Image caption: Stonefly nymph, an aquatic insect that is sensitive to changes in water quality.

Biological and Ecological Consequences of Sub-Lethal Ion Concentrations on Microbial and Macroinvertebrate Detritivores

Sally Entrekin[1*], Brooke Howard-Parker[2], Michelle Evans-White[2], and Natalie Clay[3]

[1]Department of Biology, University of Central Arkansas, [2]Department of Biological Sciences, University of Arkansas, [3]Scool of Biological Sciences, Louisiana Tech University
*Corresponding author

Abstract: Freshwater detritivores are essential to stream productivity, carbon cycling, and subsidies to terrestrial systems. Gradual low-level, sub-lethal increases in ion concentrations such as sodium (Na), chloride (Cl), and bicarbonate (HCO_3) are common, but their impacts on freshwater detritivores and stream processes are not well understood. However, these ions may impact leaf litter decomposition in various ways. We tested each of the pathways in stream mesocosms by amending water with one of 3 NaCl and 3 $NaHCO_3$ treatments: natural (from a local stream), low (16 mgL^{-1} Na added), medium (32mgL^{-1} Na added), and high (64 mgL^{-1} Na added) and measuring stonefly growth, respiration, and consuption, and fungi and algal growth over 8 weeks. Similarly, we measured the same variables for isopods that were raised in stream water but fed leaf discs amended with Na as above. Salt treatments had little effect on microbial-mediated leaf litter decomposition and the associated fungal and algal community; however, microbial respiration tended to be elevated on the leaves incubated in $NaHCO_3$ throughout the 134-day study with the lowest $NaHCO_3$ concentration having the greatest stimulatory effect. Further, algal growth also showed a pattern of increase from HCO_3 that may have been an added food resource for macroinvertebrate detritivores in the previous studies but these changes in microbial activity did not change decomposition rates. The stonefly Amphinemura increased in biomass and respired more in Na- (both Cl and HCO_3) amended water without increased leaf consumption. Na-incubated leaf discs resulted in decreased isopod Lirceus growth relative to stream water with little change in respiration and leaf consumption in Na-amended treatments. Together, these results demonstrate that low-level, non-lethal NaCl impacts detritivores both directly and indirectly even at concentrations that are near the chloride reference values for different ecoregions in Arkansas Regulation 2 (ranges from 6 to 36 mgL^{-1} depending on the ecoregion). Other ions, like HCO_3, have a similar effect on detritivores but are not currently considered in State regulations despite their prevalence in the environment from waste water.

Key Points:

- Anthropogenic activities can cause subtle increases in ion concentrations in freshwaters of Arkansas.
- Sub-lethal increases in ions can cause stress in organisms due to challenges regulating water and salt balances.
- Sub-lethal increases in NaCl and $NaHCO_3$ can affect microbial activity, leaf litter quality, and carbon cycling in detrital streams.
- A better understanding of sub-lethal ion concentrations is important when considering water quality standards.

Introduction

Ion increases in Arkansas streams are from a combination of agriculture, wastewater effluent and development associated with urbanization and resource extraction (Griffith, 2014; Musto, 2013). Small amounts of Na and Cl are essential for animals, bacteria, and fungi to maintain hormone signaling pathways, generate electrical cell potentials and regulate bodily fluids (Kaspari et al., 2009). However, increased Na and Cl concentrations have the potential to alter rates of leaf litter decomposition and subsequent carbon cycling in streams by three pathways: 1) directly altering heterotrophic fungi and bacteria consumption, respiration, and growth that colonize and decompose leaf litter from osmoregulatory changes, 2) directly altering macroinvertebrate detritivore consumption and respiration from osmoregualtory changes or 3) indirectly altering macroinvertebrate detritivore feeding rates via changes in litter quality. Greater fungal and bacterial biomass increases the nutritional value of detritus for macroinvertebrate detritivores and typically results in increased leaf litter decomposition rates. Macro-detritivores both, directly and indirectly, increase leaf litter decomposition rates via leaf consumption and by increasing surface area for microbial colonization. Thus, changes in stream ions can have large impacts on freshwater ecosystems through these direct and indirect effects on detrital processing.

Sodium and chloride ions play a key role in osmoregulatory processes of freshwater organisms, and ion imbalances between organisms and their environment can negatively impact freshwater organisms and ecosystems through increased energy expenditure to maintain osmotic balance. Arkansas streams and rivers have among the lowest natural ion concentrations in the U.S. (Griffith, 2014). However, our past studies have documented small, but increased ion concentrations from sodium (Na: 0.7-7.0 mgL^{-1}) and chloride (Cl: 0.8-21.2 mgL^{-1}) in 20 wadeable streams. Additionally, the Arkansas Department of Environmental Quality (ADEQ) has measured a range in Cl concentrations from 0.4 to over 150 mgL^{-1} in Arkansas Valley streams (ADEQ database accessed 27Oct15). Sodium bicarbonate ($NaHCO_3$) has also increased in streams in the Illinois River Basin (Scott et al., 2016). Our study will inform ecological impacts of rising ions that are below documented toxicity levels but are 1) below-, 2) near- and 3) more than- state-set chloride concentrations and quality standards detailed in Arkansas State Regulation 2 (as low as 6 mgL^{-1} depending on the site and ecoregion; APCEC 2014) . We aim to investigate how detrital organisms and their associated processes change in response to sub-lethal increases in common ions; specifically, Na, Cl and bicarbonate (HCO_3). Changes in litter processing rates in combination with altered detritivore growth will support stream ecosystem responses to modified surface water quality.

