

FIELD SURVEYS AND EXPERIMENTAL TRANSMISSION OF *PLEUROGONIUS MALACLEMYS* (DIGENEA: PRONOCEPHALIDAE), AN INTESTINAL PARASITE OF THE DIAMONDBACK TERRAPIN *MALACLEMYS TERRAPIN*

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abstract: Life history stages of *Pleurogonius malaclemys* were investigated in wild populations of the eastern mudsnail (*Ilyanassa obsoleta*) and diamondback terrapin (*Malaclemys terrapin*) in New Jersey, New York, and Rhode Island between 2011 and 2015, and laboratory experiments investigating the settling preference of metacercarial cysts of *P. malaclemys* were conducted. Cysts of *P. malaclemys* were found on mudsnails on the north and south shores of Long Island, New York and in Rhode Island, approximately 280 km farther north than previously reported. The cysts were found on mudsnails year round, but cyst prevalence increased during the summer months, reaching maximum levels (~70%) in November. Nearly 58% of Jamaica Bay, New York mudsnails had cysts; mean intensities were 2.63 cysts/mudsnail. Although cyst prevalence was high, only 11 mudsnails (0.28%) were found to have the internal redial stages of *P. malaclemys*, the stage of infection preceding external cysts. In addition to mudsnails, *P. malaclemys* could encyst on other biological substrates, including common terrapin prey species. The majority of wild adult terrapins from Stone Harbor, New Jersey were infected with the adult stage of *P. malaclemys* (80.30%, \bar{x} = 36.36 trematodes/terrapin, n = 66). Juvenile terrapins were experimentally infected with *P. malaclemys* and on average 22.5% of the consumed cysts successfully developed into adult trematodes. Studies on the life cycle of *P. malaclemys* are important because previous research has shown that the frequency of cysts of *P. malaclemys* on mudsnails can be used as an indirect measure of terrapin abundance.

The eastern mudsnail *Ilyanassa obsoleta* (Say) (hereafter noted as mudsnails) is host to 9 species of trematodes along the East Coast of the United States. Most of these trematodes infect a range of fishes (4 species) and birds (3 species, plus 1 that is suspected to be a bird parasite) as their definitive hosts (Blakeslee et al., 2012; Phelan et al., 2015). However, the trematode *Pleurogonius malaclemys* Hunter, 1961 is known only from the diamondback terrapin *Malaclemys terrapin* (Schoepff) (hereafter noted as terrapins). Whereas some of the trematodes that parasitize birds and fishes can be found in high prevalences in snail intermediate hosts (often >5%) (e.g., Curtis and Hurd, 1983; Curtis, 1985; Altman, 2010; Rossiter and Sukhdeo, 2012; Shim et al., 2013; Altman and Byers, 2014), *P. malaclemys* is generally found in low prevalence (<1%) within eastern mudsnails (McDermott, 1951; Byers et al., 2011; Blakeslee et al., 2012). The bird and fish trematodes have been extensively studied and their negative impacts (e.g., behavioral, physiological, reproductive) on some of their hosts are well documented (see references above and citations therein), but the impacts of *P. malaclemys* on its intermediate and definitive hosts remain largely unknown (McDermott, 1951; Hunter, 1961, 1967; Byers et al., 2011).

Although the internal asexual stages of *P. malaclemys* are found at low frequencies, the metacercarial cysts are relatively common on mudsnails (often on over 50% of mudsnails) and ideally suited as an indicator of terrapin populations (Byers et al., 2011) because the presence of the parasite on snails is directly linked to the presence of turtles. Adult *P. malaclemys* reside in the intestines of terrapins and release eggs with host feces (Hunter, 1961). The eggs are presumably ingested by mudsnails, and miracidia of *P. malaclemys* hatch from the eggs and penetrate and invade mudsnail gonadal and digestive tissue (McDermott, 1951). Here they develop into the redial stage, which undergoes asexual

reproduction, each producing 2 or 3 monostome cercariae (McDermott, 1951; Hunter, 1967). The cercariae leave the rediae and continue their maturation in mudsnail tissue spaces (Hunter, 1967). After rupture from the mudsnail, the cercariae settle either on the same mudsnail host or on a new mudsnail host, on the operculum or on the shell (Hunter, 1967). Cercariae may also encyst on other substrates and even the meniscus of the water (McDermott, 1951), but this has not been observed in the field. Upon encystment, cercariae enter a quiescent metacercarial cyst stage (hereafter referred to as cysts). The life cycle is completed when terrapins feed on the mudsnails or other substrates with cysts. Within its definitive host, the juvenile trematode is released from the cyst and migrates to the terrapin's intestine where it matures and sexual reproduction occurs.

Terrapins are endemic to the brackish waters of the Atlantic from Cape Cod, Massachusetts through the Gulf coasts of the United States (Ernst and Lovich, 2009) and maintain high fidelity to their natal location (Tucker et al., 2001). The purpose of this work was to explore aspects of the natural history of *P. malaclemys* and investigate potential pathways of *P. malaclemys* into terrapins through laboratory experiments investigating the settling preference of cercariae. Aside from work on mudsnails (Hunter, 1967), there are no studies on other potential substrates that could allow for uptake of cysts by terrapins. Terrapins feed extensively on marine invertebrates (Tucker et al., 1995, 1997; Ernst and Lovich, 2009; Underwood et al., 2013), and in Jamaica Bay (JB), New York, terrapins consume sea lettuce *Ulva rigida* and a wide range of marine invertebrates, including bivalves, gastropods, crustaceans, and other prey items (Erasmus, 2012). Although the mudsnail population at JB has relatively high intensities of cysts of *P. malaclemys*, mudsnails appear to only make up a small percentage of JB adult female terrapin summer diets overall (Erasmus, 2012), additional work indicates more generally that adult terrapins in this region rarely eat mudsnails (A. B. Bragin and R. C. Wood, unpubl. data). This suggests that *P. malaclemys* infection may also occur through ingestion of cysts on other substrates that are included in the diverse diets of terrapins.

