FastTracked

Effects of major ions on natural benthic communities: an experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity

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Abstract: Elevated concentrations of HCO_3^-, Cl^-, SO_4^{2-}, Mg^{2+}, Na^+, and Ca^{2+} in freshwater ecosystems are often associated with anthropogenic disturbances. The US Environmental Protection Agency developed a field-based specific conductance (SC) benchmark of 300 μS/cm for streams affected by mountain-top mining operations. The benchmark has been criticized because of the potential influence of confounding variables and difficulty in demonstrating a causal relationship between elevated SC and macroinvertebrate responses. We conducted 4 stream mesocosm experiments to quantify the effects of major ions on aquatic insect assemblages. We exposed insects from streams with low (60–72 μS/cm) and moderate (200–250 μS/cm) SC to major ions at values bracketing 300 μS/cm. We measured community metabolism, macroinvertebrate drift, community composition, and survival. Sixty-six taxa were exposed to $NaHCO_3$, $MgSO_4$, and NaCl in 4 mesocosm experiments, and 8 dominant families/subfamilies occurred in sufficient densities to develop SC-response relationships. Significant SC-response relationships occurred for each major ion tested. Drift increased and community metabolism decreased with increasing SC. Ephemeroptera were highly sensitive, whereas Trichoptera and Diptera were relatively tolerant. EC20 values (the SC that resulted in a 20% difference from controls) ranged from 151 to 3615 μS/cm and were >300 μS/cm for most endpoints. Mayfly drift, abundance of baetid and heptageniid mayflies, total mayfly abundance, and community metabolism were affected at SC levels near or <300 μS/cm. EC20 values were lower for $NaHCO_3$ and $MgSO_4$ than for NaCl, indicating greater toxicity of these 2 salts. Effects were greater on communities from the low- than the high-SC stream. Thus, accounting for context-dependent responses may be important when establishing contaminant benchmarks or thresholds. The 300-μS/cm benchmark is protective of aquatic insect communities in naturally low-conductivity streams.

Key words: mountaintop mining, salts, context-dependent responses, toxicity, mesocosms

Salinization of streams and rivers is considered one of the most important threats to the ecological integrity of freshwater ecosystems (Kaushal et al. 2005) and is recognized as a stressor of global concern (Cañedo-Argüelles et al. 2013). Natural variation in total dissolved solids is strongly influenced by local geology and patterns of precipitation and evaporation (Griffith 2014), but anthropogenic disturbances significantly increase concentrations of major ions (e.g., HCO_3^-, Cl^-, SO_4^{2-}, Mg^{2+}, Na^+, and Ca^{2+}) in streams. Sources of major ions include road deicing, industrial discharges, agricultural irrigation, produced waters from oil and gas wells, salt mines, and mountaintop mining valley fill (MMVF) operations, a process whereby coal seams are exposed and the resulting overburden materials are discarded into surrounding watersheds (Palmer et al. 2010).

Toxic effects of major ions on aquatic insects are highly variable (Table 1), but generally are associated with increased ionic stress and the metabolic cost associated with osmoregulation (Williams et al. 1999, Lob and Silver 2012, Cormier et al. 2013b). The effect of major ions on streams is relatively widespread because of the ubiquitous distribution of salts in the environment. In a national-scale assessment of road-salt effects in the USA, Corsi et al. (2010) reported that Cl^- concentrations exceeded the US EPA chronic water-quality criterion (230 mg/L) at 55% of sites in northern metropolitan areas during winter. However, toxic effects of salt on *Ceriodaphnia dubia*, a typical laboratory test species, occurred at 1050 mg Cl^-/L. These findings suggest that daphnids and other crustaceans are relatively tolerant to major ions, a finding that has been reported consistently

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Table 1. Examples of studies showing variation in the toxicity of major ions to aquatic insects based on laboratory experiments, mesocosm experiments, and field surveys. LC50 and EC20 = conductivity level that resulted in 50% mortality or 20% difference from controls, respectively; MMVF = mountaintop mining valley fill.

Source of contamination	Endpoints (duration of exposure)	Effect levels (μS/cm)	Reference
Road salt (laboratory)	Survival of limnephilid caddisflies (96 h LC50)	3526–7700	Blasius and Merritt (2002)
Road salt (laboratory)	Mean survival of many insect species (72 h LC50)	29,000	Kefford et al. (2012)
Salt mining (mesocosm)	Reduced density and diversity; increased drift (72 h)	>5000	Cañedo-Argüelles et al. (2012)
Road salt (laboratory)	*Chironomus riparius* survival and emergence (67 d)	5000	Lob and Silver (2012)
Brine salt (laboratory)	Growth of early instar *Neocloeon triangulifer* (20 d)	672	Johnson et al. (2015)
Reconstituted MMVF waters (laboratory)	Survival of early instar *Neocloeon triangulifer* (35 d)	800–1300	Kunz et al. (2013)
MMVF (field)	Alterations in community composition of aquatic insects	300–500	Pond et al. (2008), Cormier et al. (2013a)
Various salts (mesocosm)	Total abundance of mayflies (10 d EC20)	280–1287	Present study

in the literature (Dunlop et al. 2008, Kefford et al. 2012). However, in field studies, aquatic insects, especially mayflies, are quite sensitive to major ions (Pond et al. 2008, Pond 2010, Cormier et al. 2013b). Tolerance to salts is inversely related to the length of time since a group evolved from ancestors in the marine environment (Hart et al. 1991), so we would expect greater sensitivity of some aquatic insects, especially Ephemeroptera, because of their ancient evolutionary lineage. In addition to the influence of evolutionary history on salt tolerance, some investigators have reported significant regional variation in salinity effects on macroinvertebrates. Species in arid regions with greater natural salinity generally are more tolerant to major ions than are species in regions with naturally low salinity (Dunlop et al. 2008, Kefford et al. 2012, Cañedo-Argüelles et al. 2014). Because effects of salinity probably will be context-dependent and species-specific, macroinvertebrate assemblages from different streams with different background conductivities should be included in tests of toxicity of major ions.

