BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
AMENDMENTS TO THE GENERAL USE	)	R18-32
WATER QUALITY STANDARDS	)	Rulemaking – Water
FOR CHLORIDE	)	

NOTICE OF FILING

To: Don Brown, Clerk
Illinois Pollution Control Board
James R. Thompson Center
100 West Randolph Street, Suite 11-500
Chicago, IL 60601

PLEASE TAKE NOTICE that I have today electronically filed with the Illinois Pollution Control Board, Huff & Huff, Inc.'s testimony of James E. Huff, P. E., Roger Klocek, and David J. Soucek, Ph. D. in support of the Petition in R18-32, a copy of which is herewith served upon you.

Dated: December 21, 2018

Huff & Huff, Inc.

By: ______________________

James E. Huff, P.E.
Huff & Huff, Inc.
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CERTIFICATE OF SERVICE

I, the undersigned, certify that on December 21, 2018, I served electronically the attached PRE-FILED TESTIMONY OF JAMES E. HUFF, ROGER KLOCEK AND DAVID J. SOUCEK to the participants listed on the attached SERVICE LIST.

James E. Huff

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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AMENDMENTS TO THE GENERAL USE	)	R18-32
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PRE-FILED TESTIMONY OF JAMES E. HUFF, P.E.

Introduction

My name is James E. Huff, and I am a Senior Consultant of Huff & Huff, Inc., a subsidiary of GZA. I am a registered Professional Engineer in the State of Illinois and the principal author of the proposed rule change. I have been involved with the development of water quality standards in Illinois since 1971. I prepared comments on the Total Dissolved Solids proposed standards in 1971/72 and performed economic impacts/cost-benefit analyses for the State of Illinois in the mid-70s through 1981, including a number related to chlorides, sulfates, and Total Dissolved Solids. Most recently, I testified during the Chicago Area Waterway proceedings (R08-09) regarding the difficulty in meeting a not-to-exceed 500 mg/L chloride standard during de-icing runoff periods and provided an alternative winter chloride water quality standard for the Chicago Sanitary & Ship Canal. The Board adopted this alternative winter standard for the Ship Canal. The difficulty in meeting a 500 mg/L not-to-exceed water quality standard for chlorides is a concern not only on the Chicago Area Waterways, but on all urban streams in Illinois. I have worked on the implementation of Best Management Practices (BMPs) for over the past decade on behalf of the Illinois Tollway, the Citgo Refinery, and for a variety of watershed groups, including Hickory Creek, the DuPage River/Salt Creek Workgroup, and the Lower Des Plaines River. A copy of my resume is included in Attachment 1.

Highway de-icing practices cannot simply be changed. Alternatives to chloride de-icing are not technically feasible on a region-wide basis, when considering safety and mobility. This was demonstrated by the Connecticut Department of Transportation, where for a seven-year period a mixture of sand-salt (7:2) was used and accident results were compared to a seven-year period with just salt. A 19 percent increase in nonfatal injuries and a 33 percent increase in total accidents were observed with the sand-salt mixture compared to just salt.[1] So while a reduction in salt usage is certainly an achievable goal, it is technically infeasible to reduce its use for de-icing practices sufficiently to consistently meet the 500 mg/L not-to-exceed water quality standard without compromising safety.

In the Chicago Area Waterways proceedings (R08-09D) the Board adopted a winter chloride standard of 500 mg//L for the Chicago Area Waterways, excluding the Chicago Sanitary & Ship Canal, but allowed a three-year period before the effective date for the water quality standard would go into effect. During that period, the Illinois Environmental Protection Agency (Agency) took the lead in promoting watershed variances for the winter months, not only on the Chicago Area Waterways, but for all urban streams in Illinois. This has led to a series of Time-Limited Water Quality Variance requests being filed before the Pollution Control Board. The theory behind these Time-Limited Water Quality Variance requests was that through the implementation of BMPs by entities that use salt for de-icing, the receiving streams would be able to reach a not-to-exceed 500 mg/L chloride water quality limit during the variance period. While implementation of BMPs for de-icing is a good practice from an environmental perspective, and their implementation is improving water quality, it has become apparent that there is no basis to believe BMPs by themselves will result in achieving a 500 mg/L not-to-exceed winter standard. Smaller urban streams still have peak chloride concentrations in excess of 1,500 mg/L, and it is not realistic to expect the 67 percent reduction in salt usage that would be required during the worse runoff events in order to achieve compliance.