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TABLE I. Categories of Jamaica Bay mudsnails (*Ilyanassa obsoleta*) compared in independent-sample two-tailed *t*-tests. The means \pm SD are shown for each category. The feature being compared and *t*-test results are shown. 1 = Mudsnails with internal infection by *Pleurogonius malaclemys*. 2 = Mudsnails without any internal infection by *P. malaclemys*. 3 = Mudsnails with other internal trematode infections (not *P. malaclemys*). 4 = Mudsnails with metacercarial cysts of *P. malaclemys*. 5 = Mudsnails without cysts of *P. malaclemys*.

<i>t</i> -test	Mean (SD)	Feature	Results		
			<i>t</i>	df	<i>P</i>
1 vs. 2	10.53 (7.51) vs. 2.29 (1.83)	Number of cysts	11.85	39.18	<0.0001
1 vs. 3	10.53 (7.51) vs. 3.20 (2.17)	Number of cysts	11.08	39.93	<0.0001
2 vs. 3	2.29 (1.83) vs. 3.20 (2.17)	Number of cysts	5.95	339.54	<0.0001
2 vs. 3	13.76 (1.54) vs. 13.04 (1.58)	Shell length	6.83	1,597	<0.0001
4 vs. 5	12.91 (1.76) vs. 13.16 (1.40)	Shell length	−2.82	1,152.94	0.0048

Another purpose of this research was to examine the life cycle and ecology of the trematode *P. malaclemys* in its intermediate and definitive hosts. The prevalence and intensity of the internal stages of *P. malaclemys* and of cysts on mudsnails were quantified through field studies and compared with experimental studies of cercarial settlement. Similarly, the prevalence and reproduction of adult *P. malaclemys* in natural terrapin populations were compared with experimentally infected terrapins. These studies provide valuable applications to terrapin conservation management strategies.

MATERIALS AND METHODS

Study sites

To evaluate the prevalence and intensity of *P. malaclemys*, the intermediate (mudsnail) and definitive hosts (terrapin) were examined for cysts and internal adult parasites. Our main northern site was JB, New York (40°36′54.61″N, 73°49′50.61″W), on the southwestern end of Long Island. JB water temperatures and salinity vary annually and seasonally; water temperature ranges from 19.4 to 26.9 °C and ranges in salinity from 16 to 32‰ (G.W. Frame, pers. obs.). Terrapin carcasses were collected as available from JB and Great South Bay (GSB), New York (40°38′7.20″N, 73°48′15.31″W) from vehicular mortality, but because of the limited sample size in these bays, studies on adult stages of *P. malaclemys* primarily came from Stone Harbor (StH), New Jersey (39°3′27.41″N, 74°46′30.83″W) terrapin populations located on the SE edge of the Cape May Peninsula (187 km SW of JB).

Pleurogonius malaclemys and other parasitic trematodes in and on mudsnails

Cyst intensities on JB and StH mudsnails were compared to observe the differences in number of cysts between 2 sites with large terrapin populations. Mudsnails were collected by hand at JB from June 2011 to February 2013 either haphazardly (sampling 5–10 mudsnails at locations separated by at least 1 m along a shoreline of about 4,000 m²) to determine the prevalence of all trematodes, or were nonrandomly selected (only mudsnails with >5 cysts of *P. malaclemys* collected) to determine the relationship between cyst load and probability of internal *P. malaclemys* infection. Smaller, prereproductive mudsnails were not sampled during nonrandom collections. Shell and aperture lengths of mudsnails were measured with calipers and the prevalence and intensity of cysts on shells and opercula were recorded. After cracking the shells with a porcelain mortar and pestle, the presence of internal stages of trematode parasites was recorded using a dissecting scope. Mudsnails were collected haphazardly by hand from StH (June 2012 to August 2012) as described above but only data on cysts were recorded (mudsnails were not examined for internal stages of trematodes). Intensities of cysts on uninfected and internally infected mudsnails were compared using independent 2-tailed *t*-tests with *F*-tests to determine variance equality (Table I). The influence of mudsnail shell size and date of collection on the likelihood of cyst presence (prevalence) was tested using logistic regression with an iterative maximum likelihood procedure. This analysis was repeated with the addition of a shell size–date of collection interaction term. A Hosmer–

Lemeshow test evaluated how well mudsnail shell size and days of collection explained variation of cyst prevalence. The relationship between mudsnail shell lengths (predictor variable) and number of cysts (response variable) of *P. malaclemys* was examined using linear regression analysis. Chi-square analysis was used to determine whether cercarial encystment occurs at a higher frequency than would be expected on the basis of average available surface area of shells and opercula. We measured and used mean shell and operculum lengths to calculate the surface area of a cone and circle to estimate mudsnail shell surface area (85%) and operculum surface area (15%) of JB mudsnails.

In addition to the June 2011 to February 2013 collections at JB, mudsnails were collected and examined for cysts as indicated above at 8 additional sites on Long Island, New York from December 2013 to April 2014, and at 1 site in Barrington, Rhode Island (41°45′57.348″N, 71°18′52.20″W) in August 2015. The additional Long Island sites were Lido Beach, Tobay Beach, Hubbard Creek, Scallop Pond, Sag Harbor, Cedar Beach, Mt. Sinai, and Oyster Bay (see Supplementary Appendix for coordinates). The Long Island sites were chosen because they were inhabited by terrapins in the past and the most recent survey of New York terrapin populations (Morreale, 1992) and we wanted to correlate cyst variation with terrapin presence. The Rhode Island site was chosen because of a large local terrapin population (Mitro, 2003).

Field studies of metacercarial cysts on alternative substrates

Five terrapin prey species that may serve as alternative substrates for cysts of *P. malaclemys* were collected from JB from June 2012 to August 2012 from along the shoreline covering approximately 2,000 m² (a portion of the region as defined for snail collections above): sea lettuce, shells of *M. arenaria*, *Geukensia demissa*, *M. mercenaria*, and tube caps of the polychaete worm *Diopatra cuprea* (Bosc). The shells of these substrates were collected from the intertidal zone and chosen because they are common substrates at JB, they are easily accessible, and there was a substantial percent frequency of occurrence of each substrate in terrapin diets (Erazmus, 2012). The number of cysts on each shell and the total surface area available for encystment on each of these substrates was determined from digital pictures taken in the laboratory using ImageJ.