The toxicity of NaCl to aquatic organisms has been well studied, but much less is known about the effects of major ions from other sources. One of the more pervasive sources of major ions to streams in the USA central Appalachians is mountaintop coal mining (Palmer et al. 2010, Lindberg et al. 2011). Discharges from MMVF operations contain a complex mixture of major ions, the toxicity of which varies significantly with the specific ionic composition (Mount et al. 1997). The major ions associated with MMVF operations include Ca^+, Mg^+, HCO_3^-, and SO_4^{2-} (Griffith et al. 2012), among which the anions HCO_3^- and SO_4^{2-} are considered the most toxic to aquatic organisms (Mount et al. 1997). Investigators of MMVF operations in the central Appalachian Mountains estimate that ~10% of this region is disturbed by mining (Palmer et al. 2010), which has resulted in the burial of >2000 fluvial km of headwater streams (Bernhardt and Palmer 2011). Ecological effects of MMVF operations include degradation of water quality and significant alterations in the structure of macroinvertebrate and fish communities (Pond et al. 2008, Griffith et al. 2012, Hitt and Chambers 2014). In an assessment of the cumulative negative effects of mountaintop mining in central Appalachia, Lindberg et al. (2011) reported a 30 to 40× increase in SO_4^{2-} and a 9× increase in conductivity in a mined watershed compared to unmined sites. Degradation of water quality increased with the areal extent of mining and effects continued to persist for 2 decades after reclamation.

Effects of MMVF discharges on stream benthic communities have received attention in the literature. The US Environmental Protection Agency (EPA) used a novel field-based approach to develop a conductivity benchmark for Appalachian streams affected by MMVF operations (USEPA 2011, Cormier et al. 2013a). Species sensitivity distributions derived from 2210 stream samples in West Virginia (USA) were used to develop a 300-μS/cm benchmark for conductivity which, in theory, should protect 95% of the species in the region. This benchmark has been criticized (Roark 2013), in part because of the difficulty demonstrating a causal relationship between conductivity and macroinvertebrate responses based on field data (Gensemer et al. 2013). To address this criticism, a formal weight-of-evidence approach was developed (Cormier et al. 2013b) based on a set of criteria derived from epidemiological methods (Hill 1965). The 6 criteria used to support this causal relationship included: 1) co-occurrence of the stressor and responses, 2) an identified causal pathway between major ions and responses, 3) physical interaction between major ions and macroinvertebrates, 4) evidence that macroinvertebrate communities are changed by major ions, 5) evidence that exposure to major ions was sufficient to produce the observed effect, and 6) evidence that cause preceded the effect. This analysis provided support for the hypothesis that elevated conductivity altered benthic communities in the field, but we think that the relationship could be significantly strengthened by the addition of direct experimental evidence.

The objectives of our research were to investigate effects of major ions on natural assemblages of aquatic insects in stream mesocosms and to develop predictive relationships between specific conductance (SC) and benthic community metrics. We estimated effect values for several structural and functional measures and compared these to the 300-μS/cm benchmark for SC developed by the EPA (USEPA 2011, Cormier et al. 2013a). Some investigators have shown that previous exposure to major ions influences sensitivity (Dunlop et al. 2008, Kefford et al. 2012), so we also investigated context-dependent responses to salinization by comparing effects on communities from streams with different background SC. We tested the hypotheses that: 1) major ions are toxic to aquatic insects, 2) the 300-μS/cm benchmark for SC is protective of aquatic insect communities, and 3) responses to SC vary among communities from different streams.

METHODS

We conducted a series of 4 mesocosm experiments to assess the effects of major ions on natural aquatic insect communities. We used 3 experiments to examine the effects of $MgSO_4$, $NaHCO_3$, and NaCl on communities collected from the South Fork of the Michigan River (MR), a 3rd-order stream (elevation = 2761 m) in Routt National Forest, Colorado. The headwaters of the MR are in the Never Summer Wilderness Area, and the stream has no history of contamination. Background SC (60–72 μS/cm) and water hardness (30–40 mg $CaCO_3$/L) in the MR were within the range of values reported by Pond et al. (2008) for West Virginia reference streams (34–133 μS/cm, 17–72 mg $CaCO_3$/L) and were typical of undisturbed mountain streams in this region (Griffith 2014). We conducted a 4th mesocosm experiment with NaCl and communities collected from the Cache la Poudre River (PR) to examine the influence of community composition and background SC on responses to major ions. Our study site on the PR, a 5th-order stream at an elevation of 1573 m, was 15 km northwest of Fort Collins, Colorado. Background levels of SC (200–250 μS/cm) and water hardness (70–100 mg $CaCO_3$/L) were 3–4× greater in the PR than in the MR.

We conducted all mesocosm experiments during the ice-free season from autumn 2013 to summer 2014 (Table 2). We collected aquatic insect communities with 10 × 10 × 6-cm colonization trays filled with small cobble and pebble. We deployed trays in the field for 30 to 40 d, and they were colonized by a diverse assemblage of aquatic insects. The composition of communities colonizing these trays is similar to communities on natural substrate (Clements 1999). To assess initial community composition, we collected colonized trays from the MR (October 2013) and PR (April 2014) at the start of the mesocosm experiments. These day-0 samples consisted of 4 randomly selected individual trays, which were combined and washed through a 350-μm sieve in the field. Replicate samples (n = 3–5) were preserved in 80% ethanol. We also sampled benthic communities (n = 3) in the PR with a 0.1-m^2 Hess sampler (350-μm mesh) and compared them with communities on trays. We processed Hess samples as described above for day-0 trays.

We collected colonized trays for mesocosm experiments and placed them in small insulated containers (4 trays/container) for transport to the Stream Research Laboratory (SRL) at the Colorado State University Foothills Campus. The SRL consists of 18 stream mesocosms housed in a greenhouse that receives natural water directly from a deep, mesotrophic reservoir. We have used the SRL to quantify responses of aquatic insect communities to several natural and anthropogenic stressors, including trace metals (Clements et al. 2013), acidification (Courtney and Clements 2000), and UV-B radiation (Kashian et al. 2007). Water in control mesocosms was characterized by low hardness (30–38 $CaCO_3$ mg/L) and dissolved organic C (2.5–3.0 mg/L), cool temperatures (9.0–12.9°C), circumneutral pH (7.7–8.1), and low SC (74–79 μS/cm). Current in the 20-L mesocosms was provided by paddlewheels that maintained a constant current velocity of 0.45 m/s. Each flow-through stream received water from a headbox at 0.5 L/min, resulting in a turnover time of ~40 min. We placed the 4 colonized trays in each container in a single mesocosm, which was randomly assigned to a treatment. Mesocosm standpipes were covered with a screen to prevent emigration of organisms. After a 24-h acclimation period, we used peristaltic pumps that dripped stock solutions from 20-L carboys (10 mL/min) to dose streams in triplicate (n = 3) with major ions for 10 d. Six target concentrations (based on the respective major anions) ranged from 0 to 1000 mg/L in the $NaHCO_3$ and $MgSO_4$ experiments (Table 2). We anticipated that NaCl would be less toxic to aquatic insects than the other salts, so target Cl^- concentrations in MR (NaCl-mr) and PR (NaCl-pr) experiments ranged from 0 to 1200 mg/L and 0 to 1800 mg/L, respectively.