Methods

Experiment 1 (micro-detritivores):

We tested low-level NaCl and $NaHCO_3$ additions on heterotrophic fungal biomass on leaf litter. First, sweet gum leaves were cut into standard-sized discs, leached, and incubated in one of 3 NaCl and 3 $NaHCO_3$ treatments: natural (from a local stream), low (16 mgL^{-1} Na added), medium (32mgL^{-1} Na added), and high (64 mgL^{-1} Na added). Each salt treatment was represented by 10 growth chambers, and each chamber had 10 leaf discs (N=70). Conductivity and total dissolved solids increase with mineral concentrations and they were measured and interpreted along with effects from salt additions. Chambers were aerated each day to prevent low oxygen conditions and kept in a greenhouse for normal day-night cycles. Leaf discs were incubated for about 4.5 months to allow for possible microbial adaptation. Respiration was measured at the end of weeks 1, 4, 7, 10, 13, 17, and 19 following at least 2 hours of dark incubation using a Membrane Inlet Mass Spectrophotometer (MIMS; Halvorson et al., 2016). Fungal biomass was measured by solid-phase extraction (SPE) of ergosterol followed by high pressure liquid chromatography (HPLC) (Gessner, 2005). Leaf mass was measured before and after the experiment to estimate amount remaining. Finally, chlorophyll a was estimated after observing growth on leaf discs late in the experiment using ethanol extraction and standard spectrophotometric methods (Steinman, 1996).

Experiment 2 (macro-detritivore exposed to salts and fed naturally conditioned leaves):

We tested if experimental addition of salts reduce macro-detritivore growth and litter consumption from an increase in osmoregulatory stress. We used the same salt concentrations as in experiment 1. The common macro-detritivore, Amphinemura, was collected from a local stream that has low stream water conductivity (<50 $\mu S\ cm^{-1}$), sorted into size class to the nearest 2 mm, weighed, and placed in one of two salt types and one of the 4 treatments (natural, low, medium, and high). The detritivores were placed in their own growth chamber (10 chambers per treatment; 2 salt types x 3 concentrations +1 stream water x 10 growth chambers, N=70) and fed microbial conditioned leaf litter incubated for 30 days in natural stream water. Leaf discs were replaced each week after 7 days to estimate consumption and to prevent starvation. Detritivores were weighed at the end of 4 weeks. Macro-detritivore growth was expressed as (final-initial mass)/final mass*100. Initial leaf mass was measured from subsampled leaf discs and final leaf mass was measured after the 7-day exposure to detritivores upon experiment termination. Leaf disc respiration and fungal biomass were measured as described in experiment 1.

Experiment 3 (macro-detritivore not exposed to salts but fed salt-incubated leaf discs):

We measured the effects of long-term, low-level salt additions used in the other two experiments on litter quality and macro-detritivore growth. First, we used the same common macro-detritivore, Amphinemura, as in experiment 2, collected from a local stream, separated by size class and placed in natural stream water with no added salts. Unfortunately,

because of an unusually warm winter, the stoneflies emerged after a week into the experiment. We set-up a second experiment with the Isopod, Lirceus. The detritivores were then fed sweet gum discs from one of the above 2 salts and 3 salt concentrations after a 30-day incubation period. Detritivores were separated by size class as above and randomly placed in one chamber. Experimental design was as above except 5 isopods were placed in each chamber and their average growth was used as the unit of replication (2 salt types x 3 concentrations +1 stream water x 10 growth chambers, N=70). A sub-sample of detritivores that did not get placed in chambers were dried and weighed and their size class was recorded. Final detritivore dry mass was measured for all individuals. Macro-detritivore growth was measured as (final-initial mass)/final mass*100. Leaf mass lost was measured using the same methods as above.

Statistical Analysis

We used one-way analysis of variance to compare salt treatments effects on response variables (e.g. growth, biomass, leaf mass loss) for each of the proposed experiments and Student's t post-hoc pairwise comparison if main model a≤0.05. Repeated measures ANOVA was used to test differences in leaf disc respiration with a Tukey's honest significance test. If data did not follow parametric assumptions, then Wilcoxon test was used with a follow-up Wilcoxon each pair post-hoc test when a≤0.05.

Results

Experiment 1 (micro-detritivore; Figures 1-4 & Tables 1-4).

Overall, salt treatments had little effect on leaf litter decomposition and the associated fungal and algal community; however, respiration tended to be greater on the leaves incubated in $NaHCO_3$ throughout the 134-day study with the lowest $NaHCO_3$ concentration having the greatest stimulatory effect. Both salt treatment and time had significant main effects on microbial respiration (p=<0.001, 0.013), but did not interact (p=>0.005, Table 1). Salt treatment appeared to be the primary driver of microbial respiration and respiration varied across time (Figure 1). During week 1, low $NaHCO_3$ and NaCl treatments elicited greater respiration than moderate and high $NaHCO_3$ and high NaCl treatments on discs compared to stream water (SW). Low NaCl also resulted in significantly greater respiration than moderate NaCl on leaf discs. During week 19, low and moderate $NaHCO_3$ elicited a significantly greater respiration response than SW, high $NaHCO_3$, and all NaCl treatments; low $NaHCO_3$ respiration was significantly greater than moderate $NaHCO_3$. Despite differences in respiration, there were no statistically significant differences in dry mass remaining across salt treatments (Table 2). However, percent dry mass remaining in $NaHCO_3$ treatments tended to be greater than in SW and peaked at the medium $NaHCO_3$, suggesting the least amount of microbial activity (Figure 2). Fungal biomass did not differ statistically across treatments either (Table 3), but tended to increase with salt concentrations where it peaked in medium salt treatments and then decreased below fungal biomass on leaves incubated in SW (Figure 3). Algal biomass also did not differ across treatments statistically (Table 4) but NaCl treatments tended to have lower algal biomass than SW (Figure 4). Leaf discs incubated in $NaHCO_3$ treatments showed a pattern of increasing algal biomass where it was most variable at the