Laboratory study of cercarial encystment on alternative substrates

Mudsnails were collected from JB by hand 16 July 2012 to 2 August 2012 during low tide. Mudsnails were nonrandomly sampled; only mudsnails having high cyst intensities (>5 cysts) on either their shells, opercula, or both were collected. Mudsnails (*n* = 6) were individually housed in 7.5-cm-diameter dishes with 30 parts per thousand (ppt) artificial ocean water and a 3 × 3-cm piece of sea lettuce for food, and observed daily for release of cercariae from *P. malaclemys*. The number of cercariae that were either swimming, encysted on a potential substrate (mudsnail, mudsnail feces, glass, meniscus of the water, or sea lettuce), or found dead was counted once a week for 2 wk, for 5 different trials. Although the swimming cercariae have the potential to encyst, the counts were performed at a single time each week. Chi-square analysis was used to determine whether cercarial encystment occurs at a higher frequency on a given substrate than would be expected on the basis of available surface area of the different JB substrates. This was calculated by estimating the surface areas available for encystment; expected proportions (% of available surface area) for each of the 5 substrates was determined.

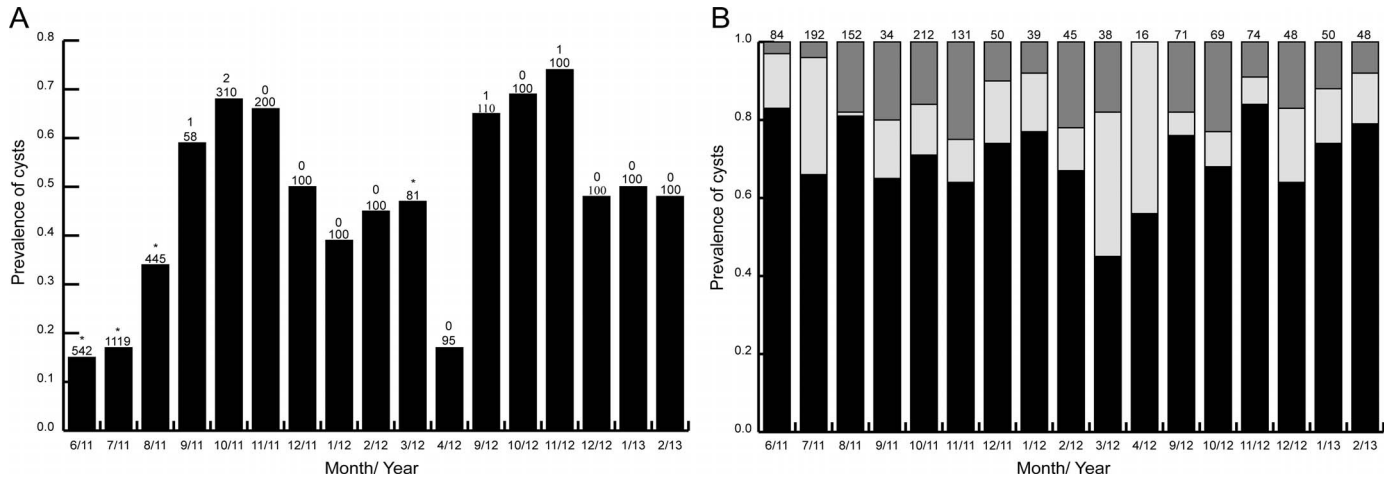


FIGURE 1. Seasonal prevalence of cysts of *Pleurogonius malaclemys* on mudsnails (*Ilyanassa obsoleta*) at Jamaica Bay. (A) Overall monthly prevalences of cysts. The top numbers over the bars indicate the number of mudsnails with internal stages of *P. malaclemys*. Asterisks indicate that internal stages were not examined. The bottom numbers over the bars represent sample sizes. (B) Monthly prevalence and location of cysts on mudsnails shell (black), opercula (light gray), or on both the shell and opercula (dark gray). The numbers across the top represent sample sizes.

Mudsnail surface area (62%) was determined using the above-mentioned methods. The water meniscus area was determined using the diameter of the glass dish; the estimated proportion was 17%, the same as the glass dish area (cysts were not found on the sides of the dish, thus not included in the surface area calculation). The surface area of a square was used to calculate sea lettuce surface area (4%). Mudsnail feces were estimated to make up 1% of the amount of area available.

Sea lettuce, mudsnails, and shells of soft-shell clam and ribbed mussel were collected from JB on 12 October 2012 to test encystment on different substrates. The mudsnails did not have current cysts of *P. malaclemys*. One of each of the substrate types (representing approximately equal surface areas of 5.6 cm²) were housed in 10-cm glass dishes with sand from JB and 30 ppt artificial ocean water. About 25 cercariae from an infected mudsnail were collected and immediately added to each glass dish containing these substrates. Data on cyst settlement was collected 1 wk after the addition of cercariae. Mudsnail operculum cysts, shell cysts, and substrates where new cysts settled were recorded and divided by the total number of cercariae added.

Prevalence and intensity of adult *Pleurogonius malaclemys* in wild terrapins

Adult female terrapin carcasses (n = 66) from StH were dissected within 6 hr of road mortality in June 2012 and June 2013. These terrapins were collected during 3 (2013) to 4 (2012) daily road surveys through StH, Avalon, Sea Isle City, and Strathmere, New Jersey over 61 km of road with heavy vehicle traffic. Parasites were examined using a dissecting microscope after manual extraction of the intestines from adult carcasses. Data on terrapin plastron length (predictor variable) and adult parasite loads (response variable) were compared using linear regression. Some samples were excluded from this analysis because shell damage did not permit length measures. Prevalence was determined by dividing the number of infected terrapins by the total number of terrapins that were dissected. The intensity and location of the trematodes in the intestine of terrapins were recorded. Chi-square analyses were used to determine whether trematode location within the intestine occurred at a higher frequency than would be expected on the basis of available surface area in the small intestine, large intestine, and the junction. This was calculated by estimating the surface areas available for trematode attachment; expected proportions (% of available surface area) of the small intestine, large intestine, and the junction were calculated using the surface area of a rectangle. Samples without trematodes were excluded from analyses.

