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Poor Correlation between Diamondback Terrapin Population Estimates Using Two New Estimation Methods

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Abstract: Reliable estimates of animal and plant population sizes are necessary to track trends in populations through time. Diamondback terrapins are an ecologically unique keystone species that are globally declining. Conservation efforts for this species rely on accurate estimates of population sizes; however, diamondback terrapin population size estimates are difficult to measure with precision or accuracy. Terrapin collection methods are often labor-, time-, and cost-intensive. The present study compares two recently developed rapid assessment methods for measuring diamondback terrapin abundances. Since mark–recapture or similar data were unavailable, we could not test the accuracy of either method directly; instead, we compared the two methods. If the methods produce similar estimates of terrapin population size, this would increase confidence in these methods. We measured the abundance of diamondback terrapins at 77 sites in Long Island, New York, using headcount surveys and surveys of parasitic trematodes that can be used as a proxy for terrapin abundance. We used random forest analyses to test whether the variation in diamondback terrapin abundance measured using headcount surveys could be explained by either the prevalence or the abundance of trematode parasites. The most variation explained by any of the models was 7.77%, indicating that trematode prevalence and abundance could not explain the variation in terrapin abundance measured using headcounts. This poor correlation between terrapin census methods indicates that one, or both, of the census measures are inaccurate, at least in the habitats found across Long Island, NY. A technique that accurately estimates the abundance of diamondback terrapin populations is critical to understanding their population fluctuations and trends. The only way to evaluate the status of the species is to have information on population numbers and trends across the species' range, which might not be possible without a more accessible survey method.

Keywords: headcount survey; Long Island; New York; *Malaclemys terrapin*; mud snail; *Pleurogonius malaclemys*; *Tritia obsoleta*; visual encounter survey

1. Introduction

Sampling procedures like mark–recapture [1] and line transect methods [2] are often used to estimate the density of populations. However, these methods might be prohibitively time- and labor-intensive in some situations. In such cases, an index correlated with the underlying population abundance might be preferable to direct density estimates [3]. Such indices are useful for making comparisons between populations and assessing trends, even if they cannot estimate the actual number of organisms in an area. For any species, ideal census methods would be rapid, easy to conduct, low cost, precise, and accurate. Such methods do not currently exist for diamondback terrapins (*Malaclemys terrapin*) [4].

Diamondback terrapins are medium-sized turtles that live in coastal, brackish marshes and swamps along over 6000 km of the East and Gulf coasts of the United States from Cape Cod, Massachusetts, to Corpus Christi Bay, Texas [5,6]. Populations in many parts of the range crashed in the early 1900s due largely to over-harvest for human consumption [7]. Terrapin populations have not fully recovered and are possibly declining because of continued harvest, incidental trapping, vehicle and boat strikes, predation, habitat loss, coastal development, and pollution [8,9]. The current status of terrapin populations is difficult to assess because there are few published studies containing terrapin population sizes [10–13].

The current decline in diamondback terrapins throughout their range is particularly troubling because they are the only American turtles that regularly inhabit tidal waters with salinity ranges from fresh to near sea water [5,6]. Terrapins are also thought to be a keystone species because of their influence on invertebrate populations, their top-down control on *Spartina* herbivores, their ability to disperse seeds for marine plants, and their ability to move nutrients from water to land [6,14]. As terrapin populations continue to decline, there is an increasing need for affordable and accurate ways to measure their abundance, especially if they can involve citizen scientists who have the ability to monitor large areas for longer periods than scientists alone could cover.

Diamondback terrapin abundance is difficult to estimate with precision or accuracy because terrapins do not consistently enter traps or bask in the open [15], ruling out techniques that are frequently successful with other turtle species [16]. Terrapins are commonly censused via mark–recapture methods, with captures obtained using modified crab pots, hand-capture, and seine netting [15]. However, modified crab pots are labor-intensive, require motor boats, and tend to yield low capture rates; seine netting is also labor-intensive, and hand-captures are limited to nesting females (adults) while on land [15].

