Article

Effects of High Salinity Wastewater Discharges on Unionid Mussels in the Allegheny River, Pennsylvania

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Abstract

We examined the effect of high salinity wastewater (brine) from oil and natural gas drilling on freshwater mussels in the Allegheny River, Pennsylvania, during 2012. Mussel cages ($N = 5$ per site) were deployed at two sites upstream and four sites downstream of a brine treatment facility on the Allegheny River. Each cage contained 20 juvenile northern riffleshell mussels *Epioblasma torulosa rangiana*). Continuous specific conductance and temperature data were recorded by water quality probes deployed at each site. To measure the amount of mixing throughout the entire study area, specific conductance surveys were completed two times during low-flow conditions along transects from bank to bank that targeted upstream (reference) reaches, a municipal wastewater treatment plant discharge upstream of the brine-facility discharge, the brine facility, and downstream reaches. Specific conductance data indicated that high specific conductance water from the brine facility (4,000–12,000 µS/cm; mean 7,846) compared to the reference reach (103–188 µS/cm; mean 151) is carried along the left descending bank of the river and that dilution of the discharge via mixing does not occur until 0.5 mi (805 m) downstream. Juvenile northern riffleshell mussel survival was severely impaired within the high specific conductance zone (2 and 34% at and downstream of the brine facility, respectively) and at the municipal wastewater treatment plant (21%) compared to background (84%). We surveyed native mussels (family Unionidae) at 10 transects: 3 upstream, 3 within, and 4 downstream of the high specific conductance zone. Unionid mussel abundance and diversity were lower for all transects within and downstream of the high conductivity zone compared to upstream. The results of this study clearly demonstrate in situ toxicity to juvenile northern riffleshell mussels, a federally endangered species, and to the native unionid mussel assemblage located downstream of a brine discharge to the Allegheny River.

Keywords: mussels; endangered; conductivity; chloride; toxicity; brine; wastewater

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Introduction

Oil and natural gas development and extraction have occurred in western Pennsylvania for >150 y. Natural gas extraction increased from 2008 to 2012 in Pennsylvania, Ohio, and West Virginia as a result of technological advances in drilling. In Pennsylvania alone, 30,784 new drilling permits for oil, gas, and coal bed methane were issued between 2008 and 2012 compared to 12,324 in 2000 to 2004 (Pennsylvania Department of Environmental Protection [PADEP] 2013). Extraction of petroleum produces high salinity water that flows to the surface in advance of the oil or gas. This sodium–calcium–chloride solution (hereafter “brine”) is more concentrated than seawater and is produced by more than 95% of the Pennsylvania oil and gas wells sampled to date (Dresel and Rose 2010). Discharge of brine from oil and gas drilling has been linked anecdotally to dramatic declines of mussels of the family Unionidae (Williams 1969), yet substantiating field studies are lacking. Considering the continued and expanding development of oil and natural gas reserves, it is important to understand the effects of permitted discharges of brine treatment wastewater and accidental releases of brine on unionid mussels, particularly at-risk mussels, some of which are confined to streams that continue to receive substantial discharges of high salinity wastewater.

The PADEP is evaluating a statewide chloride water quality criterion for the protection of aquatic life based on U.S. Environmental Protection Agency (EPA) and Iowa evaluations. The current EPA 304(a) national criteria for chloride are 860 mg/L for acute exposures and 230 mg/L for chronic exposures (EPA 1988). The EPA has obtained additional toxicity testing data (EPA 2008). The acute toxicity of chloride to four freshwater invertebrate species (water flea *Ceriodaphnia dubia*, fingernail clams *Sphaerium simile* and *Mucsulium transversum*, planorbid snail *Gyraulus parvus*, and tubificid worm *Tubifex tubifex*) was determined under different levels of water hardness and sulfate concentrations (Soucek et al. 2011). The fingernail clam *S. simile* was most sensitive to chloride. Increased hardness was found to ameliorate the acute toxicity of chloride for three species, whereas sulfate appeared to have a negligible additive effect on chloride toxicity to *C. dubia* (Soucek et al. 2011). Although EPA has not acted on these new data, Iowa Department of Natural Resources (IADNR) incorporated them with data from 31 other species to derive new chloride criteria of 254.3 (acute) and 161.5 mg/L (chronic) with hardness and sulfate corrections (IADNR 2009). In 2013, Indiana adopted this same approach in the Indiana Administrative Code (IAC 2013). Given that a fingernail clam was the most sensitive species tested for chloride toxicity and that water quality criteria are not designed to protect the most sensitive species (EPA 2012), it is necessary to evaluate the degree to which recently adopted or proposed criteria are protective of unionid mussels, particularly those that are listed as threatened and endangered pursuant to the US Endangered Species Act (ESA 1973, as amended).

The Canadian Council of Ministers of the Environment (CCME) developed chloride criteria using a species sensitivity distribution approach (CCME 2011). The substantial difference from the EPA approach is the inclusion of toxicity data for larval unionid mussels (glochidia), obligate parasites on fish for several weeks before transforming to free-living juvenile mussels. The resulting CCME acute and chronic chloride criteria are 640 and 120 mg/L, respectively. In contrast to the Iowa criteria, the CCME criteria are not adjusted for sulfate or hardness because the number of suitable chronic exposure studies for these factors did not meet data requirements. The CCME criteria are also designed to protect the most sensitive life stage of the most sensitive species. As these chloride criteria are based on multiple species of unionid mussels, they may be more relevant to waters supporting threatened and endangered unionid mussels (ESA 1973), such as the Allegheny River in Pennsylvania.

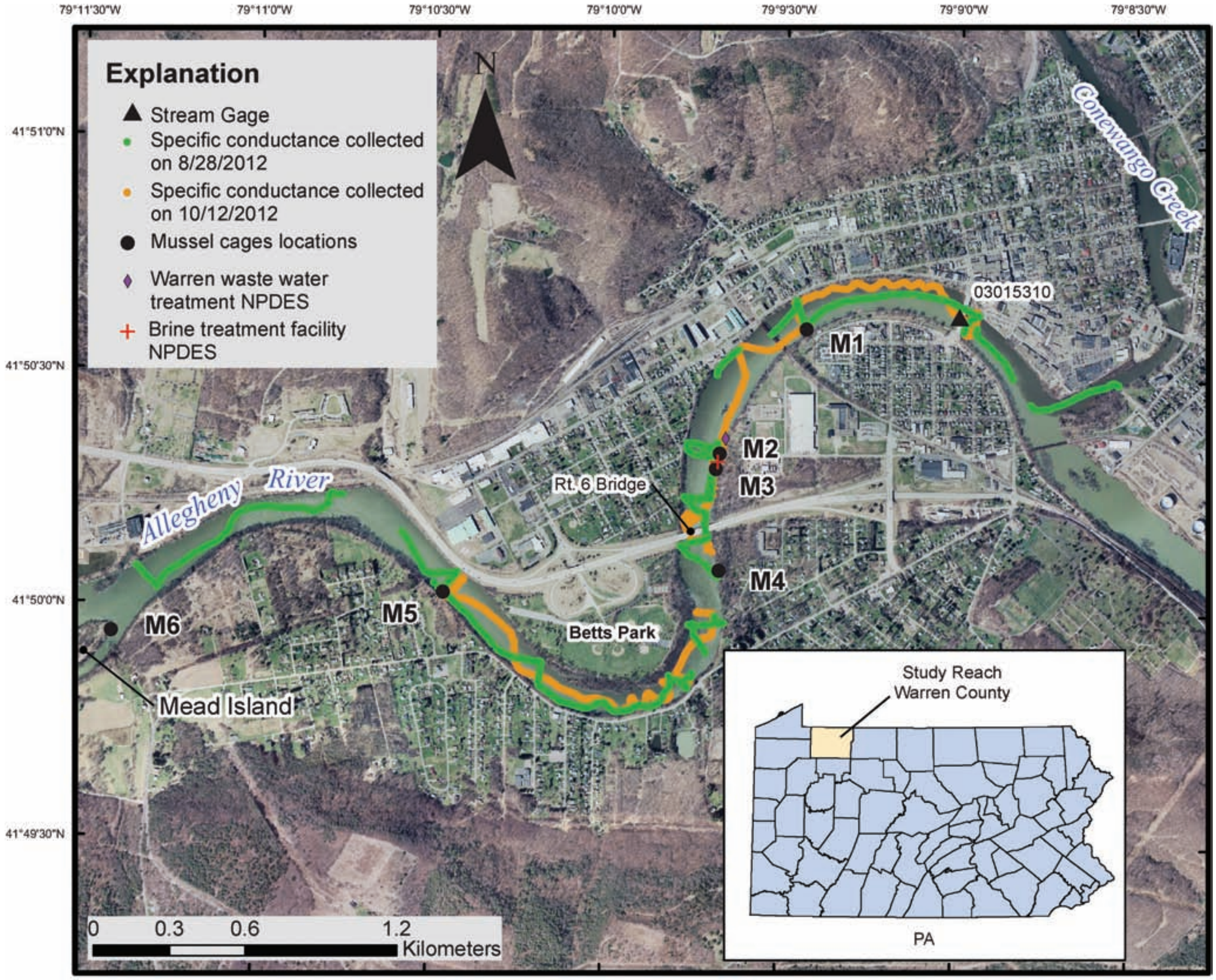
Studies examining the toxicity of chronic chloride exposures to unionid mussels are limited. All life stages are highly sensitive to elevated chloride concentrations in acute exposures (Bringolf et al. 2007), but sensitivity varies substantially among unionid species and glochidia are particularly sensitive to acute exposures (Gillis 2011; Echols et al. 2012). Glochidia of three *Epioblasma* species were nearly an order of magnitude more acutely sensitive to methylmercuric chloride than *Villosa iris* glochidia (Valenti et al. 2006). Fewer studies have been conducted using juvenile unionids to evaluate a chronic (28-d) exposure period for chloride. Survival of juvenile *V. iris* was reduced by 56% after exposure for 28 d to brine at 2,625 μS/cm (chloride not reported; Echols et al. 2012). Chronic exposure studies are unavailable for juvenile northern riffleshell mussel *Epioblasma torulosa rangiana*; northern riffleshell, hereafter NRS). Juvenile unionid mussel studies are lacking for exposures greater than 28 d that would be more representative of chronic exposures in the wild.

