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Influences of Acid Mine Drainage and Thermal Enrichment on Stream Fish Reproduction and Larval Survival

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Abstract - Potential effects of acid mine drainage (AMD) and thermal enrichment on the reproduction of fishes were investigated through a larval-trapping survey in the Stony River watershed, Grant County, WV. Trapping was conducted at seven sites from 26 March to 2 July 2004. Overall larval catch was low (379 individuals in 220 hours of trapping). More larval White Suckers were captured than all other species. Vectors fitted to nonparametric multidimensional scaling ordinations suggested that temperature was highly correlated to fish communities captured at our sites. Survival of larval Fathead Minnows was examined in situ at six sites from 13 May to 11 June 2004 in the same system. Larval survival was lower, but not significantly different between sites directly downstream of AMD-impacted tributaries (40% survival) and non-AMD sites (52% survival). The lower survival was caused by a significant mortality event at one site that coincided with acute pH depression in an AMD tributary immediately upstream of the site. Results from a Cox proportional hazard test suggests that low pH is having a significant negative influence on larval fish survival in this system. The results from this research indicate that the combination of low pH events and elevated temperature are negatively influencing the larval fish populations of the Stony River watershed. Management actions that address these problems would have the potential to substantially increase both reproduction rates and larval survival, therefore greatly enhancing the fishery.

Introduction

Early life stages (ELS) of fishes are highly sensitive to perturbations in the surrounding environment, both natural and anthropogenic (Henry et al. 1999, Houde 1989b, Mion et al. 1998, Sandstrom et al. 1997). Consequently, the strength of any year class and the overall population size are subject to events and conditions that influence survival of those ELS. In impaired systems, survival may be low, and entire year classes may be absent from the population (Leis and Fox 1994). Several successive years of extremely low survival may result in the extirpation of species from an impaired system (McCormick et al. 1989). Reduced fish populations in lotic systems may result from the inability of ELS fish to survive anthropogenically induced stressors. When multiple anthropogenic stressors are present in a system, the effects of each individual stressor may be uncertain. In coal-bearing regions, coal-fired power plants are often located close to mines to minimize coal transportation costs. As a result,

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in areas with bituminous coal seams, thermal enrichment from power plants and acid mine drainage (AMD) often occur simultaneously, and the combined disturbance on the biota may be unclear.

Thermal enrichment has been shown to negatively influence successful spawning and recruitment in various temperate fishes through several mechanisms. Oogenesis in fishes can occur abnormally early in thermally enriched systems, resulting in egg re-absorption and/or degradation before spawning occurs (Luksiene et al. 2000). At high temperatures, fertilized eggs may metabolize energy stores before hatching can occur (Sandstrom et al. 1997). This hyper-metabolism can also impact larvae, causing yolk-sac absorption before external feeding components are fully developed (Houde 2002). Additionally, spring-spawning temperate fishes often spawn early in thermally enriched systems (Cooke et al. 2003, Paller and Saul 1996, Sandstrom et al. 1995), exposing ELS fish to environmental conditions that may reduce survival. These include high river discharges that can scour young fish from nursery habitats, acid pulses from snow melt, and thermal shock caused by rapidly changing temperatures (Mion et al. 1998, Shuter et al. 1980). Also, spawning is often timed with the availability of an ephemeral food source (i.e., plankton bloom), and larvae that hatch out of synchrony with this food source can experience starvation (Houde 2002).

Acid mine drainage streams have three general characteristics that can reduce ELS fish survival: increased acidity, elevated dissolved ion concentrations (including metals), and metal precipitates (particularly iron and aluminum hydroxides) (Gray 1996). Severity of AMD varies widely within and among impacted streams, but all can negatively affect fish (Gensemer and Playle 1999). Direct acid toxicity occurs by interfering with ion regulation at the gills (McDonald et al. 1989, Verbost et al. 1995). Excess H^+ ions compete with essential ion exchange (Na^+, Cl^-, and Ca^{2+}), leading to ion imbalance and, in extreme cases, death (Gensemer and Playle 1999, Potts and McWilliams 1989). In AMD waters, metal ion toxicity functions similarly to, and occurs in conjunction with, acid toxicity, causing ionoregulatory malfunctions (McDonald et al. 1989). The most detrimental characteristic of mild AMD to fishes is the polymerization and precipitation of aluminum onto the gills, which leads to gill necrosis and asphyxia (Henry et al. 1999, Verbost et al. 1995, Witters et al. 1996). Aluminum precipitation is particularly lethal in mixing zones of acidic and circumneutral waters, and is often more toxic than just high acidity in streams alone (Henry et al. 1999, Poleo et al. 1994, Witters et al. 1996).

In systems with both thermal and AMD pollutions, synergism may reduce larval fish survival. Aluminum toxicity can increase with increasing temperature as elevated rates of aluminum speciation and polymerization occur at higher temperatures (Gensemer and Playle 1999, Lydersen et al. 1990, Poleo et al. 1991). In systems influenced by both AMD and thermal elevation, mortality events may occur more quickly and at lower aluminum and acid concentrations than they would under non-elevated temperatures.

The objective of this study was to quantify and assess the influences of AMD and thermal enrichment on the stream reproduction of fishes and larval survival. We hypothesized that the effects of multiple stressors (AMD and temperature) would be greater than individual effects on larval fish survival. To evaluate this hypothesis, we conducted a larval survey and in situ larval fish assays along an environmental gradient representative of both thermal and AMD disturbances in the same watershed.