As an alternative to mark–recapture, Harden developed a diamondback terrapin assessment technique using rapid, low-cost headcount surveys, which provide an index of terrapin abundance. When they compared terrapin abundance estimates from headcount surveys to aquatic sampling surveys, they found a strong positive relationship ($R^2 = 0.538$) [17]. Headcount surveys are a type of visual encounter method where an observer conducts counts of turtles by seeing how many turtles are out basking or how many heads emerge from a water body in a given amount of time or along a transect. This rapid assessment technique could be suitable for determining terrapin population estimates throughout their range with much lower human effort and cost compared to other techniques, but it is only useful if it is accurate.

As a second alternative to mark–recapture, sampling parasite abundance, has been proposed as a proxy for host abundance, though this has rarely been tested in reptiles. Parasites might be able to provide more information about their host than can be obtained by direct measurement of the host itself [18,19], when the abundance of parasites can be correlated with the abundance of their hosts [20–23]. Sampling parasite abundance as a proxy for host abundance could be a quick and affordable way to census species that have a parasite with high host specificity, like the diamondback terrapin [24].

Diamondback terrapins are the only known host of the adult stage of the trematode parasite *Pleurogonius malaclemys* [25]. Adult *P. malaclemys* live in the intestines of terrapins, releasing eggs that are expelled with terrapin feces [25]. The eggs are then likely ingested by mud snails (*Tritia obsoleta*) where they hatch and invade the gonadal and intestinal tissue of the mud snail [26]. In that tissue, they undergo asexual reproduction and continue to develop, eventually rupturing from the mud snail to settle and encyst upon the operculum or shell of the same mud snail, another mud snail, or possibly another substrate in the environment [25–27]. Terrapins then eat infected mud snails or other substrates *P. malaclemys* forms cysts upon. After ingestion by terrapins, the juvenile trematodes are released from the cysts and travel to the terrapin's intestines where they mature and undergo sexual reproduction, completing their life cycle [27].

As terrapins are the only known definitive host of *P. malaclemys*, the variability in terrapin abundance should affect the abundance of its parasite [24]. Byers et al. found that the prevalence and mean abundance of *P. malaclemys* cysts in mud snails (*T. obsoleta*), along with salinity, could explain most ($\geq 59\%$) of the variation in terrapin abundance (determined using mark–recapture methods) [24].

To compare these two alternative census methods, we collected headcount survey data (as per [17]) and *P. malaclemys* cyst abundance on mud snails (as per [24]) at sites with resident diamondback terrapins. We used random forest analyses to test whether the variation in diamondback terrapin abundance measured using headcount surveys could be explained by either of the two measures of *P. malaclemys* abundance in mud snails introduced by Byers et al. [24]. We did not have other population estimate data (e.g., from a mark–recapture study), so we could not test the accuracy of either method directly. Instead, we hypothesized that if both methods are reliable, the percentage of mud snails with *P. malaclemys* cysts and the total number of cysts on mud snails would explain most of the variation in diamondback terrapin abundance measured from headcount surveys. We made no prior assumptions that either census method was more accurate than the other in determining the true terrapin population sizes.

2. Materials and Methods

2.1. Terrapin Headcount Surveys

We conducted diamondback terrapin headcount surveys via kayak or motorized boat (where necessary) at 77 sites in Long Island, New York (Table 1; Figure 1). We took the kayak up (run one) and then down (run two) a creek and counted the number of terrapins on the surface of the water or on land in each run. We carried out these surveys at the end of May through mid-August and at the beginning of September through mid-October in 2015 and 2016. The same two researchers performed the surveys in 2015 and 2016 using the same routes. We only conducted surveys if the air temperature was >18 °C. In both years, we recorded weather conditions, air temperature, wind direction and strength, tide position, and terrapin behavior. We additionally recorded water surface temperature and salinity in 2016. We combined the terrapin counts from both runs for analysis.

