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HOW A STATEWIDE STREAM SURVEY CAN AID IN UNDERSTANDING FRESHWATER MUSSEL (BIVALVIA: UNIONIDAE) ECOLOGY: EXAMPLES OF UTILITY AND LIMITATIONS FROM MARYLAND

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ABSTRACT

Gaps in our knowledge of freshwater mussel life history, distribution, and ecology remain even though their study has increased considerably over the past few decades. These studies have traditionally taken place within a population, river, or larger drainage unit, but rarely across a broad landscape, such as a state. Given the imperiled status of a majority of unionid species alternative opportunities to collect valuable data cannot be overlooked. We present results from a statewide biological monitoring program (Maryland Biological Stream Survey) that has incorporated a visual survey for mussels, several example analyses using mussel-bioassessment data, and discuss the utility and limitations of incorporating freshwater mussels into stream assessments. Since 2007, we encountered 11 of the 16 mussel species extant in Maryland during assessments of wadeable streams by using an informal visual survey and recording incidental observations. On several occasions, we have discovered new populations of imperiled mussels or extended a species distribution. The biological and physiochemical data collected at sites coincident with freshwater mussels have allowed us to hypothesize factors potentially limiting species distribution, such as fish-host dynamics, habitat quality, nutrient concentration, and catchment land use. We feel that the addition of a survey effort into a biological monitoring program, invaluable data can be collected that can assist resource managers, malacologists, and researchers answer a variety of questions. Further investigation into the cost-benefits of an appropriate level of sampling effort is needed as this could vary markedly among molluscan faunal regions and by objectives.

KEY WORDS Freshwater mussels, Unionidae, biological monitoring, Maryland Biological Stream Survey

INTRODUCTION

The diversity of freshwater mussels (Bivalvia: Unionidae) in North America is unmatched globally, yet they are among the most imperiled aquatic fauna on the continent (Williams et al., 1993; Bogan, 2008). The high rate of imperilment and extinction in mussels has been linked to habitat and flow alteration, invasive species, loss of host fish, increased siltation, and dam construction (Brim Box & Mossa, 1999; Strayer, 1999a; Vaughn & Taylor, 1999; Watters, 2000). Poor land use practices and pollution have further disrupted freshwater ecosystems ultimately leading to the decline of mussels (Bogan, 1993). This decline has likely had major implications on functioning aquatic ecosystems along with the management, conservation, and restoration of aquatic species. Even though the study of freshwater mussels has increased over the past few decades their conservation still faces several challenges. Foremost, basic life history and distributional information of mussels are lacking for many species (Neves, 1993; Strayer, 2006). Potentially exacerbating this problem is that subjective observations about ecological factors that govern unionid presence typically do not agree with results from quantitative studies (Strayer, 2008). In spite of this, research into the life history and autecology of freshwater invertebrates has declined (Resh & Rosenberg, 2010).

Unionids have long been considered indicators of good water quality (Ortmann, 1909; Neves, 1993), but there is little guidance for resource agencies on ways to utilize them in assessments of stream health (Grabarkewicz & Davis, 2008). The Maryland Biological Stream Survey (MBSS) is a statewide biological monitoring and assessment program administered by the Maryland Department of Natural Resources' Monitoring and Non-Tidal Assessment Division, which has incorporated freshwater mussels into standard operation procedures (Stranko et al., 2007). Objectives of the MBSS are to assess the condition of aquatic resources, identify physiochemical and anthropogenic stressors such as acidification, land alteration, and climate change, and provide an inventory of biodiversity in Maryland's streams (Klauda et al., 1998; Stranko et al., 2005). This is primarily accomplished through a probabilistic design to make unbiased estimates on the condition of the states' (1st-4th order) wadeable streams (Heimbuch et al., 1999), but has recently included other sampling designs tailored to meet resource management needs.