Field-site Description

The Stony River located in Grant County, WV (Fig. 1) is a high-elevation (650–1200 m), high-gradient (≈1.4%) system that drains into the North Branch Potomac River. Both thermal and AMD influences are present in the watershed (Horn 2005). Recent surveys in the system have shown that fish populations are small and species diversity is low (Hoar 2005), likely due to anthropogenic disturbances. Species captured during electrofishing surveys by Hoar (2005) included *Campostoma anomalum* (Rafinesque) (Central

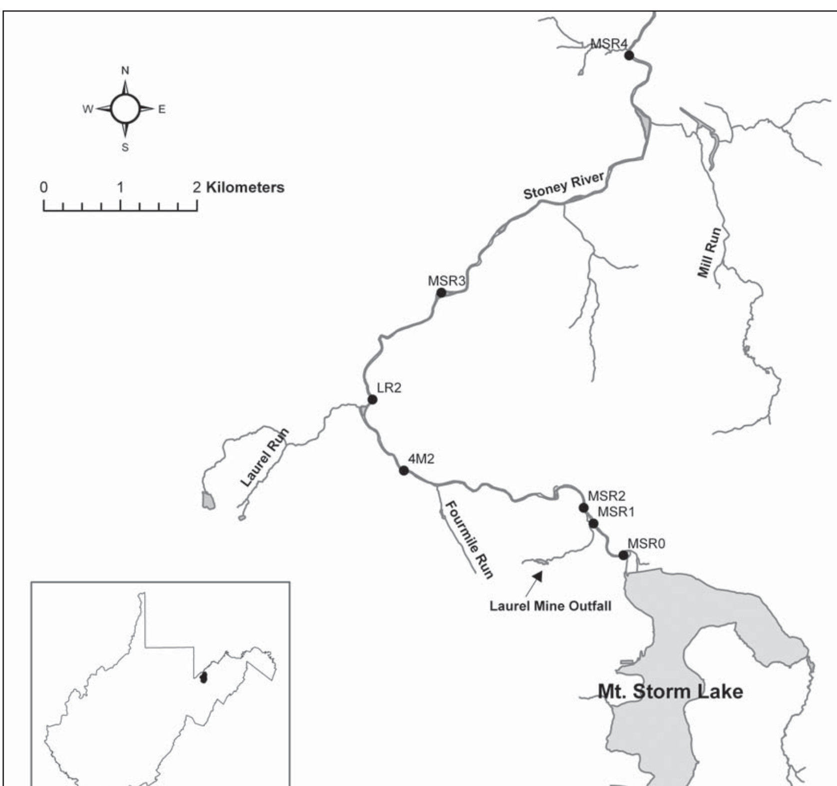

Figure 1. Sites in the Stony River watershed utilized during larval fish studies. All sites except MSR4 were used in both trapping and in situ bioassays. MSR4 was used only for trapping.

Stoneroller), *Cyprinella spiloptera* (Cope) (Spotfin Shiner), *Semotilus atromaculatus* (Mitchill) (Creek Chub), *Cottus bairdi* Girard (Mottled Sculpin), *Etheostoma flabellare* Rafinesque (Fantail Darter), *Ictalurus punctatus* (Rafinesque) (Channel Catfish), *Lepomis cyanellus* Rafinesque (Green Sunfish), *Micropterus dolomieu* Lacepède (Smallmouth Bass), and *Micropterus salmoides* (Lacepède) (Largemouth Bass).

A coal-fired electric generating station obtains cooling water from an impoundment of the river (Mt. Storm Lake), and heated discharge from the lake elevates the thermal regime in the Stony River year-round. Lake discharges are highly variable and cease periodically because of low water levels, thereby decreasing river temperatures. Subsequent precipitation events can increase lake levels, inducing a thermally elevated discharge, resulting in temperature increases in the Stony River. Moving downstream, the severity of thermal discharge lessens with the entry of groundwater and surface-water tributaries, but water temperatures do remain elevated throughout the river (≈20 km) to the confluence with the North Branch Potomac River on the West Virginia and Maryland border.

Three tributaries downstream of the dam are AMD influenced, with two of the three being treated prior to confluence with the Stony River. For the sake of simplicity, all mine-influenced tributaries (treated or not) are termed AMD from here on, even though chemical conditions may not be similar to untreated AMD water. The first tributary to enter is the Laurel Run mine outfall. This water is treated to reduce acidity and dissolved metals, and treatment is effective and consistent. The next downstream tributary, Fourmile Run, undergoes AMD treatment, but treatment is highly variable, and the tributary is prone to large fluctuations in pH and dissolved metal concentrations. Fourmile Run creates the most noticeable and severe AMD conditions of any tributary to the Stony River. The third AMD tributary, Laurel Run, is not treated and remains acidic (pH 4.0–6.0) year round, but is relatively small and has less influence on the water quality of the Stony River than does Fourmile Run.

Methods

The following description of study sites is in upstream to downstream order (Fig. 1). Sites MSR0 and MSR1 are nearest the dam, and are influenced by thermal enrichment only. Site MSR2 is downstream of the first AMD input (Laurel Run mine outfall). The next site, 4M2, is downstream of the AMD tributary Fourmile Run. Continuing downstream, LR2 is directly downstream of the small AMD tributary, Laurel Run. The remaining sites, MSR3 and MSR4, represent diminishing thermal and AMD influences along the river continuum.