Table 1. One-way repeated measures ANOVA (=0.05) output for microbial respiration across time. Salt factor includes 7 levels: filtered stream water at ambient salinity (3 mg/L Na); filtered stream water amended to low, medium, and high sodium bicarbonate concentrations (16, 32, and 64 mg/L Na); and filtered stream water amended to low, medium, and high sodium chloride concentrations (16, 32, and 64 mg/L Na). Repeated measures were carried out on weeks 1, 4, 7, 10, 13, 17, and 19.

	Factor	df	F	*p*
Dry Mass	Salt	6	6.299	**<0.001**
	Time	6	2.738	**0.013**
	Salt*Time	36	1.159	0.247
AFDM	Salt	6	2.973	**0.007**
	Time	6	1.901	0.079
	Salt*Time	36	0.717	0.889

Figure 1. Mean microbial respiration expressed per unit dry mass over time. Salt treatments were: SW-3=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high NaHCO3 treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Both salt treatment and time had significant main effects on microbial respiration (p=<0.001,0.013), but did not interact (p=>0.005). Salt treatment appeared to be the primary driver of microbial respiration responses and respiration varied across time.

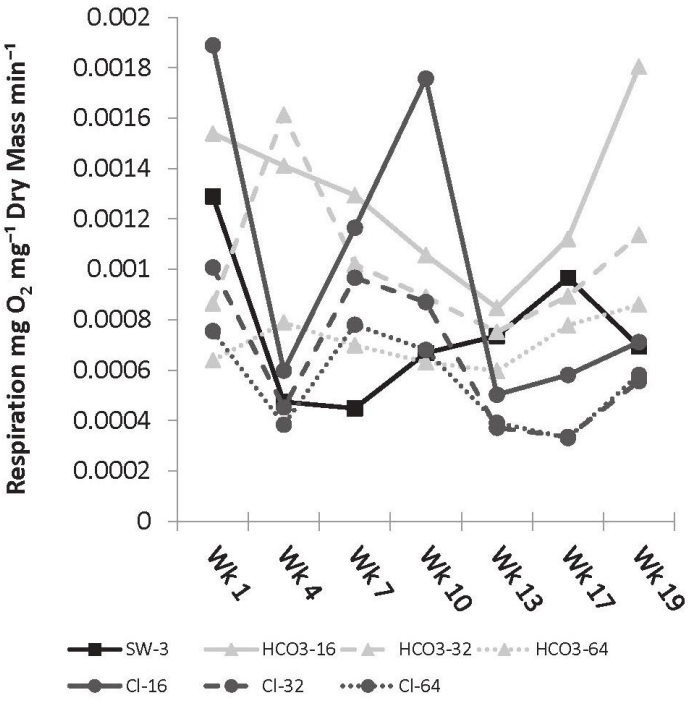

Table 2. One-way ANOVA (=0.05) output for % leaf litter remaining at termination (week 19, day 134).

	df	F	*p*
Dry Mass	6	1.577	0.169
AFDM	6	0.389	0.884

Table 3. One-way ANOVA (=0.05) output for fungal biomass at termination (week 19, day 134).

	df	F	*p*
Dry Mass	6	0.517	0.793
AFDM	6	1.115	0.364

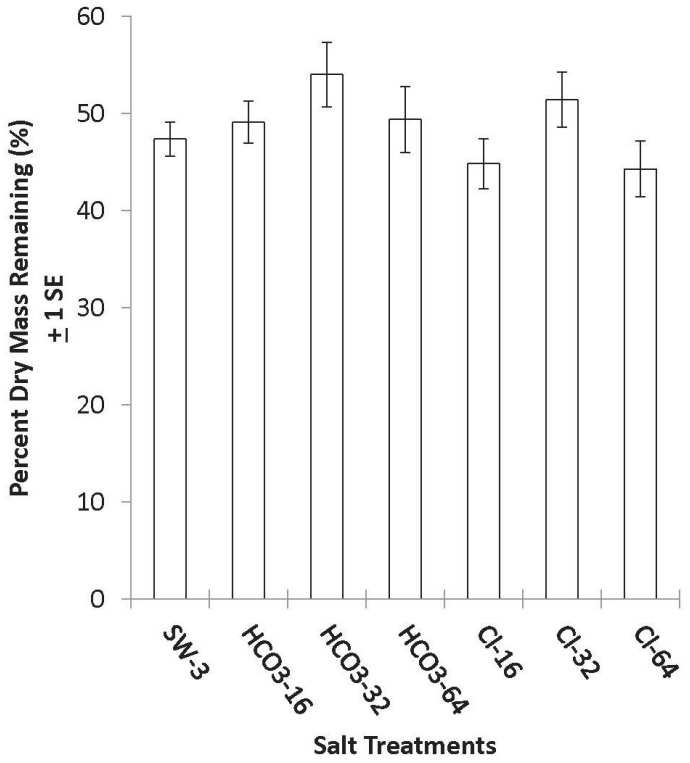

Figure 2. Mean (+1 SE) percent dry mass of litter remaining. There were no statistically significant differences in percent dry mass remaining across salt treatments, although percent dry mass remaining in NaHCO3 treatments tended to be greater than in ambient (3mg/L) stream water. Additionally, percent dry mass remaining showed an increasing pattern with increasing salt concentration for NaHCO3 treatments until peaking at median salt and then decreasing at the two greatest salt concentrations.