Experimental infection of juvenile and subadult terrapins in the laboratory

Juvenile captive-raised terrapins (n = 25) hatched from JB eggs were initially raised in communal tanks until each terrapin was moved to

individual housing in 45.5 cm × 24.5 cm × 20 cm tanks at the start of the experiment. Mudsnails with cysts of *P. malaclemys* were collected on June 2012 to February 2013. Mudsnail opercula with 1–20 naturally occurring cysts were fed to 17 terrapins to determine if we could experimentally induce infection. Similarly, 8 terrapins were fed 5–15 opercula, totaling 100 cysts/terrapien to determine the average number of cysts that develop into adults. Terrapins were fed ReptoMin® (commercial turtle food) ad libitum pre- and postinfection; they were monitored to ensure that they ate regularly throughout the experiment.

Terrapins were maintained for 10 wk postinfection to allow *P. malaclemys* to grow to adulthood. Terrapins were measured (carapace, plastron, and shell height), then euthanized and dissected. Trematode intensity was determined by counting the number of adult *P. malaclemys* in terrapin intestines using a dissecting scope.

Reproduction of adult *Pleurogonius malaclemys*

Adult *P. malaclemys* were sampled from laboratory-fed terrapin intestines and relaxed in a 7% magnesium chloride (MgCl₂) solution. Reproduction of *P. malaclemys* was then quantified by counting eggs found within the hermaphroditic adults from digital pictures of adult trematodes taken with a digital camera attached to a dissecting microscope; ImageJ was used to measure adult parasite lengths. The relationship between adult trematode size (predictor variable) and numbers of eggs (response variable) was examined using linear regression analysis.

RESULTS

Pleurogonius malaclemys and other parasitic trematodes in mudsnails

A total of 3,922 JB mudsnails was examined over 21 mo (Fig. 1A); 11 (0.28%) mudsnails contained the internal radial stage of *P. malaclemys*. Mudsnails containing internal *P. malaclemys* stages were found in June (2, 1.96%), July (2, 2.56%), August (2, 1.96%), September (2, 1.19%), October (2, 0.95%), and November (1, 1.00%) (n, % of snails internally infected with *P. malaclemys* of the total mudsnails collected that month). In addition, selective collections (mudsnails containing >5 cysts) over 5 mo revealed 31 of 553 (6%) mudsnails containing the internal radial stage of *P. malaclemys*. Mudsnails with internal *P. malaclemys* from selective collections were found during January (16, 14.97%), February (6, 13.95%), July (6, 2.55%), and December (3, 4.62%). Internal *P. malaclemys* were not found in

TABLE II. Prevalence (%) of trematode species found within Jamaica Bay mudsnails (*Ilyanassa obsoleta*) collected either haphazardly (sampling 5–10 mudsnails at locations separated by at least 1 m along a shoreline of about 4,000 m²) or by nonrandom selective (only mudsnails with >5 cysts of *P. malaclemys* collected) methods. Internal larval stages (sporocysts or rediae) and cercariae of each species verified with light microscopy. Taxonomic authorities for trematode species and their definitive hosts can be found in Phelan et al. (2015).

Trematode species	Date range of collections	Collection method	Prevalence % (# infected of total # sample)
<i>Himasthla quissetensis</i>	Jun. 2011 to Feb. 2013	Haphazard	2.83 (111 of 3,922)
<i>Zoogonus lasius</i>	Jun. 2011 to Feb. 2013	Haphazard	2.27 (89 of 3,922)
<i>Austrobilharzia variglandis</i>	Jun. 2011 to Feb. 2013	Haphazard	1.68 (66 of 3,922)
<i>Stephanostomum tenue</i>	Jun. 2011 to Feb. 2013	Haphazard	0.18 (7 of 3,922)
<i>Lepocreadium setiferoides</i>	Jun. 2011 to Feb. 2013	Haphazard	0.1 (4 of 3,922)
<i>Gynaecotyla adunca</i>	Jun. 2011 to Feb. 2013	Haphazard	0.03 (1 of 3,922)
<i>Diplostomum nassa</i>	Jun. 2011 to Feb. 2013	Haphazard	0.03 (1 of 3,922)
Unidentified species	Jun. 2011 to Feb. 2013	Haphazard	0.18 (7 of 3,922)
<i>Himasthla quissetensis</i>	Jun. 2012 to Feb. 2013	Selective	5.06 (28 of 553)
<i>Zoogonus lasius</i>	Jun. 2012 to Feb. 2013	Selective	9.22 (51 of 553)
<i>Austrobilharzia variglandis</i>	Jun. 2012 to Feb. 2013	Selective	2.0 (11 of 553)
Unidentified species	Jun. 2012 to Feb. 2013	Selective	1.08 (6 of 553)

mudsnails during March, April, or May. Hosts with >5 cysts were found to be more often internally parasitized by *P. malaclemys* than predicted (on the basis of expected equal distribution of parasites among haphazard and selective collection hosts [$\chi^2 = 147.83$, $n = 4,475$, $df = 1$, $P < 0.0001$]). Thus, selective sampling for external cyst-infected snails revealed 21× greater prevalence of infection of internal *P. malaclemys* in mudsnails collected nonrandomly than by haphazard sampling (6 to 0.28%).

In addition, 286 (7.29%) of haphazardly collected JB mudsnails were internally infected with other trematodes (Table II). Of the selective collections of JB mudsnails, 96 (17.36) mudsnails were infected with other trematodes (Table II). Hosts with >5 cysts were internally parasitized by these trematodes more often than predicted by chance (on the basis of expected equal distribution of parasites among haphazard and nonrandom collection hosts [$\chi^2 = 133.18$, $n = 4,464$, $df = 1$, $P < 0.0001$]).

Field prevalence of metacercarial cysts of *Pleurogonius malaclemys* on mudsnails and other substrates

Jamaica Bay, New York collections: The overall prevalence of cysts on mudsnails was 57.9% (957/1652); mean cyst intensity (\pm SD) for mudsnails with cysts was 2.63 ± 2.37 ($n = 1,123$). Cysts of *P. malaclemys* were found on mudsnails throughout the year, but cyst frequencies varied by season, with low cyst prevalence in April–June and a peak from September to November (Fig. 1A, B). The overall prevalence increased during the summer months and reached maximum levels of ~70% in November, and then prevalence declined (Fig. 1A, B). Cercariae preferentially encysted on opercula rather than shells ($\chi^2 = 10,415.21$, $n = 2,948$, $df = 1$, $P < 0.0001$); overall 81.3% of cysts were found on opercula and 18.7% of cysts were found on shells.