Such spatially extensive, readily available data sets may be useful in developing empirical models of multiple stressors that can guide future studies with more detailed and costly methods that test mechanistic hypotheses of mussel conservation and ecology (Strayer, 2008). In this study, we present results from the MBSS and offer simple analytical examples that could be conducted with mussel-bioassessment data that can address gaps in freshwater mussel ecology and conservation (National Native Mussel Conservation Committee, 1998; Strayer, 2006). Additionally, we discuss the utility of incorporating mussels into stream monitoring and assessment programs and the limitations that such an endeavor faces.

METHODS

Water chemistry grab samples were collected from the upstream extent of 75-m-long sites during spring (March through April) base-flow conditions and analyzed for pH, acid neutralizing capacity (μeq/L), specific conductance (μS/cm), chloride (mg/L), sulfate (mg/L), total nitrogen (mg/L), ammonia (mg/L), nitrate (mg/L), and total phosphorus (mg/L), using methods described by the U.S. EPA (1986). Water temperature was recorded at 20 minute intervals from June to September with Hobo data-loggers (Onset Corporation) deployed at each site. From these data, we calculated an average of the daily mean temperature ($N \approx 92$). Gradient (% slope) was calculated as the change in water surface height between the up and downstream extent of a site using a surveyor's level and metric stadia. Benthic macroinvertebrate samples were collected with a 540 μm D-net from 20, 0.3 m^2 areas of proportionally available optimal habitat to calculate a benthic macroinvertebrate index of biotic integrity (Stribling et al., 1998).

During summer base-flow conditions, we collected fishes within each site using two-pass depletion with backpack electrofishing units (one anode/3 m of wetted stream width) to calculate a fish index of biotic integrity based on a scale of 1-5 (very poor < 2, poor 2 < 3, fair 3 < 4, and good > 4) (Southerland et al., 2007). From these data, we also calculated the abundance of freshwater mussel host-fishes (Kneeland & Rhymer, 2008; Cummings & Watters, 2010). We visually estimated physical habitat quality using five metrics scored on a 0-20 scale (poor = 0-5, marginal 6-10, suboptimal 11-15, and optimal 16-20): instream habitat, epifaunal substrate, velocity depth diversity, pool-glide quality, and riffle-run quality. Scores of each metric are meant to characterize aspects of habitat important to stream biota. For example, the instream habitat metric relates to the quality and quantity of fish habitat, while the epifaunal substrate metric rates the suitability of benthic macroinvertebrate habitat. Scores for velocity depth diversity and quality of pool-glide and riffle-run habitats are based on the heterogeneity and extent of those habitats. Riffle embeddedness was determined by estimating the percentage of gravel and larger substrates surrounded by fine sediment (< 2 mm). Average stream width (m) was calculated from the wetted width taken at four equally distant transects within the sample reach. Stream flow was measured with a Marsh McBirney FloMate 2000 on a top-setting metric wading rod. Discharge (m^3/sec) was then calculated from the cross sectional area of the stream. We hand digitized the catchment upstream from each site based on United States Geological Survey 7.5 minute quarter quad topographic maps using ArcMap 9.3, and calculated drainage area (km^2). We then intersected satellite-derived land cover (2001 NLCD; Homer et al., 2007) to catchments and calculated the percent of major land cover types (urban, agriculture, and forest) within catchments.

While at each MBSS site, we searched suitable unionid habitats for ≥ 15 minutes to determine the presence of mussels. Additionally, we searched animal middens when present and noted incidental observations of unionids while sampling other aquatic fauna. When a live mussel was encountered, it was identified and returned to the location of its collection. Representative shells were retained to verify field identifications. A subset of these vouchers were independently verified by the state zoologist and deposited in several museum collections (Delaware Museum of Natural History, Illinois Natural History Survey, and North Carolina Museum of Natural Sciences). The remaining vouchers were housed at MDNR offices in Annapolis, MD and Frostburg, MD for training purposes. Freshwater mussel taxonomy in Maryland follows Turgeon et al. (1998). Annually, field crew managers and leaders receive thorough training in mussel identification along with other taxonomic groups for which data are recorded during MBSS sampling.

