

REGULAR ARTICLE

# COMPARISON OF SURFACE- AND PORE-WATER QUALITY BETWEEN TWO MARYLAND STREAMS WITH THE ENDANGERED DWARF WEDGEMUSSEL (*ALASMIDONTA HETERODON*)

Alfred E. Pinkney<sup>1\*</sup>, Kathleen M. Kline<sup>2</sup>, and Raymond P. Morgan II<sup>2</sup>

<sup>1</sup> United States Fish and Wildlife Service, Chesapeake Bay Field Office, 177 Admiral Cochrane Drive, Annapolis, MD 21401 USA

<sup>2</sup> University of Maryland Center for Environmental Science Appalachian Laboratory, 301 Braddock Road, Frostburg, MD 21532 USA

---

## ABSTRACT

Degraded water quality, particularly elevated concentrations of ammonia, chloride, and toxic metals, can be harmful to freshwater mussels. We investigated whether the contraction in Dwarf Wedgemussel (*Alasmidonta heterodon*) distribution that occurred between 2002 and 2012 within Browns Branch (BB), a stream within a predominantly agricultural watershed on the Coastal Plain of Maryland, was associated with these and other water-quality factors. We measured surface- and pore-water concentrations of different forms of nitrogen, orthophosphate, anions, and dissolved metals at two sites in BB for 7 mo in 2014. The upstream site (BBUP) represented the lower extent of the current Dwarf Wedgemussel population, and the downstream site (BBDO) represented the lower extent of the species' distribution observed in a 2002 survey. As a comparison, we also sampled one site in Nanjemoy Creek (NANJ), a largely forested Coastal Plain watershed where Dwarf Wedgemussel distribution exhibited no change over the same 10-yr period. We tested the hypothesis that concentrations of potentially toxic analytes were significantly higher at BBDO than at BBUP and NANJ. Total ammonia nitrogen (TAN) was the only analyte consistent with this hypothesis in both surface and pore water. Concentrations of pore-water un-ionized ammonia (UIA-N) at BBDO were below the U.S. Environmental Protection Agency's Ambient Water Quality Criterion, but they frequently exceeded 0.2 µg/L, a concentration previously associated with a lack of mussel recruitment. We recommend conducting a new mussel survey of BB to assess current condition. If range contraction is still evident, more frequent and extended sampling should be performed, including capturing high-flow events to determine if pulses of ammonia and other pollutants occur.

**KEY WORDS:** *Alasmidonta heterodon*, water quality, ammonia, pore water

---

## INTRODUCTION

Water quality degradation is commonly suspected of adversely affecting freshwater mussel populations (e.g., Brim Box and Mossa 1999; Strayer et al. 2004; Gascho Landis et al. 2012; Haag 2012; Gillis et al. 2017). Determining the precise physicochemical factors that negatively affect mussel populations is difficult because their complex life history makes them vulnerable to environmental stressors at multiple stages over

long periods. Identifying stressors to mussels is a critical information need for effective conservation (Haag and Williams 2014).

Mussels are highly sensitive to ammonia; ions such as chloride, potassium, and sulfate; and metals such as copper, nickel, and zinc (Newton et al. 2003; Gillis 2011; Johnson et al. 2014; Wang et al. 2017). The un-ionized form of ammonia (UIA-N) is acutely toxic to early life stages of mussels (Newton et al. 2003; Wang et al. 2008), and elevated concentrations of UIA-N in pore water have been associated

---

\*Corresponding Author: fred\_pinkney@fws.gov

with mussel recruitment failure (Strayer and Malcom 2012). Consequently, the U.S. Environmental Protection Agency recently lowered its freshwater ambient water quality criteria for ammonia to be more protective of mussels (USEPA 2013). Nitrogenous pollution in streams primarily results from atmospheric deposition, point source effluent discharges, and agricultural practices that deliver pollution via surface-water runoff or groundwater infiltration (Boynton et al. 1995; Kemp et al. 2005).

The Dwarf Wedgemussel (*Alasmodonta heterodon*) once ranged from New Brunswick, Canada, to North Carolina, USA, but it now inhabits less than half of its formerly occupied streams, and most surviving populations are small (Strayer et al. 1996). In Maryland, it is found in a handful of Coastal Plain streams, including Nanjemoy Creek (NANJ) and Browns Branch (BB) (Bogan and Ashton 2016). Surveys in BB detected a contraction in the distribution of Dwarf Wedgemussel between 2002 and 2012 (Ashton et al. 2013). Dwarf Wedgemussel disappeared from the lower section of BB (hereafter BBDO) during this time, but its abundance nearly doubled in the upper section (hereafter BBUP). Mussel species richness and the distribution of most other species also declined in BBDO between 2002 and 2012. NANJ continues to support a population of Dwarf Wedgemussel, and no changes in its range or mussel species richness were observed in this stream over the last 25 years (J. M. McCann, Maryland Department of Natural Resources (MDNR), unpublished data).

We characterized surface-water and pore-water chemistry in BBUP, BBDO, and NANJ in 2014 to evaluate potential causes of the Dwarf Wedgemussel decline in BBDO. We hypothesized that concentrations of pollutants would be greater at BBDO, where Dwarf Wedgemussel abundance declined, than at BBUP and in NANJ, where Dwarf Wedgemussel has not declined. For later discussion, we abbreviate this hypothesis as BBDO > BBUP = NANJ.

## METHODS

### Study Area

BB and NANJ are on the Atlantic Coastal Plain in Maryland and flow into Chesapeake Bay (Fig. 1). The surface geology of the two watersheds is similar and relatively homogenous. Stream valleys are underlain by Tertiary sands, clays, and silts, and uplands are underlain by Quaternary sands, gravels, and clays (Cleaves et al. 1968). We extracted major land use categories within the upstream catchment of each study site from the 2001 and 2011 National Land Cover Dataset (Homer et al. 2007, 2015), following Ashton (2012). At all three sites, land use changed little between 2001 and 2011. In both time periods, the BBDO (2,409 ha) and BBUP (694 ha) catchments were primarily agricultural (about 70% of land cover, mainly in row crops, but also including pasture and poultry operations), with 19–26% forest and 1–8% urban. The Nanjemoy Creek catchment (4,106 ha) was predominantly

forested (about 80%), with 7–14% agriculture and 2–6% urban.

In BB, we sampled water chemistry at one site in BBUP, at the lowermost extent of habitat occupied by the Dwarf Wedgemussel in 2012, and at one site in BBDO, 3.6 river km downstream, representing the downstream extent of habitat occupied by Dwarf Wedgemussel in 2002, prior to range contraction (Ashton et al. 2013; J. M. McCann, MDNR, unpublished data). At BBUP, the stream is approximately 2–5 m wide, and substrate consists of silt, fine sand, and fine gravel. At BBDO, the stream is 5–8 m wide, and substrate consists of sand, silt, and gravel. The study site in NANJ was located at the approximate center of the Dwarf Wedgemussel population in that stream, where the stream is 4–10 m wide and substrate is coarse sand and gravel.

### Water Sampling and Analysis

We sampled each site about every 30 d from late April to early December 2014 (Table 1), to encompass critical periods of Dwarf Wedgemussel life history, including host-fish infection, juvenile metamorphosis and recruitment, and spawning (Michaelson and Neves 1995). We measured water temperature (°C), dissolved oxygen (DO, mg/L), pH, and conductivity (μSiemens/cm) with a YSI Model 55 multimeter (YSI Inc., Yellow Springs, OH, USA) at midchannel and middepth. We then collected two surface-water samples in trace-metal-clean-certified polyethylene 1-L bottles, one for anions and nutrients and the other for metals. Sample collection and handling procedures followed those of the Maryland Biological Stream Survey (MBSS 2007), such that neither filtration nor acidification was performed in the field. We placed samples on ice, maintained them in a refrigerator, and shipped them via express carrier in iced coolers to the University of Maryland Center for Environmental Science Appalachian Laboratory (UMCES, Frostburg, Maryland) for processing within 48 h of collection.