The purpose of this study was to assess the mixing of oil and gas high salinity wastewater discharges and determine its toxicity to mussels in the Allegheny River in Pennsylvania. This river is of particular interest because it has low water hardness, which reduces the potential for amelioration of chloride toxicity (Soucek et al. 2011), and it supports a diverse unionid mussel assemblage including several species listed as endangered under the ESA (Villella and Nelson 2006). Specifically, we used a triad approach to 1) document brine concentrations and mixing throughout the river channel, 2) determine chronic exposures to ambient brine concentrations that are lethal to juvenile NRS based on in situ exposures and, 3) assess the effects of brine on adult unionid mussel distribution.

Study Site

The study was conducted during 2012 in the Allegheny River at Warren, Pennsylvania, from the confluence of Conewango Creek to Mead Island (Figure 1). This location met the study criteria of 1) available water quality data obtained from probes deployed on August 11, 2011, as part of regional and national EPA studies; 2) the presence of suitable unionid mussel habitat based on previous surveys; 3) a high saline discharge; and 4) an established U.S. Geological Survey (USGS) streamflow-gaging station where stage and discharge are continuously recorded. The study area receives effluent from the Warren municipal wastewater treatment plant as well as the Waste Treatment Corporation brine treatment plant (hereafter referred to as brine treatment facility), which is authorized to receive and discharge high saline wastewater. The high saline wastewater is generally discharged Monday through Friday for 8–15 h a day. The timing and duration of the discharge vary with the amount of brine received by the treatment plant.

The average monthly flow of the Allegheny River varies from generally less than 56.5 cubic meters per second (cms; about 2,000 cubic feet per second [cfs]) to more than 396 cms (about 14,000 cfs) in some years, as measured at USGS streamflow-gaging station 03015310 Allegheny River below Conewango Creek at Warren. Streamflow in the study reach is affected by regulation from the Allegheny Reservoir (station 03012550 Allegheny



Orthoimage - Pennsylvania Mapping Program 2003 - 2006

Figure 1. Allegheny River study area at Warren, Pennsylvania, depicting northern riffleshell mussel *Epioblasma torulosa rangiana* cage sites M1 (upstream), M2 (Warren wastewater treatment discharge), M3 (brine treatment facility wastewater discharge), M4 (first downstream location), M5 (second downstream location), and M6 (final downstream location), Warren wastewater treatment discharge, brine treatment facility wastewater discharge, and specific conductance transect locations during the study period (August 11–October 16, 2012).

River at Kinzua Dam, 18.9 km upstream of 03015310) and streamflow from Conewango Creek (station 03015000 Conewango Creek at Russell, approximately 13 km upstream of the junction of Conewango Creek and the Allegheny River). Flow from these three stations during the study period from August 11 to October 16, 2012, is available in Supplemental Material (Figure S1). Instantaneous discharge at station 03015310 ranged from 34.83 to 78.15 cms and was generally steady and near long-term median values until September 22, after which time flow was below long-term median values (http://nwis.waterdata.usgs.gov/pa/nwis/uv/?cb_00060=on&format=gif_default&period=&begin_date=2012-08-11&end_date=2012-10-16&site_no=03015310).

In the study location, the river is about 100 m wide and consists of a series of pools and riffles. Based on previous surveys cataloged by the Pennsylvania Natural Heritage Program (PNHP 2014), mussels inhabit the river upstream, within, and below the study reach in areas with gravel and cobble substrate. During the surveys, the water depth was generally between 0.5 and 1 m, with the riffle areas less than 0.3 m deep. Pools were 3–5 m deep throughout the reach. Water quality samples were collected at the US Route 6 bridge, about 3 km upstream of the Conewango Creek, six times per year as part of the PADEP Water Quality Network monitoring and analyzed for parameters such as specific conductance, pH, hardness, major and minor ions, and nutrients (http://nwis.waterdata.usgs.gov/pa/nwis/qwdata/?site_no=03012600&agency_cd=USGS&); these data indicate chloride concentrations commonly are less than 20 mg/L at this upstream site on the Allegheny River.

We established six sampling sites denoted M1–M6 within the study reach (Figure 1) for collection of water quality data and placement of cages for in situ juvenile

NRS exposure trials. The sites were located upstream (M1) and downstream (M2) of the Warren municipal wastewater treatment plant, downstream of the brine treatment facility jetty that effectively limits exposure to only the brine effluent (M3), and at three sites at increasing distances downstream of the discharges (M4–M6).

Methods

Basic water quality sampling

Specific conductance probes were deployed to provide data for selection of study sites and to determine the relationship between conductance and ion concentrations. We evaluated hourly specific conductance and water temperature data from EPA's regional monitoring probes (HOBO U24-001 conductivity data logger; Onset) deployed on August 11, 2011 (these locations became M1, M4, and M6 in this study; Figure 1). The USGS deployed In-Situ Aqua Troll 100 specific conductance and temperature probes at sites M2, M3, M4 (redundant probe used to demonstrate consistency between probe models), and M5 on August 1, 2012. The EPA and USGS probes were set to record at 15-min intervals from the beginning of the mussel exposure period (day 0 on August 14, 2012) and collected when the mussels were removed (day 63 on October 16, 2012). During the course of the study, we cleaned and transferred data from the probes three times (August 1 [EPA probes only], August 28, and October 26). We measured instantaneous specific conductance and water temperature at deployment, each data transfer and at final retrieval with a model 6820V2 multiparameter (YSI) or MS-5 multiprobe (Hydrolab). Meters were calibrated with conductance standards to permit adjustment of the probe data with the instantaneous readings in accordance with agency protocols (Wagner et al. 2006).

Water grab samples were collected on two separate occasions by EPA and PADEP. The EPA analyzed water samples collected on October 4, 2012, from all six mussel cage sites (A. Bergdale, EPA R3, personal communication). The PADEP collected water samples on October 18, 2012, at five locations from immediately upstream of the municipal wastewater discharge to immediately upstream of M4, including a location between M2 and M3 (Brancato and Williams 2013). We used analytical water quality data from these samples to define the relationship between conductance and ion sum (chloride, bromide, sulfate, nitrate, lithium, sodium, ammonium, magnesium, and calcium) and conductance and chloride.

Distribution and mixing of brine contamination

To define the zone of high conductance and areas of mixing, we measured conductance along transects in the river channel during two separate low flow events. We sampled the transects on August 28 and October 12, 2012, when the mean discharge was 57.48 cms (2,030 cfs) and 45.87 cms (1,620 cfs), respectively (Figure S1); these values are less than the average monthly mean for August of 80.14 cms (2,830 cfs) for 1988–1998. Based on a typical low-flow day of 49.27 cms (1,740 cfs) the travel time of the brine plume from M3 to M6 is approximately 2.5 h (graphs for this analysis are shown in Figure S3). We began transect sampling after the brine plant had been discharging for at least 2 h (as evidenced by a rise in conductance). The exact location of transects differed slightly between sampling dates, but both encompassed nearly the entire length of the study reach from the mouth of Conewango Creek downstream to M5 or M6 (Figure 1). Upstream of the brine treatment plant, transects generally proceeded longitudinally down the river channel (roughly parallel to flow). Downstream of this point, we sampled bank to bank in a downstream direction to cover as much of the cross-sectional area of the channel as possible. In addition, we sampled across the channel (perpendicular to flow) at each sampling site and at likely water mixing areas, such as downstream of riffles and bridges.