We described environmental conditions at sites with *Elliptio fisheriana* (Lea, 1838) to sites where they were apparently absent throughout their range with non-parametric pair-wise comparisons. Continuous variables (water chemistry, habitat, and land use) were compared with Kolmogorov-Smirnov tests and categorical variables (physical habitat metrics and biological multi-metric index scores) with Mann-Whitney U tests. We chose this species as an example as it is restricted to one physiographic region (Coastal Plain); therefore, conditions should be relatively homogenous (Stribling et al., 1998; Southerland et al., 2007). Absence was presumed if no mussels were detected and present if live or dead specimens were collected. At sites that were sampled on more than one occasion, a species was also assumed present if it was previously encountered. For this study, we defined a species range as the sites within watersheds (Maryland 8-digit) in which we encountered at least one individual of that species. To investigate the role of known and potential fish-hosts on patterns of *E. fisheriana* presence, we calculated the frequency of occurrence for stream fishes collected during MBSS surveys.

RESULTS

From 2007 to 2009, we encountered unionids at 117 of the 595 MBSS sites sampled (20%). At a minimum, 148.75 person-hours were expended searching for mussels. We made 133 observations of live freshwater mussels or dead shell material representing 11 species (Table 1); however most species were encountered infrequently. *Elliptio complanata* (Lightfoot, 1786) was by far the most widely distributed and frequently encountered unionid during MBSS sampling. *Elliptio fisheriana* was the second most encountered species, followed by *Pyganodon cataracta* (Say, 1817), and *Alasmidonta heterodon* (Lea, 1830). The remaining six species were found at < 5 MBSS sites since 2007. Five species, including the state endangered *Alasmidonta varicosa* (Lamarck, 1819) and *Lasmigona subviridis* (Conrad, 1835), have yet to be found during stream assessments.

Freshwater mussel richness in wadeable streams throughout Maryland's 8-digit watersheds was generally low (Figure 1). The most diverse assemblages were generally found in coastal streams on Maryland's Eastern shore, although two Potomac River watersheds also had relatively diverse assemblages for Maryland streams. We rarely found live mussels or spent valves at sites in central and western Maryland. Some notable distributional records resulting from MBSS surveys include the first records of *Elliptio producta* (Conrad, 1836) in the Upper Patuxent River watershed, the range extension of *Alasmidonta undulata* (Say, 1817) in the Patapsco River, and discovery of a relic population of *A. heterodon* in the Upper Choptank River watershed.

Significant differences were found for most physiochemical and biological variables compared between sites with *E. fisheriana* and sites where they were apparently absent (Table 2). When *E. fisheriana* was encountered at MBSS sites, pH, ANC, and nutrient concentrations were higher. These streams were also on average several meters wider, had considerably larger upstream catchments, lower gradient, and greater discharge compared to streams where *E. fisheriana* was not found. Physical habitat metrics were consistently several points higher and often in categories that represented better conditions. Fish and benthic macroinvertebrate community indices were also higher at sites with *E. fisheriana* compared to other sites in their range. Differences observed in the amounts and types of catchment land use were likely representative of prevailing land use patterns than a biological response.

Previously confirmed fish-hosts were frequent to absent at sites where *E. fisheriana* was encountered and uncommon to frequent at sites where they were not encountered (Table 3). Two of these species (Bluegill and Largemouth Bass) are not native, while the other (Johnny Darter) is not native to Maryland's Atlantic Slope. While Largemouth Bass were frequently collected at sites with *E. fisheriana*, only a few bass were typically found at a site. Several native fishes (Tesselated Darter, Pumpkinseed, and Redbreast Sunfish) that are congenerics of *E. fisheriana* host-fish had rates of occurrence as high to nearly double their respective non-native relative. In addition, these native fishes were infrequently collected at sites where *E. fisheriana* was also not found. Other than American Eel, we rarely collected host-fish of congeneric mussels at sites along with *E. fisheriana*.