We placed drift nets (20 × 15 cm) downstream from the trays in each mesocosm immediately after dosing began and removed them 24 h later. The drift nets captured all aquatic insects drifting in the water column. We rinsed drift samples through a 350-μm sieve and preserved organisms in 80% ethanol.

We estimated community metabolism by measuring diurnal changes in dissolved O_2 concentration (DO) in each mesocosm at the end of the experiment. Measures of

Table 2. Routine physicochemical characteristics measured during each of the 4 stream mesocosm experiments and target and measured anion (HCO_3^-, SO_4^{2-}, and Cl^-) concentrations in each experiment. NaCl experiments were conducted with communities from the Cache la Poudre River (PR; NaCl-pr) and Michigan River (MR; NaCl-mr).

Salt (Date of experiment)	Target concentration (mg/L)	Measured concentration (mg/L)	Conductivity (μS/cm)	pH	Temperature (°C)	Dissolved O_2 (mg/L)
$NaHCO_3$ (October 2013)	0	33.3	74.3	8.1	11.7	8.5
	125	170.0	270.7	8.4	11.6	8.5
	250	324.5	463.7	8.6	11.4	8.4
	500	588.0	861.1	8.7	11.5	8.4
	750	746.6	1120.3	8.7	11.6	8.2
	1000	843.0	1351.5	8.7	11.5	8.3
$MgSO_4$ (October 2013)	0	5.7	75.5	7.7	10.7	8.5
	125	116.7	318.9	7.6	10.7	8.5
	250	276.7	561.4	7.6	10.6	8.5
	500	440.0	814.6	7.6	10.6	8.5
	750	713.3	1212.7	7.5	10.7	8.4
	1000	760.0	1401.3	7.5	10.6	8.4
NaCl-pr (April 2014)	0	2.6	78.4	8.0	9.0	10.0
	75	92.8	441.2	7.9	9.1	9.8
	150	175.0	699.3	7.9	8.9	9.8
	300	321.7	1134.7	7.8	9.0	9.8
	600	526.7	1897.9	7.8	9.1	9.8
	1200	978.3	3395.6	7.7	9.1	9.8
NaCl-mr (August 2014)	0	2.9	79.3	8.0	12.9	8.8
	75	86.0	363.6	7.8	12.7	8.8
	150	168.3	625.8	7.7	12.6	8.8
	300	310.0	1107.5	7.6	12.6	8.6
	600	545.0	1815.3	7.6	12.9	8.6
	1800	1433.3	4433.9	7.3	12.8	8.5

DO in daylight reflect both respiration and photosynthesis, whereas measures at night reflect autotrophic and heterotrophic respiration (Odum 1956). Although this approach does not account for abiotic factors that influence DO and is not a direct measure of total metabolic activity, we assumed that differences between light and dark would provide a reasonable estimate of whole community metabolism.

We measured SC daily and pH, temperature, and DO on days 2, 6, and 10 in each mesocosm with handheld YSI meters (models 550A and 63; Yellow Springs Instruments, Yellow Springs, Ohio). To verify target anion concentrations, we collected 50-mL water samples and analyzed for Cl^- and SO_4^{2-} using ion chromatography (USEPA 1993). We estimated HCO_3^- from our measurements of $CaCO_3$ by titration. After 10 d, we drained the streams, removed the trays, removed the organisms by rinsing the substrate, and sieved all surviving organisms through a 350-μm mesh. We preserved organisms in 80% ethanol.

Data analysis

We developed SC-response relationships for macroinvertebrate drift, community metabolism, abundance of dominant taxa, abundance of macroinvertebrate orders, and total abundance. We used SC instead of major anion concentrations for these analyses to facilitate comparisons among the 3 salts and because previous research has shown that SC is the best predictor of stream condition below mine discharges (Pond et al. 2008). We used regression analyses instead of the more-traditional analysis of variance (ANOVA) with multiple comparisons because of criticisms of ANOVA in ecotoxicological research (Laskowski 1995) and because we were interested in estimating the SC that caused a specific effect (the SC that reduced macroinvertebrate abundance or community metabolism by 20% compared to controls [EC20]). We conducted all analyses with SAS (PROC REG, version 9.3; SAS Institute, Cary, North Carolina) on $\log_{10}(x)$-transformed response variables. We estimated EC20 values from regression equations for all significant ($p < 0.10$) relationships. We selected a slightly higher p-value than the usual 0.05 a priori because of the expected high within-treatment variation and to protect against Type II errors (e.g., incorrectly concluding there was no effect of major ions). Avoidance of Type II errors is especially important in applied research where the societal risks of incorrectly concluding no effects of an anthropogenic stressor are high (Brosi and Biber 2009). Note that lower EC20 values indicate greater (more toxic) effects of major ions. The EC20 value for macroinvertebrate drift was defined as the SC that increased drift by 20% compared to controls. We used a similar approach to estimate EC20 values based on measured anion concentrations (Table S1). We tested for differences in mean EC20 values among the 4 experiments with 1-way ANOVA.

RESULTS

Physicochemical conditions in stream mesocosms

Measured anion concentrations increased with treatment levels but generally were lower than target concentrations in the higher treatments (Table 2). These lower concentrations were caused by the difficulty of keeping salts in solution in the 20-L dosing carboys. Routine physicochemical characteristics in stream mesocosms varied among the 4 experiments, reflecting natural seasonal fluctuations in the source-water quality, but were generally similar among treatments within an experiment (Table 2). The only exception to this pattern was pH in the $NaHCO_3$ experiment, which was greater in treated mesocosms than in controls. SC ranged from 74 µS/cm in control mesocosms to 4434 µS/cm in the highest NaCl treatment and was highly correlated (r^2 > 0.99) with measured anion concentrations in each experiment.

