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Using Parasitic Trematode Larvae to Quantify an Elusive Vertebrate Host

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Abstract: *Digenean trematode parasites require multiple host species to complete their life cycles, and their abundance can often be strongly correlated with the abundance of their host species. Species richness and abundance of parasites in easily sampled host species may yield an accurate estimate of the species richness and abundance of other hosts in a parasite's life cycle that are difficult to survey directly. Accordingly, we investigated whether prevalence and mean abundance of trematodes could be used to estimate the abundance of one of their host species, diamondback terrapins (*Malaclemys terrapin*), which are difficult to sample and are designated as near threatened (by the International Union for Conservation of Nature [IUCN Red List]) along some U.S. coasts. As an adult the trematode* Pleurogonius malaclemys *is specific to terrapins. Its larval stages live first inside mud snails (*Ilyanassa obsoleta*) and are subsequently shed into the environment where they form external metacercarial cysts on hard surfaces such as snail opercula. The life cycle of* P. malaclemys *is completed when terrapins ingest these cysts. At 12 sites along the coast of Georgia (U.S.A.), we determined the prevalence of internal* P. malaclemys *larvae in mud snails (proportion of infected snails in a population) and the prevalence and mean abundance of external trematode cysts. We examined whether these data were correlated with terrapin abundance, which we estimated with mark-recapture methods. The abundance of external cysts and salinity explained ≥59% of the variability in terrapin abundance. We suggest that dependent linkages between the life stages of multihost parasites make them reliable predictors of host species' abundance, including hosts with abundances that are challenging to quantify directly.*

Keywords: biological monitoring programs, coastal estuaries, diamondback terrapin, ecological parasitology, *Malaclemys terrapin*, parasite ecology, threatened species, trophic transmission

Utilización de Larvas de Trematodos Parásitos para Cuantificar a Un Hospedero Vertebrado Elusivo

Resumen: *Los trematodos digeneos parásitos requieren de múltiples especies de hospederos para completar sus ciclos de vida, y su abundancia a menudo puede estar fuertemente correlacionada con la abundancia de su especie hospedera. La riqueza y abundancia de especies de parásitos en especies hospederas fácilmente muestreadas puede producir una estimación precisa de la riqueza y abundancia de otras especies hospederas en el ciclo de vida de un parásito que son difíciles de muestrear directamente. En consecuencia, investigamos sí la prevalencia y abundancia promedio de trematodos puede ser utilizada para estimar la abundancia de una de sus especies hospederas,* Malaclemys terrapin*, que es difícil de muestrear y está designada como casi amenazada (por la Unión Internacional para la Conservación de la Naturaleza [Lista Roja de la IUCN]) a lo largo de algunas costas de E. U. A. Como adulto, el trematodo* Pleurogonius malaclemys *es específico de* M. terrapin. *Sus etapas larvarias primero viven en caracoles (*Ilyanassa obsoleta*) y subsecuentemente son liberados al ambiente donde forman quistes metacercarios externos sobre superficies duras como los*

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opérculos de caracoles. El ciclo de vida de P. malaclemys *se completa cuando las tortugas ingieren estos quistes. Determinamos la prevalencia de larvas de* P. malaclemys *en caracoles (proporción de caracoles infectados en la población) y la prevalencia y abundancia promedio de quistes externos en 12 sitios a lo largo de la costa de Georgia (E.U.A.). Examinamos si esos datos se correlacionaron con la abundancia de tortugas, que fue estimada con métodos de marca-recaptura. La abundancia de quistes externos y la salinidad explicaron ≥59% de la variabilidad en la abundancia de tortugas. Sugerimos que las relaciones dependientes entre las etapas de vida de parásitos multihospederos las hace pronosticadores confiables de la abundancia de las especies, incluyendo hospederos cuya cuantificación directa de la abundancia es un reto.*

Palabras Clave: ecología de parásitos, especies amenazadas, estuarios costeros, *Malaclemys terrapin*, parasitología ecológica, programas de monitoreo ecológico, tortuga, transmisión trófica

Introduction

Several studies have examined whether the abundance of parasites is correlated with the abundance and distribution of their hosts (reviewed in, e.g., Lafferty 1997; Huspeni & Lafferty 2004; Hechinger & Lafferty 2005). Under some circumstances parasites can provide clearer and more easily obtained information about the history or status of populations of host species than direct measures of the host itself (Criscione et al. 2006; Blakeslee et al. 2008). Researchers have used both species richness (Blakeslee & Byers 2008) and genetic diversity of parasites (Criscione et al. 2006; Blakeslee et al. 2008) to increase understanding of a host species' ecology, such as its past spatial distributions and genetic bottlenecks. Thus, the close relation between parasites and their hosts may make parasites useful sources of information on host ecology and population dynamics, especially when little is known about the host.