DISCUSSION

By instituting a simple visual survey at all MBSS sites we have been able to readily collect valuable distributional data concurrent with an array of biological and physiochemical data that address several continuing challenges to freshwater mussel conservation (National Native Mussel Conservation Committee, 1998). Our basic analysis illustrates just one example how the information garnered from stream assessments that include measures of freshwater mussels can be used and lays the foundation for more rigorous hypothesis development. The growing data set will be instrumental in addressing aspects of freshwater mussel management and conservation, such as describing species habitat associations and tolerances to anthropogenic stressors, such as nutrients and urbanization (MDNR, 2005). Within the context of streams on Maryland's Coastal Plain, it appears as though *E. fisheriana* cannot tolerate the conditions of naturally acid, blackwater (i.e. low pH and ANC) or headwater streams, and marginal to poor physical habitat. Our pair-wise comparisons also proved useful in describing broad conditions that typify mussel presence; larger streams with flowing water, and relatively low nutrient concentrations (Watters, 1992; Watters, 2000; Morgan & Kline, 2011). However,

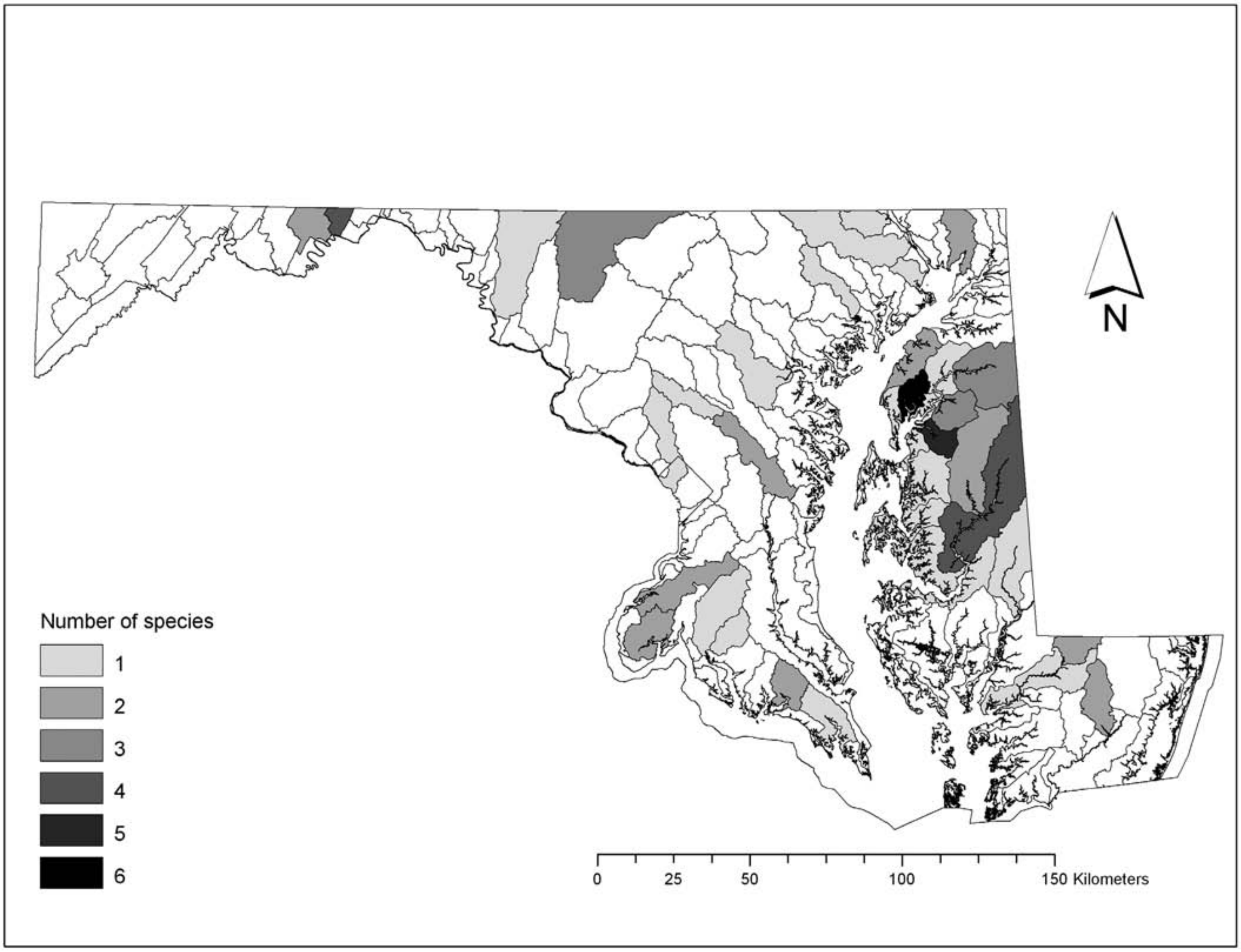

FIGURE 1

Freshwater mussel richness by Maryland 8-digit watershed as observed during the Maryland Biological Stream Survey, 2007-2009.

we recognize that many of these variables are often correlated with one another and must be accounted for to more rigorously hypothesize determinants of distribution. Variables with considerable overlap between sites of mussel absence and presence further illustrate the confounding nature of mussel-habitat associations as they relate to species distribution (Strayer, 2008).