Initial community composition

Total macroinvertebrate abundance and number of taxa in trays collected at the start of the experiment (day 0) were similar to values in control mesocosms after 10 d (Fig. 1A, B). Total macroinvertebrate abundance was greater and number of taxa was slightly lower in the Cache la Poudre River (PR) compared to the Michigan River (MR). Abundance and number of taxa measured in natural substrate at the PR was also similar to values in day-0 trays. Abundance of dominant macroinvertebrate groups was variable among the 4 experiments, reflecting natural spatial and seasonal differences in community composition (Fig. 1C). Communities from the $NaHCO_3$ and $MgSO_4$ experiments were dominated by mayflies, stoneflies, and dipterans, whereas dipterans (primarily orthoclad chironomids and blackfly larvae) accounted for 76 to 86% of total individuals in the 2 NaCl experiments.

Effects of major ions

Macroinvertebrate drift and community metabolism Mayflies (Ephemeroptera) were the dominant organisms in drift samples from the $MgSO_4$, $NaHCO_3$ and NaCl-mr experiments, and accounted for 43% of all organisms across all treatments. In contrast, blackflies (Simuliidae) accounted for 86% of the organisms in drift samples from the NaCl-pr experiment. Moderate increases in SC caused significant in-

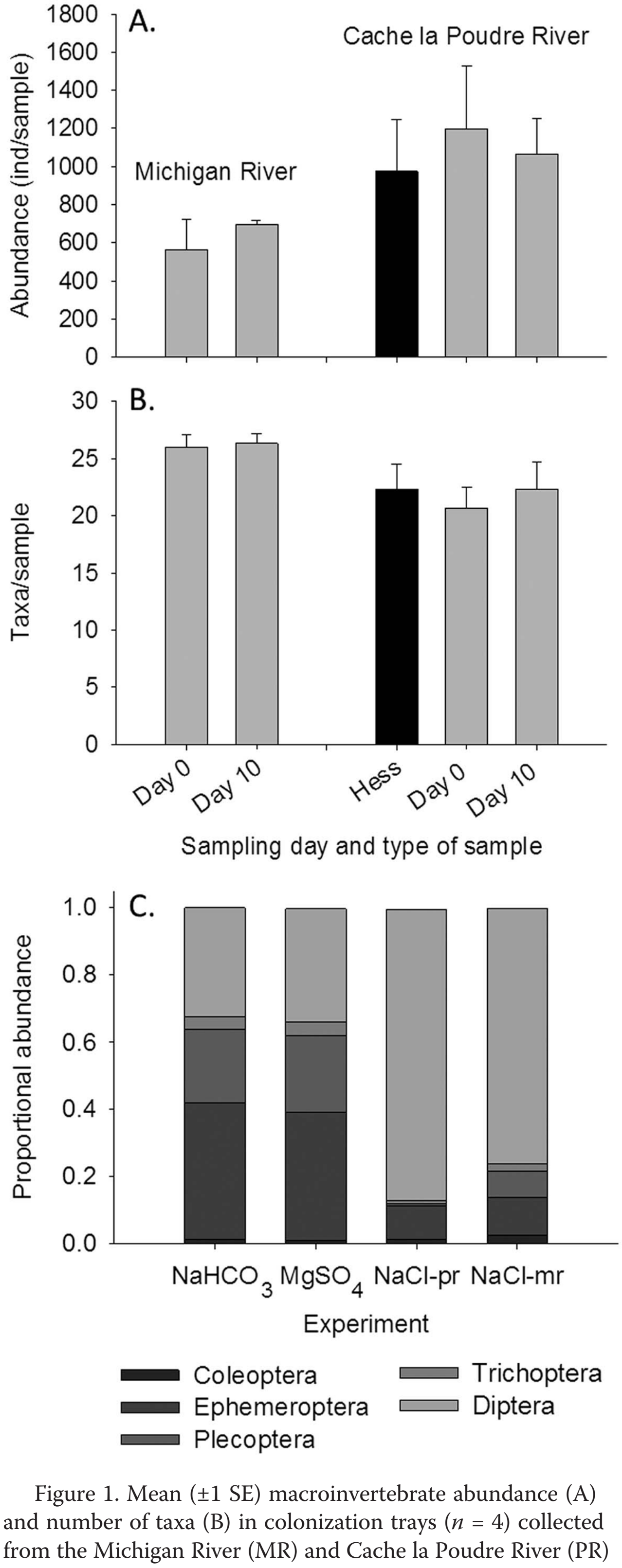

Figure 1. Mean (±1 SE) macroinvertebrate abundance (A) and number of taxa (B) in colonization trays (n = 4) collected from the Michigan River (MR) and Cache la Poudre River (PR) at the start of the mesocosm experiments (day 0), in control mesocosms at the end of the experiments (day 10), and from natural substrate in Hess samples from the PR, and proportional abundance of major macroinvertebrate groups in control mesocosms at the end of each experiment (C). Ind = individuals.

creases in mayfly drift in all experiments except for NaCl-pr (Fig. 2A–D). The strongest response was observed for the $MgSO_4$ experiment, in which total mayfly drift increased by ~2× when SC was >300 μS/cm (Fig. 2B).

Community metabolism was sensitive to SC and decreased significantly with increasing concentrations of all major ions (Fig. 2E–H). Responses varied among salts, but effects were greatest in the $MgSO_4$ and $NaHCO_3$ experiments. Estimated EC20 values for SC in the $MgSO_4$ and $NaHCO_3$ experiments were 285 μS/cm and 369 μS/cm, respectively (Table 3). We observed significant relationships between NaCl and community metabolism, but these effects were much less compared to the other salts, particularly on communities from the PR.

Macroinvertebrate abundance and community composition
Significant SC-response relationships were observed for each mesocosm experiment, but the strength of these relationships varied among taxa and among major ions. Total macroinvertebrate abundance was not affected in the NaCl-pr experiment, but significant SC-response relationships were observed in the other 3 experiments (Fig. 3A–D). Mean EC20 values for total abundance ranged from 390 to 1026 μS/cm (Table 3). In contrast to the patterns for macroinvertebrate abundance, total number of taxa was relatively insensitive to major ions in our experiments and was reduced only in the NaCl-pr experiment (r^2 = 0.59, p = 0.0748).