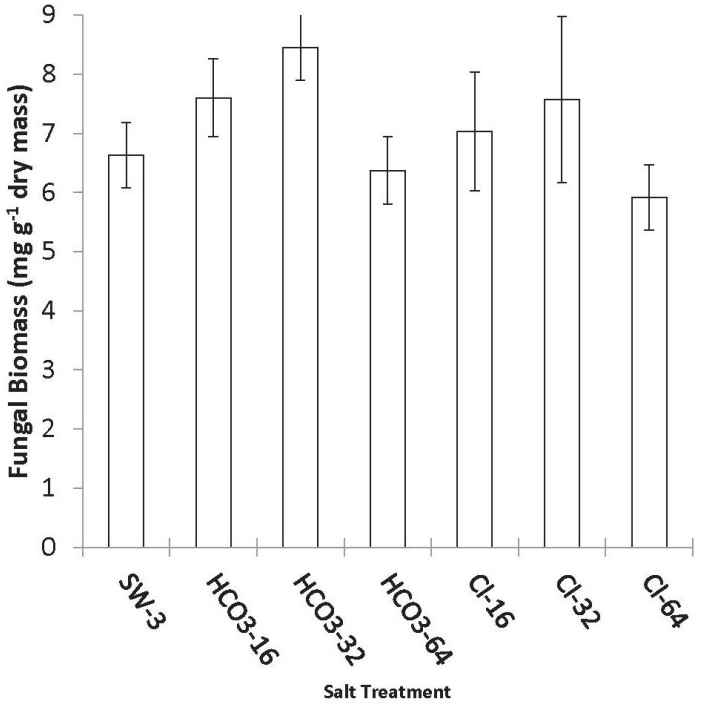

Figure 3. Mean fungal (+1 SE) expressed per unit litter dry mass across salt treatments. Fungal biomass tended to increase with salt concentrations peaking in moderate salt treatments (32mg/L) and then decreasing in the highest salt treatments (64mg/L) to levels below that found in ambient salinity controls for NaCl and NaCO3 salts (p>0.05).

greatest $NaHCO_3$ concentration that was likely from the more basic pH that supports optimal algal growth (Brock, 1973).

Experiment 2 (macro-detritivores exposed to added salts in streamwater and fed naturally conditioned leaves; Figures 5-6).

Overall, salt amendments to SW tended to stimulate stonefly growth, respiration, and fungal biomass on leaf discs. Stoneflies in stream water gained about 50% mass over the month long experiment compared to ~60% increase for stoneflies in low and high NaCl and $NaHCO_3$ amended water (p=0.04). Stoneflies in the medium salt treatments gained about the same mass as those in SW (p>0.05). Added low and high salts resulted in ~10% increase in mass (Figure 5A). Stonefly respiration was measured on day 30 of the experiment. Stonefly respiration in salt-amended water was ≥ stonefly respiration for individuals in SW (p=0.02). Stonefly respiration was ~ 3 times faster for individuals in the highest $NaHCO_3$ treatments and the low and medium NaCl than for stoneflies in SW (Figure 5B). Leaf litter mass remaining after 7 days in stonefly chambers did not differ across treatments (p=0.73). Leaf discs lost 20-30% of their mass over the week-long feeding period (Figure 6A). Leaf discs placed in salt amended water with stoneflies gained fungal biomass particularly in NaCl amendments from 1 mg/g on leaves in SW up to an average of 9 mgg^{-1} on leaves in the lowest NaCl added treatment (p=0.04, Figure 6B). The increase in fungal biomass on leaves fed to stoneflies incubated in added salt treatments may be from added nutrients provided by stonefly excretion and the overall positive stonefly growth response is probably from this added fungal biomass as a more nutritious food resource (Ferreira et al., 2014).

Table 4. One-way ANOVA (=0.05) output for algal biomass at termination (week 19, day 134).