The sample for anions and nutrients was vacuum filtered using a 0.45-μm membrane filter and divided between two 125-ml polyethylene bottles. The anions sample was stored at 4°C, and the nutrients sample was stored at –20°C until analysis within recommended holding times. The anion sample was analyzed for chloride (Cl), nitrate-N (NO<sub>3</sub>-N), and sulfate (SO<sub>4</sub>) using ion chromatography (USEPA 1987) with a Dionex DX-120 instrument. The nutrient sample was analyzed for nitrite-N (NO<sub>2</sub>-N), total ammonia-N (TAN), and orthophosphate (PO<sub>4</sub>) using flow-injection colorimetry with a Lachat QuikChem 8000 (APHA 1998) (see Table 2 for detection limits). For dissolved-metals analysis, samples were withdrawn from the collection bottle in the lab using a sterile, 60-cc polyethylene syringe and filtered for dissolved metals with single-use 0.45-μm membrane syringe filters into trace-metal-clean polyethylene bottles. Filtered samples were acidified to a pH < 2 with Optima-grade concentrated nitric acid. The following dissolved metals were measured by inductively coupled plasma mass spectrometry, using an

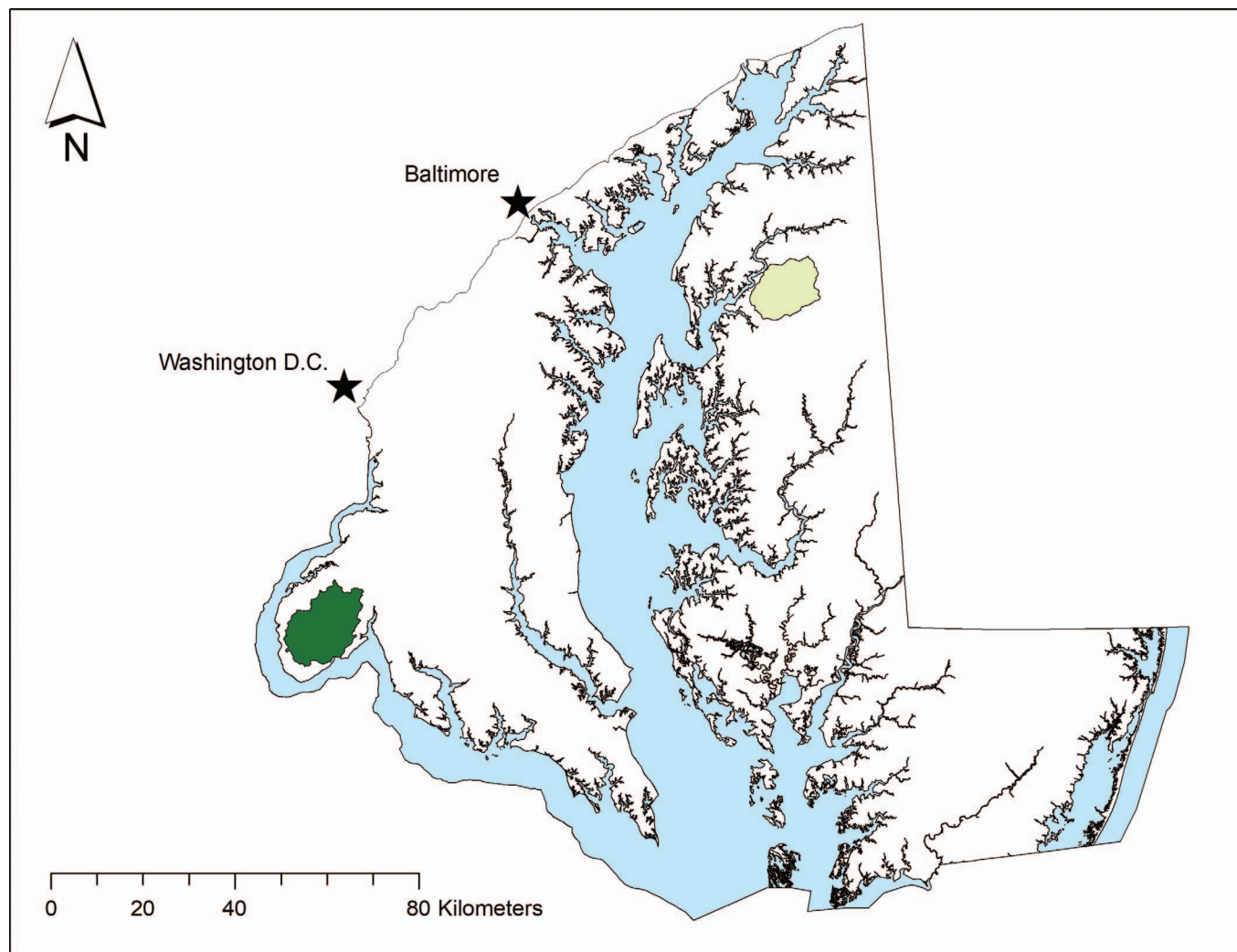


Figure 1. Atlantic Coastal Plain of Maryland, USA, showing Nanjemoy Creek (dark green) and Browns Branch (light green).

Agilent 7900 instrument equipped with an octopole reaction system to remove polyatomic interferences (USEPA 1998): aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), thallium (Tl), vanadium (V), and zinc (Zn) (see Table 3 for detection limits). UMCES employs a rigorous quality assurance/quality control program; results for a 10-sample proficiency test conducted during our project time period and analysis of an independent control sample for metals are presented in Appendix Tables A1 and A2.

We sampled pore water using sediment peepers (Teasdale et al. 1995; Strayer and Malcom 2012). The peepers (Fig. 2) were constructed from 225-ml polyethylene centrifuge tubes by drilling a 5-mm hole in the cap, beneath which we inserted a 1.2- $\mu$ m polycarbonate filter. The filter was supported by vinyl mesh on the interior and protected from external damage by fiberglass mesh affixed to the cap with cyanoacrylate adhesive. Prior to deployment, the UMCES lab filled the

peepers with deoxygenated, deionized water and shipped them to the U.S. Fish and Wildlife Service Chesapeake Bay Field Office laboratory (Annapolis, Maryland) in iced coolers. We maintained the peepers in a refrigerator for several days before taking them to the sites in iced coolers. Over the deployment period, pore water displaced the deoxygenated, deionized water by passive diffusion.

We deployed peepers by strapping three 225-ml peepers to a 100  $\times$  10 cm high-density polyethylene slab with cable ties. We also strapped one 500-ml peeper to the slab so that we could insert the YSI probe into the peeper for water quality measurement. During our initial April sampling, we buried the slab about 10 cm deep in the substrate and anchored it in place with 1.2-cm-diameter steel rebar.

We retrieved peepers every 30 d, coincident with water sampling. On each sampling date, we exposed the slab and removed the peepers. We opened the 500-ml peeper and immediately measured pore-water temperature, pH, conductivity, and DO with the YSI probe. For the 225-ml peepers, we replaced the filter caps with solid caps, placed the peepers on

Table 1. Monthly surface- and pore-water temperature, dissolved oxygen, and ammonia data for Nanjemoy Creek (NANJ), lower Browns Branch (BBDO), and upper Browns Branch (BBUP). Abbreviations and units: Temp = water temperature (°C), DO = dissolved oxygen (mg/L), TAN = total ammonia-N (mg/L), UIA-N = un-ionized ammonia nitrogen (µg/L), AWQC = USEPA acute and chronic ambient water quality criteria for TAN (mg/L, USEPA 2013).