Continuous data-collection units (Datasonde 4 multi-probe hydrolab, Hach Environmental, Loveland, CO) were maintained at all seven of the sites mentioned above by Dominion Environmental, the environmental quality subsidiary of Dominion Resources, Inc., Richmond, VA, which owns the power plant. These units recorded in-stream temperature, pH, and specific conductivity hourly. Our experiments were conducted within 200 m of these data-collection units to allow comparison of results with water quality parameters.

Larval trapping survey

A larval trapping survey was undertaken in 2004 to determine the timing and relative reproductive success of fishes in the Stony River (below Mt. Storm Lake). Larvae were captured with shallow-water quatrefoil light traps (Aquatic Research Instruments, Lemhi, ID) using a green, six-inch Cyalume® chemical lightstick (Omniglow Corp., West Springfield, MA) as a larval attractant during nighttime (Kissick 1993). Previous research by Gerhke (1994) showed that green light sticks glowed brighter during the first hour of sampling than other colored light sticks and produced sufficient light to attract fish larvae. Chemical light traps have been reported to sample larval fishes as efficiently as electric light traps (Gerhke 1994, Kissick 1993).

Trapping was conducted from 26 March to 2 July 2004 at all seven sites mentioned previously. Over this period, ten trapping events occurred. During each trapping event, two traps were set at each site in areas of low water velocity and depth of >0.5 m (pool-type habitats). Traps were activated sequentially (beginning at 2200 hours), remained active for approximately two hours (range = 1.83–2.20 hours), and were retrieved in the same sequence as set. Captured larvae were preserved in 10% buffered formalin (Floyd et al. 1984) and later measured (total length) and identified to species in the laboratory using the methods described by Auer (1982). Because light emitted from chemical light sticks diminishes over time, capture efficiency bias was minimized by keeping the trapping-effort times consistent. Other factors, such as water turbidity, could also affect catch efficiency of light traps. However, turbidity was similar across sites on each sample date, so turbidity effects on capture efficiency would be shared across sites for a trapping event. Temperature and water quality data for the trapping period were obtained from Dominion Environmental's data loggers.

The structure of the larval fish community data for all seven sites was analyzed by a nonparametric multidimensional scaling (NMDS) ordination technique (McCune and Grace 2002). Because effort was similar across all sites (range = 31.57–32.09 hrs), total catch was used for NMDS ordination. The vegan package (Oksanen et al. 2008) in program R was used to run the NMDS. Sites that plot close together in the ordination space are more similar than sites that plot farther apart (Merovich and Petty 2007). To assess the influences of thermal and AMD enrichment on reproduction of stream fishes, function Envfit (part of the vegan package in program R) was used to fit vectors for summary statistics (minimum, maximum, standard deviation, and average) of each environmental variable (pH, temperature, and conductivity) and plot them onto the NMDS ordination. The number of permutations was set to 10,000, and only vectors with *P*-values < 0.05 were plotted. Bray-Curtis distance metrics were used for all NMDS ordinations (Hawkins and Norris 2000).

In situ bioassay

To examine larval fish survival, a four-week in situ bioassay was conducted from 13 May to 11 June 2004. Six of the seven described sites were chosen for bioassays: MSR0, MSR1, MSR2, 4M2, LR2, and MSR3. Each of these sites represents a point of change in the environmental gradient along the Stony River continuum and has an associated in-stream water quality data-collection unit.

Pimephales promelas Rafinesque (Fathead Minnows) were chosen as a representative warmwater fish for the bioassays because they are a standardized test organism that is endorsed by the US EPA (Milam et al. 2000). Fertilized Fathead Minnow eggs were obtained from the US EPA laboratory in Cincinnati, OH. Upon arrival, eggs were thermally acclimated, then transferred to water from the site MSR1 (temperature = 24 °C, pH = 7.5, specific conductivity = 160 $\mu S/cm^3$) for hatching in the laboratory. After hatch, larvae (<24 hours old) were transported in aerated, insulated coolers to test sites. Larvae were acclimated to river temperatures at each site by immersing transport containers into the river until thermal equilibrium was reached. Twenty-five larvae were placed into each of 14 test containers at each site, for a total of 350 larvae per site. One test container from site MSR1 was omitted from statistical analysis, as handling errors reduced the survival of that replicate.

Larval containers were constructed from 3.7-L clear plastic jars (model 3314, Rubbermaid Home Products, Fairlawn, OH). Three 7.5-cm diameter holes were drilled into the sides of each container along the median horizontal axis, and covered with 0.4-mm mesh nitex screen (Sefar Filtration Inc., Dewey, NY). Each of these 14 larval containers was placed into a holding rack made from untreated pine wood, which was housed in one of two 85-L plastic tubs (model 25487, Sterilite Corporation, Townsend, MA). Water depth in each tub was maintained to immerse each larval container >95%. Tubs were partially buried and anchored in the stream channel, near the stream bank. Water was gravity-fed to the tubs via a pair of 15-mm diameter rubber hoses from 10–30 m upstream. The intake ends of hoses were covered in coarse-mesh aluminum screen to prevent clogging. Flow through each tub was adjusted daily to ≈5 L/min (Hulsman et al. 1983) via a valve fitted to each feeder hose. Within each tub, a HOBO® Water Temp Pro (Onset Computer Corp., Bourne, MA) temperature-collection unit was set to record temperature every hour for the duration of the test.