	df	F	*p*
Dry Mass	6	1.167	0.336
AFDM	6	1.664	0.145

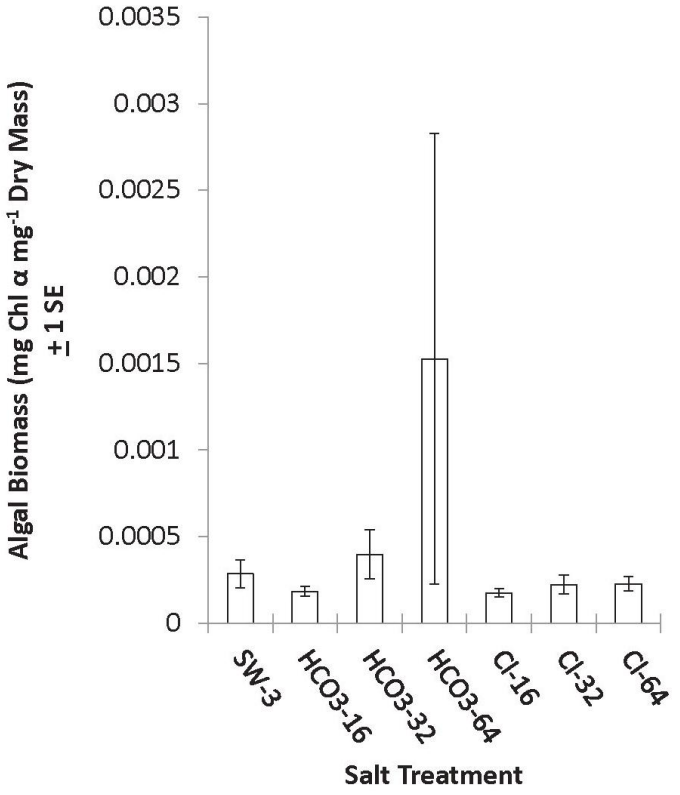

Figure 4. Mean algal biomass measured as chlorophyll a (chl a ± 1 SE) expressed per unit litter dry mass across salt treatments. NaCl treatments tended to have lower algal biomass than ambient stream water (p>0.05). NaHCO3 treatments had increasing algal biomass with increasing salinity, but only moderate (32mg/L) to high (64mg/L) NaHCO3 treatments had higher algal biomass than ambient (3mg/L) steam water (p>0.05). The greatest variation occurred in high (64mg/L) NaHCO3 treatments.

Experiment 3 (macro-detritivore not exposed to added salts in streamwater but fed added salt-incubated leaf discs; Figures 7-8).

Overall, feeding isopods leaves that were incubated in some of the added salt treatments suppressed isopod growth and respiration compared to isopods that were fed leaves incubated in SW alone. Isopods fed leaves incubated in SW increased their mass by 70%. In contrast, isopods fed leaves incubated in medium $NaHCO_3$ and NaCl grew 20% less. Isopods fed leaves incubated in 32mgL^{-1} NaCl amendments grew about 28% less than those fed SW-incubated leaves (Figure 7A). Isopod respiration was equal to or greater than respiration of isopods fed leaves incubated in SW compared to salts. Isopods that were fed leaves from low NaCl incubations respired the least (and gained the least amount of mass) with nearly 3x lower respiration than isopods fed leaves from SW and medium $NaHCO_3$ and NaCl (p=0.03, Figure 7B). There was no measurable difference in leaf mass remaining across salt treatments (p=0.13). All leaf discs lost 20-40% of their mass over the week-long feeding period. Although not statistically significant, the trend was more leaf mass was lost in the low NaCl incubated leaf discs where isopod growth and respiration were lowest (Figure 7A&B, 8A). Fungal biomass on discs incubated and then fed to isopods had variable biomass ranging from 2 to 6 mgg^{-1} and there was no treatment effect (p=0.41).

Conclusions

These results demonstrate the complexities of nutrient subsidies on stream processes. In spite of the lack of significance for fungal biomass estimates, low level salts, especially $NaHCO_3$, appear to stimulate microbial respiration. Considering there were no significant differences in percent dry mass remaining across treatments, higher microbial respiration rates may be indicative of microbial energy diverted toward osmoregulation in the presence of ionic stress instead of growth and consumption. Increased algal biomass and fungal biomass can provide added resources to detrital invertebrates, which may initially help mitigate macro-detritivore osmoregulatory stress from increased ion concentrations. Amphinemura increased growth rates and respired more in Na- (both Cl and HCO_3) amended water without increased leaf consumption. Conservation of mass suggests that stoneflies may be feeding on an alternative resource like fungi or algae when NaCl or $NaHCO_3$ is present. However, diet switching could have long term effects on resource availability (Brown et al., 2004). In addition to potential osmoregulatory stress caused by water ion concentrations, changes to detritus from salts resulted in decreased Lirceus growth relative to stream water with little change in respiration and leaf consumption in salt-amended treatments. This suggests that salts impact the quality of detritus. Although non-lethal, ion increases may impact stream ecosystem processes 1) directly via changes in fungi biomass and respiration, 2) directly by altering macroinvertebrate detritivore consumption, respiration, and growth, and 3) indirectly by altering litter quality. Together, these results demonstrate that low-level, non-lethal NaCl and $NaHCO_3$ impacts detritivores both directly and indirectly even at concentrations that are near the existing chloride standards in Arkansas. Other ions, like HCO_3, have a similar effect on detritivores but are not currently considered in state and federal regulatory standards despite their prevalence in the environment from waste water treatment and release (Canedo-Arguelles et al., 2016).

Acknowledgements

This material is based upon work supported by the U.S. Geological Survey under grant agreement No. G16AP00040 and administered by the Arkansas Water Resources Center. The views and conclusions contained in this document are those of