Date	Surface Water					Pore Water <sup>a</sup>				
	Temp	DO	TAN	UIA-N	AWQC	Temp	DO	TAN	UIA-N <sup>b</sup>	AWQC
<b>NANJ</b>										
24-Apr	12.8	10.50	0.013	0.007	41, 3.3	—	—	—	—	—
21-May	17.6	9.13	0.047	0.037	27, 2.4	17.8	6.91	0.056	0.045	27, 2.4
26-Jun	23.8	7.60	0.048	0.060	16, 1.6	23.4	0.83	0.039	0.048	18, 1.8
22-Jul	22.3	8.50	0.034	0.038	19, 1.9	22.2	0.43	0.267	<b>0.302</b>	19, 1.9
18-Sep	16.9	6.72	0.015	0.011	29, 2.6	17.0	2.43	0.225	0.172	29, 2.6
23-Oct	12.4	11.34	0.006	0.003	44, 3.6	12.6	0.93	0.047	0.026	41, 3.3
4-Dec	6.9	12.67	0.008	0.003	51, 4.9	6.9	—	0.007	0.002	51, 4.9
<b>BBDO</b>										
23-Apr	13.2	12.08	0.035	0.085	30, 3.0	—	—	—	—	—
20-May	16.2	10.38	0.124	0.379	23, 2.4	16.0	6.82	0.134	<b>0.403</b>	23, 2.4
25-Jun	20.3	9.52	0.092	0.381	17, 1.9	20.6	0.53	0.501	<b>2.12</b>	15, 1.8
22-Jul	22.2	9.45	0.072	0.341	14, 1.7	22.0	1.49	0.639	<b>2.99</b>	14, 1.7
28-Aug	20.0	7.86	0.068	0.275	17, 1.9	20.9	3.30	0.295	<b>1.27</b>	15, 1.8
1-Oct	16.8	10.40	0.094	0.301	21, 2.3	16.9	2.87	0.020	0.065	21, 2.3
5-Nov	10.8	10.52	0.089	0.181	35, 4.1	11.0	1.04	0.951	<b>1.96</b>	35, 4.1
<b>BBUP</b>										
23-Apr	12.3	12.65	0.007	0.033	24, 2.7	—	—	—	—	—
20-May	14.4	10.85	0.087	0.484	20, 2.4	14.4	8.10	0.106	<b>0.589</b>	20, 2.4
25-Jun	20.7	9.69	0.033	0.292	11, 1.5	21.0	6.40	0.003	0.027	11, 1.5
22-Jul	22.9	8.90	0.027	0.280	9.5, 1.3	21.7	1.12	0.091	<b>0.865</b>	10, 1.4
28-Aug	20.8	7.82	0.160	1.424	11, 1.5	— <sup>c</sup>	—	0.012	0.109	11, 1.5
1-Oct	17.2	10.33	0.014	0.096	16, 2.0	17.2	5.95	0.021	0.144	16, 2.0
5-Nov	11.0	10.24	0.022	0.094	26, 2.9	11.0	2.05	0.027	0.116	26, 2.9

<sup>a</sup>Pore-water samples were not collected in April and from NANJ in August.

<sup>b</sup>UIA-N pore-water concentrations exceeding the Strayer and Malcom (2012) threshold of 0.2 µg/L are in bold; those >2.0 are in bold italics.

<sup>c</sup>Pore-water temperature not measured; surface-water temperature used to estimate pore-water UIA-N.

Table 2. Mean (± 95% CI) ion and nutrient concentrations in seven, monthly surface-water samples from Nanjemoy Creek (NANJ), lower Browns Branch (BBDO), and upper Browns Branch (BBUP). Sites with different letters in parentheses have significantly different means (Tukey's HSD,  $P < 0.05$ ). All units are mg/L except UIA-N (µg/L).

Analyte <sup>a</sup>	NANJ	BBDO	BBUP
Cl <sup>b</sup>	10.52 ± 2.89 (a)	20.16 ± 0.46 (b)	20.05 ± 3.43 (b)
TAN <sup>c, d</sup>	0.02 ± 0.02 (a)	0.08 ± 0.03 (b)	0.03 ± 0.02 (a)
UIA-N	0.02 ± 0.02 (a)	0.28 ± 0.10 (a)	0.39 ± 0.45 (a)
NO <sub>3</sub> -N <sup>d</sup>	0.04 ± 0.03 (a)	5.55 ± 0.66 (b)	4.99 ± 0.57 (b)
NO <sub>2</sub> -N <sup>d</sup>	0.003 ± 0.001 (a)	0.020 ± 0.010 (b)	0.020 ± 0.010 (b)
PO <sub>4</sub> <sup>d</sup>	0.005 ± 0.002 (a)	0.010 ± 0.010 (a)	0.040 ± 0.010 (b)
SO <sub>4</sub> <sup>d</sup>	2.54 ± 1.36 (a)	15.04 ± 1.41 (b)	30.02 ± 3.44 (c)

<sup>a</sup>Minimum detection limits (mg/L): Cl—0.020, TAN—0.002, NO<sub>3</sub>-N—0.0019, NO<sub>2</sub>-N—0.0019 mg/L, PO<sub>4</sub>; 0.0011, SO<sub>4</sub>—0.020.

<sup>b</sup>USEPA (2018) AWQC (mg/L): 860 (acute), 230 (chronic).

<sup>c</sup>See Table 1 for USEPA (2013) ammonia criteria.

<sup>d</sup>Southerland et al. (2005) categories (mg/L; L = low, M = Moderate, H = High): TAN: L < 0.03, M 0.03–0.07, H > 0.07; NO<sub>3</sub>-N: L < 1.0, M 1.0–5.0, H > 5.0; NO<sub>2</sub>-N: L < 0.0025, M 0.0025–0.01, H > 0.01; PO<sub>4</sub>: L < 0.008, M 0.008–0.03; H > 0.03.

ice, and shipped them to UMCES. For the samples collected in May through July, UMCES analyzed anions in one peeper, nutrients in a second, and metals in a third. From August through the end of the study, UMCES followed the same procedure for anions and metals, but they analyzed nutrients from all three peepers (see Data Analysis). After retrieving the peepers, we replaced them with fresh ones and reburied the slab, attempting to minimize turbidity. We measured surface-water-quality parameters and collected surface-water samples before working with the peepers.

We monitored water temperature at each site throughout the study with Hobo Pro V2 data loggers (Onset Computer Corporation, Bourne, Massachusetts) programmed to record at 20-min intervals. We drove a piece of rebar into the substrate and affixed with cable ties one data logger about 5 cm above the substrate surface (for surface-water temperature) and one data logger buried about 5 cm below the substrate surface (for sediment temperature). We measured temperature because it influences the toxicity of chemical stressors, particularly ammonia (USEPA 2013), and because high temperatures can be lethal to mussels. We retrieved the loggers on the last day



Table 3. Mean ( $\pm$  95% CI) dissolved metals concentrations ( $\mu\text{g/L}$ ) in seven, monthly surface-water samples from Nanjemoy Creek (NANJ), lower Browns Branch (BBDO), and upper Browns Branch (BBUP). Sites with different letters in parentheses have significantly different means (Tukey's HSD,  $P < 0.05$ ). Concentrations that exceed a USEPA AWQC<sup>a</sup> are in bold.

