	0.5	0	0.5
Bird video	Birds	High	Moderate	Video equipment	Low	30[b]	0.5[c]	2	2.5
Plant survey	Plants	Low	High	Transect tapes	Low	1	1	0	1
Larval trematode survey	Birds, benthos, fishes	High	High	Calipers, mesh bags, hammer, microscope	Low	1	0.5	3	3.5

[a] Person-hours equal the number of workers multiplied by number of hours required to sample a site from our protocols for estuary assessment; estimated efforts for each method do not include travel times to and from sites or the effort involved in transporting required equipment to each site.

[b] This is for sampling two seasons, with 15 visits each season.

[c] Field time for video method also includes equipment setup and removal (divided by 30 visits).

been proposed (and are being used) based on the logic that conditions that are favorable for a particular indicator (e.g., spionid polychaetes) might also be good for other species in the community. By comparison, the conditions that are favorable for larval trematodes in snails *are* the host organisms. This is because abundant and rich larval trematode communities are impossible without abundant and diverse host communities. It is also important to consider that diverse and abundant trematode communities, while they reflect abundant hosts, might not always reflect positive environmental conditions. For example, some sites with large host populations (such as dump sites that attract gulls) are considered degraded yet may lead to prevalent trematode infections in snails (Bustnes and Galaktionov, 1999; Bustnes et al., 2000).

The ease of use and applicability of larval trematode assessments will vary by hosts available in a region and habitat, the number of trematode species infecting these hosts, and the work to date on descriptions of larval trematodes from particular hosts. In these respects, we submit that in estuarine habitats in the tropics and subtropics, *Cerithidea* spp. offer good potential. So too do species of *Cerithium* and *Batillaria*. In some cases (e.g., *Cerithium* spp.), the larval trematodes infecting a host snail may encompass two different types of final host (e.g., fishes and birds). In these cases, larval trematode infections give information about very different final hosts. Because trematodes are ubiquitous in many aquatic habitats (Dawes, 1946; Schell, 1970; Yamaguti, 1971; Yamaguti, 1975), larval trematodes are potentially powerful indicators in far more than just estuarine habitats.

The comprehensive information provided by larval trematode assessments comes at a relatively low cost. Collection of hosts and identification of larval trematodes requires less work than many traditional community survey techniques (Table 19.2). We also emphasize that the work associated with identifying species or operational taxonomic units for larval trematodes is no more laborious, and requires no more expertise, than similar identifications of invertebrates, fishes, or plants.

The use of larval trematode communities should not completely supplant traditional taxonomic surveys, especially in cases where particular species are of interest. Rather, larval trematode community assessments can be used to inform these lists and highlight trophic linkages between them. However, since they have a high information yield and low cost, using larval trematode bioindicators might be given high priority when considering how to use limited resources in a monitoring project.

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