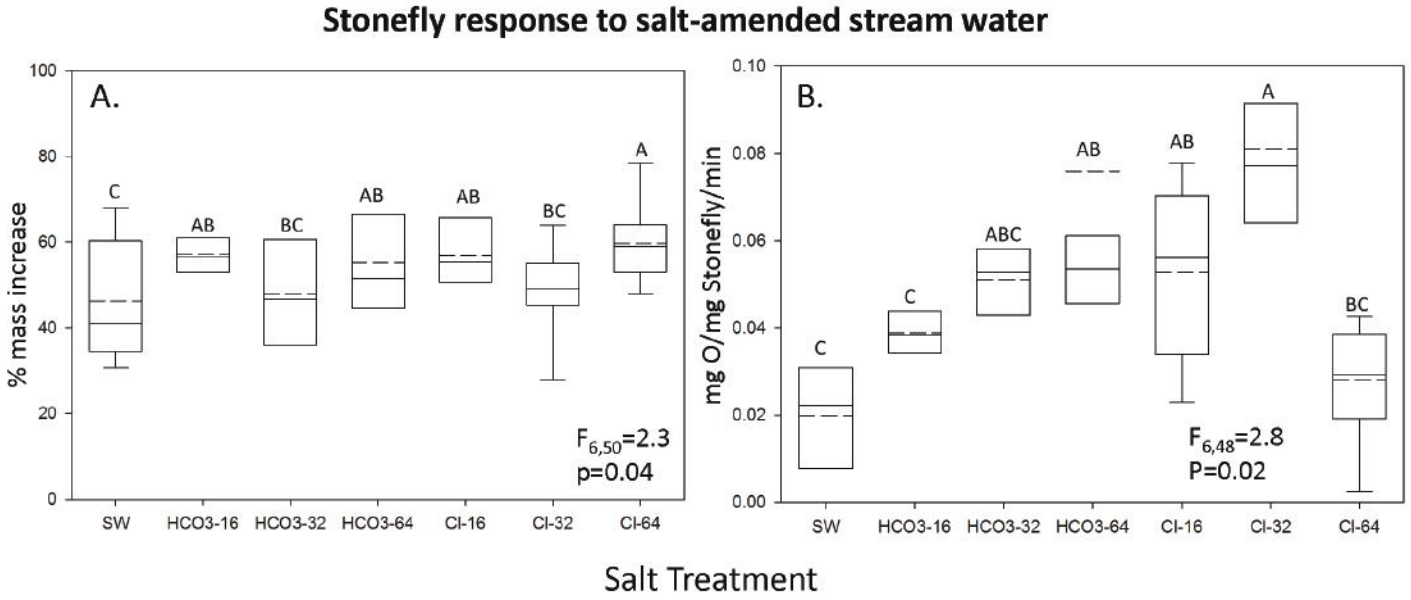

Figure 5. Stoneflies (Amphinemura sp.) were fed sweet gum leaves incubated in stream water and reared in chambers with stream water amended with salts. Salt treatments were: SW=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high NaHCO3 treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Box plots show the upper value as the top whisker that is not an outlier, upper quartile, then a dashed line represents the average and the solid line is the median. Lower box is the lower quartile and the lower whisker is the minimum value excluding outliers. When whiskers are not present it is because they equal the upper and lower quartile, respectively. Panel A. is stonefly growth. Panel B. is stonefly respiration measured on the final day of the experimentDifferent letters represent statistical significance at =0.05.

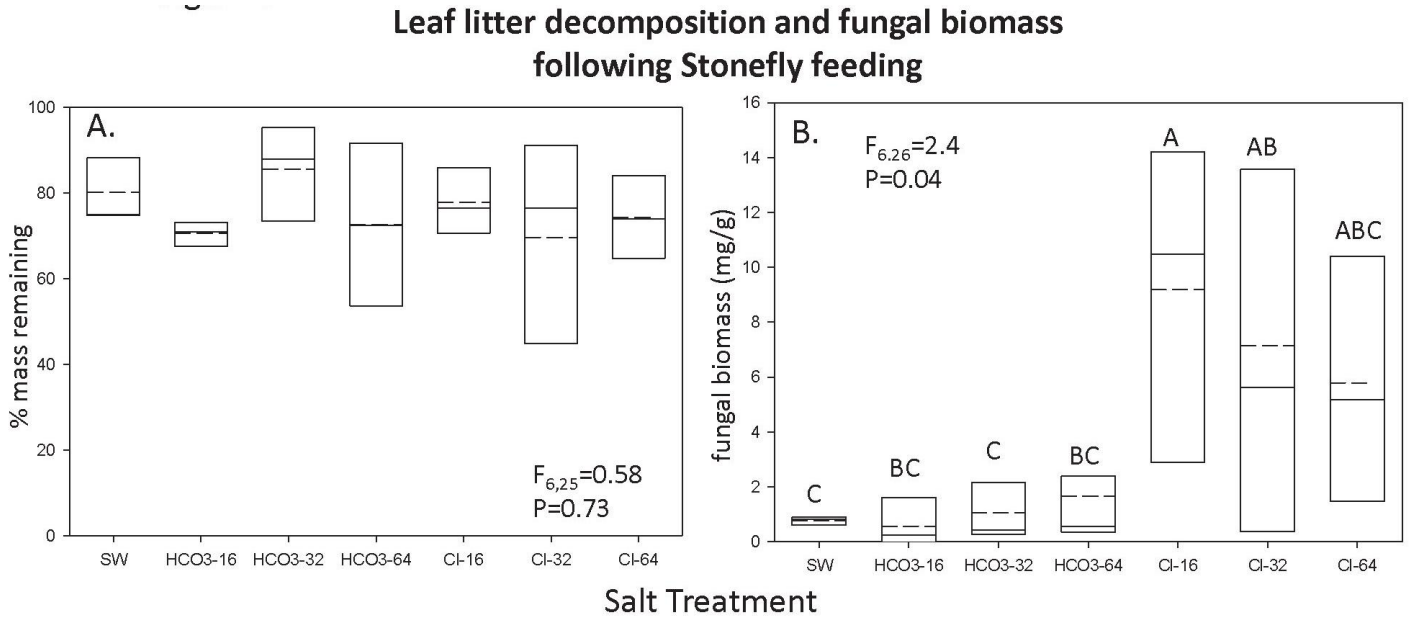

Figure 6. Stoneflies (Amphinemura sp.) were fed sweet gum leaves incubated in stream water and reared in chambers with stream water amended with salts. Salt treatments were: SW-3=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high NaHCO3 treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Box plots show the upper value as the top whisker that is not an outlier, upper quartile, then a dashed line represents the average and the solid line is the median. Lower box is the lower quartile and the lower whisker is the minimum value excluding outliers. When whiskers are not present it is because they equal the upper and lower quartile, respectively. Panel A is leaf disc mass remaining on final discs. Panel B is fungal biomass on leaf discs following the final stonefly feeding period. Different letters represent statistical significance at =0.05.