Analyte <sup>b</sup>	NANJ	BBDO	BBUP
Al	77.1 $\pm$ 44.4 (b)	23.1 $\pm$ 12.0 (a)	18.2 $\pm$ 9.7 (a)
Sb	0.02 $\pm$ 0.02 (a)	0.03 $\pm$ 0.03 (a)	0.02 $\pm$ 0.01 (a)
As	0.51 $\pm$ 0.16 (a)	0.36 $\pm$ 0.06 (a)	0.38 $\pm$ 0.07 (a)
Ba	31.8 $\pm$ 3.2 (a)	98.8 $\pm$ 9.7 (c)	79.1 $\pm$ 3.9 (b)
Be	0.06 $\pm$ 0.03 (b)	0.03 $\pm$ 0.01 (a)	0.04 $\pm$ 0.02 (a, b)
Cd	0.01 $\pm$ 0.01 (a)	0.04 $\pm$ 0.02 (a)	0.10 $\pm$ 0.05 (b)
Cr	0.34 $\pm$ 0.18 (a)	0.18 $\pm$ 0.08 (a)	0.21 $\pm$ 0.09 (a)
Co	0.63 $\pm$ 0.40 (a)	0.36 $\pm$ 0.21 (a)	0.38 $\pm$ 0.28 (a)
Cu	1.78 $\pm$ 0.87 (b)	0.79 $\pm$ 0.38 (a)	0.80 $\pm$ 0.42 (a)
Fe	<b>1,103 <math>\pm</math> 398</b> (b)	201 $\pm$ 130 (a)	196 $\pm$ 131 (a)
Pb	0.35 $\pm$ 0.11 (b)	0.07 $\pm$ 0.04 (a)	0.05 $\pm$ 0.04 (a)
Mn	118.0 $\pm$ 65.0 (b)	54.0 $\pm$ 22.9 (a)	38.1 $\pm$ 21.1 (a)
Ni	1.29 $\pm$ 0.60 (a)	1.56 $\pm$ 0.55 (a)	2.77 $\pm$ 0.68 (b)
Se	0.10 $\pm$ 0.04 (a)	0.45 $\pm$ 0.05 (b)	0.49 $\pm$ 0.09 (b)
Ag	0.002 $\pm$ 0.00 (a)	0.002 $\pm$ 0.00 (a)	0.003 $\pm$ 0.00 (a)
Sr	30.0 $\pm$ 6.6 (a)	155.0 $\pm$ 7.0 (b)	251.0 $\pm$ 29.0 (c)
Tl	0.005 $\pm$ 0.00 (a)	0.02 $\pm$ 0.00 (b)	0.04 $\pm$ 0.01 (c)
V	0.59 $\pm$ 0.22 (a)	0.31 $\pm$ 0.06 (a)	0.42 $\pm$ 0.08 (a, b)
Zn	5.59 $\pm$ 2.48 (a)	3.20 $\pm$ 1.53 (a)	4.14 $\pm$ 1.83 (a)

<sup>a</sup>USEPA (2018) AWQC ( $\mu\text{g/L}$ ) (acute, chronic): Al, Cu: could not be calculated due to lack of required water-quality parameters, As: 340, 150; Cr<sup>III</sup>: 570,74; Cr<sup>VI</sup>: 16,11; Fe: 1000 (chronic); Cd: 0.30, 0.16 (NANJ); 1.22, 0.48 (BBDO); 1.57, 0.59 (BBUP); Pb: 7.77, 0.30 (NANJ); 40.97, 1.60 (BBDO); 55.48, 2.16 (BBUP); Ni: 93.40, 10.41 (NANJ); 326.3, 36.31 (BBDO); 412.0, 45.84 (BBUP); Zn: 22.39, 22.97 (NANJ); 76.43, 79.19 (BBDO); 96.09, 99.74 (BBUP); Se: 3.1 (30-day); Ag: 3.2.

<sup>b</sup>Minimum detection limits ( $\mu\text{g/L}$ ): Al, 0.200; Sb, 0.005; As, 0.006; Ba, 0.008; Be, 0.010; Cd, 0.003; Cr, 0.011; Co, 0.004; Cu, 0.281; Fe, 0.320; Pb, 0.019; Mn, 0.043; Ni, 0.011; Se, 0.018; Ag, 0.002; Sr, 0.028; Tl, 0.005; V, 0.051; Zn, 0.109.

of monitoring at each site and trimmed the data to June 1, 2014, through October 31, 2014, to include only the full months when all sites were monitored.

### Data Analysis

At the beginning of the study, we composited all peeper samples to ensure that we had adequate sample volume to complete all of the analyses. After the July sampling, we realized that we could complete the nutrient analyses with smaller sample volumes than expected, which allowed us to assess the variability between individual peepers. Thus, we began analyzing the three peepers for separate nutrient samples in August. We calculated the trimean (TM =  $0.5 [Q_2 + 0.5 (Q_1 + Q_3)]$  where Q = Quartile), instead of the arithmetic mean, to generate a more representative single estimate of pore-water nutrients for each sampling event.

We summarized monthly water-chemistry data as means with 95% confidence intervals (CI) in package *Rmisc* (Hope 2013) in R (R Core Team 2014). Distributions of 11 of the 75 analyte estimates (25 analytes at each site) for surface-water chemistry deviated from normality (Kolmogorov-Smirnov test,  $D = 0.10$ – $0.38$ ;  $P = 0.003$ – $0.99$ ). Distributions of 18 of the 75 analyte estimates for pore-water chemistry deviated from normality ( $D = 0.11$ – $0.49$ ,  $P = 0.00005$ – $0.99$ ). Most variables that exhibited non-normal distributions were dis-

solved metals with very low concentrations over a narrow range of values. However, more than half of the analytes had unequal variances among sites for both surface-water analytes (Levene's test,  $F = 0.12$ – $26.01$ ,  $P = 0.000005$ – $0.89$ ) and pore-water analytes ( $F = 0.60$ – $23.37$ ;  $P = 0.0001$ – $0.56$ ). Heterogeneous variances were observed for anions, nutrients, and metals.



Figure 2. Photograph of peeper assembly.

Since our objective was to determine if water-chemistry data fit a particular pattern and not if means were equal across all sites, we avoided the omnibus ANOVA and substituted multiple comparison tests to assess differences in chemical parameters between sites. We calculated Tukey HSD tests using the R package *multcomp* (Hothorn et al. 2008) from mean-square results of one-way ANOVAs with adjustment for heteroscedastic data (Long and Ervin 2000) using the R package *car* (Fox and Weisberg 2011). Due to the small experimental sample size and multiple statistical comparisons, we would expect to find significant differences in at least some chemical concentrations among sites that fit our hypothesized pattern of a stressor by chance under a null-hypothesis testing framework with a rigid alpha (e.g.,  $\alpha = 0.05$ ). Therefore, we placed similar weight on confidence intervals of mean concentrations in determining whether or not an analyte fits the pattern of BBDO > BBUP = NANJ. That is, results from HSD tests were not meant solely to firmly accept or reject, but instead to provide support for focusing on specific analytes to investigate in a more intensive study.

We compared mean analyte concentrations at each site with Ambient Water Quality Criteria (AWQC) (USEPA 2018). For hardness-dependent criteria (e.g., Ag, Cd, Cu, Ni, Pb, and Zn), we used hardness values measured previously by MBSS at our sites (NANJ = 16 mg/L as  $\text{CaCO}_3$ ; BBDO = 66; BBUP = 87; <https://dnr.maryland.gov/streams/Pages/dataRequest.aspx>; accessed May 14, 2019). We compared TAN concentrations with AWQC for each sample event. We calculated these concentrations based on pH and temperature. We used temperature measurements obtained when our surface-water samples were collected. We did not use pH values collected simultaneously with surface-water samples because our values were not consistent with prior measurements at the sites by MBSS, which suggested probe malfunction. Instead, we calculated mean ( $\pm$  95% CI) values from the antilog of pH measured previously by MBSS. The pH values we used for AWQC were 6.38 ( $\pm$  0.22) for NANJ, 7.01 ( $\pm$  0.11) for BBDO, and 7.33 ( $\pm$  0.20) for BBUP.