Table 1. Average number of turtles observed from headcount surveys, average percent of snails infected with *Pleurogonius malaclemys*, and average number of *P. malaclemys* cysts on mud snails per research site.

Site	Year	Average Headcount	Average Percent Infected Snails	Average Number of Cysts
1	2015 and 2016	0.25	0.00	0.00
2	2015 and 2016	0.00	0.01	1.00
3	2015 and 2016	1.25	0.00	0.00
4	2015 and 2016	6.25	0.01	1.00
5	2016	0.00	0.00	0.00
6	2016	0.00	0.01	1.00
7	2016	0.00	0.00	0.00
8	2015 and 2016	1.50	0.01	2.00
9	2016	0.00	0.01	1.00
10	2016	0.50	0.02	3.00
11	2015 and 2016	0.25	0.01	1.50
12	2015 and 2016	0.50	0.03	32.00
13	2015 and 2016	5.50	0.34	118.50
14	2016	1.00	0.03	3.00
15	2015 and 2016	8.50	0.37	151.00
16	2015 and 2016	17.75	0.15	27.50
17	2015 and 2016	10.50	0.13	25.50
18	2015 and 2016	4.50	0.17	1.50
19	2015 and 2016	0.75	0.09	18.50
20	2016	6.00	0.00	0.00

Table 1. Cont.

Site	Year	Average Headcount	Average Percent Infected Snails	Average Number of Cysts
21	2015	9.50	0.31	98.00
22	2015	12.00	0.69	544.00
23	2015 and 2016	1.67	0.26	162.33
24	2015	0.00	0.07	19.00
25	2016	4.00	0.03	3.00
26	2015	6.00	0.13	31.00
27	2015 and 2016	0.33	0.34	151.50
28	2016	23.00	0.22	27.00
29	2015	1.00	0.03	12.00
30	2015	6.50	0.69	372.00
31	2015	1.00	0.03	6.00
32	2016	0.00	0.02	2.00
33	2015 and 2016	0.00	0.00	0.00
34	2016	0.00	0.11	14.00
35	2015 and 2016	23.00	0.41	711.00
36	2016	0.00	0.02	2.00
37	2015 and 2016	1.75	0.11	29.00
38	2015 and 2016	3.00	0.23	107.50
39	2015 and 2016	3.00	0.09	16.50
40	2016	1.50	0.00	0.00
41	2016	2.50	0.00	0.00
42	2016	0.00	0.01	1.00
43	2016	0.00	0.01	1.00
44	2016	0.00	0.00	0.00
45	2016	0.00	0.00	0.00
46	2016	0.50	0.01	2.00
47	2016	0.50	0.00	0.00
48	2016	0.00	0.00	0.00
49	2016	0.00	0.01	1.00
50	2015 and 2016	0.00	0.13	25.00
51	2015 and 2016	0.25	0.03	6.50
52	2015 and 2016	0.00	0.01	1.00
53	2015 and 2016	7.75	0.17	50.00
54	2015 and 2016	0.50	0.03	7.00
55	2016	0.50	0.00	0.00
56	2015	0.00	0.65	453.00
57	2015	17.50	0.60	282.00
58	2016	2.00	0.01	2.00
59	2016	16.00	0.01	1.00
60	2015	0.00	0.03	5.00
61	2015 and 2016	11.25	0.05	10.00
62	2015 and 2016	0.00	0.00	0.00
63	2015 and 2016	1.50	0.03	27.00
64	2015 and 2016	3.25	0.02	2.50
65	2015 and 2016	11.00	0.07	9.00
66	2015 and 2016	0.00	0.00	0.00
67	2015 and 2016	0.00	0.00	0.00
68	2016	0.00	0.00	0.00
69	2015 and 2016	48.25	0.09	24.00
70	2015 and 2016	9.50	0.02	5.50
71	2015 and 2016	15.25	0.43	446.00
72	2015 and 2016	0.50	0.17	59.00
73	2015 and 2016	3.50	0.08	23.50
74	2015 and 2016	14.75	0.36	250.50
75	2015 and 2016	2.00	0.21	101.00
76	2016	0.50	0.00	0.00
77	2015 and 2016	92.50	0.30	154.50