During the Chicago Area Waterway hearings (R08-09), while deriving a unique chloride limit for the Chicago Sanitary & Ship Canal, I began to question how the acute and

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[1] Mahoney, JU. D.S. Larsen, and E. Jackson. Reduction in nonfatal injury crashes after implementation of anti-icing technology. Transportation Research Record: *Journal of the Transportation Research Board*, No. 2613. Transportation Research Board of the National Academies, Washington, D.C., 2017, pp. 77-86. See Attachment 4

chronic chloride criteria were developed. There was some data in the literature that indicated chlorides are less toxic at colder temperatures. The metabolism of many organisms slows down at colder temperatures, reproduction for other organisms does not occur, and many organisms literally become dormant. For many these organisms, it is appropriate to question even the need for a chronic chloride water quality standard during the winter months for the reasons listed.

I believe the implementation of BMPs is a worthwhile endeavor; however, I believe the efforts will fall far short of achieving a 500 mg/L not-to-exceed chloride level. This will leave all of our urban streams listed as *impaired waters* for chlorides, and, therefore, any future growth involving additional roadways, parking lots, or driveways, will be virtually impossible. Offsets of the chlorides will be required, but these offsets cannot be the same BMPs being implemented as part of the Time-Limited Water Quality Variances. Therefore, taking a more in-depth look at cold temperature toxicity of chloride seemed like an approach that could contribute greatly to resolving this problem. As a result, I reached out to the USEPA[2] at the end of the UAA proceedings about the possibility of funding cold temperature toxicity testing, as they had recently funded chloride toxicity testing on several sensitive aquatic species. Unfortunately, USEPA indicated they had no interest in such studies.

Without the leadership in the regulatory agencies to critically look at this issue and scientifically evaluate how effective BMPs can be in reducing peak chloride stream concentrations, I elected to see if I could raise funds from the regulated community to conduct cold temperature chloride toxicity tests. The response was sufficient to undertake this work, with a consortium of highway departments (local, county, and Tollway), industries, sanitary districts, the Salt Institute, and watershed groups. Dr. David Soucek from the Illinois Natural History Survey was enthusiastic about this effort and agreed to conduct much of the work. New England Bioassay was retained to conduct the cold temperature testing on *Ceriodaphnia dubia.* A work plan was drafted and sent to the USEPA and the Illinois EPA on August 2, 2016. A copy of the transmittal letter

[2] Personal Communication with Candice Bauer, USEPA, 2016.

welcoming any comments before we initiated the work, along with the work plan are included in Attachment 2. No comments on the work plan were received from USEPA. Scott Twait from the Agency called on September 15, 2016, and he indicated the sole comment from the Agency was they would like to see testing at more temperatures. He also indicated that the Agency would send me a written comment. I pointed out to Mr. Twait that the reason the testing was limited to temperatures representative of winter conditions was that the USEPA historically has not accepted standards that would vary based on the stream temperature at the time of sampling. The USEPA has historically only approved standards based on defined seasons, as was done with ammonia. I also told the Agency that if it could come up with additional funding for the research, we would be happy to conduct these additional temperature studies. No follow-up correspondence was received after Mr. Twait's phone call, so we proceeded to carry out the work plan as drafted.

In addition to my testimony, Dr. David Soucek will testify on the findings from his testing, and Roger Klocek, Senior Biologist at Huff & Huff, will summarize the literature findings, including a recent publication from the Stroud Water Research Center, and discuss the acute-to-chronic ratio used for deriving the chronic standard for chlorides proposed in this proceeding. Finally, Stephen McCracken from the DuPage River/Salt Creek Workgroup will be submitting written comments providing stream biology data from Salt Creek, and the East and West Branches of the DuPage River. This watershed group found reaches of river meeting the aquatic life threshold for macro invertebrates despite showing frequent winter exceedances of 500 mg/L. Summer values of less than 200 mg/L appeared more important in supporting benthic communities. This is an important finding that strongly supports the winter chloride proposal in this proceeding.