We estimated the fraction of un-ionized ammonia (UIA-N  $\mu\text{g/L}$ ) in surface and pore water following Thurston et al. (1979). In addition to AWQC, we compared pore-water UIA-N with the 0.2  $\mu\text{g/L}$  UIA-N pore-water threshold for recruitment failure in *Elliptio complanata* proposed by Strayer and Malcom (2012). For nutrients, we compared surface-water concentrations with low, moderate, and high categories for Maryland streams (Southerland et al. 2005).

## RESULTS

### Temperature, Conductivity, and DO

Mean monthly (June through October) surface-water and sediment temperatures were nearly identical at BBDO (surface and sediment both = 18.9°C) and BBUP (surface = 18.9°C, sediment = 18.8°C). Mean monthly temperatures were about 1.0–1.5°C higher at NANJ (surface = 20.4°C; sediment =

20.2°C). The maximum surface-water temperatures observed at each stream were NANJ, 27.1°C; BBDO, 25.7°C; and BBUP, 24.6°C. Maximum sediment temperatures were NANJ, 25.3°C; BBDO, 24.8°C; and BBUP, 24.2°C.

Conductivity ( $\mu\text{S/cm}$ ) in surface and pore water ranged from 39 to 149 at NANJ, 190 to 214 at BBDO, and 226 to 299 at BBUP. Surface-water DO exceeded Maryland's water quality criterion of 5 mg/L (MDE 2019) on all sample dates at all sites. In contrast, pore-water DO rarely exceeded this criterion, and all sites had values less than 3 mg/L (Table 1).

### Ammonia

Surface-water and pore-water TAN concentrations did not exceed the AWQC at any site on any date (Table 1). The two highest-observed values were in pore water at BBDO (0.951 and 0.639 mg/L) and were 23% and 38% of the chronic AWQC, respectively. The maximum pore-water concentration at NANJ was 0.267 mg/L (14% of the chronic AWQC) and 0.106 mg/L (4% of the chronic AWQC) at BBUP. Compared with the Southerland et al. (2005) categories for Maryland MBSS surface-water data, TAN concentrations were low (<0.03 mg/L) and moderate (0.03 to 0.07 mg/L) at NANJ, moderate and high (>0.07 mg/L) at BBDO, and mostly low at BBUP.

Differences in mean TAN concentrations among sites for both surface water and pore water supported our hypothesis of BBDO > BBUP = NANJ (Tables 2 and 4). Differences in mean pore-water UIA-N concentrations among sites were consistent with our hypothesis, but differences in surface-water UIA-N were not. Pore-water UIA-N concentrations exceeded the 0.2  $\mu\text{g/L}$  threshold of Strayer and Malcom (2012) in five of six measurements at BBDO, including two observations >2.0  $\mu\text{g/L}$  (Table 1). Two of six measurements at BBUP (0.59 and 0.86  $\mu\text{g/L}$ ) and one of six at NANJ (0.30  $\mu\text{g/L}$ ) exceeded the threshold. In general, UIA-N concentrations were substantially lower in surface water than in pore water, except for two events at BBUP and one at BBDO.

### Nitrate-N, Nitrite-N, and Phosphate

There are no AWQC for  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ , or  $\text{PO}_4$ . Differences in  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ , and  $\text{PO}_4$  concentrations among sites did not support our hypothesis of BBDO > BBUP = NANJ. Mean surface-water  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  were both significantly higher at both BB sites compared with NANJ, and there were no significant differences between BBDO and BBUP (Table 2). For  $\text{NO}_3\text{-N}$ , both BB sites were categorized as high following Southerland et al. (2005). Mean pore-water  $\text{NO}_3\text{-N}$  at BBUP was significantly higher compared with results from BBDO and NANJ, which did not differ from each other (Table 4). Mean pore-water  $\text{NO}_2\text{-N}$  did not differ among sites. The mean surface-water  $\text{PO}_4$  concentration at BBUP was significantly higher than the concentrations at NANJ and BBDO, which were similar to each other (Table 2). Pore-water  $\text{PO}_4$  concentration was intermediate at BBDO and was not

Table 4. Mean ( $\pm$  95% CI) ion and nutrient concentrations in six, monthly pore-water samples from Nanjemoy Creek (NANJ), lower Browns Branch (BBDO), and upper Browns Branch (BBUP). Sites with different letters in parentheses have significantly different means (Tukey's HSD,  $P < 0.05$ ). All units are mg/L except UIA-N ( $\mu\text{g/L}$ ). See Table 2 for detection limits.

Analyte	NANJ	BBDO	BBUP
Cl	5.89 $\pm$ 2.55 (a)	10.57 $\pm$ 4.71 (a)	18.56 $\pm$ 2.20 (b)
TAN	0.11 $\pm$ 0.12 (a)	0.42 $\pm$ 0.36 (b)	0.04 $\pm$ 0.05 (a)
UIA-N <sup>a</sup>	0.10 $\pm$ 0.12 (a)	<b>1.47 <math>\pm</math> 1.16</b> (b)	<b>0.31 <math>\pm</math> 0.35</b> (a)
NO <sub>3</sub> -N	0.01 $\pm$ 0.01 (a)	0.82 $\pm$ 1.35 (a)	4.03 $\pm$ 1.28 (b)
NO <sub>2</sub> -N	0.002 $\pm$ 0.001 (a)	0.06 $\pm$ 0.11 (a)	0.05 $\pm$ 0.05 (a)
PO <sub>4</sub>	0.004 $\pm$ 0.002 (a)	0.03 $\pm$ 0.02 (a, b)	0.05 $\pm$ 0.02 (b)
SO <sub>4</sub>	0.96 $\pm$ 1.11 (a)	5.15 $\pm$ 3.65 (a)	25.02 $\pm$ 4.18 (b)

<sup>a</sup>Mean ion and nutrient concentrations exceeding the Strayer and Malcom (2012) threshold of 0.2  $\mu\text{g/L}$  are in bold.

significantly different from those concentrations at NANJ or BBUP (Table 4).

### Chloride and Sulfate

Chloride concentrations did not exceed the acute or chronic AWQC in any samples. The maximum concentration was 26.2 mg/L in a surface-water sample from BBUP, about 10% of the chronic criterion of 260 mg/L (Table 2). Differences in mean Cl concentrations among sites did not support our hypothesis of BBDO > BBUP = NANJ. Surface-water mean concentrations were not significantly different between the two BB sites, but both were significantly higher (about double) than that at NANJ. Pore-water Cl mean concentrations were significantly higher at BBUP compared with those at BBDO and NANJ (Table 4).

There are no AWQC for SO<sub>4</sub>. Differences in SO<sub>4</sub> concentrations among sites did not support our hypothesis of BBDO > BBUP = NANJ. In surface water, SO<sub>4</sub> differed significantly among all three sites with the highest value at BBUP and a much lower value at NANJ (Table 2). SO<sub>4</sub> varied similarly in pore water, but it was significantly higher at BBUP than at BBDO and NANJ, both of which had low values and did not differ from each other (Table 4).