Digenean trematodes are potentially useful as measures of the status of the multiple host species that they require to complete their life cycles. If any host species required for a given stage of a trematode's life cycle has low abundance or is absent, the trematode also will have low abundance or be absent. Thus, species richness and abundance of parasites in easily sampled host species may yield an accurate estimate of the species richness and abundance of other hosts in a parasite's life cycle that are difficult to quantify directly. Furthermore, because transmission of parasites between hosts depends on ecological processes and species interactions not often examined with traditional biological indicator variables, multihost parasites may allow for simultaneous assessment of the abundance of interacting species.

Hechinger et al. (2007) demonstrated statistically significant positive correlations between the species richness of trematode species that infect snail hosts and the species richness of free-living benthic hosts. Similarly, results of several studies show strong positive correlations between definitive host densities and the prevalence of larval stages of a parasite in intermediate hosts (i.e., the proportion of the host population that is infected) (e.g., Smith 2001; Fredensborg et al. 2006; Byers et al. 2008). In a small number of cases, however, the correlation is weak, presumably due to a lack of host specificity or strong influence of abiotic factors on parasite abundance (Kube et al. 2002; Latham & Poulin 2003). Overall, although results of previous studies suggest trematode abundance and species richness should reflect abundance and species richness of their host populations, to the best of our knowledge, no one has specifically tested their utility for predicting the abundance of a host population of conservation concern.

Study System

Diamondback terrapins (*Malaclemys terrapin*) are an estuarine turtle with a range from Cape Cod, Massachusetts, to eastern Texas (U.S.A.). Terrapins appear to remain within the same small tidal creeks (Gibbons et al. 2001) such that their abundance is primarily a function of local creek conditions and abundance differences among creeks are generally consistent over time (Dorcas et al. 2007). Terrapins are a species of conservation concern throughout their range, primarily because they were intensively harvested, currently are bycatch in crab traps (Roosenburg et al. 1997; Dorcas et al. 2007; Grosse 2009), and have high mortality on coastal roads (Wood & Herlands 1997; Tucker et al. 2001). Several states monitor spatial or temporal patterns of terrapin abundance; however, reliable estimates of site occupancy and abundance are difficult to generate. Common terrapin sampling techniques include trapping in modified crab pots, which yields low capture rates and thus poor estimates of abundance; visual surveys ("head counting" Harden et al. 2009), which are a poor measure of abundance because sampling effectiveness differs among sites, individuals cannot be identified, sex cannot be determined, and sampling is limited to sites accessible by boat at low tide; and capture in seine nets, which is labor intensive and limited to sites at which a person can walk at low tide. Therefore, there is a need for a reliable, low-cost method for assessing terrapin abundance among many, varied sites.

Terrapins are hosts of the adult stage of the host-specific trematode *Pleurogonius malaclemys* (Hunter 1961; Werner 2003) (Fig. 1). No other naturally occurring host of the adult trematode has been documented.