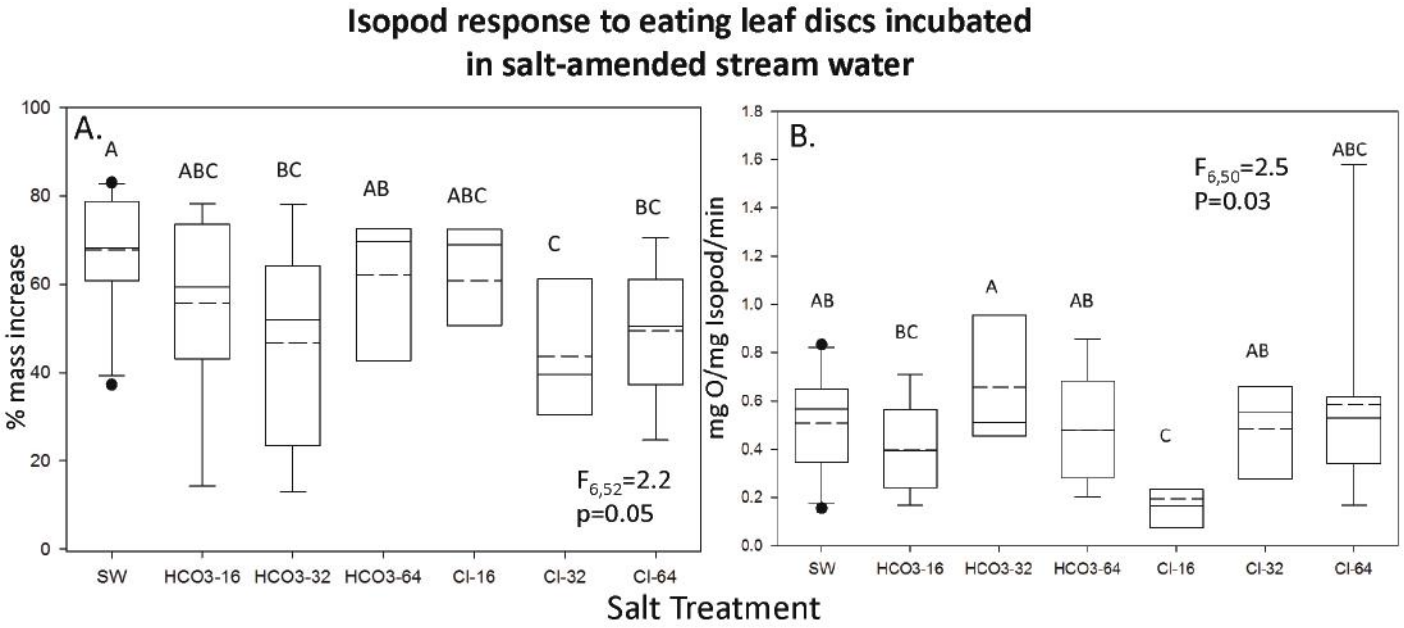

Figure 7. Isopods were fed leaves incubated in stream water amended with salts and chambers had only stream water. Salt treatments that leaves incubated in prior to being offered to isopods were: SW-3=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high NaHCO3 treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Box plots show black circles as outliers, the upper value as the top whisker that is not an outlier, upper quartile, then a dashed line represents the average and the solid line is the median. Lower box is the lower quartile and the lower whisker is the minimum value excluding outliers. When whiskers are not present it is because they equal the upper and lower quartile, respectively. Panel A is isopod growth about one month after being fed salt-incubated leaves. Panel B is isopod respiration per mg of their body mass (mg). Different letters represent statistical significance at =0.05.