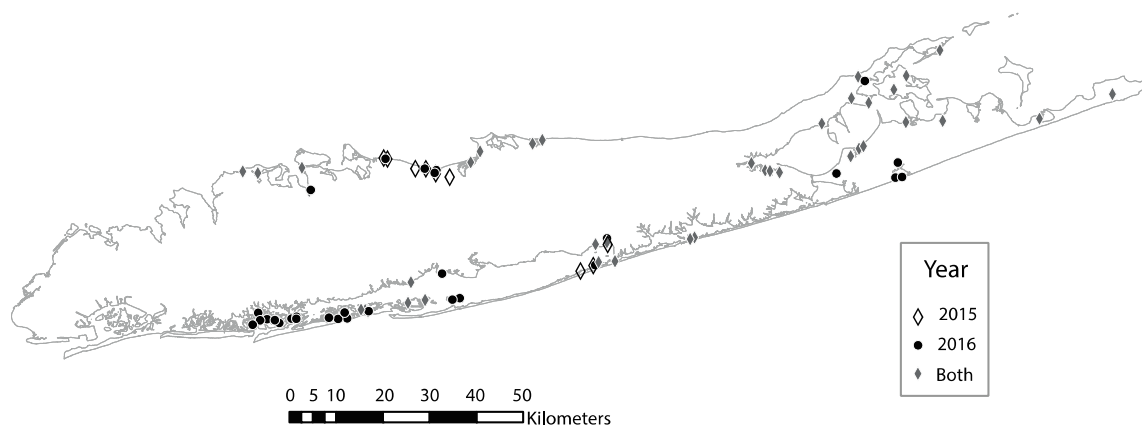


Figure 1. Location of diamondback terrapin and snail surveys in 2015, 2016, and in both 2015 and 2016 in Long Island, NY.

2.2. Trematode Surveys

We collected mud snails (*T. obsoleta*) during low tide on beaches and mud flats within 300 m of terrapin survey locations. A researcher walked along the waterline and collected 5–10 snails every two strides until they had collected 200 snails. We recorded the starting and stopping GPS coordinates, as well as the number of strides taken. We excluded small, pre-reproductive snails from collections. We stored the snails in 75% ethanol. In the lab, we measured the shell and aperture lengths along with the number of trematode (*P. malaclemys*) cysts on the shell and operculum of the mud snails (Figure 2).



Figure 2. Photograph of mud snail (*Tritia obsoleta*) with arrows pointing to two cysts of the trematode parasite *Pleurogonius malaclemys*. Photograph taken by Dr. Russell Burke.

2.3. Statistical Analysis

We used random forest (RF) analyses to explore the relationship between snail infection rates and terrapin visual counts. RF analysis combines the predictions from many classification trees to produce

classifications with very high accuracy [28]. RF analysis also has the advantages of a novel method of calculating variable importance, and the ability to model complex predictor variable interactions; for both classification and regression, RF analysis is either competitive or superior to most methods in common use [28]. RF analyses allowed us to assess the importance of each predictor variable on terrapin visual counts.

RF begins by selecting many bootstrapped samples from the original data; those observations that do not occur in a bootstrapped sample are called out-of-bag (OOB) observations. Classification trees are built using the bootstrapped samples, then those trees are used to predict the OOB observations. The importance of each variable is calculated by permuting the values of OOB observations for a variable and then passing those through the tree to get new predictions. The difference between the original and permuted misclassification rates, divided by the standard error, is a measure of variable importance [28].