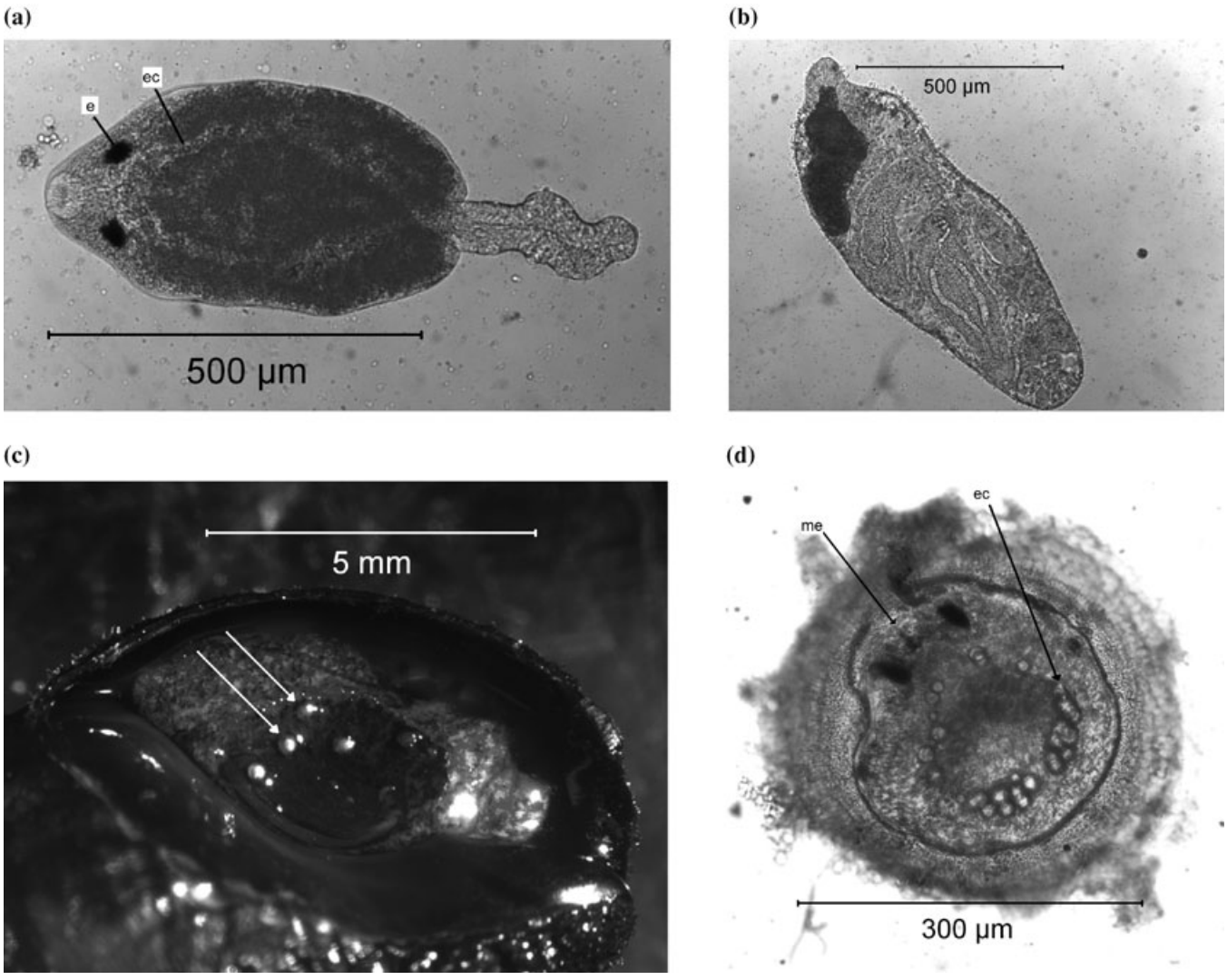

Figure 1. Life stages and distinguishing characteristics of the trematode Pleurogonius malaclemys *(a) cercaria (straight tail slightly longer than the body when extended) and (b) redia are both internal infection stages in the first intermediate snail host (*Ilyanassa obsoleta*) (100× magnification). Rediae typically have two to three cercariae inside. (c, d) External metacercarial cyst stage of* P. malaclemys*, which is always this characteristic size and opaqueness: (c) cysts on the operculum of* I. obsoleta *(10× magnification) and (d) a single cyst (200× magnification). Key to features in photographs: e, eyespots; ec, excretory canal (e and ec are present in both the [a] cercaria and [d] metacercarial cyst and are identifying morphological features of* P. malaclemys*); me, median eyespot.*