During both events, we measured specific conductance and water temperature at midcolumn depth every 5 s with a calibrated MS-5 probe located at the front of a jon boat equipped with a 2.5-horsepower engine. At depths greater than 1.5 m, we measured vertical specific conductance profiles to assess any differences throughout the water column. At no time was a difference seen in the vertical column, indicating vertical mixing; therefore, we did not change the sampling strategy based on depth. Due to the draft of the boat, we did not collect data where the water was very shallow (e.g., flowing over riffles) and when water velocity was great enough to force the probe out of the water. The geographic position of the probe was recorded with a RiverSurveyor M9 (Sontek M9; Sontek) system, an acoustic Doppler current profiler (ADCP) equipped with a global positioning system (GPS) that also records water velocity and depth. We deployed the Sontek M9 in a tethered float off the front of the boat. Due to separation of the GPS antenna and the water quality probe, the probe reading locations have a precision of ±1.5 m.

In situ juvenile mussel exposures

Juvenile NRS were cultured at the USFWS White Sulphur Springs National Fish Hatchery, White Sulphur Springs, West Virginia. Female mussels were collected in April from French Creek, Crawford County, Pennsylvania. At the hatchery, glochidia were extracted from females and infested on mottled sculpins *Cottus bairdi*. Juvenile NRS were excysted from their host fish 3–4 wk later and reared until mid-August, at which time the 2-mo-old juveniles were approximately 2 mm in length. On August 14, 2012, we placed 20 randomly selected juvenile NRS into each cage chamber.

We assessed survival of juvenile NRS under ambient brine concentrations by installing cages at each sampling site (M1–M6). Cages are flow-through, screened chambers (3 in. × 4 in. [7.6 × 10.1 cm] [diameter × height]) housed in a domed concrete base (8 in. × 6 in. [20.3 cm × 15.2 cm]) that are used for culturing juvenile unionid mussels (Barnhart 2006). We installed five cages at each of the six sampling sites. At each site, cages were placed about 1.5 m apart in a triangular array, and we placed a probe in the middle of the array for continuous water quality monitoring at the site (see Figure S2). Mean values of specific conductance during cage exposure trials were computed using only data points collected during brine discharge periods at M3 and M4 (i.e., intermittent exposure).

We assessed NRS survival, condition, and behavior at days 3, 6, 9, 14, 22, 29, 44, and 63 after installation. Each juvenile was recorded as live if we observed organ activity or presence of food through the translucent valves, foot movement, or gaping to feed during a 10-min period while the cage was held in a container of site river water. We ensured adequate water flow through the cages by cleaning the mesh and cage opening of algae and debris during each visit. Surviving juveniles were photographed at day 63 to evaluate growth. Final length of each individual from M1, M5, and M6 was measured from digital images using digital caliper software (Pixel Stick). On day 64, we released all surviving juveniles back to the site of the adult female collection in French Creek. We compared data on survival between sites using repeated measures analysis of variance with the R statistical package (R Core Team 2012). The Tukey's honestly significant difference test (de Mendiburu 2014) was used to identify significant differences between sites at days 29 and 63. Growth data were evaluated at day 63 using analysis of variance. We evaluated the relationship between survival and conductance using the Finney method of probit analysis in the BioStat (AnalystSoft, Inc. 2012) and R (R Core Team 2012) statistical software packages to identify the no adverse effect concentration (NOAEC) relative to the upstream reference (i.e., no added mortality above background).

Unionid mussel surveys

We described unionid mussel diversity, species relative abundance, and total abundance at several locations within the study reach to examine the relationship between unionid mussel assemblages and brine discharge. Using methods modified from Smith et al. (2000, 2001) we sampled unionid mussels along 10 transects located throughout the study reach; each transect was about 1 m wide and spanned the river perpendicular to flow (Figure 2). Three transects were located upstream of wastewater discharges (T1–T3), three were located immediately downstream of the discharges (T4–T6), and the other four (T7–T10) were located farther downstream. We surveyed these transects August 27–31, 2012, using a pair of divers. Each transect was divided into 10-m segments, and each diver sampled approximately 0.5 m of substrate on each side of the transect line for a minimum of 7 min. The actual sampling time necessary to search the entire segment was recorded, but some segments were not sampled due to high water velocity that made diving hazardous. When feasible, we overturned larger rocks and disturbed the upper 5–10 cm of sediment to find buried unionid mussels. We placed all collected unionid mussels in mesh bags and brought them to the surface for identification and measurement (anterior-posterior shell length) before returning them to the river. Because some species are difficult to detect (particularly small species), we augmented transect sampling data with searches of shell middens on shore to construct a species list for the area. Substrate size and composition were estimated and depth was recorded for each 10-m segment along all transects.

Results

Continuous recording probes documented significant differences in specific conductance between study sites as determined by a Wilcoxon rank-sum test. Background-specific conductance for the Allegheny River ranged from 103 to 188 μS/cm (mean 151 μS/cm) based on data collected at M1. Specific conductance measured at M2 (municipal wastewater discharge) ranged from 122 to 755 μS/cm (mean 294 μS/cm). All of the probes located downstream of the brine facility documented the daily and weekly signature of brine treatment facility (i.e., operates Monday through Friday, 8–15 h a day). When the facility was actively discharging, specific conductance at M3 ranged from 4,000 to12,000 μS/cm (mean 7,846 μS/cm). At M4, the specific conductance was 1,050 to 5,270 μS/cm (mean 3,863 μS/cm) when the brine discharge was reaching this site. The specific conductance at the most distant sites (i.e., M5 and M6) ranged from 158 to 275 μS/cm (Figure 3). The differences in the sites can be graphically represented by specific conductance duration curves. Figure 4 shows the percent of time an indicated specific conductance is exceeded at each site. For example, sites M3 and M4 exceeded 1,000 μS/cm approximately 40% of the time that the caged NRS were in situ. Data for probe graphs are included in Table S1.

The August 28, 2012, and October 12, 2012, specific conductance surveys revealed a similar pattern of low background specific conductance upstream of the brine facility, high values immediately downstream of the discharge, and an attenuation of specific conductance values in a downstream direction (Figure 5). These data also showed that the high specific conductance zone is largely restricted to a plume that parallels the left descending bank. Stream flow in the Allegheny River can affect the mixing and dilution of the high specific conductance discharge. This is illustrated by the specific conductance difference between the two surveys downstream of S4 and subsequent decreased dilution of the brine wastewater. Specific conductance data for Figure 5 are included in Supplemental Material (Table S2).

Juvenile NRS cumulative survival after 63 d showed a strong response to discharges from both brine and wastewater treatment plants (Figure 6). Mean cumulative survival (of 20 juveniles in each of five cages) upstream of both discharges (M1) after 63 d was 84%. Survival declined dramatically to 21% immediately below the wastewater discharge (M2) and to 2 and 34% immediately downstream of and approximately 0.4 km downstream of the brine discharge (M3 and M4, respectively). Mean survival at downstream sites M5 and M6 (91% for both sites) was not statistically different from M1 ($P < 0.05$) throughout the study, showing a dilution of wastewater and brine effluents. Mean survival differed significantly between M1 and M2 and M1 and M4 by day 63. The difference between M1 and M3 was significant at day 29 and persisted at day 63. Survival data for Figure 6 are included in Supplemental Material (Table S3). NRS shell length (in millimeters) was statistically lower at M1 (mean 4.6) than M5 (mean 5.1;