We performed three RF analyses using R statistical software [29]. One was performed on the data from 2015, one on those of 2016, and one on the data from the two years combined. We split the analysis among years to determine if these population estimation methods could be used for rapid assessment, or if multiple survey years were required for the methods to be accurate. We chose the number of variables to be available for splitting at each tree node (mtry parameter) using the tuneRF function in the randomForest package [30]. Each forest was constructed with 10,001 trees, because a higher number of trees leads to better stability in the variable importance score [31]. We chose an odd number of trees so potential ties could be broken.

We used the initial forests to select which variables went into our final RF by eliminating those variables with negative variable importance scores. A negative variable importance score means that random permutations of that variable performed better than the original values, so that variable is increasing the OOB error rate. Only the results from the final RF are reported.

3. Results

3.1. 2015

We eliminated three predictor variables (month of survey, tide position, and weather conditions) from the final RF analysis because they had negative variable importance scores in the initial RF analysis (Figure 3a). The final RF analysis included the total number of cysts present on snails, the percentage of snails with cysts, air temperature, and wind intensity as predictor variables. The model could explain 4.43% of the variation, meaning none of the predictor variables used in the model were able to predict terrapin visual counts.

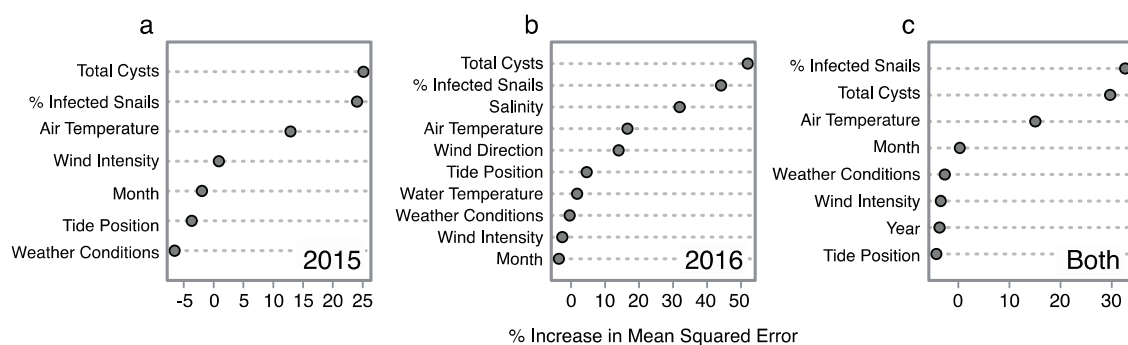


Figure 3. Variable importance plots for predictor variables from random forest (RF) classifications used for predicting terrapin visual counts in (a) 2015, (b) 2016, and (c) both 2015 and 2016 combined. The mean decrease in accuracy from the permuted out-of-bag (OOB) observations compared to the original values is reported as percent increase in mean squared error. Higher values of percent increase in mean squared error indicate variables that are more important to the classification.

3.2. 2016

We did not include three predictor variables (month of survey, wind intensity, and weather conditions) in the final RF analysis because they had negative variable importance scores in the initial RF analyses (Figure 3b). We included the total number of cysts present on snails, the percentage of snails with cysts, air temperature, water temperature, salinity, tide position, and wind direction as predictor variables in the next RF analysis. The model could explain 5.26% of the variation in terrapin abundance. In this model, water temperature had a negative variable importance score, so we removed this variable from the final RF analysis, which explained 7.77% of the variation in terrapin abundance.

3.3. Combined Data from 2015 and 2016

We removed wind intensity, year of survey, tide position, and weather conditions from the RF analysis because they had negative variable importance scores in the initial RF analyses (Figure 3c). We included the total number of cysts present on snails, the percentage of snails with cysts, air temperature, and month of survey as predictor variables in the next RF analysis. The model could explain 7.31% of the variation. In this model, month of survey had a negative variable importance, but removing it lowered the amount of variation the model could explain, so we included this variable in the final RF.