For mudsnails with cysts, higher cyst intensities were found on mudsnails infected with internal *P. malaclemys* compared with uninfected mudsnails ($t = 11.85$, $n = 906$, $df = 39.18$, $P < 0.0001$) and higher than mudsnails that were infected with other trematode species ($t = 11.08$, $n = 280$, $df = 39.93$, $P < 0.0001$). Mudsnails internally infected with any trematode species other than *P. malaclemys* had significantly higher cyst intensities ($t = 5.95$, $n = 1,106$, $df = 339.45$, $P < 0.0001$) and longer shells ($t = 6.83$, $n = 1,599$, $df = 1,597$, $P < 0.0001$) than mudsnails without

internal trematode infection. Mudsnails with cysts had longer shell lengths than mudsnails without any cysts ($t = -2.82$, $n = 1,328$, $df = 1,152.94$, $P = 0.0048$). Cyst intensities increased significantly but very weakly with mudsnail shell size ($y = 0.1557x - 0.437$, $r^2 = 0.009$, $t = 2.82$, $n = 894$, $df = 892$, $P = 0.00245$; Fig. 2). The addition of the interaction term into the logistic regression did not improve the power of the model; the -2 log likelihood of shell size–data of collection models was 2,136.702, whereas that of the model with the interaction term was 2,136.364. Shell size and date of collection influenced likelihood of infection ($P < 0.001$, $P = 0.006$, respectively; Table III; Hosmer and Lemeshow test $\chi^2 = 18.936$, $df = 8$, $P = 0.015$). Mean numbers of cysts on the opercula (2.84 ± 2.30 , $n = 845$) versus shells (1.99 ± 2.50 , $n = 278$) were significantly different for snails from JB ($t = 5.02$, $df = 441.12$, $P \leq 0.0001$; Fig. 3).

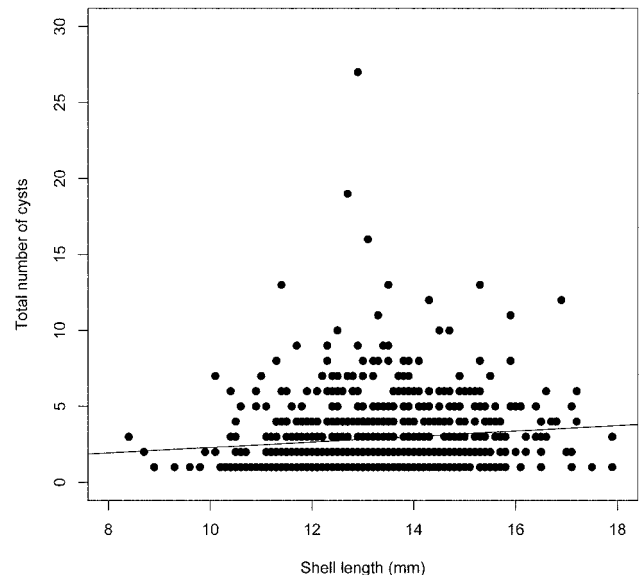


FIGURE 2. Cysts on mudsnails (*Ilyanassa obsoleta*) at Jamaica Bay, New York. Number of cysts of *Pleurogonius malaclemys* on mudsnail shells and opercula regressed against the shell length ($r^2 = 0.00881$, $n = 894$, $P = 0.00245$). An outlier with a shell length of 14.3 mm and 39 cysts was found but not included in this regression.

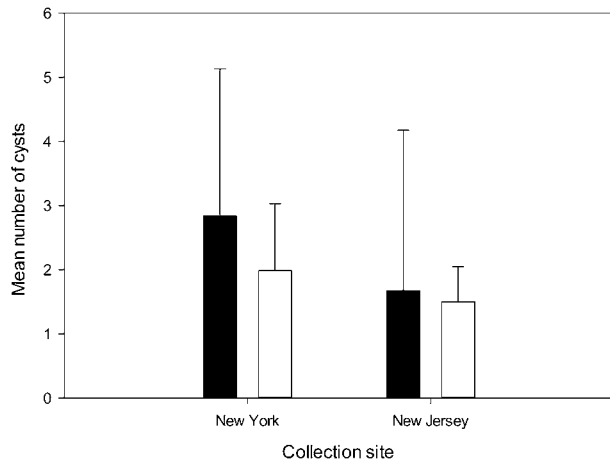


FIGURE 3. Mean number of cysts of *Pleurogonius malaclemys* found on mudsnail (*Ilyanassa obsoleta*) opercula (black) and shells (white) in both Jamaica Bay, New York ($n = 845$; $n = 278$) and Stone Harbor, New Jersey ($n = 6$; $n = 123$) populations, where n is the number of mudsnail individuals with infection. The error bars represent standard deviation from the mean.

In the field, no cysts were found on 990 cm² of sea lettuce or 1,172 cm² of any mollusc shell substrates (soft-shell clam, ribbed mussel, hard clam) sampled from July 2012 to November 2012.

Additional New York and Rhode Island collections: Metacercarial cysts were found at 7 of the 9 sites sampled on Long Island, New York (Fig. 4); 1 site that lacked cysts was on the south shore (Tobay Beach) and the other was on the north shore (Oyster Bay). Prevalence of cysts on mudsnails at the other sites ranged from 21% (Lido Beach) to 52% (Sag Harbor); the prevalence of cysts at Jamaica Bay (49%) was similar to that found in previous years. The overall intensity of cysts on mudsnails at these sites ranged from 0 to 2 with an overall mean of 1.04 ± 0.06 ($n = 3,532$). Metacercarial cysts were found on 11 of 103 (10.7%) mudsnails examined from Barrington, Rhode Island.