### Metals

Iron was the only metal detected that exceeded the AWQC. For surface water, the mean Fe concentration (1,103  $\mu\text{g/L}$ ) exceeded the chronic AWQC of 1,000  $\mu\text{g/L}$  (there is no acute criterion) at NANJ, but values at BBDO and BBUP were much lower (Table 3). For pore water, the mean Fe concentration exceeded the chronic AWQC at NANJ and BBDO but not at BBUP (Table 5). Concentrations of no other metals closely approached AWQC. There were significant differences in mean concentrations of many metals among sites (Tables 3 and 5), but none supported our hypothesis of BBDO > BBUP = NANJ.

Table 5. Mean ( $\pm$  95% CI) dissolved metals concentrations ( $\mu\text{g/L}$ ) in six, monthly pore-water samples from Nanjemoy Creek (NANJ), lower Browns Branch (BBDO), and upper Browns Branch (BBUP). Sites with different letters in parentheses have significantly different means (Tukey's HSD,  $P < 0.05$ ). See Table 3 for detection limits. Concentrations that exceeded a USEPA AWQC (see Table 3) are in bold.

Analyte	NANJ	BBDO	BBUP
Al	79.6 $\pm$ 154.0 (a)	18.8 $\pm$ 30.4 (a)	23.8 $\pm$ 22.4 (a)
Sb	0.65 $\pm$ 0.70 (a)	0.18 $\pm$ 0.14 (a)	0.11 $\pm$ 0.11 (a)
As	2.24 $\pm$ 1.69 (a)	2.38 $\pm$ 2.46 (a)	0.80 $\pm$ 1.01 (a)
Ba	45.1 $\pm$ 21.3 (a)	61.5 $\pm$ 21.1 (a)	66.8 $\pm$ 8.1 (a)
Be	0.05 $\pm$ 0.05 (a)	0.01 $\pm$ 0.01 (a)	0.03 $\pm$ 0.01 (a)
Cd	0.01 $\pm$ 0.01 (a)	0.03 $\pm$ 0.04 (a, b)	0.06 $\pm$ 0.03 (b)
Cr	0.18 $\pm$ 0.23 (a)	0.11 $\pm$ 0.06 (a)	0.14 $\pm$ 0.07 (a)
Co	3.37 $\pm$ 3.06 (b)	1.89 $\pm$ 1.49 (a, b)	0.20 $\pm$ 0.17 (a)
Cu	0.56 $\pm$ 0.45 (a)	0.27 $\pm$ 0.06 (a)	0.59 $\pm$ 0.30 (a)
Fe	<b>1,220 <math>\pm</math> 1,192</b> (a)	<b>1,753 <math>\pm</math> 2,958</b> (a)	79 $\pm$ 71 (a)
Pb	0.37 $\pm$ 0.50 (a)	0.10 $\pm$ 0.12 (a)	0.09 $\pm$ 0.07 (a)
Mn	1,249 $\pm$ 1,021 (b)	406 $\pm$ 375 (a, b)	16 $\pm$ 18 (a)
Ni	1.05 $\pm$ 0.80 (a, b)	0.96 $\pm$ 0.55 (a)	1.99 $\pm$ 0.59 (b)
Se	0.06 $\pm$ 0.06 (a)	0.10 $\pm$ 0.03 (a)	0.31 $\pm$ 0.11 (b)
Ag	0.003 $\pm$ 0.01 (a)	0.002 $\pm$ 0.01 (a)	0.006 $\pm$ 0.01 (a)
Sr	19 $\pm$ 8 (a)	69 $\pm$ 28 (b)	203 $\pm$ 27 (c)
Tl	0.005 $\pm$ 0.01 (a)	0.01 $\pm$ 0.01 (a)	0.04 $\pm$ 0.02 (b)
V	0.57 $\pm$ 0.53 (a)	0.40 $\pm$ 0.30 (a)	0.47 $\pm$ 0.23 (a)
Zn	20.1 $\pm$ 9.9 (a)	17.5 $\pm$ 9.6 (a)	15.4 $\pm$ 5.8 (a)

### DISCUSSION

Nutrient concentrations in the agriculturally dominated landscape of eastern Maryland are among the highest in the nation (Denver et al. 2004). The region contains most of the state's extant Dwarf Wedgemussel populations, and most historical (but extirpated) populations in Maryland occurred in the region (Bogan and Ashton 2016). Concentrations of ammonia in sediment often increase in agriculturally dominated landscapes with high reactive nitrogen load (Strayer 2014), such as Browns Branch. We note, however, the similar percentages of agriculture in catchments of BBUP and BBDO in 2001 and 2011 and are thus unable to link land-use differences or changes with the observed range contraction.

Although TAN concentrations at BBDO did not exceed AWQC, conditions stressful to juvenile freshwater mussels may have occurred, based on pore-water UIA-N concentrations that exceeded the threshold of 0.2  $\mu\text{g/L}$  (five of six measurements) proposed for *E. complanata* (Strayer and Malcom 2012). UIA-N concentrations also exceeded this threshold at BBUP and NANJ, but the frequency and magnitude of exceedance were much lower than at BBDO. Thus, elevated UIA-N can be considered a potential cause of Dwarf Wedgemussel range contraction in BB because it supports our hypothesis about the spatial distribution and concentrations of such a factor (BBDO > BBUP = NANJ). Furthermore, the concentrations reported in the peepers are long-term averages and may underestimate transient peak



concentrations to which juvenile mussels may be exposed (Strayer and Malcom 2012).

Mussels at BBDO may have been exposed to the combined stressors of high UIA-N and low DO in pore water. Low pore-water DO favors the long-term presence of ammonia because higher oxygen conditions are needed for oxidation of ammonia to nitrate (Kinsman-Costello et al. 2015). Low oxygen in sediments can result in death of juvenile mussels or increase their susceptibility to predators (Sparks and Strayer 1998). Although low DO was detected in pore water at all sites, TAN concentrations were much higher at BBDO. In general, the lower-DO pore water contained higher concentrations of ammonia than the higher-DO surface water. The highest UIA-N concentrations occurred in summer, but no sites experienced temperatures approaching the 29°C thermal limit proposed for Dwarf Wedgemussel (Campbell 2014).

Other factors not measured in this study could influence Dwarf Wedgemussel distribution and merit further examination. The only documented host fish of Dwarf Wedgemussel that co-occurs in Maryland is the Tessellated Darter (Ashton 2010). On average, their abundance was three times lower at BBDO compared with BBUP (M. J. Ashton, MDNR, unpublished data), which may limit Dwarf Wedgemussel reproduction and dispersal (McClain and Ross 2005). Additionally, many herbicides are commonly detected in agricultural streams of eastern Maryland (Denver et al. 2004). The effects of current-use pesticides on mussels are not as well studied as nutrients or metals (but see Bringolf et al. 2007). *Elliptio complanata* also has been found to integrate transgenic material via bacteria uptake near cornfields, which may weaken its immune system (Gagne et al. 2006; Douville et al. 2009). Whether this could occur in Dwarf Wedgemussels is unknown.

There are several sources of uncertainty in the study. First, the strength of our conclusions is limited because measurements were made only monthly within a single year. Additional sampling is necessary to further characterize inter- and intra-annual variation in pore-water and surface-water quality. Furthermore, the Strayer and Malcom (2012) threshold has not been replicated in other studies with *E. complanata* or examined for other species. Thus, Dwarf Wedgemussel may be more or less sensitive than *E. complanata* to pore-water UIA-N.

We recommend conducting a survey of Browns Branch to assess the current condition of the mussel fauna. If range contraction is still evident, more frequent and extended water-quality parameter and surface- and pore-water sampling should be performed, including capturing high-flow events to determine if pulses of ammonia and other pollutants occur. Sampling tributaries and areas of groundwater input also could identify sources of pollutants.