4. Discussion

Diamondback terrapins are a keystone species that are thought to be declining in parts of their range [32–34]. Accurate population estimates remain difficult to obtain for this species, but terrapin headcounts [17] and *P. malaclemys* cyst abundance measures [24] offer two new affordable and potentially reliable alternatives to more traditional survey methods [34]. The accuracy of each of these techniques has only been demonstrated once in a single test case in a small area: terrapin headcount surveys (as per [17]) at Kiawah Island, South Carolina, and *P. malaclemys* cyst abundance on mud snails (as per [24]) at 12 sites along the coast of Georgia. Neither of these methods has been tested in the northern part of the terrapin's range. Lacking independent and reliable population estimates, we could not test accuracy directly, either. However, we indirectly tested the two methods by comparing them to each other; if these two methods provided accurate population estimates for the diamondback terrapin, the estimates obtained with these methods would correlate with each other. We found no correlation between these indices, indicating that one or both techniques are not accurately determining the true underlying terrapin abundances on Long Island, NY, USA.

In all the random forest analyses, one of the two trematode abundance measurements (total number of cysts present on snails or the percentage of snails with cysts) was the most important variable in the model, and the two trematode measurements always explained most of the variation in terrapin abundance (Figure 3). However, the most variation explained by any of the models was 7.77% using data from 2016 alone. Neither trematode abundance measure has a clear correlation with terrapin abundance (Figure 4). This outcome indicates that none of the covariates collected over the two years could explain the variation in terrapin visual counts observed over those two years. This result differs from both that of Byers et al. [24], who found that the abundance of trematode cysts and salinity could explain 59% of the variability in terrapin abundance, and that of Harden et al. [17], who found that terrapin abundance from headcount surveys had a strong, positive correlation ($R^2 = 0.538$) with abundance measures from aquatic sampling.