Stone Harbor, New Jersey collections: The overall prevalence of cysts of *P. malaclemys* on StH mudsnails was 24.9% (129/519); mean cyst intensity was 1.7 ± 1.03 ($n = 129$). StH mudsnail shell lengths were significantly longer (20.1 ± 2.56 , $n = 519$) than those from JB (13.20 ± 1.59 , $n = 1,599$) ($t = 58.16$, $df = 653.30$, $P < 0.0001$). StH mudsnails also had longer apertures (9.60 ± 1.87 , $n = 519$) than JB mudsnails (5.50 ± 1.20 , $n = 1,600$) ($t = 46.90$, $df = 662.52$, $P < 0.0001$). Despite the smaller size of JB mudsnails, total cyst intensities on mudsnails were significantly higher on JB mudsnails (2.63 ± 2.37) than on StH mudsnails (1.70 ± 1.03) ($t =$

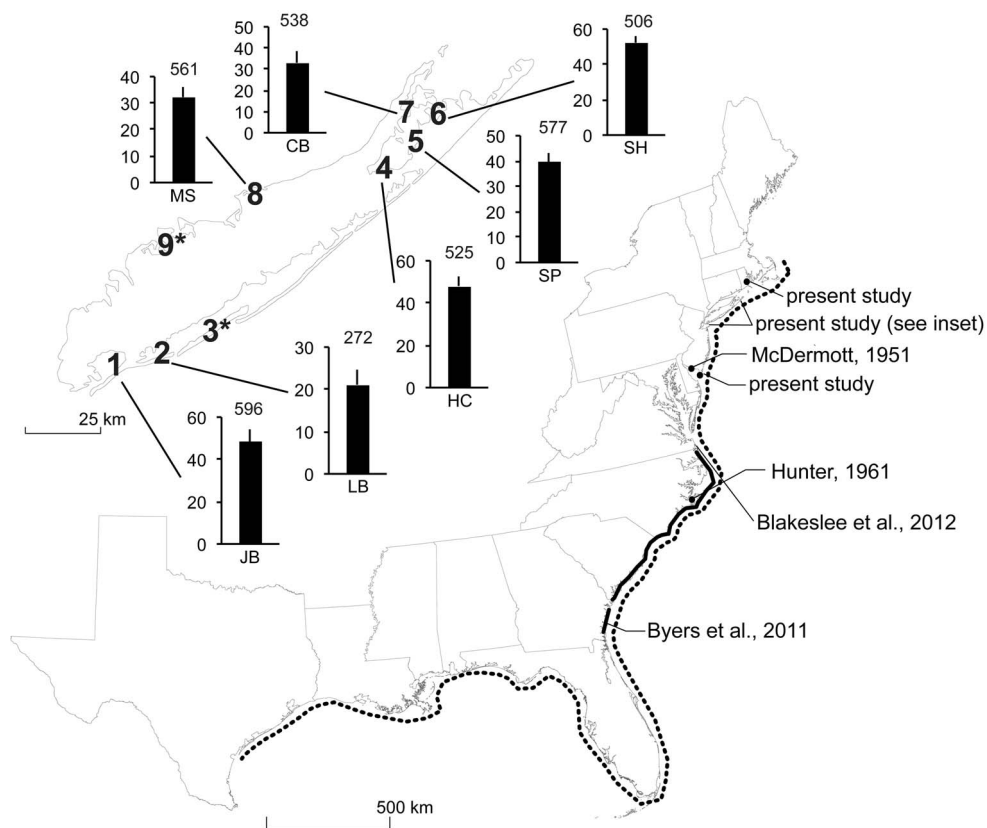


FIGURE 4. Prevalence of *Pleurogonius malaclemys* from 9 sites around Long Island, New York between December 2013 and April 2014: Jamaica Bay (JB, #1), Lido Beach (LB, #2), Hubbard Creek (HC, #4), Scallop Pond (SP, #5), Sag Harbor (SH, #6), Cedar Beach (CB, #7), and Mt. Sinai (MS, #8). * Metacercarial cysts were not found on snails at 2 sites (Tobay Beach [TB, #3] and Oyster Bay [OS, #9]). Error bars indicate standard deviation; numbers above graphs indicate total number of snails sampled per site. Larger map of United States shows the coastal range of *Malaclemys terrapin* (represented by the dashed line) and the sites where *Pleurogonius malaclemys* (single sites represented solid circles; multiple sites represented by a solid line) has been documented from mudsnails or within terrapins (New Jersey, McDermott, 1951; South Carolina, Hunter, 1961; Georgia, Byers et al., 2011; North and South Carolina, Blakeslee et al., 2012; New York, New Jersey, and Rhode Island, present study).

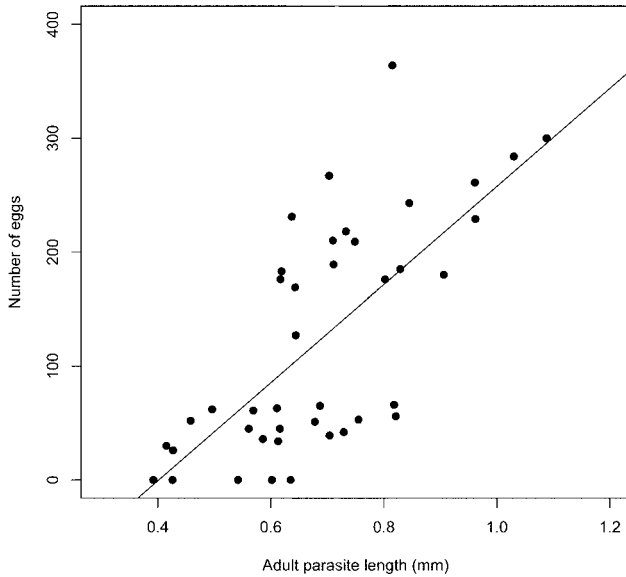


FIGURE 5. Reproduction of the trematode *Pleurogonius malaclemys*. Number of eggs within individual adult *P. malaclemys* ($n = 41$) regressed against adult length.

-8.35 , $n = 1,252$, $df = 320.23$, $P < 0.0001$; Fig. 3). Mean numbers of cysts on the opercula (1.67 ± 1.04 , $n = 123$) versus shells (1.50 ± 0.55 , $n = 6$) were not significantly different for snails from StH ($t = 0.41$, $df = 127$, $P = 0.68$; Fig. 3).