Although the specific biological effects of *Pleurogonius* in terrapins are not well known, adult trematodes at typical low to moderate infection intensities seldom have lethal effects on their hosts (Lafferty & Kuris 2002). The adult trematode lives in a terrapin's intestine. Trematode eggs are shed in the feces of infected terrapins and are incidentally ingested by foraging mud snails (*Ilyanassa obsoleta*), the only known intermediate host of *P. malaclemys*. Larval trematodes usually reside in the gonads and digestive glands of snails, and they typically castrate their snail host but do not kill it (Kuris 1990). Thus, infections are typically maintained throughout a snail's life, which for *Ilyanassa* can be a decade or more (Curtis et al. 2000; Curtis 2003). Within the first intermediate snail host, trematodes reproduce asexually, producing free-swimming cercarial larvae that are periodically shed from the snail. In the external environment, the released cercariae rapidly encyst on substrates such as snail opercula (Hunter 1967) or vegetation (McDermott 1951). Opercula are readily available and appropriate sites for encystment because transmission to the definitive terrapin host occurs through ingestion of metacercarial cysts, and

mud snails are a component of terrapin diets (M. Erickson, J. Maerz, & A. Grosse, unpublished data).

Because terrapins remain mostly within small tidal creeks throughout their lives and co-occur with *I. obsoleta*, the life cycle of *P. malaclemys* can be completed within a relatively small area (Roosenburg et al. 1999; Gibbons et al. 2001; Butler 2002). Therefore, the variability in terrapin abundance in space should affect the abundance of all life stages of the terrapin-specific trematode. We investigated the relation between trematode prevalence in mud snails and terrapin abundance. We hypothesized that terrapin abundance determines the prevalence of trematode larvae and cysts in snail populations, and in turn that prevalance of larval infection and cyst abundance can accurately estimate terrapin abundance. At 12 sites distributed along the Georgia coast (U.S.A.), we used mark-recapture methods to estimate terrapin abundance, and we quantified *P. malaclemys* larval infections in mud snails and *P. malaclemys* metacercarial cysts in the environment. By quantifying the prevalence and abundance of this host-specific trematode, we sought to determine whether these data collected through less labor-intensive means could serve as an indirect measure of terrapin abundance.

Methods

We estimated terrapin abundance at 29 randomly selected estuarine sites in coastal Georgia that were used in a related study (Grosse 2009). At 14 sites in spring 2007 and at 15 sites in spring 2008, we conducted mark-recapture surveys of terrapins. In each tidal-creek site, we pulled by hand 2, 10-m seine nets (2.54-cm mesh) with bags to capture terrapins, following the methods of Dorcas et al. (2007). We began seining immediately prior to low tide and pulled the nets from the start of each sampling area to the end of the creek (mean distance of 0.44 km [SD 0.15]). Occasionally we pulled the seines onto the mud bank to remove captured *M. terrapin* and other animals. We then seined back along the course we had come to the starting point. We seined each creek at low tide every 20 days between 1 April and 30 June, which is the most effective period for capturing terrapins (Gibbons et al. 2001), for a total of five mark-recapture periods per creek. We sexed; measured carapace length, plastron length, and shell width and depth; aged (number of scute rings) when possible (Sexton 1959); and uniquely marked with scute notches (Cagle 1939) each terrapin we caught.

In some creeks we did not recapture one or both sexes, so we could not estimate sex-specific capture probabilities for each site independently. Therefore, we assumed terrapin capture and recapture probabilities were similar among creeks and pooled all data into two data sets, one for males and one for females. We used each data set to estimate universal capture and recapture probabilities for each sex. We used Program CAPTURE in Program MARK (White & Burnham 1999) to determine the most appropriate model for measuring sex-specific probabilities of terrapin capture. We assumed a closed population for each creek and used sex-specific capture probabilities and the mean number of each sex captured over the five sampling periods to estimate male and female abundance. We summed the male and female abundance estimates to generate the terrapin abundance estimate for each creek. We also calculated a juvenile-specific capture–recapture probability in the same manner in which we calculated sex-specific probabilities, but only for juvenile females. Juvenile females are easily distinguished from adult females, which are considerably larger.