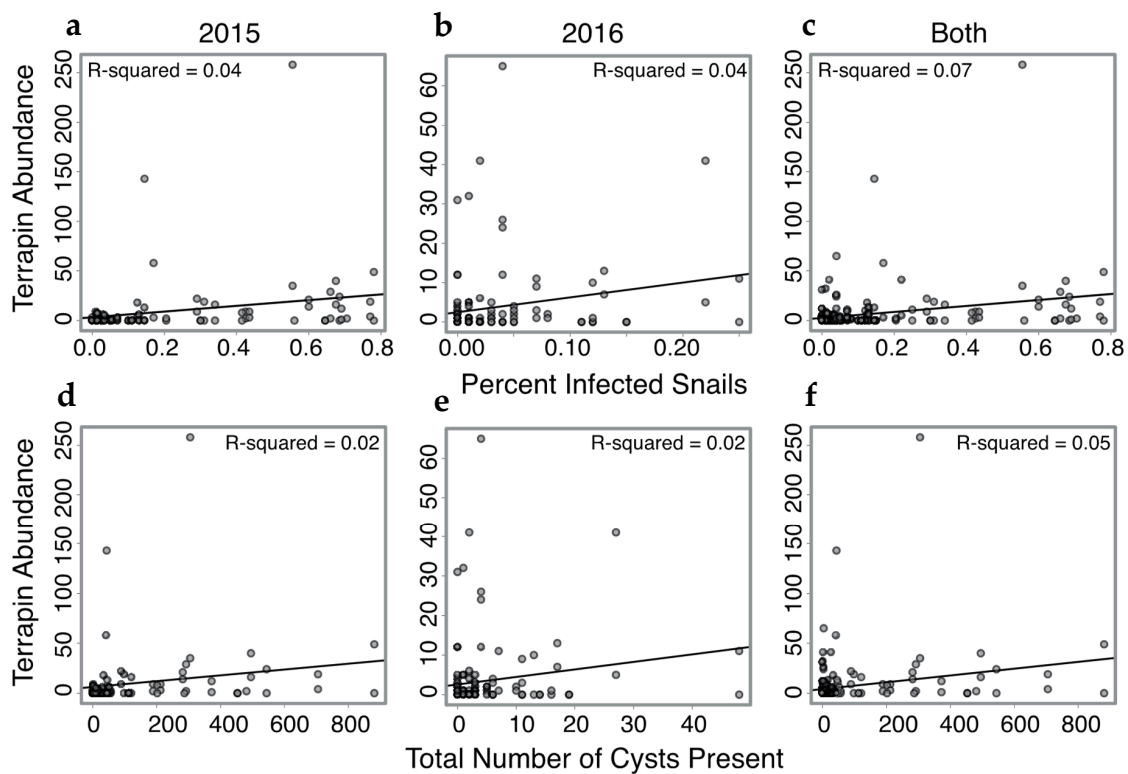


Figure 4. Bivariate plots of terrapin abundance, from headcount surveys, and trematode abundance measured using the percent of infected snails (a–c) and the total number of cysts on snails (d–f).

This poor correlation between terrapin census methods indicates that one, or both, of the census measures are inaccurate, at least in the habitats found across Long Island, NY, USA. Byers et al. conjectured that the use of trematode abundance as a proxy for terrapin abundance estimates would not work if terrapin abundance was rapidly declining, as trematode abundance might lag behind the decline in terrapin abundance [24]. There is not sufficient evidence to indicate that terrapin populations are rapidly changing in this region, although populations appear to be declining. We are not able to fully rule out the possibility that trematode abundance measures did not work in this region because of declining terrapin abundances.

Harden et al. and Byers et al. suggested that more variation could potentially be explained if water temperature were added to the analysis, but we did not find that to be the case (Figure 3b) [17,24]. Air temperature, however, was the third or fourth most important variable in every model (Figure 3). Harden et al. additionally suggested adding weather conditions and time of year (month) as predictors in a headcount survey model to increase accuracy, but these factors had very little effect on our models (Figure 3) [17].

Our results could be different from those of Harden et al. and Byers et al. for a number of reasons [17,24]. First, we examined 77 sites, whereas Byers et al. looked at 12, and Harden et al. had only 5 creeks. It is possible that if only a few of our sites had been examined, our results could have strongly supported the work of Harden et al. and Byers et al., but at this larger scale, the relationship is diminished. It is also possible that the habitats of the subspecies of terrapins in New York are different to those of Georgia [24] or South Carolina [17], and for that reason, one or both of the survey methods are less effective in this region.

While the results of this study indicate a poor correlation between these survey methods when trying to determine terrapin abundance, both are still useful for determining terrapin presence and absence. If terrapins were present at a site, 58% of the time cysts were present as well. If terrapins were absent, 13% of the time cysts were also absent. Cysts gave a false positive signal of terrapin presence 18% of the time and a false negative signal 10% of the time. So, overall, cyst presence correctly predicted

terrapin presence at 71% of sites. Environmental DNA (eDNA) has been increasingly used to detect cryptic turtle species of conservation concern [6]. This method is useful, but expensive, so trematode sampling might provide a reasonable alternative if the only goal of the study is to determine terrapin presence or absence. However, if a terrapin population were rapidly declining, the trematode survey method would not be able to detect local extinction as cysts could be present for some time as mud snails are long-lived, and their trematode infections are generally retained throughout their life [35].

Future studies should compare both terrapin estimation methods tested here to abundance estimates obtained from mark–recapture data. More studies are needed to determine the accuracy of both headcount terrapin surveys and the use of trematode parasite surveys as a proxy for terrapin abundance. Both techniques need additional tests in more areas, and until such tests are conducted, management programs should not rely solely on abundances estimated with these methods.

A rapid assessment technique that accurately predicts the abundance of diamondback terrapin populations is critical to understanding their population fluctuations and trends. Long-term mark–recapture studies provide valuable and accurate population trends but are often prohibitively time-consuming and expensive. The only way to evaluate the status of the species is to have information on population numbers and trends across the species' range, which might not be possible without a more accessible survey method.

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