## ACKNOWLEDGMENTS

We appreciate the assistance and coordination provided by Julie Slacum of the Chesapeake Bay Field Office, U.S. Fish

and Wildlife Service, which provided the funding for the project. Matt Ashton, Maryland Department of Natural Resources, provided his expertise on mussel ecology and statistical analysis. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

## LITERATURE CITED

- APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater. 20th ed. American Public Health Association, Washington, DC. 874 pp.
- Ashton, M. J. 2010. Freshwater mussel records collected by the Maryland Department of Natural Resources' Monitoring and Non-Tidal Assessment Division (1995–2009): Investigating environmental conditions and host fishes of select species. RAS-MANTA-AIM-10-01. Maryland Department of Natural Resources, Annapolis. Available at [https://dnr.maryland.gov/streams/Publications/AIM\\_10\\_01\\_mussels.pdf](https://dnr.maryland.gov/streams/Publications/AIM_10_01_mussels.pdf) (accessed December 12, 2019).
- Ashton, M. J. 2012. How a statewide stream survey can aid in understanding freshwater mussel (*Bivalvia*: Unionidae) ecology: examples of utility and limitations from Maryland. *Freshwater Mollusk Biology and Conservation* 15:1–11.
- Ashton, M. J., J. Zimmerman, and J. M. McCann. 2013. Distribution and status of Dwarf Wedgemussel (*Alasmidonta heterodon*) in Browns Branch and Southeast Creek UT, Queen Anne's County, MD. Report to the Chesapeake Bay Field Office, U.S. Fish and Wildlife Service, Annapolis, Maryland.
- Bogan, A. E., and M. J. Ashton. 2016. Manual of the freshwater bivalves of Maryland. CBWP-MANTA-EA-96-03. Maryland Department of Natural Resources, Annapolis. Available at <https://dnr.maryland.gov/streams/Publications/Manual-Freshwater%20Mussels%20of%20MD.pdf> (accessed December 12, 2019).
- Boynton, W. R., J. H. Garber, R. Summers, and W. M. Kemp. 1995. Inputs, transformations, and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. *Estuaries* 18:285–314.
- Brim Box, J., and J. Mossa. 1999. Sediment, land use, and freshwater mussels: Prospects and problems. *Journal of the North American Benthological Society* 18:99–117.
- Bringolf, R. B., W. G. Cope, C. B. Eads, P. R. Lazaro, M. C. Barnhart, and D. Shea. 2007. Acute and chronic toxicity of technical-grade pesticides to glochidia and juveniles of freshwater mussels (unionidae). *Environmental Toxicology and Chemistry* 26:2086–2093.
- Campbell, C. A. 2014. Identifying the elusive Dwarf Wedgemussel habitat through modeling and field approaches. Doctoral dissertation. University of Maryland, College Park. Available at [https://drum.lib.umd.edu/bitstream/handle/1903/15322/Campbell\\_umd\\_0117E\\_15132.pdf;sequence=1](https://drum.lib.umd.edu/bitstream/handle/1903/15322/Campbell_umd_0117E_15132.pdf;sequence=1) (accessed June 3, 2019).
- Cleaves, E.T., J. Edwards, Jr., and J. D. Glaser. 1968. Geologic map of Maryland. Maryland Geological Survey, Baltimore.
- Denver, J. M., S. W. Ator, L. M. DeBrewer, M. J. Ferrari, J. R. Barbaro, T. C. Hancock, M. J. Brayton, and M. R. Nardi. 2004. Water quality in the Delmarva peninsula, Delaware, Maryland, and Virginia, 1999–2001. Circular 1228. U.S. Geological Survey, Washington, DC. Available at <https://pubs.usgs.gov/circ/2004/1228> (accessed June 3, 2019).
- Douville, M., F. Gagne, C. Andre, and C. Blaise. 2009. Occurrence of the transgenic corn *cryIAb* gene in freshwater mussels (*Elliptio complanata*) near corn fields: Evidence of exposure by bacterial ingestion. *Ecotoxicology and the Environment* 72:17–25.
- Fox, J., and S. Weisberg. 2011. An R Companion to Applied Regression. 2nd ed. Sage Publishers, Thousand Oaks, California. Available at <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion> (accessed February 11, 2020).