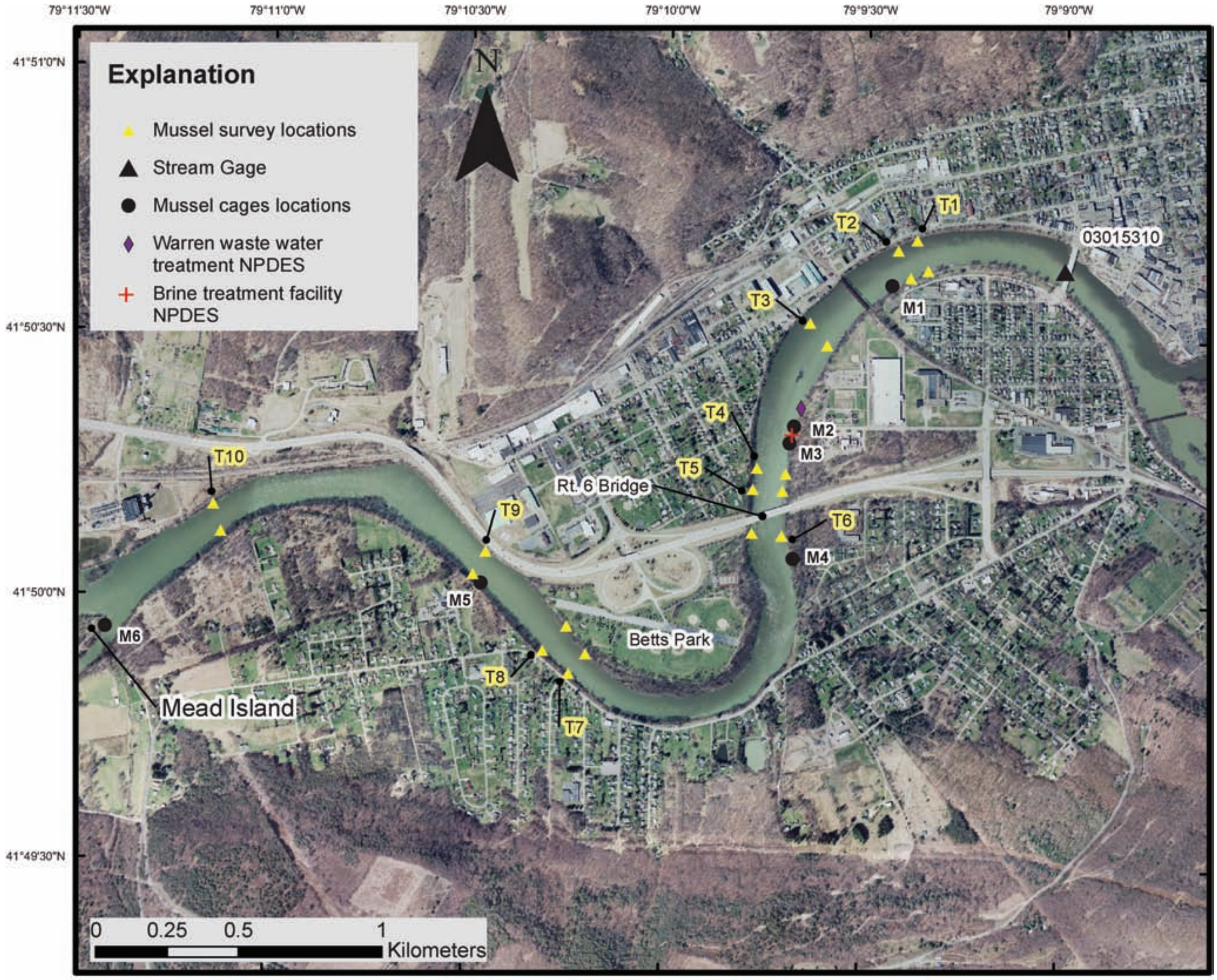


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Figure 2. Native unionid mussel transects upstream (T1–T3), within discharge (T4–T6), downstream (T7–T9), and Mead Island (T10) on the Allegheny River near Warren, Pennsylvania, surveyed by divers August 27–31, 2012.

$P < 0.001$) and M6 (mean 5.0; $P < 0.001$), but the biological relevance of this difference is unknown. Insufficient numbers of live NRS were available for M2, M3, and M4 to evaluate growth.

Variability in survival between cages and over time provides additional insight into effluent toxicity. Survival varied widely from 0 to nearly 70% among cages at M2 downstream of the municipal wastewater discharge. Due to cage placement, cages furthest into the river likely received less exposure to the plume as higher mortality was observed in the immediate vicinity of the discharge pipe (see Figure S2). This observation is consistent with benthic macroinvertebrate assessments by PADEP showing impairment at M2, but substantial recovery immediately upstream of the brine treatment discharge (Brancato and Williams 2013). In contrast, high-to-severe NRS mortality was observed in all cages at sites M3 and M4 downstream of the brine discharge, as well as severe impairment of the benthic invertebrate community (Brancato and Williams 2013). Mortality increased substantially from day 14 to day 63 at M2 and M3. The delayed and chronic nature of this rise in mortality and our observations of live juvenile NRS suggest that mussels attempted to avoid exposure by remaining closed. Unlike M2 and M3, live juvenile NRS at M1, M4, M5, and M6 were routinely agape, evidently respiring and feeding. Time series evaluation of specific conductance readings indicates that M2 discharge did not contribute significant ions to M3. In addition, our observations of minor algal growth at M3, the absence of chironomids common to sewage treatment effluent in the cages, observations of normal feeding behavior of M3 NRS when brine was not discharging, substantial recovery of benthic invertebrates between M2 and M3 (Brancato and Williams 2013), and evaluation of maximum potential WWTP contaminant exposure indicated that the severe toxicity at M3 resulted primarily from exposure to the brine discharge.

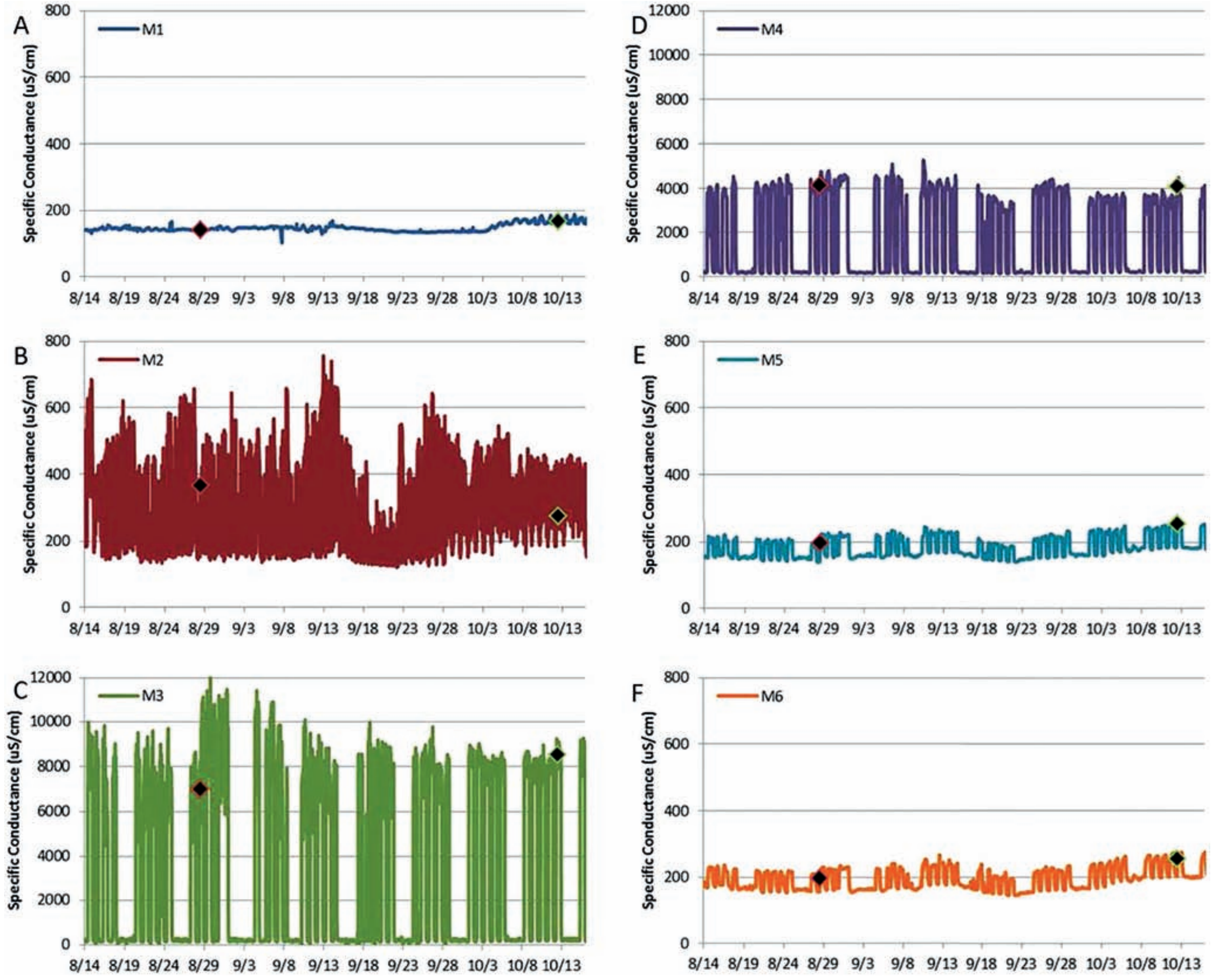

Figure 3. Specific conductance readings from probes deployed at mussel sites M1 (**A**), M2 (**B**), M3 (**C**), M4 (**D**), M5 (**E**) and M6 (**F**) on the Allegheny River near Warren, Pennsylvania, during the study period (August 11–October 16, 2012). M1, M2, M5, and M6 are shown at the same scale to show the specific conductance outside the brine plume. M3 and M4 are shown at the same scale to show the specific conductance within the brine plume. The dates when the specific conductance surveys were conducted are shown as black diamonds on each graph. All sites are significantly different from each other at the 95% confidence level as determined by a Wilcoxon rank-sum test.