We sampled trematodes in 12 of the 29 sites in which we sampled terrapins (six that were sampled in 2007 and six in 2008). These 12 sites represented the range of terrapin abundances we found. In August and September of 2008 during low tide, we collected mud snails in a 50-m transect along both banks of the downstream portion of the creeks where we sampled terrapins. Because trematode occurrence in snails can be spatially heterogeneous, we collected one snail approximately every meter (Sousa 1990; Kuris & Lafferty 1994; Torchin et al. 2005; Curtis 2007). Also, because salinity influences the physiologies of terrapins (e.g., Davenport & Macedo 1990; Davenport & Ward 1993), mud snails (e.g., Richmond & Woodin 1996), and trematodes (e.g., Koprivnikar & Poulin 2009), we quantified salinity at each site in May 2008 with a hydrometer placed in the center of each creek at low tide.

In the lab we examined snails for internal larvae and external cysts. We measured snail lengths from the apex of the spire to the tip of the siphonal canal with vernier calipers. Because metacercariae encyst on external surfaces in the environment (McDermott 1951; Hunter 1967;), we wanted to ascertain where on a snail the cysts occurred most often. In an initial examination of a subset of snails selected haphazardly from multiple sites (~25 individuals from each of three sites), cysts were present only on snail opercula and not on the surface of snail shells. Thus, we focused on quantifying the presence and abundance of cysts only on the operculum, which is a primary area of encystment of *P. malaclemys* (Hunter 1967). We removed the operculum from snails with forceps and used a small paintbrush to gently clear both sides of the operculum of associated debris. We examined each operculum under a stereomicroscope (40× magnification) and counted the metacercarial cysts of *P. malaclemys* on the outside and underside margins of the operculum (Fig. 1).

For each site we examined a minimum of five cysts, each removed from a different snail, under a compound microscope (200×) to verify, at a higher level of magnification, that cyst characteristics matched those described

for *P. malaclemys*. Snails from one site had only three cysts, and we examined all of them microscopically. *Pleurogonius malaclemys* is the only known digenean trematode in the region that produces cysts of the size and morphology we observed (Hunter 1967; Yamaguti 1975; Schell 1985; R. Heard, personal communication). All the cysts we found were morphologically similar and consistent with descriptions of *P. malaclemys*.

We examined the gonad and digestive gland from each snail under 40× magnification to determine the occurrence of *P. malaclemys* and other trematode larvae (i.e., rediae and cercariae) in these tissues. We made wet mounts of all trematodes encountered and observed them under a compound microscope. We based identification of trematodes on McDermott (1951), Hunter (1967), Yamaguti (1975), and Stunkard (1983).

For the mud snail populations at each site we determined (1) the prevalence of internal infections (i.e., proportion of snails infected with rediae and cercariae) of *P. malaclemys*, (2) prevalence of internal infections by all trematode species, (3) prevalence of external *P. malaclemys* cysts, (4) average number of cysts among snails that had at least one cyst (i.e., intensity), and (5) mean abundance of cysts per snail among all snails examined.

Data Analyses

We transformed data on terrapin abundance to the natural log to normalize the data (data on juvenile abundance were $\ln[x+1]$ transformed). To normalize data on prevalence of *P. malaclemys* and all other trematode larvae (rediae and cercariae), we transformed the data to the Anscombe arcsine square root. With the exception of salinity, which did not require transformation, we normalized data on all independent variables with natural-log transformation. Our initial data exploration indicated the prevalence of *P. malaclemys* cysts (number 3 above) and the average number of cysts among snails with at least one cyst (number 4) were highly collinear ($R = 0.80$). Furthermore, the product of these two variables is represented by the mean abundance of cysts per snail among all snails examined (number 5). Thus, to proceed with model selection using the most parsimonious suite of variables, we removed prevalence of *P. malaclemys* cysts (number 3) and the average cyst number among snails with at least one cyst (number 4) from our initial set of five independent variables.

We used multiple regression to analyze the relation between abundance of terrapins, which we estimated from mark-recapture data, and enumerations of external and internal trematode infections of mud snails. Because terrapins are the definitive host of *P. malaclemys*, we determined whether terrapin abundance limits, and is therefore correlated with, parasite abundance and prevalence in mud snails. We examined whether terrapin abundance and salinity were associated with the prevalence of infection and the abundance of external cysts in mud snails.