- Gagne, F., T. Edge, M. Douville, and C. Blaise. 2006. Effects of commercial microbial products on the immune system of *Elliptio complanata* mussels. *Bulletin of Environmental Contamination and Toxicology* 76:848–854.
- Gascho Landis, A. M., W. R. Haag, and J. A. Stoeckel. 2012. High suspended solids as a factor in reproductive failure of a freshwater mussel. *Freshwater Science* 32:70–81.
- Gillis, P. L. 2011. Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters. *Environmental Pollution* 159:1702–1708.
- Gillis, P. L., R. McInnis, J. Salerno, J., S. R. de Solla, M. R., Servos, and E. M. Leonard. 2017. Freshwater mussels in an urban watershed: Impacts of anthropogenic inputs and habitat alterations on populations. *Science of the Total Environment* 574:671–679.
- Haag, W. R. 2012. *North American freshwater mussels: Natural history, ecology, and conservation*. Cambridge University Press, Cambridge, United Kingdom.
- Haag, W. R., and J. D. Williams. 2014. Biodiversity on the brink: An assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia* 735:45–60.
- Homer, C. C., J. Dewitz, J. Fry, M. Coan, N. Hossain, C. Larson, N. Herold, A. McKerrrow, J. N. VanDriel, and J. Wickham. 2007. Completion of the 2001 National Landcover Database for the conterminous United States. *Photogrammetric Engineering and Remote Sensing* 73: 337–341.
- Homer, C., J. Dewitz, L. Yang, S. Jin, P. Danielson, G. Xian, J. Coulston, N. Herold, J. Wickham, and K. Megown. 2015. Completion of the 2011 National Land Cover Database for the conterminous United States—Representing a decade of land cover change information. *Photogrammetric Engineering and Remote Sensing* 81:345–354.
- Hope, R. M. 2013. Rmisc: Ryan Miscellaneous. R package version 1.5. Available at <https://CRAN.R-project.org/package=Rmisc> (accessed February 11, 2020).
- Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50:346–363.
- Johnson, G. C., J. L. Krstolic, and B. J. Ostby. 2014. Influences of water and sediment quality and hydrologic processes on mussels in the Clinch River. *Journal of the American Water Resources Association* 50:878–897.
- Kemp, W. M., W. R. Boynton, J. E. Adolf, D. F. Boesch, W. C. Boicourt, G. Brush, J. C. Cornwell, T. R. Fisher, P. M. Gilbert, J. D. Hagy, L. W. Harding, E. D. Houde, D. G. Kimmel, W. D. Miller, R. I. E. Newell, M. R. Roman, E. M. Smith, and J. C. Stevenson. 2005. Eutrophication of Chesapeake Bay: Historical trends and ecological interactions. *Marine Ecology Progress Series* 303:1–29.
- Kinsman-Costello, L. E., J. M. O'Brien, and S. K. Hamilton. 2015. Natural stressors in uncontaminated sediments of shallow freshwaters: The prevalence of sulfide, ammonia, and reduced iron. *Environmental Toxicology and Chemistry* 34: 467–479.
- Long, J. S., and L. H. Ervin. 2000. Using heteroscedasticity consistent standard errors in the linear regression model. *American Statistician* 54:217–224.
- MBSS (Maryland Biological Stream Survey). 2007. Sampling manual: Field methods. CBWP-MANTA-EA-07-01. Maryland Department of Natural Resources, Annapolis. Available at [https://dnr.maryland.gov/streams/Publications/ea-07-01b\\_fieldRevMay2007.pdf](https://dnr.maryland.gov/streams/Publications/ea-07-01b_fieldRevMay2007.pdf); (accessed January 3, 2020).
- McClain, D. C., and M. R. Ross. 2005. Reproduction based on local patch size of *Alasmidonta heterodon* and dispersal by its darter host in the Mill River, Massachusetts, USA. *Journal of the North American Benthological Society* 24:139–147.
- MDE (Maryland Department of the Environment). 2019. Water quality criteria specific to designated uses. COMAR 26.08.02.03-3. MDE, Annapolis. Available at <http://www.dsd.state.md.us/comar/comarhtml/26/26.08.02.03-3.htm> (accessed December 19, 2019).
- Michaelson, D. L., and R. J. Neves. 1995. Life history and habitat of the endangered Dwarf Wedgemussel *Alasmidonta heterodon* (Bivalvia: Unionidae). *Journal of the North American Benthological Society* 14:324–340.
- Newton, T. J., J. W. Allran, J. A. O'Donnell, M. R. Bartsch, and W. B. Richardson. 2003. Effects of ammonia on juvenile unionid mussels (*Lampsilis cardium*) in laboratory sediment toxicity tests. *Environmental Toxicology and Chemistry* 22:2554–2560.
- R Core Team. 2014. R: A language and environment for statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at <https://www.R-project.org/> (accessed February 11, 2020).
- Southerland, M. T., L. A. Erb, G. M. Rogers, and P. F. Kazyak. 2005. Maryland biological stream survey 2000–2004 volume 7: Statewide and tributary basin results. DNR-12-0305-0109. Maryland Department of Natural Resources, Annapolis. Available at <http://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=A07C0ADDE34AC68A90940A6712A0A1C5?doi=10.1.1.599.3529&rep=rep1&type=pdf> (accessed May 5, 2019).
- Sparks, B. L., and D. L. Strayer. 1998. Effects of low dissolved oxygen on juvenile *Elliptio complanata* (Bivalvia: Unionidae). *Journal of the North American Benthological Society* 17:129–134.
- Strayer, D. L. 2014. Understanding how nutrient cycles and freshwater mussels (Unionoida) affect one another. *Hydrobiologia* 735:277–292.
- Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton, and J. S. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* 54:429–439.
- Strayer, D. L., and H. M. Malcom. 2012. Causes of recruitment failure in freshwater mussel populations in southeastern New York. *Ecological Applications* 22:1780–1790.
- Strayer, D. L., S. J. Sprague, and S. Claypool. 1996. A range-wide assessment of populations of *Alasmidonta heterodon*, an endangered freshwater mussel (Bivalvia: Unionidae). *Journal of the North American Benthological Society* 15:308–317.
- Teasdale, P. R., G. E. Batley, S. C. Apte, and I. T. Webster. 1995. Pore water sampling with sediment peepers. *Trends in Analytical Chemistry* 14:250–256.
- Thurston, R. V., R. G. Russo, and K. Emerson. 1979. Aqueous ammonia equilibrium tabulation of percent un-ionized ammonia. EPA-600/3-79-091. U.S. Environmental Protection Agency, Duluth, Minnesota.
- USEPA (U.S. Environmental Protection Agency). 1987. *Handbook of Methods for Acid Deposition Studies: Laboratory Analyses for Surface Water Chemistry*. USEPA, Washington, DC. 376 pp.
- USEPA (U.S. Environmental Protection Agency). 1998. Method 6020A (SW-846): Inductively coupled plasma-mass spectrometry, revision 1. USEPA, Washington, DC.
- USEPA (U.S. Environmental Protection Agency). 2013. Aquatic life ambient water quality criteria for ammonia—Freshwater. 822-R-13-001. USEPA, Washington, DC.
- USEPA (U.S. Environmental Protection Agency). 2018. National recommended water quality criteria—aquatic life criteria table. USEPA, Washington, DC. Available at <https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table#table> (accessed December 19, 2019).
- Wang, N., R. J. Erickson, C. G. Ingersoll, C. D., Ivey, E. L. Brunson, T. Augspurger, and M. C. Barnhart. 2008. Influence of pH on the acute toxicity of ammonia to juvenile freshwater mussels (fatmucket, *Lampsilis siliquoidea*). *Environmental Toxicology and Chemistry* 27:1141–1146.
- Wang, N., C. D. Ivey, C. G. Ingersoll, W. G. Brumbaugh, D. Alvarez, E. J. Hammer, C. R. Bauer, T. Augspurger, S. Raimondo, and M. C. Barnhart. 2017. Acute sensitivity of a broad range of freshwater mussels to chemicals with different modes of toxic action. *Environmental Toxicology and Chemistry* 36:786–796.

## Appendix

The University of Maryland Center for Environmental Science Appalachian Laboratory (UMCES, Frostburg, MD)

Table A1. Summary of UMCES results from 2014 proficiency test.

Analyte	Rating
Total ammonia nitrogen	Ideal
Nitrate-N	Ideal
Sulfate	Ideal
Chloride	Flagged low on 1 sample

employs a rigorous Quality Assurance/Quality Control (QA/QC) program, which includes ongoing monitoring and evaluation of precision and accuracy (analysis of duplicates, matrix spikes, method blanks, and independent control samples with acceptance criteria that must be met to accept analytical results), as well as participation in blind audits, proficiency tests (PT), and split-sample programs. Most of

these practices are outlined in a report of the Chesapeake Bay Program Data Integrity Workgroup (EPA Chesapeake Bay Program 2017). Analysis of method blank, matrix spike, laboratory duplicate, and independent control sample results indicates acceptable laboratory performance. The results for some of the measured constituents from a 10-sample PT study conducted during the project time period (Table A1) and analysis of the independent control sample for metals (Table A2) help document the quality of UMCES' performance.

## APPENDIX REFERENCE

Environmental Protection Agency (EPA) Chesapeake Bay Program. 2017. Methods and Quality Assurance for Chesapeake Bay Water Quality Monitoring Programs. CBP/TRS-319-17. EPA, Annapolis, Maryland. Available at <https://www.chesapeakebay.net/documents/CBPMETHODSMANUALMAY2017.pdf> (accessed January 10, 2020).

Table A2. Summary of UMCES results from the analysis of the independent control sample for metals. RSD: Relative standard deviation.

Analyte	Target (µg/L)	Mean	Count	Standard Deviation	Minimum	Maximum	% RSD
Be	10	10.4	17	0.34	9.9	10.9	3.27
Al	40	43.6	17	1.06	41.0	45.7	2.44
V	60	61.6	17	1.16	59.8	63.6	1.88
Cr	30	30.4	17	0.50	29.8	31.2	1.65
Mn	90	90.6	17	1.31	88.4	92.4	1.44
Fe	80	80.8	17	1.06	79.1	82.9	1.31
Co	60	61.9	17	0.98	60.1	63.1	1.59
Ni	80	82.6	17	0.56	81.7	83.6	0.68
Cu	40	41.8	17	0.37	41.1	42.3	0.88
Zn	50	51.3	17	0.73	50.2	52.6	1.41
As	20	20.7	17	0.35	20.3	21.4	1.68
Se	10	10.3	17	0.13	10.1	10.5	1.21
Sr	40	40.1	17	1.37	37.7	41.6	3.41
Ag	20	20.4	17	1.93	19.0	27.7	9.43
Cd	20	19.9	17	0.45	19.2	20.6	2.25
Sb	10	10.0	17	0.22	9.71	10.4	2.20
Ba	10	9.94	17	0.30	9.49	10.5	2.97
Tl	10	10.1	17	0.22	9.75	10.4	2.19
Pb	40	40.8	17	0.80	39.6	41.9	1.96