Procedure for Deriving Numerical Water Quality Criteria

In 1985, the USEPA published *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephen, et al., 1985). This document outlines the methodology for deriving water quality criteria, and notes for the chronic criteria that a four-day averaging period is appropriate, and that a 20 to 30-day period is unacceptable. The four-day period was intended to “prevent increased adverse effects on sensitive life stages by limiting the durations and magnitudes of exceedances of the CCC.” (CCC is the Criterion Continuous Concentration, or chronic criterion.) (pg. 5.)

The Criterion Maximum Concentration (CMC, or acute criterion) is derived by taking 50 percent of the Final Acute Value (FAV), so there is the equivalent of a 100 percent safety factor in this step alone. The FAV is derived where there are less than 59 species in the database, using only the four most sensitive species, so there is an additional safety factor for the less sensitive species beyond the 100 percent from the conversion of the FAV to the CMC.

For nearly all aquatic species, the bioassays are conducted at between 20 and 25 degrees C (or 69 and 77 degrees F), which are more representative of mid-summer temperatures. As the four most sensitive species drive the derivation of the FAV, and subsequently the CMC and CCC, the work plan selected the four species most sensitive to chlorides for toxicity testing. The approach was to substitute the results from these four species and similar organisms in the list of Genus Mean Acute Values (GMAV), leaving the remaining species results as published, without temperature adjustment and then recompute the new FAV with this mixed temperature list. This would be a conservative approach for calculating winter standards if any other species now became one of the four most sensitive species based on results at 20 to 25 degrees C. Such an approach would provide the basis to establish a conservative, new winter water quality criterion for chlorides.

Under the USEPA protocol, once the FAV is computed, the CCC or chronic criterion is calculated based on an acute-to-chronic ratio, or ACR. Where results at 10 degrees C failed to show any chronic impacts, using the ACR in the literature (derived at 20 to 25 degrees C) provides a very significant safety factor for the chronic criterion. The absence of testing other species at 10 degrees C should not be a reason for delaying the adoption of the standards proposed, as further species toxicity results at 10 degrees C would only result in calculating higher water quality chloride standards.

Literature Search

Once the consortium was formed, Huff & Huff, Inc. conducted a literature review on cold temperature toxicity tests. Roger Klocek and Larissa Herrera of Huff & Huff were the principal authors of the literature search, and Mr. Klocek will present a summary of the literature search, which was included as an appendix to the Technical Support Document attached to the petition and describe some recent results from the Stroud Water Research Center on four species of mayflies at various temperatures. What is striking from this literature review is the sparsity of toxicity studies at colder temperatures.

Selection of Appropriate Winter Temperature

As major snow storms have historically occurred in April, winter was defined as December 1st to April 30th, for our purposes, although the early snow event in late November 2018 suggests there needs to be some relief for such events outside the proposed winter dates. There is precedent for seasonal water quality standards in Illinois, with ammonia standards being the prime example. Historically, USEPA has rejected standards based on field measurements of temperature (and pH), rather requiring a definitive time period for seasonal standards. The Agency has a protocol for computing the 75th percentile temperature (and a similar approach for pH) for computing seasonal water quality standards for each specific water body for ammonia, and a similar approach was used for developing winter chloride standards. The Agency was kind enough to provide all Illinois stream temperature data from 2002 to 2016. Using just the data from December 1st to April 30th yielded a 75th percentile temperature of 9.3 degrees C. Based on these results, a temperature of 10 degrees C was selected for conducting winter temperature toxicity testing.

Duration Periods for Elevated Chlorides in Illinois Streams

Elevated chlorides tend to be transient in receiving streams, from shorter intervals on smaller streams to longer intervals on larger streams. Data from several sources were analyzed to evaluate the peak existing chloride concentrations and the duration the stream remained above the proposed chronic water quality standard of 640 mg/L, or in the case of the Chicago Sanitary & Ship Canal (CSSC) the current 620 mg/L chronic standard and 990 mg/L acute standard. On the CSSC at Romeoville, since 2006, there have been two events above the existing 990 mg/L winter acute standard; 998 mg/L in 2007 and 1,099 in 2011. This equates to one exceedance on average every 5.5 years. Over the eleven-year period, concentrations above the chronic standard of 620 mg/L extending for four or more days occurred 18 times, or 1.6 events per year. Similar results on the CSSC at Cicero were presented in the Technical Support Document, where the peak recorded chloride concentration was 1,241 mg/L in 2008, with no other results above the acute water quality standard over the eleven-year period of record. The four-day chronic 620 mg/L standard was exceeded 55 times over the eleven years or a recurrence interval of 5 events per year.