Containers were checked daily to monitor larval survival and water quality, and for maintenance. Living larvae in each container were counted to determine survival. Larvae were considered dead when no movement was observed when prodded, and deceased individuals were removed daily following the methods of Rickwood et al. (2006). Larvae in each of the 3.7-L jars received 2.5 mL of a concentrated suspension of live brine shrimp nauplii (<24 hours after hatch) daily to supplement natural food sources (Stewart et al. 1990). Each day, the screens on feeder hoses were cleaned of algae and detritus to minimize clogging, and flow rates were adjusted to ≈5 L/min.

Temperature (°C), pH, and specific conductivity (μS/cm^3) were measured daily (YSI multimeter model 650, Yellow Springs, OH) within tubs at each site. These measures were used to verify continuous data from data-collection units maintained by Dominion Environmental near each test site. Mean temperatures were calculated from HOBO® temperature loggers within each tub, while mean pH and specific conductivity were calculated from the in-stream data-collection units for each site.

Package survival (Therneau and Lumley 2008) in program R was used to estimate survival along with 95% confidence intervals for each site. The function survdiff (part of the package survival in R) was used to test for significant differences ($P \leq 0.05$) in survival curves between sites directly downstream of AMD-impacted tributaries and non-AMD sites.

A Cox proportional hazard analysis (Kleinbaum and Klein 2005) was performed to assess the effects of pH, conductivity, and temperature on hazard rates during in situ bioassays. The mean number of fish alive on each day among the 14 jars at each site was used for this statistical analysis. Covariates were measured on each day throughout the study, so they were treated as time dependent. We checked the proportional hazard assumption by correlating scaled Schoenfeld residuals to the estimated survival function and computing a chi-square significance test for the resulting correlation (Fox 2002). A test of proportionality was then obtained using the cox.zph function (part of the package survival in program R). The martingale residuals were plotted against covariates to detect nonlinearity (Fox 2002).

Results

Larval trapping survey

Larval fishes were collected in the Stony River during 2004, but overall catch was low (Fig. 2). A total of 379 larvae were captured from 26 March to 1 June 2004. No larvae were captured during the 24 June and 2 July trapping attempts, and no larvae were captured at MSR0 throughout the duration of the study. Catch peaked in April, and was dominated by *Catostomus commersoni* (Lacepède) (White Sucker) larvae, which comprised 90% of all larvae captured. Other species captured were Creek Chub, Central Stoneroller, and Smallmouth Bass. Water quality measurements recorded during the trapping period are summarized in Table 1.

Because no larvae were captured at MSR0, a small number (1.00E-30) was added to the White Sucker catch for that site. This adjustment was necessary for the NMDS ordination to work properly. The NMDS ordination with two dimensions was selected because the ease of interpretation and the stress was low (7.28). Two convergent solutions were found after four tries. Maximum temperature, average May temperature, average June temperature, and average temperature all had P-values < 0.05, suggesting correlation, and were plotted on the NMDS ordination (Table 2).

Table 1. Data collected by Dominion Environmental, Richmond, VA from the Stony River, 2002 to 2004. Data are summarized to represent major differences among sites along the river continuum during spring months. Numbers in parentheses denote standard deviation. SpC = specific conductivity (uhmos).

Site	Mean temp. (ºC) early spring[A]	Mean temp. (ºC) late spring[B]	Associated with AMD tributary	AMD precipitate present	Mean pH[C]	Mean SpC[D]	Max/min SpC[D]	Mean Al[C] in main stem (ppb)	Mean Al[E] in AMD tributary (ppb)	Mean hardness as $CaCO_3$ (ppm)[F]
MSR0	15.7 (1.1)	23.2 (2.0)	No	No	7.4 (0.2)	157 (5)	181 / 145	17.0 (13.6)		74 (14)
MSR1	14.7 (1.6)	21.8 (1.1)	No	No	7.0 (0.3)	192 (130)	572 / 125	31.2 (17.7)		68 (15)
MSR2	14.2 (1.5)	20.6 (1.8)	Yes	No	7.7 (0.3)	442 (305)	1214 / 145	30.3 (15.6)	31.3 (8.8)	372 (382)
4M2	13.8 (1.8)	20.6 (1.8)	Yes	Heavy	7.8 (0.9)	231 (358)	1373 / 167	33.5 (15.5)	239.0 (78.0)	309 (232)
LR2	12.9 (2.2)	20.1 (1.2)	Yes	Light	7.4 (0.4)	407 (281)	1223 / 137	34.3 (16.7)	107.0 (81.2)	310 (191)
MSR3	12.5 (2.4)	20.3 (1.5)	No	No	7.2 (0.9)	257 (243)	1009 / 41	31.3 (17.3)		258 (164)
MSR4	10.6 (3.0)	18.7 (0.7)	No	No	7.3 (0.4)	282 (120)	688 / 127	18.8 (12.9)		180 (57)

[A]Temperature values calculated from hourly in-stream measurements from March to April 2004.
[B]Temperature values calculated from hourly in-stream measurements from May to June 2004.
[C]pH values calculated from hourly in-stream measurements from March to June 2003 and 2004.
[D]Specific conductivity values from hourly in-stream data collected from March to June 2004.
[E]Aluminum values (total Al) calculated from four measurements from 2002 to 2004.
[F]Hardness values calculated from five measurements from 2002 to 2004.