A total of 66 macroinvertebrate taxa were exposed to major ions across the 4 experiments, and 8 dominant families/subfamilies (Baetidae, Ephemerellidae, Heptageniidae, Chloroperlidae, Capniidae, Brachycentridae, Orthocladiinae, Elmidae) occurred in sufficient densities to develop SC-response relationships (Fig. S1A–H). Baetid (Fig. S1A), ephemerellid (Fig. S1B), and heptageniid mayflies (Fig. S1C) were the most sensitive groups, but effects of major ions were consistently less in the NaCl-pr than in the other experiments. Mean estimated EC20 values for Baetidae ranged from 206 to 1104 μS/cm (Table 3). Mean EC20 values for Heptageniidae from the MR were very similar for all salts (334–394 μS/cm), but heptageniids in the NaCl-pr experiment were unaffected by major ions (Table 3). In contrast to patterns observed for mayflies, most stoneflies, caddisflies, and elmid beetles were relatively tolerant to major ions and showed little response to increased SC (Fig. S1D–G). Orthoclad chironomids, the dominant dipterans in all experiments, showed moderate sensitivity to $NaHCO_3$ and NaCl, with mean EC20 values of 429 and 821 μS/cm, respectively (Fig. S1H).

Total abundance of mayflies was highly sensitive to major ions, with significant SC-response relationships observed in each experiment (Fig. 3E–H). EC20 values for total mayfly abundance ranged from 280 μS/cm ($NaHCO_3$) to 1287 μS/cm (NaCl-pr). Effects of NaCl were considerably greater on mayfly assemblages from the MR (EC20 = 387 μS/cm) than from the PR (Fig. 3G, H). Total abundance of stoneflies (Plecoptera) responded significantly to major ions in 3 of the 4 mesocosm experiments (Table 3), but these organisms were considerably more tolerant than mayflies (Fig. S1I). Mean EC20 values for total stonefly abundance ranged from 670 to 3614 μS/cm, with $NaHCO_3$ being the most toxic salt.

Effects of major ions on aquatic macroinvertebrates differed significantly among the 3 salts and between the 2 communities that we tested (Fig. 4A). Across all endpoints, mean EC20 values were considerably lower for $NaHCO_3$ and $MgSO_4$ than for NaCl, indicating greater toxicity of these salts. We also found evidence that effects of NaCl were greater on communities from the MR than the PR. The EC20 value for NaCl was 42% lower for MR than for PR communities. We observed considerable variation among endpoints in the responses to major ions (Fig. 4B). Mean EC20 values across the 13 endpoints that showed significant SC-response relationships ranged from 370 to 2372 μS/cm. These results illustrated the greater sensitivity of mayflies to major ions because abundance and drift of these organisms consistently had the lowest EC20 values. EC20 values were >300 μS/cm for most endpoints, but several metrics (mayfly drift in the $NaHCO_3$ and $MgSO_4$ experiments, total mayfly abundance, abundance of baetid mayflies in the $NaHCO_3$ experiment and community metabolism in the $MgSO_4$ experiment) were affected at SC levels near this benchmark (Table 3).

DISCUSSION

The most significant finding of this research was that natural benthic communities collected from a low-conductivity stream were highly sensitive to major ions. Effects observed in mesocosm experiments varied among major ions, endpoints, and taxonomic groups, but mayflies were particularly sensitive to elevated SC. The intolerance of mayflies relative to other taxonomic groups has been a consistent finding reported in laboratory experiments (Kunz et al. 2013, Johnson et al. 2015) and field studies (Hartman et al. 2005, Pond et al. 2008). The extirpation of mayflies downstream from MMVF mining operations in West Virginia was an important factor contributing to the development of the 300-μS/cm SC benchmark for these watersheds (USEPA 2011, Cormier et al. 2013a). Results of our experiments suggest that this benchmark provides a reasonable level of protection for most aquatic insect groups. However, we observed increased mayfly drift, reduced community metabolism, and reduced abundance of some mayflies (Baetidae and Heptageniidae) at SC levels near this benchmark. In addition, our results suggest that SC values >~1200 μS/cm, levels commonly observed in mined watersheds throughout the central Appalachians (Lindberg et al. 2011, Griffith et al. 2012), would have major restructuring effects on stream benthic communities.