A strong dose–response relationship between juvenile mortality and specific conductance was observed. Chi-square goodness of fit for the dose–response curve (Figure 7) was significant ($P = 0.003$), and linear regression of $\log_{10}$ conductivity and probit percent mortality were predictive ($r^2 = 0.9983$). Absence of juvenile exposures in the 1,000–3,000-μS/cm specific conductance range limited our ability to identify the lowest observable effect concentration or the lethal concentration 50. However, this limitation is not critical to estimating an NOAEC with two exposures (M5 and M6) exhibiting survival comparable to a reference site (M1). Data from M2 were not included in this analysis due to high variability between replicates and rapid recovery of benthic macroinvertebrate populations, indicating spatially limited toxicity as well as predominance of nonbrine contaminants (e.g., ammonia, chlorine). Conductivity data for M3 and M4 were limited to periods of active brine discharge. The highest specific conductance resulting in no adverse effect compared to background (NOAEC; 84% survival; lethal concentration 16) after 63 d of exposure is 247 (lower confidence limit 148, upper confidence limit 370) μS/cm. When calculated over the typical chronic laboratory exposure time of 28 d, the NOAEC value was 573 (lower confidence limit 224, upper confidence limit 1,095) μS/cm. Survival data for juvenile NRS monitoring are included in Supplemental Material (Table S3).

Water sample analysis indicated that the high specific conductance and ion sum concentrations from the brine discharge are driven by chloride. The EPA water samples document that 60% of the ion sum (milligrams per liter) at the brine discharge (M3) is attributable to chloride compared to 28% at the reference site (M1; Table S4). A strong linear correlation was found between specific

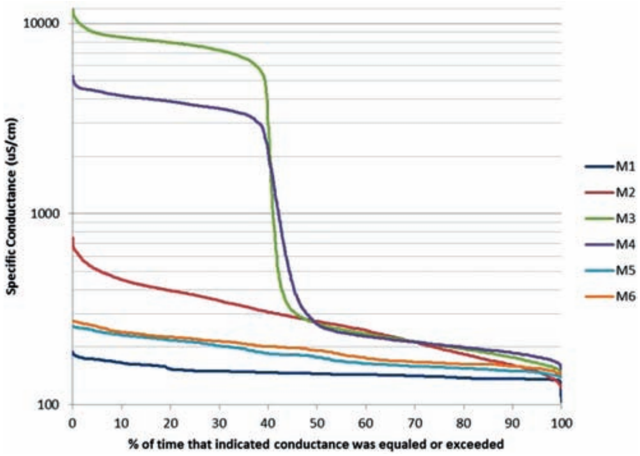

Figure 4. Graph showing the specific conductance duration curve for each cage containing northern riffleshell mussel *Epioblasma torulosa rangiana* (M1-M6) during the study period (August 11–October 16, 2012) in the Allegheny River near Warren, Pennsylvania. The percentage of time a particular specific conductance was exceeded at each silo during the study period is represented.

conductance and ion sum and between specific conductance and chloride (Figure 8). Using the NOAEC for specific conductance of 247 μS/cm, the calculated NOAECs for ion sum and chloride are 122 and 78 mg/L, respectively. Following the IADNR (2009) protocol, the chronic chloride criterion is adjusted for hardness and sulfate by the following equation:

$$\text{chronic criterion} = 161.5(\text{hardness})^{0.20579}(\text{sulfate})^{-0.0745}$$

Based on measured background hardness and sulfate at the reference site (2.338 and 0.839 mg/L, respectively; Brancato and Williams 2013), the adjusted chronic chloride criterion for the Allegheny River is 316.71 mg/L. Based on measured hardness and sulfate at the brine discharge site (4.305 and 0.779 mg/L, respectively), the adjusted chronic chloride criterion would be 541.86 mg/L. Ion concentrations for the water samples are included in Supplemental Material (Table S4).

Transect surveys further demonstrated that the unionid mussel assemblage is impaired within the plume of the brine discharge. Reduced unionid mussel abundance was evident within the discharge plume along the left descending bank downstream from the brine facility (T4–T6) through the downstream transects (T7–T9) compared to upstream (T1–T3) and Mead Island (T10; Figure 9). Mean abundance along the left descending side (0–40 m) was 7 (range 3–10) for upstream, 0.8 (0–2) near the discharge, 0.3 (0–1) downstream of the brine treatment discharge, and 9.5 (2–20) at Mead Island. Species richness was also reduced within the high specific conductance zone, as well as in mussel habitat downstream compared to upstream of the discharge (Figure 10). Mean species number was 3.6 (range 2–6) upstream, 1.5 (0–3) near the discharge, 0.3 (0–1) downstream of the discharge, and 3.5 (2–4) at Mead Island. Most of the unionid mussel species native to the Allegheny River, including NRS, are associated with clean-swept cobble and gravel substrates in riffle-and-run flow (Ortmann 1919; Parmalee and Bogan 1998; Smith and Crabtree 2010; USFWS 1994; Watters et al. 2009). With the exception of the right descending bank at Mead Island, the substrate and overall habitat conditions in all transects was comparable to that in other reaches of this river supporting large numbers of unionid mussels. Furthermore, although not the target of this study, divers reported other species often associated with Allegheny River mussel assemblages including darters (*Etheostoma* sp.) and hellbenders *Cryptobranchus alleganiensis*. Adult NRS were documented at T7 near the right descending bank and transect T10 near the left descending bank. Living and freshly dead shells of NRS were also located on the right descending bank near T7 and T8 outside of the transect survey area. Transect data are included in Table S5.

Discussion

Using a triad approach, we defined the zone of high conductivity resulting from brine discharge, demonstrated direct toxic effects to the endangered NRS (ESA 1973), and identified direct and potential indirect changes in unionid mussel communities within and downstream of the high specific conductance zone in the Allegheny River near Warren, Pennsylvania. The chemical and transect data define a zone of high specific conductance where the plume from the brine facility influences water quality. The plume was virtually unmixed along the left descending side of the river until it reaches the first pool 0.45 mi (724 m) downstream of the discharge. Daily and weekly signatures of the brine facility discharge were evident to the furthest point that we assessed over 2 mi (3 m) downstream. The area encompassed by the high specific conductance zone that we observed during this study was specific to the flow and effluent discharge conditions under which we took measurements. A change in volume or concentration of the discharge or river flow would likely alter the area of the high specific conductance zone and could result in periodic expansion and contraction of the mixing zone.

The in situ toxicity trials showed a clear negative effect of brine exposure on NRS survival, and probit analysis demonstrated that specific conductance was a suitable parameter for predicting juvenile NRS survival. Chloride is the presumed primary toxin based on known unionid sensitivity to this ion from laboratory testing (CCME 2011).

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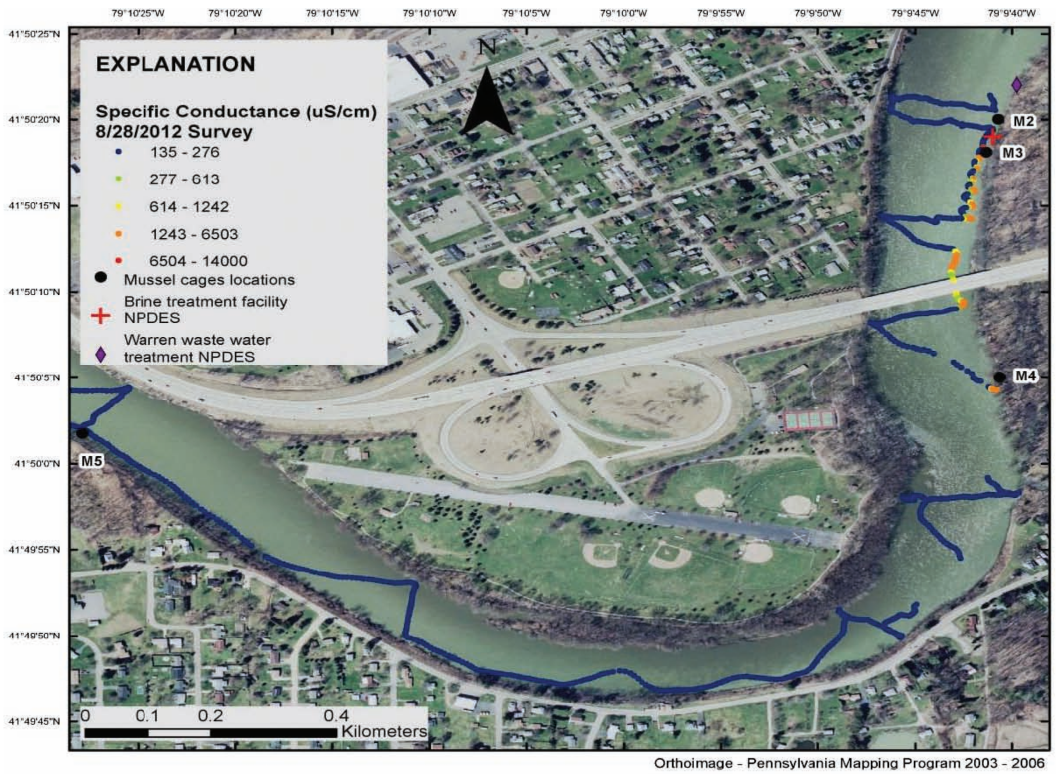
Figure 5. Specific conductance readings for the reach between M2 (Warren wastewater treatment discharge) and M5 (second downstream location) in the Allegheny River near Warren, Pennsylvania, based on the August and October 2012 transect sampling events. All values upstream of M2 and downstream of M5 were less than 246 μS/cm.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41°50'25"N
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41°50'15"N
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EXPLANATION
Specific Conductance (uS/cm)
8/28/2012 Survey
135 - 276
277 - 613
614 - 1242
1243 - 6503
6504 - 14000
Mussel cages locations
Brine treatment facility NPDES
Warren waste water treatment NPDES
N
M2
M3
M4
M5
0
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Kilometers
Orthoimage - Pennsylvania Mapping Program 2003 - 2006