Laboratory study of cercarial encystment on alternative substrates

The 6 infected mudsnails observed for cercarial release and encystment released 648 cercariae; of these, 296 (45.68%) were swimming, 230 (35.49%) were encysted on a substrate, and 122 (18.83%) cercariae were found dead. Of the cercariae that encysted, 131 (59.96%) were on sea lettuce, 69 (30.00%) were on glass, 4 (1.74%) were on mudsnail feces, 14 (6.09%) encysted back on the same mudsnail, and 12 (5.22%) were on the water surface. The cercarial encystment patterns differed significantly from an expected equal distribution ($\chi^2 = 1,769.13$, $df = 4$, $P < 0.0001$); cercariae settled on sea lettuce in numbers greater than expected and settled on mudsnails in numbers less than expected when adjusted for surface area. Cercariae were found to settle on nonliving ribbed mussel shells (5 of 75 cercariae), yet no cercariae (0 of 648) settled on sand or nonliving soft-shell clam shells.

Prevalence and intensity of adult *Pleurogonius malaclemys* in wild terrapins

Of the 66 StH terrapins dissected, 53 (80.30%) were infected with *P. malaclemys*; intensities ranged from 1 to 213 trematodes.

No other trematode species were observed. In total, 1,927 adult trematodes were found and the mean intensity for infected terrapins was 36.36 ± 51.55 ($n = 52$) trematodes/terrapin. There was no significant correlation between terrapin plastron sizes and number of trematodes in the StH wild terrapins ($y = -0.3542x + 30.125$, $n = 44$, $r^2 = 0.0001$, $t = -0.05$, $df = 43$, $P = 0.48$). Adult *P. malaclemys* were found significantly more often in large intestines than in small intestines or junctions after adjusting for surface areas ($\chi^2 = 2,282.95$, $df = 2$, $P < 0.0001$). Additionally, 2 adult terrapin carcasses from JB and 1 adult terrapin carcass from GSB were collected; only 1 adult of *P. malaclemys* was found, in the large intestine of the terrapin collected from GSB.

Experimental infection of juvenile and subadult terrapins in the laboratory

There was a significant positive association between the number of cysts fed and number of adult trematodes recovered in either the juvenile or subadult terrapins ($y = 0.2045x - 0.4294$, $r^2 = 0.62$, $t = 6.28$, $df = 24$, $P < 0.0001$). All of the terrapins fed 100 cysts each were infected after 10 wk, with a mean intensity (\pm SD) of 22.50 ± 11.05 ($n = 8$) trematodes/terrapin.

Reproduction of adult *Pleurogonius malaclemys*

Adult *P. malaclemys* contained an average (\pm SD) of 139.6 ± 96.50 ($n = 36$) eggs. There was a significant positive relationship between trematode lengths and number of eggs ($r^2 = 0.49$, $t = 6.13$, $df = 39$, $P < 0.0001$) (Fig. 5).

DISCUSSION

Pleurogonius malaclemys occurs at least as far north as Barrington, Rhode Island, approximately 280 km farther north than previously reported (McDermott, 1951). Before the present study, *P. malaclemys* was documented from southwestern New Jersey (King Pond, $39^{\circ}12'0.17''N$, $75^{\circ}8'11.74''W$; McDermott, 1951) to Georgia (approximately $32^{\circ}N$ and $81^{\circ}W$; Byers et al., 2011) (Fig. 4; McDermott, 1951; Hunter, 1961; Blakeslee et al., 2012; Byers et al., 2011). *Pleurogonius malaclemys* was not previously found within mudsnail hosts in other sites sampled from the mid-Atlantic to northern New England (including the Chesapeake Bay, Delaware, and the Long Island Sound) (Blakeslee et al., 2012; Altman and Byers, 2014); however, Blakeslee et al. (2012) only sampled the internal stages of 100 mudsnails per site and our studies show that the internal stage of *P. malaclemys* can exist at a very low prevalence within mudsnails. The overall prevalence of *P. malaclemys* internal infections in JB mudsnails was lower than McDermott's (1951) collection in King Pond, New Jersey (0.84%), and collections of Byers et al. (2011) in Georgia (up to 6%), but about 10 \times higher than some of the sites of Blakeslee et al. (2012) in the southeastern

TABLE III. Results of logistic regression analysis showing the variables used in the equation used to explain variation in cyst intensities.

Variable	β	SE	Wald	df	P value	Exp(B)
Shell size	0.127	0.033	14.376	1	<0.001	1.135
Date	0	0	7.566	1	<0.001	1
Constant	119.312	43.783	7.426	1	0.006	6.552×10^{51}

United States. In contrast to the low numbers of mudsnails with the internal stages of *P. malaclemys*, high prevalence of cysts on mudsnails (up to 52%) was achieved through amplification via asexual reproduction by these trematodes. In turn, this led to the high prevalence of adult trematodes in terrapins (~80%) via trophic transmission.

The geographical distribution and impacts of *P. malaclemys* on hosts are in need of study, especially in the northern range of its terrapin hosts (Fig. 4). Although *P. malaclemys* has not been recorded north of Barrington, Rhode Island (N. E. Karraker, unpubl. data), it is possible that this parasite exists in mudsnail populations as far north as Cape Cod, Massachusetts, the northern range limit for terrapins. Definitive host abundance has been shown to be a significant driver of parasite prevalence (Galatkionov and Bustnes, 1999; Smith, 2001; Hechinger and Lafferty, 2005; Fredensborg et al., 2006; Byers et al., 2008; Altman and Byers, 2014). Global climate change could induce a farther northward range expansion of the parasite (e.g., Poulin, 2006), particularly because the required intermediate hosts and definitive hosts are already present. Temperature increases associated with global climate change may also increase cercarial production, although cercarial viability may be compromised (Poulin, 2006; Neal and Poulin, 2012). Thus, it is important to collect baseline data on the presence/absence of the trematode in this region.

This is the first report on the seasonal variation of cyst frequencies of *P. malaclemys* on mudsnails. Seasonal peaks in cysts occur in the fall, when terrapins of both sexes are probably entering winter refugia and not feeding. However, cysts are found commonly on mudsnails throughout the winter, long after terrapins have become inactive. The viability of cysts that remain on shells during winter months is unknown (see Morley, 2015 for review of metacercaria viability in other trematode species); however, our preliminary data suggest that they may not remain viable for extended periods. During our feeding experiments, 1 subadult terrapin was fed 100 cysts found on mudsnails in February. After 10 wk, no adults were recovered in the terrapin intestine (N. Chodkowski, pers. obs.). All similar feeding experiments with mudsnails infested with cysts from other times of the year resulted in infections in terrapins. Future studies should determine whether cysts remain infective to definitive hosts in the spring.