In situ bioassay

Mean survival in the field bioassay ranged from 0–64% across all sites (Table 3). Survival was lower, but not significantly different among

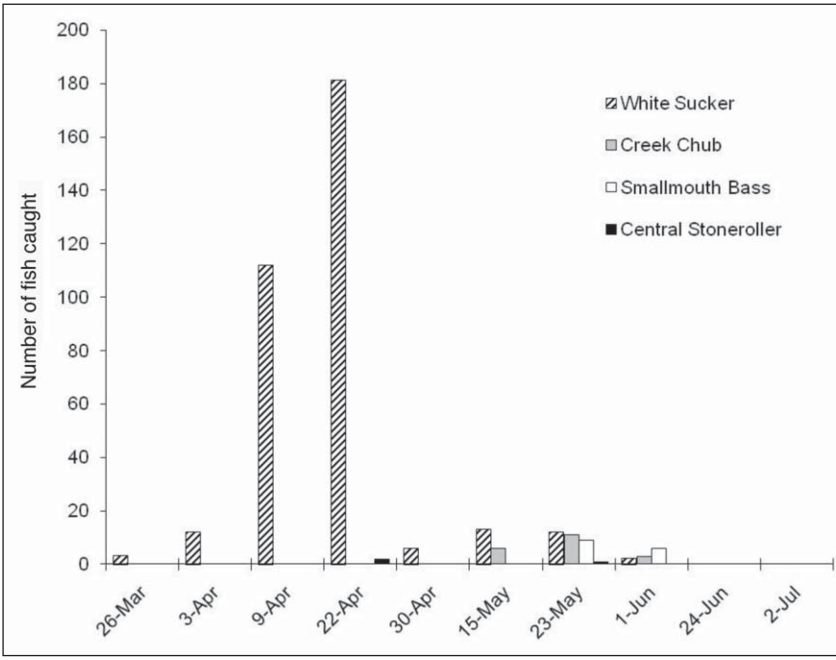

Figure 2. Total number of larval fishes caught with light traps during each sampling period.

Table 2. Vectors for summary statistics of environmental variables collected at sites MSR0, MSR1, MSR2, 4M2, LR2, and MSR3 along the Stony River, WV. The number of permutations was set to 10,000, and only vectors with P-values < 0.05 (those in bold) were plotted in the NMDS ordination.

Vectors	NMDS1	NMDS2	r^2	Pr(>r)
Average temperature	**-0.42**	**0.91**	**0.87**	**0.02**
Standard deviation temperature	-0.74	0.67	0.18	0.71
Maximum temperature	**-0.50**	**0.86**	**0.85**	**< 0.01**
Minimum temperature	-0.21	0.98	0.60	0.15
March average temperature	-0.35	0.94	0.70	0.09
April average temperature	-0.38	0.93	0.73	0.07
May average temperature	**-0.46**	**0.89**	**0.89**	**0.01**
June average temperature	**-0.54**	**0.84**	**0.78**	**0.03**
Average pH	-0.61	-0.79	0.09	0.86
Standard deviation pH	-0.91	0.41	0.22	0.60
Maximum pH	-0.70	-0.71	0.16	0.75
Minimum pH	0.94	0.35	0.46	0.28
Range pH	-0.82	-0.58	0.38	0.34
Average conductivity	0.90	-0.43	0.19	0.62
Standard deviation conductivity	0.55	-0.83	0.21	0.58
Maximum conductivity	0.39	-0.92	0.18	0.64
Minimum conductivity	-0.02	1.00	0.18	0.73

sites directly downstream of AMD-impacted tributaries and non-AMD sites ($\chi^2 = 1.7$, df = 1, $P = 0.20$, Fig. 3). Most mortality observed occurred during the first fifteen days of the test. Survival declined steadily at all sites except below Fourmile Run (4M2), which experienced an acute mortality event between the 21st and 22nd of May 2004. This mortality event coincided with

Table 3. Physiochemical conditions at in situ bioassay sites. Data for site 4M2 encompasses only the days up to the major mortality event (day 10 of test), while data for other sites covers the entire test period. Numbers in parentheses denote standard deviation.

Site	Mean temp. (ºC)	Temperature range (ºC)	Mean pH	pH range	Mean specific conductivity (µS/cm³)	Specific conductivity range (µS/cm³)	Mean survival
MSR0	23.8 (3.1)	17.5–27.8	7.7 (0.2)	7.3–7.9	185 (40)	159–278	0.44 (0.33)
MSR1	22.8 (2.6)	15.5–26.5	7.7 (0.2)	7.5–8.1	173 (22)	149–232	0.60 (0.38)
MSR2	21.9 (3.4)	14.7–26.7	7.9 (0.1)	7.7–8.1	558 (400)	104–1223	0.64 (0.38)
4M2	22.3 (2.0)	18.7–24.3	7.6 (0.5)	6.3–8.1	490 (256)	331–1170	0 (NA)
LR2	20.4 (3.2)	13.7–24.9	7.6 (0.1)	7.3–7.9	509 (320)	201–1170	0.56 (0.37)
MSR3	19.8 (2.6)	14.3–23.5	7.6 (0.1)	7.5–8.0	473 (276)	201–1042	0.52 (0.36)