79°10'25"W
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41°50'0"N
41°49'55"N
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41°49'45"N
EXPLANATION
Specific Conductance (uS/cm)
10/12/2012 Survey
135 - 276
277 - 613
614 - 1242
1243 - 6503
6504 - 14000
Mussel cages locations
Brine treatment facility NPDES
Warren waste water treatment NPDES
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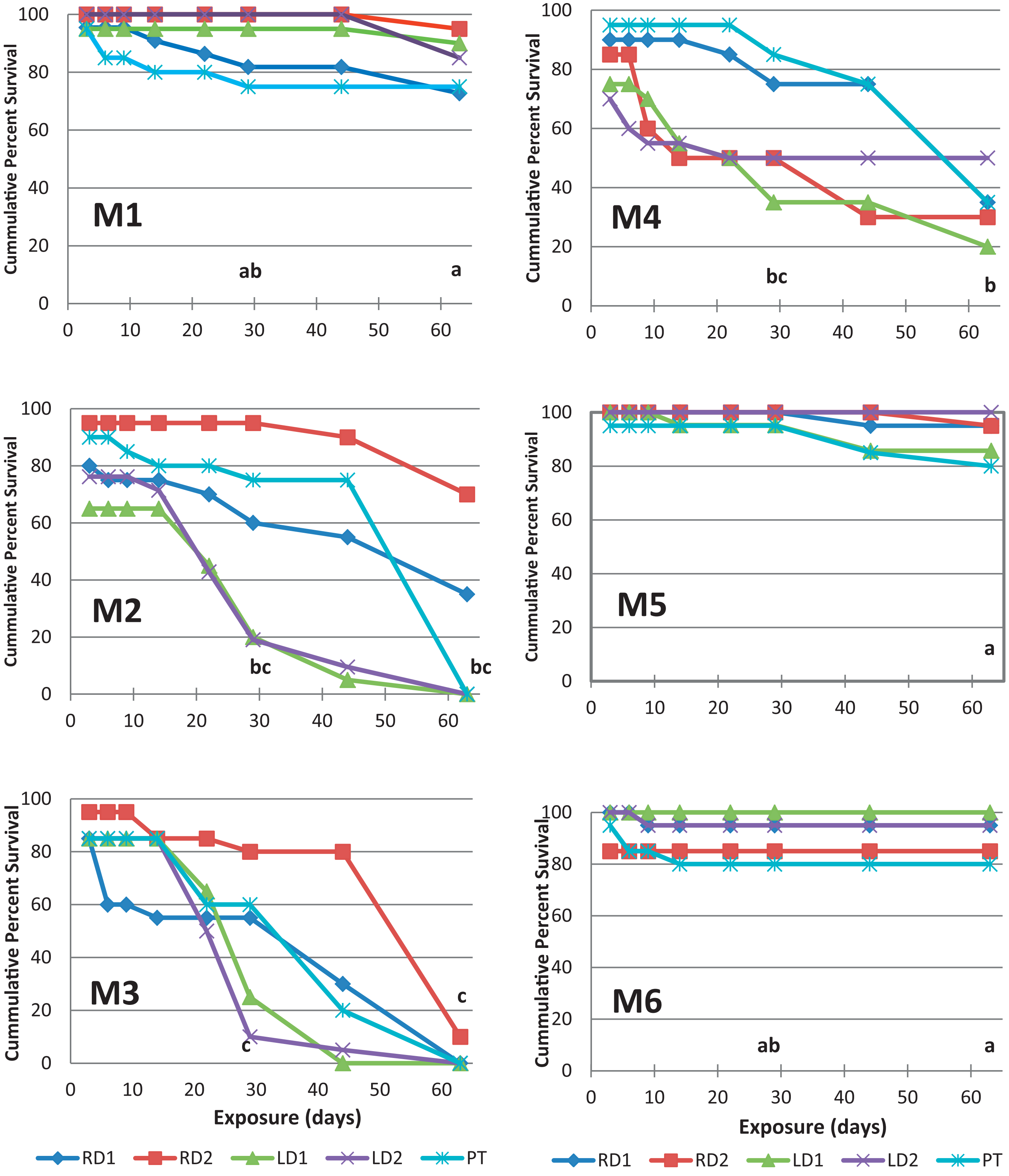

Figure 6. Cumulative percent survival of juvenile northern riffleshell mussel *Epioblasma torulosa rangiana* from days 0 to 63 by mussel cage site (M1-M6) and within cage array (see Figure S2) on the Allegheny River near Warren, Pennsylvania, for August 14–October 16, 2012. Positions in array are most upstream point (PT), first on left descending side (LD1), second on left descending side (LD2), first on right descending side (RD1), and second on right descending side (RD2). Dissimilar letters (a, b, c) denote statistically significant differences (Tukey's honestly significant difference) in cumulative survival between mussel sites at days 29 and 63.