Once the strength of the biological dependence of these relations was established (i.e., terrapin abundance as independent variable and parasite metrics as dependent variables), we then reversed the analyses so that we could examine whether parasite abundance could explain terrapin abundance. For these analyses, in four separate multiple regressions, we examined terrapin abundance, adult female abundance, adult male abundance, and juvenile abundance. In these models, in addition to our three independent trematode variables (i.e., prevalence of internal infections of *P. malaclemys*, prevalence of internal infections by all trematode species, mean abundance of cysts per snail among all snails examined), we again included salinity as a variable. Initially, we explored second-degree polynomials and interaction terms for all independent variables, but none significantly improved the fit of the models. We compared all possible regression models (i.e., linear models with all combinations of the four independent variables) and used the lowest Akaike information criterion (AIC) values to evaluate the best fit. We also used Mallow's C_p to evaluate model fit. We recognize that a model's explanatory power may be more important than its parsimony. Thus, to enable a more complete comparison, we present the two best models for each increasing number of independent variables being fitted and the full model with all four independent variables.

Results

Eight trematode species occurred in the mud snails we examined. Prevalence of all trematode species combined ranged from 1.2% to 35% across all sites (Supporting Information). The prevalence of larval *P. malaclemys* was low across sites (maximum 6%), and in six sites we found no snails infected with *P. malaclemys* larvae. *Pleurogonius malaclemys* cysts were found at every site, including sites where no larvae were detected in snails. Cyst prevalence on opercula ranged from 2% to 60%. The mean abundance of cysts per snail ranged from 0.04 to 1.79 (Supporting Information).

Terrapin abundance and salinity did not explain significant variation in prevalence of *P. malaclemys* infection in mud snails ($R^2 = 0.24$, $p = 0.28$), but were significantly and positively associated with *P. malaclemys* mean abundance of cysts per snail ($R^2 = 0.72$, $p = 0.003$). When we reversed the statistical relations to explain terrapin abundance with parasite metrics (i.e., parasite metrics as the independent variables and terrapin abundance as the dependent variable), the best models included mean abundance of *P. malaclemys* cysts per snail and salinity (Table 1). These variables explained 59% of the variability in terrapin abundance across the 12 sampled sites

Table 1. Model results for multiple regression analyses on the mark-recapture estimates of terrapin abundance (all) at each site (natural-log transformed) and the mark-recapture estimates of female terrapin abundance at each site (natural-log transformed).[a]

No. independent variables	*Model*	R^2	AIC_c	ΔAIC_c[b]	C_p[c]
All terrapins					
1	external *Pleurogonius malaclemys* cyst abundance[d]	0.21	40.00	3.27	7.84
1	internal *P. malaclemys* prevalence[e]	0.19	40.30	3.57	8.24
2	**external *P. malaclemys* cyst abundance, salinity***	**0.59**	**36.73**	**0.00**	**2.15**
2	external *P. malaclemys* cyst abundance, internal *P. malaclemys* prevalence	0.28	43.49	6.76	8.31
3	external *P. malaclemys* cyst abundance, internal *P. malaclemys* prevalence, salinity	0.63	41.88	5.15	3.41
3	external *P. malaclemys* cyst abundance, internal prevalence of all trematodes, salinity	0.60	42.81	6.08	4.00
4	external *P. malaclemys* cyst abundance, internal *P. malaclemys* prevalence, internal prevalence of all trematodes, salinity	0.65	50.00	13.27	5.00
Female terrapins[f]					
1	external *P. malaclemys* cyst abundance	0.33	38.90	3.56	10.73
1	internal *P. malaclemys* prevalence	0.32	39.21	3.87	11.23
2	**external *P. malaclemys* cyst abundance, salinity***	**0.67**	**35.34**	**0.00**	**3.40**
2	external *P. malaclemys* cyst abundance, internal *P. malaclemys* prevalence	0.47	40.95	5.61	9.01
3	external *P. malaclemys* cyst abundance, internal *P. malaclemys* prevalence, salinity	0.75	38.25	2.91	3.10
3	external *P. malaclemys* cyst abundance, internal prevalence of all trematodes, salinity	0.67	41.60	6.26	5.38
4	external *P. malaclemys* cyst abundance, internal *P. malaclemys* prevalence, internal prevalence of all trematodes, salinity	0.75	46.89	11.55	5.00