Our findings also indicate that *E. fisheriana* rarely inhabited biologically degraded streams (IBI's ≤ 3), yet were frequently collected in high quality streams (IBI's ≤ 4) (COMAR 26.08.02). This further supports the hypothesis that freshwater mussels are indicators of healthy aquatic ecosystems (Grabarkewicz & Davis, 2008). We feel this highlights a key reason to collect freshwater mussel data as part of a biological monitoring program; regulatory mechanisms (i.e., biocriteria) are in place that react to IBI scores as thresholds of stream and watershed degradation or health (COMAR 26.08.02). In addition, mussel-bioassessment data would be able to support water quality standard (e.g., ammonia and copper) revisions and could be considered for the development of conductivity standards where impacts associated with natural gas extraction are a concern.

The MBSS data set includes over 3,000 randomly selected sites sampled during three state-wide Rounds and approximately 1,000 non-random sites and has been extensively published on using fish and benthic macroinvertebrate as response organisms (e.g. Pinder & Morgan, 1995; Morgan & Cushman, 2005; Stranko et al., 2005; Stranko et al., 2008; Kilian et al., 2010; Hildebrand et al., 2010). Despite the fact that freshwater mussels are good response organisms for understanding spatial and temporal environmental patterns (Green et al., 1985), only two publications (Mynsberge et al., 2009; Stranko et al., 2010) have included MBSS-mussel observations. While the pitfalls of using readily available, large environmental data sets are known (Anderson et al., 2001; Strayer, 2008) their potential utility should not be ignored. In fact, we have several ongoing studies proposing hypotheses of mussel distribution and tolerances to environmental and anthropogenic stressors that build upon the basic relationships presented in this study (e.g., Haag & Warren, 1998; Nicklin & Balas, 2007). However, since we have no measure of detection and our data are limited to presence-absence we hesitate to test certain hypotheses without confirming the power of our survey methods. Our data may also not be appropriate for making inference about the cause of a species decline (Strayer, 1999b).