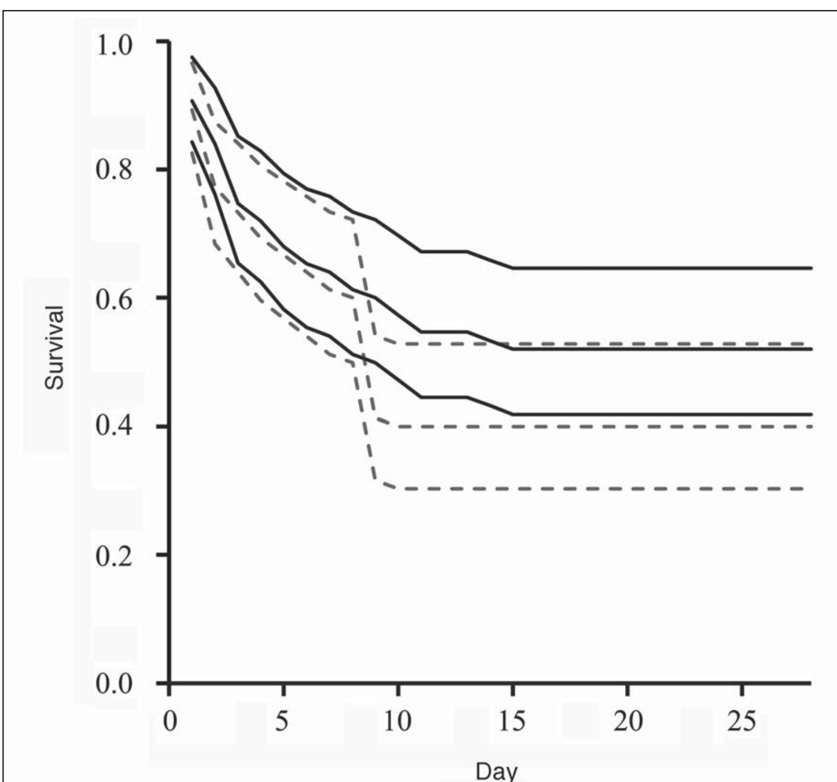

Figure 3. Comparison of daily mean survival for larval Fathead Minnows at sites directly downstream of AMD impacted tributaries and non AMD sites during in situ bioassays.

an 18-hour depression in pH to <5.0 in the tributary Fourmile Run (Fig. 4). Water quality measurements recorded at in situ bioassay locations are summarized in Table 3.

Cox proportional hazard test revealed that low pH had a strong negative influence on survival of larval Fathead Minnows in our in situ bioassay (Table 4). The log likelihood ratio test for the model including temperature, conductivity, and pH was significant (log likelihood = 45.8, df = 3, $P < 0.001$). Figure 5 summarizes predicted survival at the levels of pH, conductivity, and temperature that were present during this study. Both the proportional hazard (global model: $\chi^2 = 5.86$, df = 3, $P = 0.12$) and the non-linearity assumptions were met.

Discussion

Larval trapping survey

Trapping suggests that abundance of fish larvae in the Stony River is low. Floyd et al. (1984) captured 4549 larvae in 96 hours of light trapping out of the

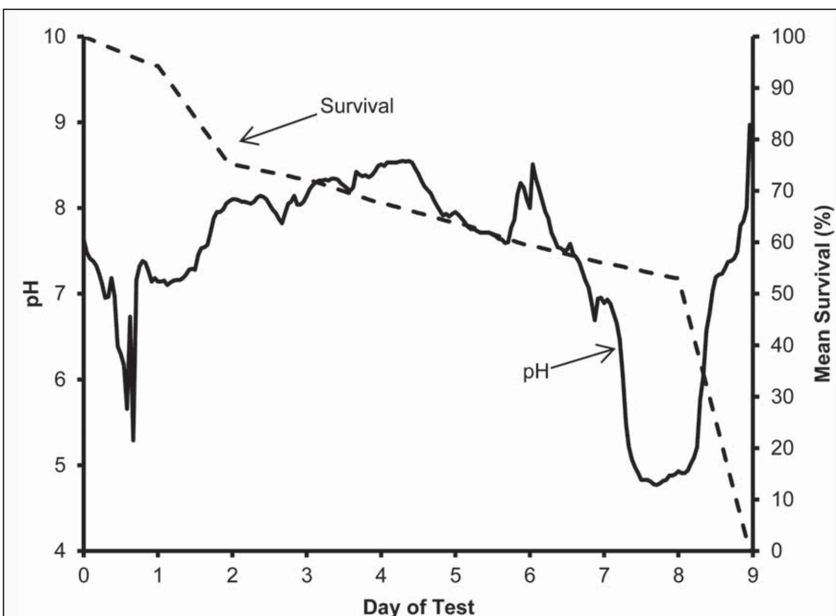

Figure 4. In situ survival of larval Fathead Minnows at site 4M2 and pH flux in Fourmile Run, which enters the Stony River directly upstream of 4M2.

Table 4. Results from the Cox proportional hazard test ($n = 150$; 25/site).