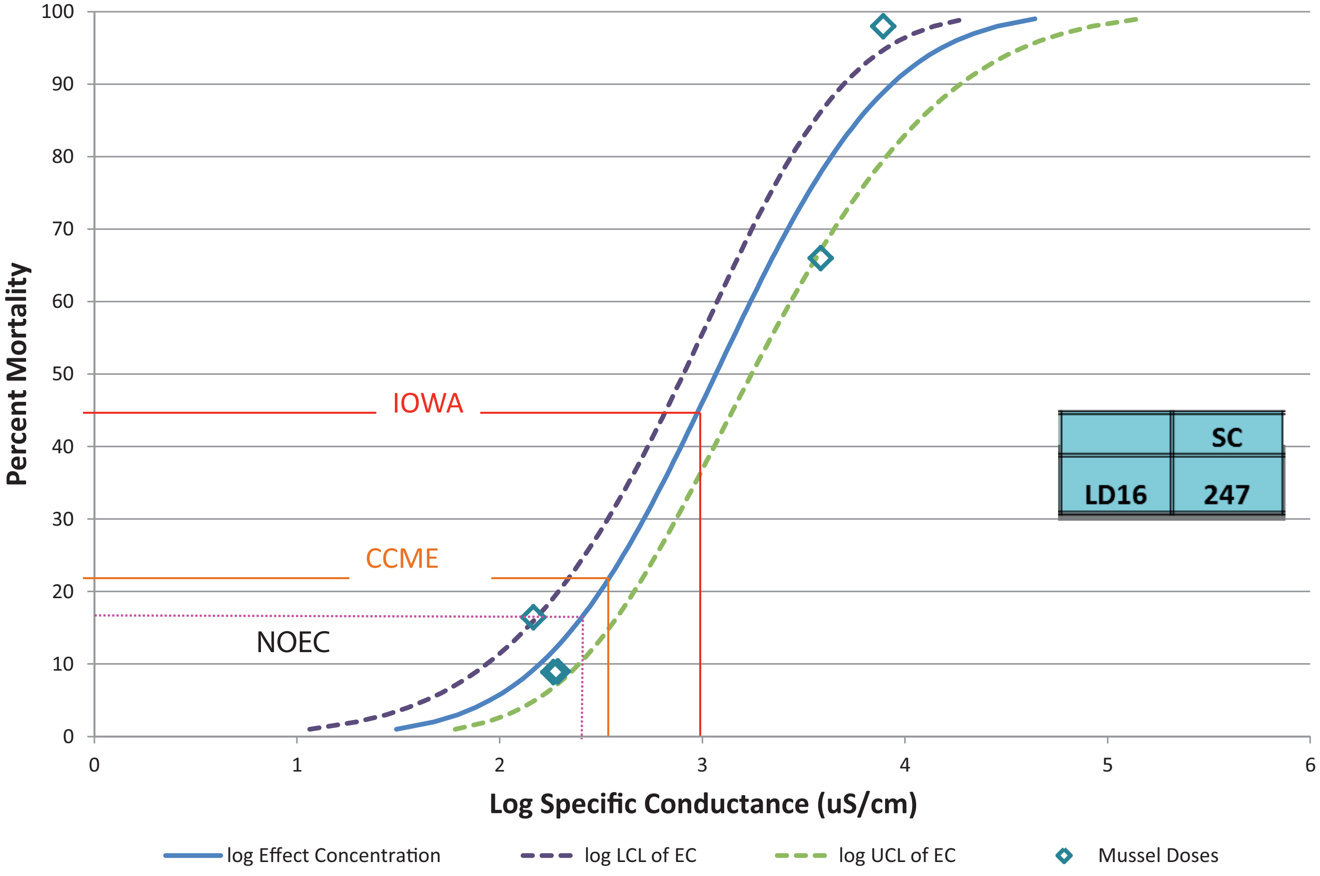

Figure 7. Mean cumulative percent mortality of northern riffleshell mussel *Epioblasma torulosa rangiana* at day 63 in response to mean specific conductance data during discharge periods from probes attached to each first left descending side cage in the array for mussel cages in the Allegheny River near Warren, Pennsylvania, from August 14 to October 16, 2012. Chi-square goodness of fit for the curve was significant ($P = 0.003$) and linear regression of $\log_{10}$ conductivity and probit percent mortality was predictive ($r^2 = 0.9983$). M2 (Warren wastewater treatment discharge) data were omitted due to potential confounding effects of nonbrine contaminants. Dissimilar letters reflect significant differences in mortality between mussel sites. NOAEC = specific conductance with predicted background mortality (lethal concentration 16), UCL = 95% upper confidence limit. LCL = 95% lower confidence limit.

Because ion concentrations of effluents can change without altering the total dissolved solids, it is more consistent to derive water quality criteria based on the presumed primary toxic constituent in this high salinity effluent (i.e., chloride). The relationship of specific conductance to chloride can be used to evaluate the protectiveness of literature-based chloride criteria for sensitive unionid species. The NRS survival data at this site indicate that the site-specific chloride criterion of 316.71 mg/L derived using the Iowa formula (IADNR 2009) would increase NRS mortality by approximately 30% compared to a 6% increase if the CCME (2011) 120 mg/L criterion were used. A chloride concentration of 78 mg/L or less would be required to maintain NRS reference survival rates and prevent added mortality of this endangered mussel (ESA 1973).

Differences between literature-based chloride criteria and criteria determined from this study could be due to the limited toxicity data with juvenile unionids, the inappropriateness of the sulfate and hardness corrections in the Iowa formula for the Allegheny River, the variability in this field study dose (i.e., discharge concentration), and the differences in the duration of exposure. The CCME (2011) species sensitivity distribution depicts NRS as the species most sensitive to chloride. However, NRS were not included in the derivation of the Iowa criteria (IADNR 2009). The CCME (2011) also found that insufficient data were available to develop a hardness relationship for chronic toxicity. Laboratory testing with NRS and other unionid species should be conducted to provide controlled chloride exposures that span our field-derived criterion. These tests should be designed to determine whether hardness and sulfate have an ameliorative effect on chloride toxicity before adoption of criteria with hardness and sulfate corrections.

The duration and pattern of exposure in this study influenced the NOAEC. Based on our observations, it is likely that juvenile NRS avoid exposure by limiting feeding and respiration. Similar behavioral changes have been observed in bivalves exposed to high sulfate concentrations (Soucek 2007). This response apparently minimized mortality during the first week of exposure and lengthened the period of chronic mortality. This behavioral adaptation has ramifications for both acute and chronic pollutant criteria determination for juvenile unionid mussels (>2 mo) that would not be observed in

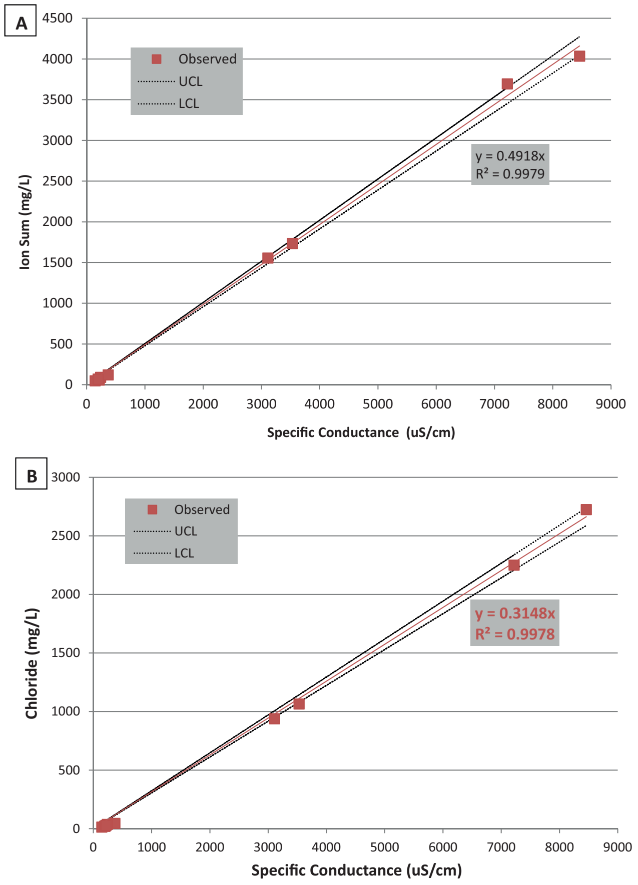

Figure 8. Relationship between specific conductance and (**A**) ion sum (TDS) and (**B**) chloride in grab samples collected by U.S. Environmental Protection Agency and Pennsylvania Department of Environmental Protection in October 2012 in the Allegheny River near Warren, Pennsylvania.

glochidia or newly transformed unionid mussels that are unable to close their valves. Cessation of our study at day 29 would have resulted in a more than two-fold higher NOAEC for specific conductance as significant mortality occurred in the period from 30 to 63 d. Although criteria derived using 28-d studies would be relevant for exposures of limited duration, they may not be applicable to long-term exposures often seen in the wild. Modifications to the duration of the current testing protocol should be considered before its use for the development of water quality standards applicable to unionid mussel habitat.

The native adult unionid mussel assemblage has reduced abundance and diversity along the left descending side of the Allegheny River below the brine treatment facility discharge compared to reaches upstream, along the right descending side of the river, and the most distant downstream site (T10). This observation adds evidence that the discharge has impaired and continues to affect mussel populations within this high specific conductance zone. The presence of a robust unionid mussel assemblage and the distribution of NRS outside of the high specific conductance zone demonstrate that the physical and chemical habitat in the vicinity of Warren is suitable for this endangered mussel (ESA 1973), as well as other species in this unionid mussel assemblage. Villella and Nelson (2006) regularly observed NRS in similar habitat at their survey sites downstream of Mead Island.

Like all the other native unionid mussel species encountered in this study (Table S5), NRS depend on transport of juveniles through an obligate parasitic life stage on fish, most likely several species of *Etheostoma* and *Percina* darters (Zanatta and Murphy 2007). Due to their relatively sedentary nature, this is the primary means of unionid mussel dispersal. Although the fish assemblage was not specifically documented, surveyors observed darters, smallmouth bass *Micropterus dolomieu*, and other potential unionid host fish during transect surveys immediately downstream of the discharges. Juvenile unionid mussels that drop off of host fish in the discharge area likely have a low chance of survival. Moreover, the absence of a robust unionid mussel assemblage within the high specific conductance zone appears to have indirect effects downstream (e.g., left descending sides of T7–T9). Although our in situ study indicated that juvenile NRS can survive in this downstream reach of the river, the lack of reproduction in the upstream high specific conductance zone could be responsible for poor recruitment and thus, reduced unionid mussel numbers and species along the left descending side of the river. Alternatively, periodic downstream expansion of the toxicity zone would repeatedly set back recolonization by increasing juvenile mortality. The unionid mussel survey data including the presence of NRS at the most downstream transect (T10) suggest that chronically toxic concentrations rarely reach mussel habitat at the upstream end of Mead Island and that any acute events are brief enough that unionid mussels can survive.

The results of this study demonstrate that this triad approach can provide information to support wastewater discharge permit limits in unionid mussel habitats. In rivers with complex hydrodynamics, specific conductance field surveys combined with continuous monitoring at a waste discharge is a quick and effective way to document the mixing. A continuous monitor is necessary to determine the discharge pattern and range of specific conductance of the source. Once a pattern is determined, the optimal time for the survey can be identified. Two surveys (to ensure repeatability) at low flow (to capture the worst-case scenario) can be accomplished to give managers a field map of the mixing zone instead of relying on model-derived maps.