[a] *For each number of independent variables the best two models (on the basis of lowest Akaike's information criterion [AIC] corrected for small sample size [AIC_c]) and the full four-variable model (external* P. malaclemys *cyst abundance, internal* P. malaclemys *prevalence, internal prevalence of all trematodes, salinity) are shown. For each response variable the model with lowest AIC_c is indicated with bold font and an asterisk (*): [ln (mark-recapture terrapins) = −0.18(salinity) + 0.97(ln external* P. malaclemys *cyst abundance) +10.7]; [ln (mark-recapture female terrapins) = −0.18(salinity) + 1.10(ln external* P. malaclemys *cyst abundance) +9.6].*

[b] *Difference between the lowest AIC_c score and the AIC_c score of each model.*

[c] *Mallow's C_p is an alternative metric for selecting a reduced model that also avoids overfitting.*

[d] *Average per capita number of* P. malaclemys *encysted on the operculum of each* I. obsoleta *snail in a population.*

[e] *Proportion of individuals in a mud snail population infected with* P. malaclemys.

[f] *Only results for females are shown because collinearity among male, female, and juvenile terrapin abundance was high. The fit of the best models of male and juvenile abundances converged on the same two-variable model as the best-fitting, most parsimonious model of female abundance (males [ln(x)], $R^2 = 0.53$; juveniles [ln(x+1)], $R^2 = 0.51$).*

(Table 1). Regression coefficients for both terms were significant and suggested a nearly one to one increase in the natural log of terrapin abundance with the natural log of mean abundance of cysts per snail (mean [SE] $\beta_{\text{cyst abundance}} = 0.97$ [0.28], $p = 0.007$; $\beta_{\text{salinity}} = -0.18$ [0.063], $p = 0.017$). The same two independent variables also explained the greatest proportion of variation in models of abundance of female ($R^2 = 0.67$), male ($R^2 = 0.53$), and juvenile ($R^2 = 0.51$) terrapins. Regression coefficients for females were very similar to those in the model for all terrapins ($\beta_{\text{cyst abundance}} = 1.10$ [0.26], $p = 0.0024$; $\beta_{\text{salinity}} = -0.18$ [0.060], $p = 0.015$). On the basis of Mallow's C_p, the best fit to the data on abundance of females was a three-variable model that also included internal prevalence of *P. malaclemys* larvae ($R^2 = 0.75$). To avoid overfitting we emphasize the AIC-selected model with the lower number of variables. In addition, although the additional variable in the three-variable model potentially added explanatory power, prevalence of *P. malaclemys* infection in mud snails is logistically more difficult and more time consuming to quantify than cyst abundance.

Discussion

The strong association between terrapin abundance and *P. malaclemys* cysts on mud snails likely reflects the biological dependence of the parasite on the terrapin population. Our results suggest that trematode cyst abundance on the easily sampled mud snail can be used to predict terrapin abundance. It is not uncommon for trematode larval and definitive host abundance to be positively correlated (e.g., Fredensborg et al. 2006; Smith 2007; Byers et al. 2008). Ours is one of few efforts, however, to use these relations to assess abundance of host populations of conservation concern (Huspeni & Lafferty 2004).

The use of parasites to assess host abundance has conceptual and logistical advantages. Because digenean parasites naturally use several interacting estuarine species

to complete their life cycles, our approach fosters an integrative approach to assessment. In addition, the use of parasites as proxies could save tremendous effort. Although trematode abundance was an indirect measure of terrapin abundance, surveying snails for trematode cysts required nearly 16-fold less effort than directly estimating abundance of terrapins. Collection of snails from all 12 sites required on average two people working 4 days for about 8 h/day to complete, and laboratory analyses of snail samples took 1 person 9 working days (1303 snails processed). Thus, in total, the parasite sampling and analysis took 17 worker days. In contrast, sampling for terrapins at the same 12 sites required 60 days by four to five people (and required five visits to each site). Thus, the total effort for sampling terrapins was ~270 worker days.

The strength of statistical correlations (R^2 values) we calculated are likely conservative. Correlations might be stronger if environmental factors other than salinity were included in the models. We used a minimum number of environmental variables in part so we could explore the extent to which terrapin abundance could be explained primarily by variables associated with trematodes. Models likely could be further improved with the addition of variables such as snail density and dispersion and water pH and temperature, but this would require additional labor (and overfitting of models would still need to be avoided).