Unfortunately, we have no direct way of evaluating the cost-per-unit-effort (site visit). We suspect actual costs were quite low because assessments would have taken place regardless of our mussel survey and the amount of effort per site was relatively minimal. Therefore, some costs (e.g., travel) were independent of the mussel search. Moreover, the importance of recording freshwater mussel observations was recognized at the inception of the MBSS (1995) and visual surveys were conducted during stream assessments in advance of dedicated funding. Considerable effort was made at the onset of the study period to train crew members in freshwater mussel identification in response to concerns over the quality of identifications (discussed in Shea et al., 2011) from prior MBSS Rounds. Annually, time was required to inspect voucher shells, obtain independent confirmation, maintain voucher collections, attend regional identification workshops, examine institutional holdings, and address potential errors in the data. Further investigation into total costs, the cost-benefits of current versus more traditional mussel surveys, and additional survey effort (e.g., timed snorkel searches) are warranted.

It should be noted our technique may not be appropriate in other parts of the country where unionid distribution, diversity, and richness differ due to differences among faunal regions. The inability to detect certain species (e.g. *A. varicosa* and *Ligumia nasuta* (Say, 1817)) was likely due a variety of factors, including their existence in streams primarily outside of the MBSS scope (> 4th order, tidal influence) or in populations with very low abundance. Although not indicated in this study's data, we have recently encountered these species through stream assessments in large river and tidal-fresh habitats as part of other studies while using the same informal visual search. Nonetheless, we realize the current methods may be insufficient to detect and characterize the true mussel richness in some habitats where species with cryptic life history traits reside (Metcalfe-Smith et al., 2001; Tiemann et al., 2009). However, a cursory comparison of our mussel richness data to that collected by the Maryland Natural Heritage Program using timed-snorkel surveys has shown good agreement between most small to medium sized streams and watersheds.

The need for basic information on freshwater mussels remains and is imperative to develop and implement effective management plans, in addition to guide regulatory agencies in the development water quality standards that are more protective of freshwater mussels (Augspurger et al., 2003; Strayer et al., 2004). While the number of resource agencies that currently employ some form of standardized, state-wide unionid survey is increasing (Howells, 2006; Sietman, 2009; Shasteen et al., 2010; Stagliano, 2010), few pair their effort with assessments of water quality and biological condition even though monitoring programs are ubiquitous. To be clear, we are not by any means discrediting the traditional species or watershed centric approach to conducting mussel research, but there are well documented limitations on applying data collected at small scales to populations outside of those studied (Hamilton et al., 1997). We feel the proper context for mussel-bioassessments such as ours is to 1) support new or strengthen existing regulatory mechanisms to be more protective of freshwater mussels, 2) collect relevant landscape and physiochemical data at large spatial scales, and 3) supplement and guide quantitative surveys of imperiled unionids and specious watersheds.

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TABLE 1

Number of Maryland Biological Stream Survey sites sampled (N = 595) where live or dead freshwater mussels were encountered, 2007-2009.

Species	Total	Live	Dead	Proportion of sites
Alasmidonta heterodon (Lea, 1830)	10	2	9	0.02
Alasmidonta undulata (Say, 1817)	3	0	3	< 0.01
Anodonta implicata Say, 1829	2	1	1	< 0.01
Elliptio complanata (Lightfoot, 1786)	76	35	55	0.13
Elliptio fisheriana (Lea, 1838)	17	9	11	0.03
Elliptio producta (Conrad, 1836)	3	2	3	< 0.01
Lampsilis radiata radiata (Gmelin, 1791)	2	0	2	< 0.01
Lampsilis sp.	3	0	3	< 0.01
Leptodea ochracea (Say, 1817)	1	1	0	< 0.01
Pyganodon cataracta (Say, 1817)	15	5	11	0.03
Strophitus undulatus (Say, 1817)	1	0	1	< 0.01

*Due to longstanding problems distinguishing between the non-native *Lampsils cardium* Rafi nesque, 1820, native L. cariosa (Say, 1817), and suspected hybrids between the two in the Potomac River basin, Lampsilis sp. is recorded when an individual resembling either species is encountered.

TABLE 2

Non-parametric comparisons of biological and physiochemical variable medians between Maryland Biological Stream Survey sites where *E. fisheriana* was present (N = 41) and absent (N = 61) in 1st-4th order streams of Maryland, 2007-2009. Sulfate, ammonia, and nitrate concentrations represent the molecules whole weight (e.g., nitrate-nitrogen). Higher physical habitat metric and biological index scores represent superior conditions.