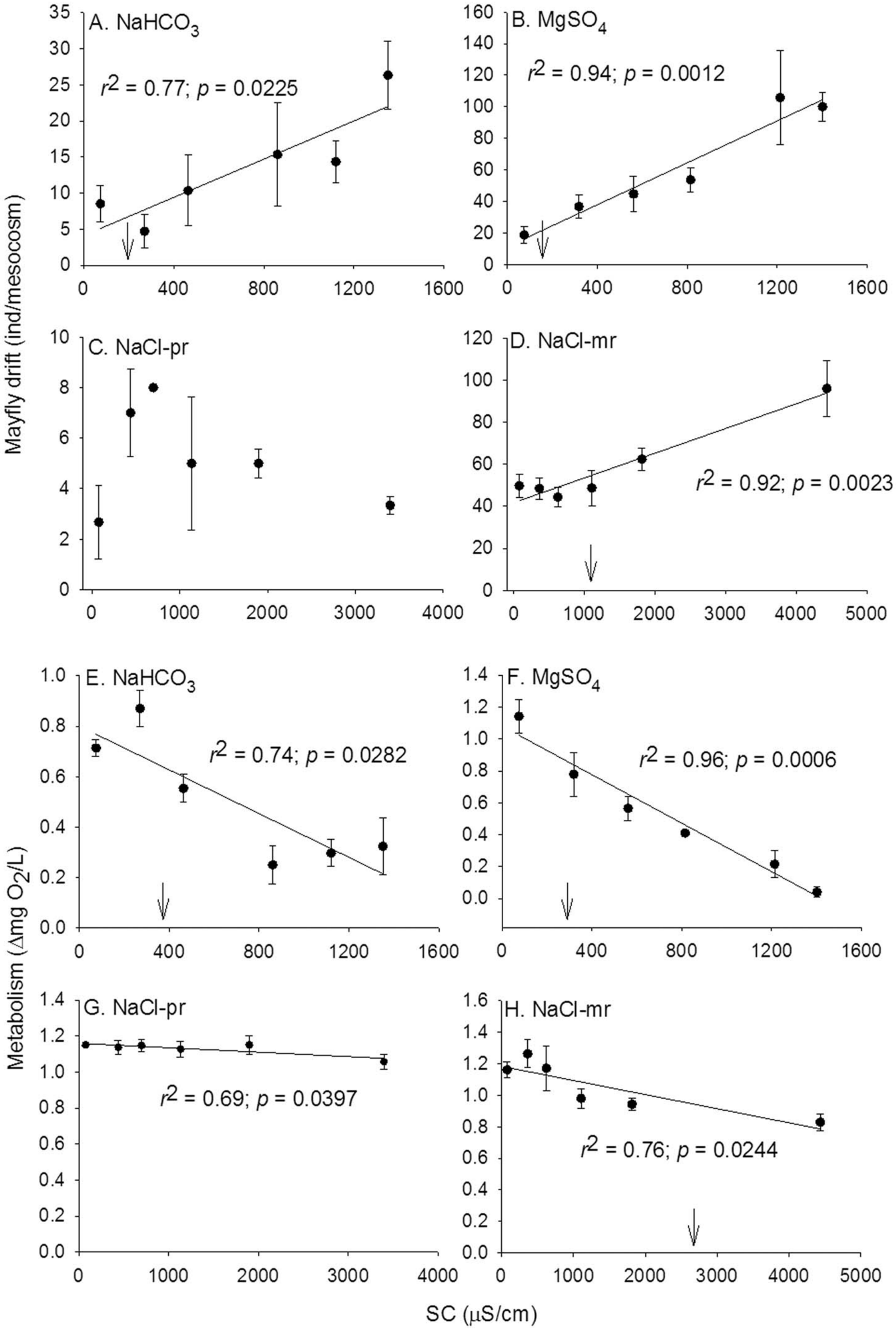

Figure 2. Mean (±1 SE) mayfly drift (A–D) and community metabolism (E–H) in stream mesocosms treated with $NaHCO_3$ (A, E), $MgSO_4$ (B, F), NaCl-pr (C, G), or NaCl-mr (D, H). NaCl experiments were conducted with communities from the Cache la Poudre River (PR; NaCl-pr) and Michigan River (MR; NaCl-mr). Regression lines, r^2 values, p values, and arrows indicating the estimated specific conductance (SC) that resulted in a 20% difference from controls (EC20) are shown for all significant ($p < 0.10$) relationships. An EC20 value is not shown for metabolism in the NaCl-pr experiment because it was beyond the range of data used to develop the regression. Ind = individuals.

Table 3. Mean (± SE) specific conductance (SC) that resulted in a 20% difference from controls (EC20) for all metrics that showed a significant ($p < 0.10$) relationship in the mesocosm experiments. NaCl experiments were conducted with communities from the Cache la Poudre River (PR; NaCl-pr) and Michigan River (MR; NaCl-mr). – indicates the relationship was not significant, * indicates the estimated EC20 value was outside the predicted range based on the regression equation (data not included in the summary of EC20s shown in Fig. 4). EPT = Ephemeroptera, Plecoptera, Trichoptera.

Metric	$NaHCO_3$	$MgSO_4$	NaCl–pr	NaCl–mr
Baetidae	206 (164, 275)	418 (313, 625)	1104 (891, 1452)	427 (334, 591)
Ephemerellidae	684 (494, 1112)	–	–	425 (361, 516)
Heptageniidae	334 (280, 413)	383 (340, 438)	–	394 (355, 443)
Capniidae	–	–	–	1006 (688, 1869)
Simuliidae	–	1049 (767, 1660)	–	1231 (977, 1663)
Orthocladiinae	429 (312, 685)	–	–	821 (682, 1031)
Total Ephemeroptera	280 (221, 382)	435 (349, 577)	1287 (1013, 1765)	387 (337, 456)
Total Plecoptera	670 (460, 1230)	–	3614 (2573, 6072)	2833 (2153, 4143)
Total Diptera	368 (252, 728)	–	–	1021 (836, 1313)
EPT abundance	384 (294, 554)	692 (567, 881)	1442 (1148, 1938)	967 (807, 1207)
Total abundance	390 (287, 609)	833 (600, 1363)	–	1026 (847, 1301)
Ephemeroptera drift	196 (153, 271)	151 (135, 172)	–	1077 (940, 1260)
Community metabolism	369 (284, 525)	284 (258, 317)	9406* (7058, 14,096)	2638 (2055, 3685)

Results of our mesocosm experiments differ from some laboratory studies that showed high tolerance of aquatic insects to salts (Blasius and Merritt 2002, Dunlop et al. 2008, Kefford et al. 2012). The relatively short duration of most laboratory toxicity tests (72–96 h) may account for these differences because significant increases in toxicity of major ions have been observed when exposure duration was extended (Hassell et al. 2006, Echols et al. 2010). However, investigators conducting longer-term exposures (e.g., 2 mo) also have shown relatively high salt tolerance for some aquatic insects (e.g., chironomids; Lob and Silver 2012). We believe the greater sensitivity of mayflies in our study was partially explained by the strong effects on smaller, early instars which were the predominant life stage in the MR during our late summer and autumn experiments. Previous investigators have reported greater sensitivity of early instars to NaCl (Kefford et al. 2004, 2007), and similar findings have been observed for metals (Kiffney and Clements 1996, Clark and Clements 2006). Because most aquatic insects cannot be cultured in the laboratory, toxicity tests typically are done with larger instars, which are easily collected in the field but are generally more tolerant to chemical stressors. We think these differences in sensitivity among instars have important implications for interpreting responses to major ions and other contaminants in the field. For example, if instar size influences sensitivity to contaminants, seasonal variation in effects will be determined largely by insect phenology. Therefore, we would expect greater effects of contaminants to occur when a population is dominated by smaller instars (Clements et al. 2013). Phenology of aquatic insects and its potential influence on sensitivity is generally not considered when developing benchmarks or water-quality criteria for contaminants.