	Coefficient	se(Coefficient)	z	*P*-value
Conductivity	0.001	0.001	1.08	0.28
pH	-2.580	0.399	-6.47	<0.01
Temperature	0.163	0.104	1.57	0.12

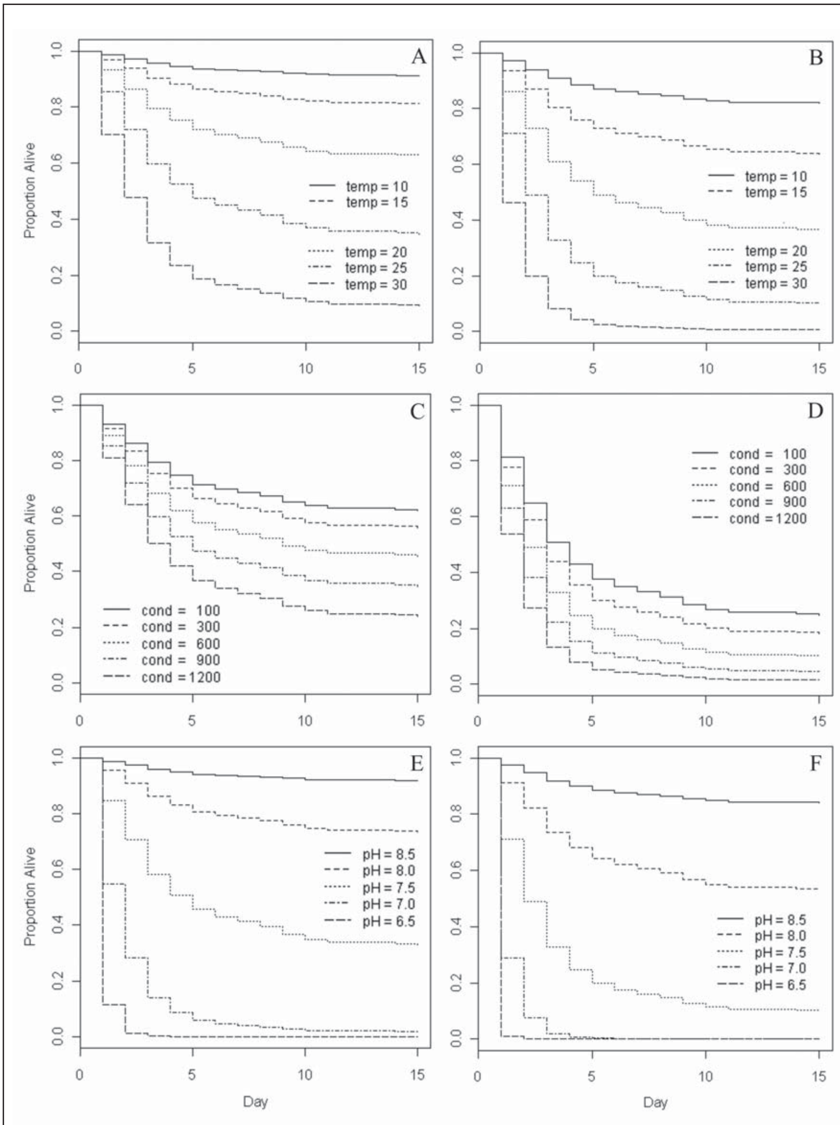

Figure 5. Cox proportional hazard survivorship curves for Fathead Minnows at the varying levels of pH, conductivity (μS/cm^3), and temperature (°C). Graphs A and B show estimated survival at different temperatures while holding conductivity and pH constant at mean values (368 and 7.72, respectively; A) and constant at values of 600 and 7.72, respectively (B). Graphs C and D show estimated survival at different conductivities while holding temperature and pH constant at mean values (21.85 and 7.72, respectively; C) and constant at values of 25 and 7.5, respectively (D). Graphs E and F show estimated survival at different pH values while holding temperature and conductivity constant at mean values (E) and constant at values of 25 and 600 (F), respectively.

Middle Fork of Drake's Creek, KY. Marchetti et al. (2004) captured 4672 larval fish from the upper mainstem Sacramento River, CA, in 120 hours of light trapping. Niles and Hartman (2007) captured 9221 larvae from the Kanawha River, WV, in 378 hours of light trapping. Using the same methods as Floyd et al. (1984), Marchetti et al. (2004), and Niles and Hartman (2007), we only captured 379 larvae in 220 hours of trapping in this study, providing evidence to suggest that overall stream fish reproduction in the Stony River is low.

A possible explanation for our low capture rates is that the spring spawning event did not entirely overlap with our trapping period (26 March to 2 July 2004). However, the peak capture rates for all four species we captured were between 22 April and 23 May. Catch rates decreased with every trapping event prior to 22 April. Catch rates for all species declined after 23 May, and no larvae were captured in the two trapping periods after 1 June. Thus, the timing of our trapping seems to have encompassed the major spring spawning event, and the low larval fish capture rates are not likely an artifact of our sampling design, but rather are most probably explained by some other factor.

Fitted vectors on the NMDS ordination provided strong evidence that high water temperatures were having a significant influence on the larval fish communities at our sites in the Stony River, WV. Early life stages of fishes have been found to be very sensitive to temperature conditions (Houde 1989a, Pepin 1991). Thus, it is likely that thermally induced mortality during the ELS of fishes in the Stony River is a major factor contributing to low catch rates.