In situ toxicity testing combined with adult unionid mussel surveys provide strong weight of evidence for effects on individuals and populations within and beyond the mixing zone. As rivers typically have multiple stressors, it is critical to locate cages and survey transects to segregate confounding influences. The study also shows that further toxicity testing with juvenile unionid mussels, particularly including longer duration tests, is needed to ensure that state and national water quality criteria for chloride are protective of unionid mussels. Test duration for juvenile unionid mussels should be reevaluated to account for variability in field exposures and potential

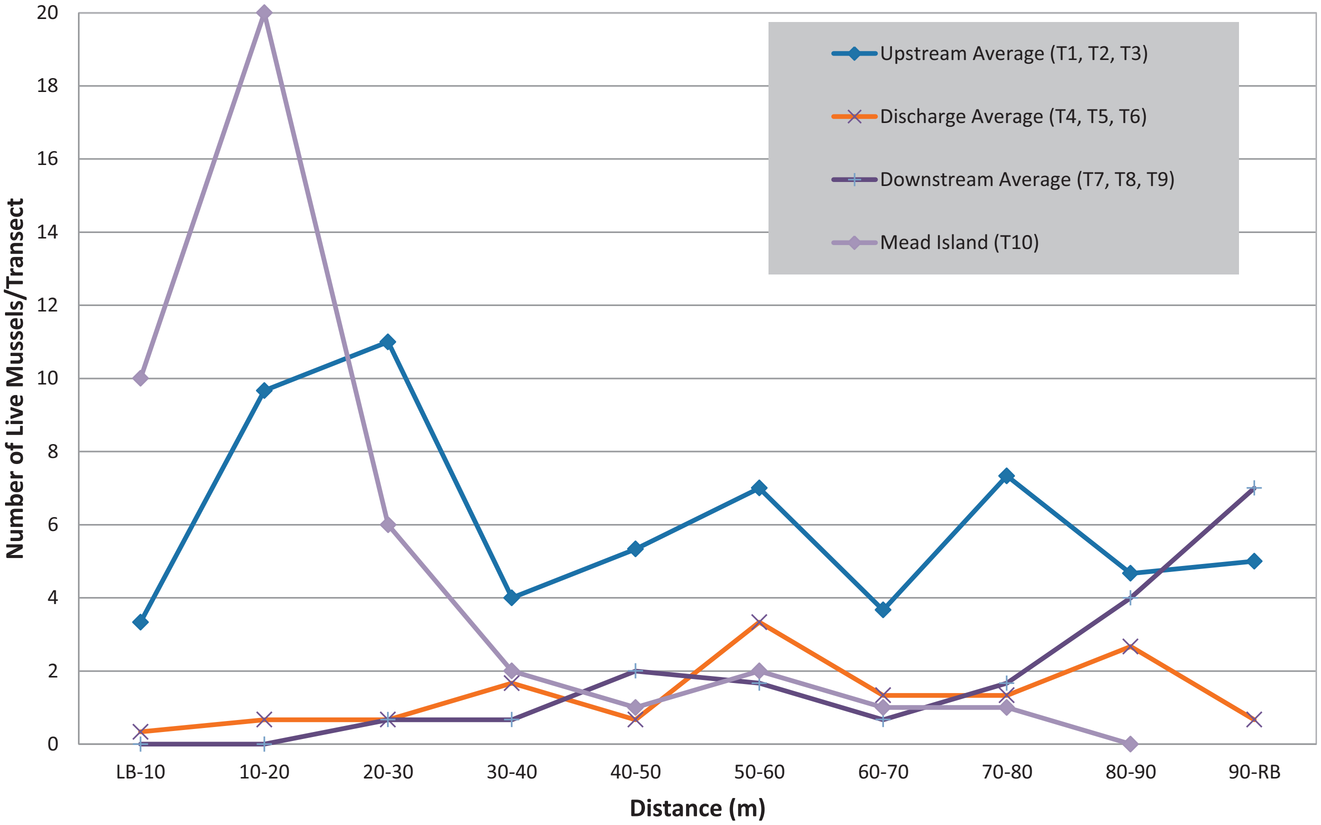

Figure 9. Native unionid mussel survey adult numbers from August 2012 for 10 transects between Conewango Creek and Mead Island from left descending bank (LB) to right descending bank (RB) on the Allegheny River near Warren, Pennsylvania. Transect data were compiled as upstream (T1–T3), discharge (T4–T6), downstream (T7–T9), and Mead Island (T10).

behavioral adaptations that reduce exposure and extend the period of chronic mortality.

Supplemental Material

Please note: The *Journal of Fish and Wildlife Management* is not responsible for the content or functionality of any supplemental material. Queries should be directed to the corresponding author for the article.

Table S1. Specific conductance data from U.S. Environmental Protection Agency and U.S. Geological Survey continuous water quality probes at cage sites for northern riffleshell mussels *Epioblasma torulosa rangiana* from upstream (M1) to downstream (M6) on the Allegheny River near Warren, Pennsylvania, from August to October 2012.

Found at DOI: 10.3996/052013-JFWM-033.S1 (948 KB XLS).

Table S2. Specific conductance readings at five second intervals for the surveys performed on August 28, 2012, and October 12, 2012, on the Allegheny River near Warren, Pennsylvania.

Found at DOI: 10.3996/052013-JFWM-033.S2 (404 KB XLS).

Table S3. Juvenile northern riffleshell mussel *Epioblasma torulosa rangiana* counts in cages from upstream (M1) to downstream (M6) on the Allegheny River near Warren, Pennsylvania, from August to October 2012.

Found at DOI: 10.3996/052013-JFWM-033.S3 (94 KB DOC).

Table S4. Ion concentrations in water samples collected by U.S. Environmental Protection Agency at cage sites for northern riffleshell mussels *Epioblasma torulosa rangiana* from upstream (M1) to downstream (M6) in the Allegheny River near Warren, Pennsylvania, in October 2012.

Found at DOI: 10.3996/052013-JFWM-033.S4 (32 KB DOC).

Table S5. Summary data for transects from upstream (T1) to downstream (T10) for the unionid mussel survey conducted on the Allegheny River near Warren, Pennsylvania, in August 2012.

Found at DOI: 10.3996/052013-JFWM-033.S5 (324 KB DOC).

Figure S1. Stream flow from August 14 to October 16, 2012, as determined by U.S. Geological Survey station 03015310 Allegheny River below Conewango Creek at Warren, Pennsylvania. The stream flow for stations 03012550 Allegheny River at Kinzua Dam and 03015000 Conewango Creek at Russell, which combine to give total flow at station 03015310 are also shown. Stream

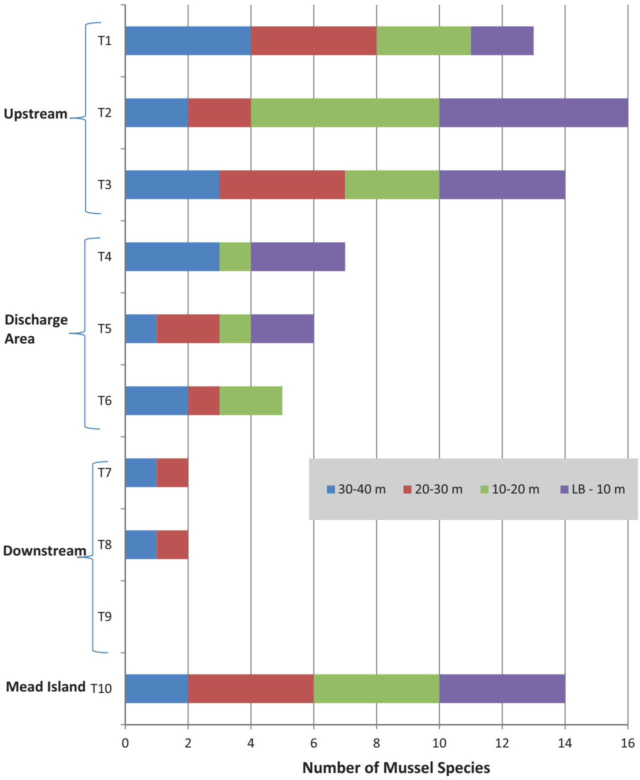

Figure 10. Native unionid mussel survey adult species richness from August 2012 for 10 transects (T1-T10) from left descending bank to 40 m into channel between Conewango Creek and Mead Island on the Allegheny River near Warren, Pennsylvania. No mussels were observed in the first 40 m on T9.

flow during the specific conductivity surveys are shown as yellow diamonds on the graph.

Found at DOI: 10.3996/052013-JFWM-033.S6 (133 KB PDF).

Figure S2. Cage array at M2 and M3 northern riffleshell mussel *Epioblasma torulosa rangiana* cage sites with discharge pipes on the Allegheny River near Warren, Pennsylvania, from August to October 2012. Cage arrays upstream (M1) and downstream (M4–M6) were comparable absent discharge pipes.

Found at DOI: 10.3996/052013-JFWM-033.S7 (457 KB PDF).

Figure S3. Temporal specific conductance data used to determine transit time for specific conductance transects from August to October 2012. The blue line shows the specific conductance readings at M3 (brine treatment plant), and the red line shows the conductance readings at M6 (furthest downstream). Note that the trailing edge of the specific conductance plume takes approximately 2.5 h to reach M6.

Found at DOI: 10.3996/052013-JFWM-033.S8 (51 KB PDF).

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