An exception to the high tolerance of aquatic insects to salts observed in laboratory experiments has been reported for the mayfly *Neocloeon triangulifer* (Table 1). Because these organisms are parthenogenetic and can be cultured in the laboratory, experiments have been conducted with very early instars (e.g., 5 d after hatching). Results of these experiments have reported an EC20 value of 672 μS/cm and significantly reduced survival at SC > 1000 μS/cm (Kunz et al. 2013, Johnson et al. 2015), values consistent with our mesocosm results. One of the often cited advantages of mesocosm experiments is the ability to quantify differences in sensitivity among species (Schäfer et al. 2011). Because our experiments exposed natural communities of aquatic insects across a wide range of size classes, another advantage of this approach is the ability to investigate effects on different developmental stages.

The greater sensitivity of mayflies relative to traditional laboratory test species (e.g., *Ceriodaphnia dubia*, *Daphnia magna*) has important implications for the development of water-quality criteria for major ions and other contaminants. Laboratory toxicity tests conducted with crustaceans showed moderate tolerance for major ions (Dunlop et al. 2008, Corsi et al. 2010), probably reflecting the shorter length of time since this group evolved from the marine environment (Hart et al. 1991). In contrast, experiments conducted with *Isonychia* sp. (Ephemeroptera) exposed to either NaCl (Echols et al. 2010) or coal-mining effluents

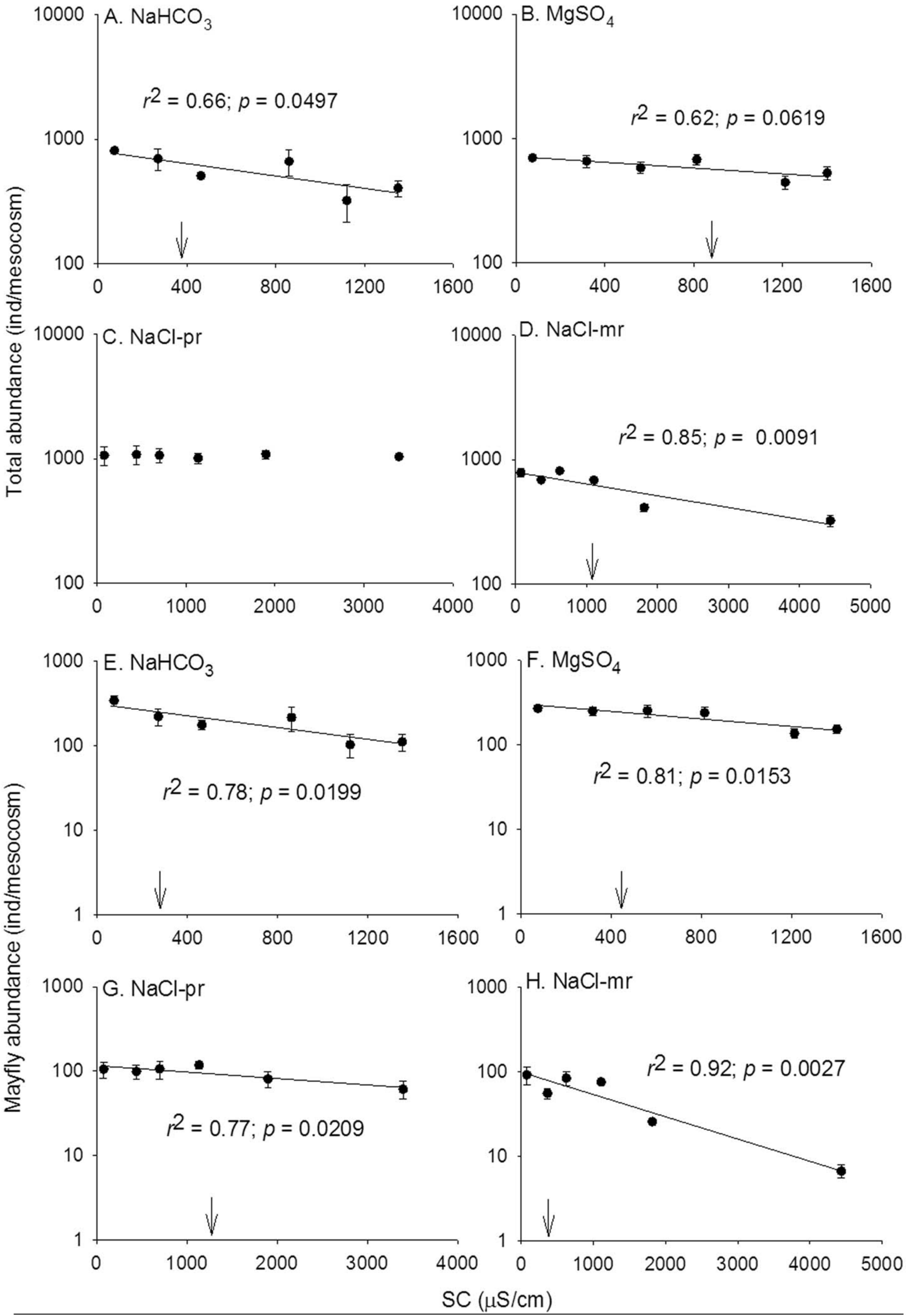

Figure 3. Mean (±1 SE) total macroinvertebrate abundance (A–D) and total mayfly abundance (E–H) in stream mesocosms treated with $NaHCO_3$ (A, E), $MgSO_4$ (B, F), NaCl-pr (C, G), or NaCl-mr (D, H). NaCl experiments were conducted with communities from the Cache la Poudre River (PR; NaCl-pr) and Michigan River (MR; NaCl-mr). Regression lines, r^2 values, p-values, and arrows indicating the estimated specific conductance (SC) that resulted in a 20% difference from controls (EC20) are shown for all significant ($p < 0.10$) relationships.