Variable	Present (Median ± SD)	Absent (Median ± SD)	*p*
pH	7.05 ± 0.46	6.45 ± 0.89	<0.001
Conductivity (µS/cm)	56 ± 79	169 ± 116	0.77
Acid neutralizing capacity (µeq/L)	495 ± 350	239 ± 446	0.002
Average daily temperature °C	21.15 ± 1.53	20.90 ± 1.41	0.42
Total phosphorus (mg/L)	0.07 ± 0.03	0.04 ± 0.05	0.02
Total nitrogen (mg/L)	2.26 ± 1.27	2.00 ± 3.20	0.04
Nitrate (mg/L)	1.96 ± 1.32	1.22 ± 2.85	0.02
Ammonia (mg/L)	0.03 ± 0.04	0.03 ± 0.10	0.77
Chloride (mg/L)	14.80 ± 10.01	16.02 ± 24.97	0.52
Sulfate (mg/L)	15.13 ± 7.17	15.02 ± 8.84	0.66
Discharge (m^3/sec)	0.04 ± 0.14	0.01 ± 0.04	<0.001
Average wetted width (m)	4.75 ± 4.15	2.80 ± 1.84	0.001
Fish-IBI	4.33 ± 0.80	3.33 ± 1.23	<0.001
Benthic macroinvertebrate-IBI	4.14 ± 0.82	3.14 ± 1.12	<0.001
Instream habitat quality	14.00 ± 3.17	11.00 ± 4.41	<0.001
Epifaunal substrate quality	13.00 ± 3.78	10.50 ± 4.44	0.001
Velocity-depth diversity	9.00 ± 2.98	7.00 ± 3.61	0.001
Pool-glide score	13.00 ± 3.04	11.00 ± 3.84	0.001
Riffle-run score	11.00 ± 5.85	6.00± 5.98	0.009
Embeddedness (%)	100 ± 30.51	100 ± 28.87	0.54
Urban land use (%)	1.60 ± 2.41	0.90 ± 7.73	0.01
Agricultural land use (%)	68.20 ± 14.85	62.00 ± 25.38	0.09
Forested land use (%)	30.10 ± 14.33	31.80 ± 24.72	0.20
Catchment size (km^2)	19.42 ± 50.76	6.73 ± 19.99	<0.001
Gradient (% slope)	0.09 ± 0.10	0.18 ± 0.19	0.05

TABLE 3

Frequency of occurrence for fishes collected at sites throughout the range of *E. fisheriana* in Maryland. An asterisk (*) indicates a non-native fish species.

		Frequency of occurrence	
		Present	Absent
Confirmed fish hosts		N = 41	N = 54
Etheostoma nigrum	Johnny Darter	0.00	0.00
*Lepomis macrochirus**	Bluegill	0.66	0.57
*Micropterus salmoides**	Largemouth Bass	0.49	0.24
Congenerics of confirmed fish hosts			
Etheostoma olmstedi	Tessellated Darter	0.93	0.48
*Lepomis cyanellus**	Green Sunfish	0.56	0.31
Lepomis gibbosus	Pumpkinseed	0.78	0.54
Lepomis auritus	Redbreast Sunfish	0.56	0.22
Luxilus cornutus	Common Shiner	0.00	0.00
Confirmed fish hosts of congeneric mussels			
Anguilla rostrata	American Eel	0.98	0.74
Dorosoma cepedianum	Gizzard Shad	0.02	0.00
Fundulus diaphanus	Banded Killifish	0.02	0.04
Gambusia holbrooki	Eastern Mosquitofish	0.22	0.11
Perca flavescens	Yellow Perch	0.15	0.09
*Pomoxis nigromaculatus**	Black Crappie	0.17	0.04
*Pomoxis annularis**	White Crappie	0.00	0.04