Our model also might be improved by elaborating on some of the independent variables already included, for example by increasing the number of mud snails collected and examined for *P. malaclemys* infection. Our analysis of approximately 100 snails at each site allowed us to determine prevalence of infection in 1% increments. We chose this level of resolution to maintain reasonable sample sizes and because we initially expected the range of prevalence of *P. malaclemys* infection to be similar to the moderate prevalence (e.g., 1–20%) of other trematode larvae that infect *I. obsoleta* (e.g., *Zoogonus rubellus* and *Lepocreadium setiferoides*) (Curtis 1997, Supporting Information). But the average prevalence of *P. malaclemys* infection across all sites was only 1.4%, and no infections were detected in six of our sites. Nevertheless, although prevalence of infection was low, the variation we measured was still adequate to contribute to the fit of some of the higher-order models of terrapin abundance.

The low prevalence of *P. malaclemys* infection inside snails underscores the importance of including the cyst stage in our study. Even among the six sites where no larval infections were found, the average prevalence of cysts on snails was 20% and average per snail abundance of cysts was 0.3. We expected external cyst abundance would be a function of local larval trematode production, which is a direct function of the number of infected snails. Because a single infected snail will potentially produce cercariae that may result cumulatively in thousands of cysts, cyst prevalence is simply an amplification of the larval infection rate. Thus, incorporating a measurement of cyst abundance into analyses greatly increased explanatory ability because it provided sufficient resolution for a manageable sample size of snails. Furthermore, enumeration of cysts on snail opercula requires less expertise and time than enumerating larval infections because cysts are readily observed and easily counted and dissection and tissue examination are not required.

Parasite cysts were strongly correlated with abundance of terrapins of both sexes and of adults and juveniles. Not only were the slopes and intercepts for all terrapin categorizations similar, but female abundance was most strongly correlated with trematode metrics. Females are a higher conservation priority than males for most dioecious species, and probability of mortality of female terrapins from some anthropogenic factors, such as collisions with vehicles on roads, is greater than of male terrapins (Szerlag-Egger & McRobert 2007).

A caveat to the use of trematodes to estimate abundance of hosts is that in areas where terrapin abundance is declining rapidly, trematode abundance on snails may lag behind the decline in terrapin abundance. Mud snails are long-lived, and their trematode infections are generally retained throughout their life (Curtis 2003). Therefore, parasite infections of mud snails may decline more slowly than abundance of a terrapin population and parasites may persist after a terrapin population has been extirpated. Nevertheless, this was not the case in our study. In fact, the regression of cyst abundance on terrapin abundance had a negative intercept, which indicates that parasite abundance declines to zero before terrapin abundance does. This pattern seems intuitive in stable (or slowly changing) terrapin populations, in which one might expect that at low terrapin abundances the probability of stochastic extirpation of the parasite would be higher. Thus, the absence of the parasite cannot resolve smaller differences in terrapin abundance at sites with few or no terrapins, but the parasite's absence could still reliably distinguish sites with few to no terrapins from those with higher terrapin abundances. Parasite presence might be used to quickly identify sites with terrapin abundance above a minimum, and cyst abundance could then be used to estimate terrapin abundance at sites with higher abundance of terrapins.

Based on our study and a small but growing literature, parasite species with obligate multihost life cycles may serve as useful measures of the abundances of their hosts. Results of several studies show significant correlations between trematode prevalence in first intermediate host snails and the abundance of definitive hosts at the same sites (Smith 2001; Huspeni & Lafferty 2004; Hechinger & Lafferty 2005; Byers et al. 2008). Results of other studies more broadly relate abundances of benthic free-living species to trematode infections in first intermediate host

snails (Hechinger et al. 2007). An advantage of the use of digenean trematodes to measure the abundance of host species is their ubiquity and substantial biomass in estuarine ecosystems (Kuris et al. 2008). Ultimately, the effectiveness of the use of parasites to estimate host population abundance provides an additional tool for conservation practitioners seeking good information on their system who have limited time and resources available.

Supporting Information

Full data by site including prevalences of all individual trematode species in mud snails, site descriptive characteristics, and all independent and response variables collected are available as part of the online article (Appendix S1). The author is responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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