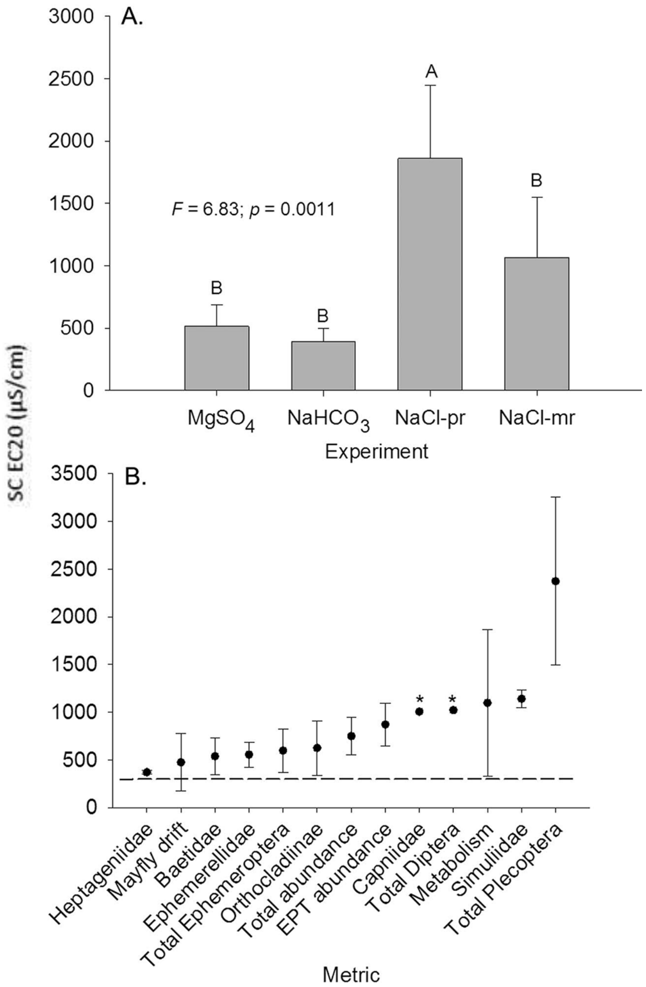

Figure 4. Mean (±1 SE) specific conductance (SC) that resulted in a 20% difference from controls (EC20) among the 4 mesocosm experiments (A) and among the 13 metrics that showed a significant ($p < 0.10$) relationship with SC (B). NaCl experiments were conducted with communities from the Cache la Poudre River (PR; NaCl-pr) and Michigan River (MR; NaCl-mr). Results of 1-way analysis of variance testing for differences among experiments are shown in A. Means with the same letter were not significantly different based on Duncan's multiple range test. Horizontal line in B represents the 300 µS/cm SC benchmark. * = these points were based on a single EC20 value. EPT = Ephemeroptera, Plecoptera, Trichoptera.

(Kennedy et al. 2004) showed that this mayfly was ~2× more sensitive to major ions than *C. dubia*. Corsi et al. (2010) reported an IC25 value (concentration resulting in a 25% inhibition) of 1050 mg Cl^-/L for *C. dubia*, a value considerably greater than the EC20s observed for mayflies in our mesocosm experiments (Table S1). At the chronic water-quality criterion for Cl^- (230 mg/L; USEPA 1988), we observed significant restructuring of aquatic insect communities after 10 d in our mesocosms, suggesting this level may not be protective of some aquatic insects from naturally low-conductivity streams.

Our experiments provided support for the hypothesis that natural differences in community composition between streams influenced responses to SC. For all endpoints that we examined, benthic communities from the moderate SC stream (PR) were consistently more tolerant to NaCl than those from the low SC stream (MR). These context-dependent responses, which are well characterized in ecology (Cardinale et al. 2000), have received limited attention in ecotoxicology (Clements et al. 2012). The specific mechanisms responsible for these differences are uncertain, but may relate to differences in the exposure history between MR and PR communities. Dunlop et al. (2008) characterized spatial variation in salinity tolerance and concluded that species that evolved in conditions of greater natural salinity were more tolerant to salt. Similarly, Kefford et al. (2012) examined global patterns of salinity tolerance and observed greater tolerance of macroinvertebrates that evolved in naturally arid regions. In our study, background levels of SC were ~3 to 4× greater in the PR compared to the MR, which may account for the greater tolerance of PR communities. Alternatively, seasonal differences in insect phenology may explain variation between the PR and MR experiments. In contrast to the MR populations described above, mayfly populations from the PR in late spring were dominated by larger, more mature instars that probably were more tolerant than smaller instars to NaCl. Regardless of the specific mechanism(s), results of our study demonstrated significant variation in the responses of different communities to the same stressor. We think that these context-dependent responses have important implications for establishing water-quality standards or benchmarks for contaminants. Although our understanding of how physicochemical characteristics (e.g., pH, water hardness, dissolved organic C) influence toxicity is relatively advanced, we know little about how ecological factors, such as differences in community composition or natural insect phenology, influence contaminant effects. Developing a better understanding of these context-dependent responses to contaminants should become a research priority in aquatic ecotoxicology.

The use of field-based data sets to develop benchmarks or standards has been criticized, primarily because of the inability of these approaches to rigorously control potential confounding variables (Gensemer et al. 2013). We generally agree that field-based approaches have limitations and that experimental studies may be required to rigorously demonstrate cause-and-effect relationships between contaminants and responses. However, for certain classes of stressors (e.g., suspended sediments, nutrients, and deposition of iron colloids), traditional laboratory-based assessments are often inappropriate or impractical (Pacheco et al. 2005). Results of laboratory toxicity tests conducted with either traditional test species or with late instars of some aquatic insects consistently show much greater tolerance to contaminants compared to patterns observed in the field or in mesocosm experiments (Clements et al.

2013). The greater tolerance of aquatic insects observed in laboratory experiments may result from the failure to account for indirect effects of contaminants, such as species interactions or reduced food availability. For example, the strong negative effects on community metabolism we observed in our experiments suggested a loss of food resources for grazing mayflies, which may partially explain the sensitivity of this group to major ions.

Standards or criteria derived exclusively from laboratory experiments may not be protective of natural benthic communities and, therefore, should be supported by alternative approaches (Pacheco et al. 2005). Mesocosm experiments provide an ecologically realistic alternative to laboratory toxicity tests while controlling for the confounding variables associated with field-based approaches. In addition to providing mechanistic insights into stressor–response relationships across different levels of biological organization, mesocosm experiments can be coupled with field assessments to address important policy issues (Benton et al. 2007). Based on SC-response relationships for behavioral (macroinvertebrate drift), structural (macroinvertebrate abundance), and functional (community metabolism) measures observed in our study, we conclude that the 300 μS/cm SC benchmark is protective of aquatic insect communities in naturally low-conductivity streams.

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