Mudsnails with internal trematode infections hosted higher cyst intensities, most likely because cercariae released from mudsnails do not swim far before settlement (J. D. Williams, pers. obs.) and can encyst on the same mudsnail from which they emerged. Mudsnails infected with other trematode species had higher cyst intensities than uninfected mudsnails, and this may be attributed to mudsnail age; older mudsnails have had more time to accumulate cysts and are typically larger, providing greater surface for cercarial encystment (Byers et al., 2008). A similar relationship between infection probability and body size was not found in terrapins; this could be due to multiple factors including the fact that terrapins grow slowly once they reach adulthood and possibly due to a short life span of adult trematodes in the terrapins. It should be tested whether behavioral changes (i.e., reduced burrowing in the mud) in infected mudsnails (as found by others: Levri, 1998, 1999; Bernot, 2003) could expose the mudsnails to higher numbers of cercariae.

Although some mudsnails have defenses that deter epibiont settlement on their shells (Wahl, 1989; Wahl and Sönnichsen, 1992), this does not appear to be the case for eastern mudsnails in

guarding against encystment by cercariae of *P. malaclemys*. Heavily parasitized mudsnails had such high levels of cysts that cysts were sometimes encysted on top of each other on the mudsnail opercula. In New Zealand, cercariae of *Philophthalmus* sp. similarly encyst on intertidal mudsnails (*Zeacumantus subcarinatus* [Sowerby II]) with high conspecific cyst intensities (Neal and Poulin, 2012). Gregarious settlement of metacercariae has been shown in several trematode species (Morley, 2015) and may occur because of settlement cues from hosts or conspecifics (Lafferty, 1999; Neal and Poulin, 2012; Morley, 2015). Such cyst aggregations would presumably increase the likelihood of infection after predation on mudsnails by terrapins.

Under laboratory conditions, cercariae of *P. malaclemys* are capable of settling and encysting on sea lettuce, ribbed mussel shells, mudsnail feces, glass, and menisci. Although dead cercariae were commonly found, once encysted the metacercariae appeared to remain viable (on the basis of movement of juveniles within the cysts) for long periods (at least weeks, possibly months). However, no cysts were found on these substrates in the field, suggesting that when the cercariae are able to choose substrates they may use chemical cues to settle preferentially on mudsnails. The settlement cues for cercariae of *P. malaclemys* are unknown, but are likely a metabolite of the host mudsnail because cercariae were aggregated mostly near the aperture, either on the shell surrounding the aperture or on the operculum. Other cercariae and miracidia have been documented to use chemical cues in locating the intended host species (Haas, 2003; Sukhdeo and Sukhdeo, 2004; Morley, 2015).

Although mudsnails seem to make up only a small percentage of the JB terrapins' diets, even infrequently eaten prey can be sufficient to maintain parasite populations. In our study, nearly 25% of the ingested cysts matured into adults. On the basis of adult trematode intensities of approximately 36 trematodes/ terrapin from StH, about 144 cysts would need to be consumed to obtain the observed intensity of adults in terrapins. Because the average number of cysts/mudsnail at JB is 0.68, approximately 212 mudsnails would need to be consumed to achieve the observed infection rates (unless snails with high intensities are preferentially ingested). Thus, even relatively low levels of mudsnail consumption may be sufficient to maintain observed levels of *P. malaclemys* infections in terrapin populations. These calculations provide an estimate of what may occur; however, there are currently no data on the life span of either the cysts or adult *P. malaclemys*.

Previous research proposed using cyst counts of *P. malaclemys* as an easy, noninvasive, and effective way to census terrapin populations where mudsnails are present (Byers et al., 2011). Our data show that there are seasonal changes in cyst counts, and thus it would be informative to examine seasonal dynamics of cysts to determine abundance estimates of terrapins. We suggest sampling during late summer to early fall (e.g., September–November on the basis of JB data) or months with high cyst intensities to avoid false negatives for terrapins. It is also important to note that only counts of operculum cysts were used in the study of Byers et al. (2011) because their initial assessment of 75 mudsnails (25 from 3 different sites) did not contain any shell cysts (Byers et al., 2011). Opercula also appeared to be the preferred area of encystment in the present study, as has been found with other trematodes (Morley, 2015). However, 28% of infected mudsnails in our study had cysts on the shell or both the shell and operculum. Thus, estimates of terrapin populations based only on the prevalence of

cysts on snail opercula may lead to conservative assessments of definitive host numbers.

We used cysts of *P. malaclemys* as a tool to assess presence/absence of terrapin populations at 9 sites around Long Island and found evidence for terrapins at 7 of these sites. Previous studies have documented terrapins at all of these sites (Morreale, 1992), but only at 4 of them (JB, Tobay Beach, Lido Beach, and Oyster Bay) have terrapins been documented recently (Feinberg and Burke, 2003; Bauer, 2004; R. L. Burke and J. D. Williams, pers. obs.). Only 1 of these sites (JB) has been sampled extensively for terrapins, and it appears to support >4,000 terrapins per 2,500 ha (R. L. Burke, unpubl. data). These observations constitute baseline data for a detailed analysis of the relationship between *P. malaclemys* and terrapin populations.

Adult *P. malaclemys* were previously found primarily in the posterior third of terrapin small intestines (Hunter, 1961), whereas we found *P. malaclemys* primarily in the large intestines of both wild and laboratory-fed terrapins. It may not be uncommon for *P. malaclemys* to reside in the large intestine, as another species of this genus, *P. tortugueroi*, was also found in the large intestine of its definitive host, green sea turtles (*Chelonia mydas*) (Santoro et al., 2007). Researchers should examine both the small and large intestines of terrapins when looking for *P. malaclemys* adult trematodes.

In conclusion, we report the northernmost record of *P. malaclemys* and document the prevalence of internal and external stages of the parasite from hosts on Long Island and New Jersey. Our findings suggest that although the cercariae can settle on multiple substrates, mudsnails act as the main pathway of the parasite into terrapins, leading to high prevalence (~80%) in these hosts. Future studies examining the viability and longevity of cysts of *P. malaclemys* and adult life stages will augment our understanding of the life cycle of *P. malaclemys* and potential impacts on terrapins.

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