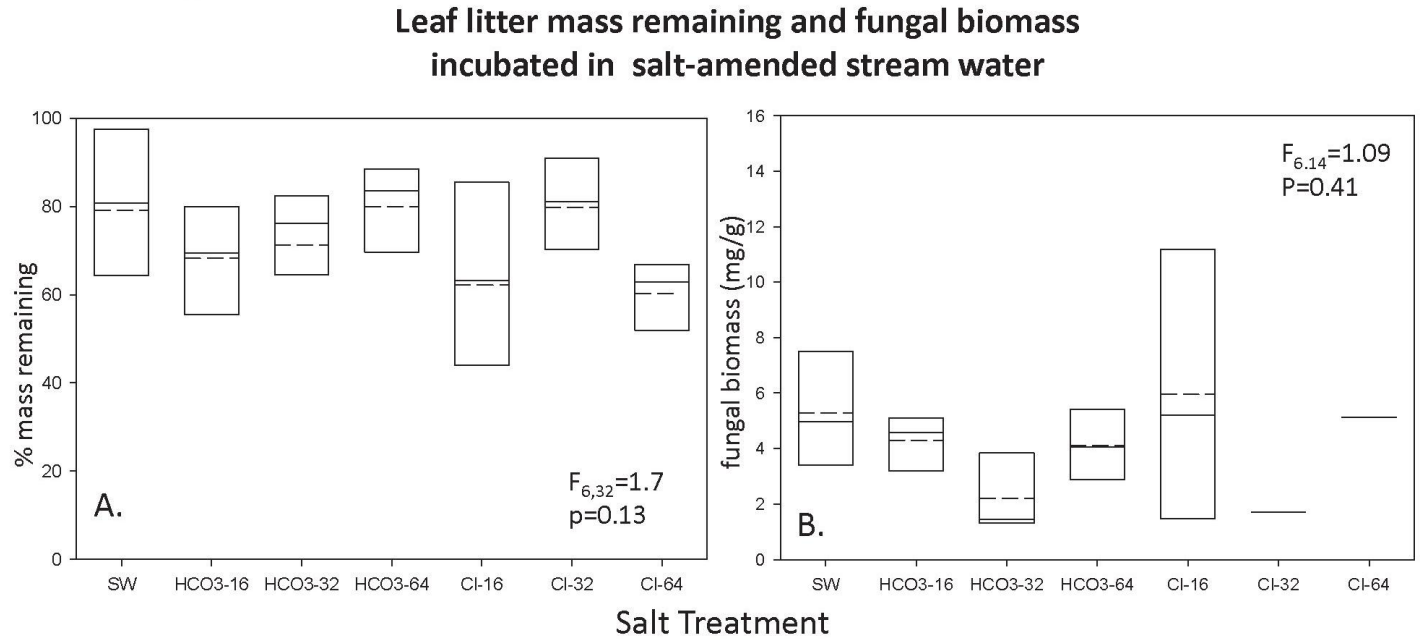

Figure 8. Salt-incubated leaf disc mass remaining and fungal biomass following the last isopod feeding period. Salt treatments that leaves incubated in prior to being offered to isopods were: SW-3=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high NaHCO3 treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Box plots show black circles as outliers, the upper value as the top whisker that is not an outlier, upper quartile, then a dashed line represents the average and the solid line is the median. Lower box is the lower quartile and the lower whisker is the minimum value excluding outliers. When whiskers are not present it is because they equal the upper and lower quartile, respectively. Panel A. is average leaf disc mass remaining on final discs following isopod feeding. Panel B is fungal biomass on final discs.

the authors and should not be interpreted as representing the opinions or policies of the U.S. Geological Survey. Mention of trade names or commercial products does not constitute endorsement by the U.S. Geological Survey.

References

APCEC, Regulation 2: Regulation Establishing Water Quality Standards for Surface Waters of the State of Arkasnas 2014, Arkansas Pollution Control and Ecology Commission (APCEC).

Arkansas Water Resources Center, 2015. Water Quality Monitoring in the Upper Illinois River Watershed and Upper White River Basin. Final Report, Project 11-500, Arkansas Natural Resources.

Brock, T. D., 1973, Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications: Science, v. 179, no. 4072, p. 480-483.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., and West, G. B., 2004, Toward a metabolic theory of ecology: Ecology, v. 85, no. 7, p. 1771-1789.

Canedo-Arguelles, M., Hawkins, C. P., Kefford, B., Schafer, R. B., Dyack, B. J., Brucet, S., Buchwalter, D. B., Dunlop, J., Fror, O., Lazorchak, J. M., Coring, E., Fernandez, H. R., Goodfellow, W., Gonzalez Achem, A. L., Hatfield-Dodds, S., Karimov, B. K., Mensah, P., Olson, J. R., Piscart, C., Prat, N., Ponsa, S., Schulz, C. J., and Timpano, A. J., 2016, Ion-specific standards are needed to protect biodiversity.: Science, v. 351, no. 6276, p. 914-916.

Ferreira, V., Castagneyrol, B., Koricheva, J., Gulis, V., Chauvet, E., and Graca, M. A., 2014, A meta-analysis of the effects of nutrient enrichment on litter decomposition in streams: Biol Rev Camb Philos Soc.

Gessner, M. O., 2005, Ergosterol as a measure of fungal biomass, Methods to study litter decomposition, Springer, p. 189-195.

Griffith, M. B., 2014, Natural variation and current reference for specific conductivity and major ions in wadeable streams of the conterminous USA: Freshwater Science, v. 33, no. 1, p. 1-17.

Halvorson, H. M., Scott, E. E., Entrekin, S. A., Evans-White, M. A., and Scott, J. T., 2016, Light and dissolved phosphorus interactively affect microbial metabolism, stoichiometry and decomposition of leaf litter: Freshwater Biology, v. 61, no. 6, p. 1006-1019.

Kaspari, M., Yanoviak, S. P., Dudley, R., Yuan, M., and Clay, N. A., 2009, Sodium shortage as a constraint on the carbon cycle in an inland tropical rainforest: Proc Natl Acad Sci U S A, v. 106, no. 46, p. 19405-19409.

Musto, A. L., 2013, Trace elements and macroinvertebrate community structure across a gradient of shale gas extraction [M.S.: University of Central Arkansas, 93 p.

Steinman, A., 1996, Biomass and pigments of benthic algae. Á In: Hauer, FR and Lamberti, GA (eds), Methods in stream ecology, Academic Press, pp. 295Á311.