No larval fishes were captured at site MSR0, while White Suckers and Central Stonerollers were captured at MSR1. Although temperature is having a significant effect on the fish communities of sites MSR0 and MSR1, the temperature regimes at both of these sites were very similar. It is therefore unlikely that temperature is the only reason that larval fish were not captured at site MSR0. Bed coursing and loss of spawning gravel below dams is a common occurrence (Kondolf 1997). All of the species captured in this study spawn over medium- to small-sized gravels (Curry and Spacie 1984, Lukas and Orth 1995, Miller 1962, Ross and Reed 1978). MSR0 is approximately 200 m from the dam, where loss of spawning habitat resulting from scouring could be contributing to the zero capture rate at this site.

Temperature effects from the thermally enriched Mount Storm Lake diminished farther downstream in the Stony River as water from coldwater tributaries and groundwater inputs entered the system. Site MSR2 is directly below a tributary which caused the average late spring temperature to be 2.6 °C cooler than MSR0, the farthest upstream site. Smallmouth Bass have been classified as a coolwater fish (Eaton and Scheller 1996), and larvae were captured in MSR2 but not in MSR1, where high temperatures were still present. This result implies that if the temperature regime of the Stony River was decreased, the habitat for larval Smallmouth Bass could be substantially improved in the section downstream of the impoundment.

Because Smallmouth Bass are sensitive to acidic conditions (Snucins and Shuter 1991), the capture of Smallmouth Bass at MSR2, an AMD-influenced site, is evidence that MSR2 is treated effectively. Larval fish commonly experience downstream drift (Brown and Armstrong 1985), so it is possible that the Smallmouth Bass captured at MSR2 had drifted from upstream. However, Smallmouth Bass were not captured at MSR1, which was only approximately 250 m upstream of MSR1. Therefore, if Smallmouth Bass were present upstream of MSR2, they should have been captured in the trapping that occurred at MSR1. The only other site where larval Smallmouth Bass were captured was MSR4, which is the site farthest downstream, where AMD effects have diminished. The capture of Smallmouth Bass at MSR2 suggests that if all AMD tributaries within the study area were treated effectively, fish populations could be improved and the Smallmouth Bass population would likely increase.

Only three larval fish were captured at 4M2, a severely AMD-influenced site. This capture rate was much lower than the capture rates at MSR1 and MSR2, the other AMD-influenced sites. Sporadic treatment failures on 4M2, like the event on 21–23 May, are the most probable reason for low capture rate at 4M2. Treatment keeps pH high the majority of the time in Fourmile Creek, but sporadic treatment failures may be enough to have a severe negative influence on the stream fish communities.

In situ bioassay

Significant larval mortality was observed in conjunction with fluctuations in AMD severity. The major mortality event that occurred at site 4M2 followed lowered pH (<5.0) in the AMD tributary, Fourmile Run. This event did not create a significant difference between survival curves of sites directly downstream of AMD-impacted tributaries and non-AMD sites because other treated sites (MSR2) mitigated the impacts. Fluctuations in the pH of Fourmile Run created acutely toxic conditions in the Stony River likely related to fluctuations in metal solubility (aluminum). Mixing zones between acidic and circumneutral streams are often highly toxic due to aluminum polymerization and precipitation (Henry et al. 1999, Poleo et al. 1994). Even when conditions are not overly acidic (pH > 6.0), high mortality can occur (Verbost et al. 1995). Thus, it seems that when an AMD tributary is treated to circumneutral pH, acute larval toxicity is reduced. However, alterations in treatment that lead to periodic depressions of pH and increased aluminum solubility can create acutely toxic conditions. Such conditions could have severely negative effects on larval fish populations when larvae encounter these mixing zones during downstream drift. Similarly, adults exposed to mixing zones during spawning migrations may experience mortality or avoid these areas, further reducing potential larval production. With 23,000 km of AMD-influenced streams in the United States (Sasowsky et al. 2000), the implications for riverine fishes are significant.

The survival rates of larvae at MSR1 and MSR2 (upstream and downstream of the Laurel Run mine outfall) suggests that effective treatment of mine wastes creates water quality conditions tolerable by larval fishes. Larval survival was also similar to MSR1 and MSR2 at site LR2, even though it is downstream of an AMD tributary that is acidic on occasion. The range in pH (7.3–7.9) at LR2 over the course of the study suggests that treatment was effective at creating stable pH levels, at least over the short term. The stable pH levels at LR2 may be the reason that the survival estimates were similar among MSR1, MSR2, and LR2. Another possible explanation is that because of physical constraints, assays were placed on the side of the Stony River opposite the confluence with the AMD tributary (LR2). Thus, any mixing-zone effects that occur near the confluence may have been missed.

Results from the Cox proportional hazard test clearly show pH largely affects survival rates of larval fishes in the Stony River. Predicted survival rates decrease substantially at low pH values. High temperature also had major influences on estimated survival rates from Cox proportional hazard models. The combination of high spring temperatures and low pH from ineffectively treated AMD tributaries is having severe negative impacts on the larval fish communities of the Stony River. Management actions that would decrease spring and summer water temperatures in combination with effectively treating AMD-impacted tributaries should greatly benefit the fish populations of the Stony River through increased larval survival and recruitment.

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