On the North Branch of the Chicago River, just above its confluence with the Chicago River, the peak measured chloride concentration of 1,160 mg/L was recorded over a seven-year period, with two recorded values above the 1,010 mg/L proposed acute standard, or once every 3.5 years. Twenty-seven days exceeded the proposed chronic standard of 640 mg/L, or a recurrence interval of four events per year. The Cal Sag Channel at Cicero over nine years did not record any chloride concentration above the proposed acute standard of 1,010 mg/L, and two days were above the proposed chronic standard of 640 mg/L, or once every 4.5 years.

Similar analyses were completed on the DuPage River and Salt Creek, based on data provided by the DuPage River/Salt Creek Workgroup. Salt Creek at Wolf Road from 2007 to 2016 exceeded the proposed chronic standard 146 days over a ten-year period, or 14.6 days per year. The peak chloride concentration recorded was 1,353 mg/L with five days above the proposed acute standard over the ten-year period, or once every two years. It should be noted that this sampling location is below a dam, so the peaks would be dampened; however, the dam also extends the duration of elevated chlorides. The trend at this location appears to be lower chloride concentrations with time, perhaps reflecting the benefits of the BMPs implemented over the past decade within this watershed.

On the East Branch of the DuPage River from 2008 to 2015, there have been 51 days above the proposed 4-day chronic standard, or 6.4 days per year. Two chloride events above the proposed acute standard of 1,010 mg/L have been recorded over the eight-year period. On the West Branch of the DuPage River, from 2007 to 2015, there have been 15 days when the 4-day chronic proposed standard was exceeded, or an average of 1.7 days per year. No exceedances of the proposed acute standard have been recorded on the West Branch of the DuPage River.

From the Technical Support Document, each exceedance was generally less than a week duration, which formed the basis for asking Dr. Soucek to run the third series of toxicity testing with exposure to elevated chloride for seven days followed by returning the chlorides to a lower concentration over a period of days. Dr. Soucek refers to these tests as “pulsed tests”.

Toxicity Testing

As noted previously, Dr. David Soucek and his associates at the Illinois Natural History Survey agreed to conduct the toxicity testing for three of the four species outlined in the original work plan, while New England Bioassay conducted the testing on the fourth species. The work plan called for running the standard USEPA protocol for acute and chronic testing, with one set of tests at 10 degrees C and the second set at the recommended water temperature (23 to 25 degrees C). The findings from the first round of chronic testing failed to find any chronic effects attributed to the chloride concentrations, as no reproduction occurred at 10 degrees C. Based on these first results, the tests were repeated, but for longer durations, 14 days for the mayfly and 28 days for the Hyalella and the clams. As Dr. Soucek will describe in his testimony, some chronic effects were observed at the colder temperatures with the extended exposure duration. However, based upon the duration of elevated chlorides in Illinois urban streams, the extended exposures to elevated chlorides is not realistic. At my request, Dr. Soucek has been running additional testing, where the exposure of the organisms to elevated chlorides was reduced to seven days, then the chloride concentration reduced over several days to a lower value, and the test extended to seven weeks. Dr. Soucek will present his findings from this additional testing, which was just recently completed. For two of the three species Dr. Soucek tested, (the amphipod and the fingernail clam) the chronic values at 10 degrees C increased for the seven-day pulsed chloride study, by a factor of 2.2 for the amphipod and 1.2 for the fingernail clam when compared to the testing at 23 to 25 degrees C.

For the *Ceriodaphnia dubia* or *C. dubia,* reproduction is used as the chronic toxicity indicator, and the test is run for 7 days. The acute test is completed over a 48-hour test period. The first test found no reproduction at 10 degrees C at any chloride concentration. The acute toxicity at 10 degrees C was greater than 1,518 mg/L, and at 25 degrees, the acute toxicity was reported at less than 759 mg/L. The test was repeated to fine tune the results, with the acute toxicity calculated to be 2,197 mg/L at 10 degrees C and 1,165 mg/L at 25 degrees C, or a ratio of 1.9 for the temperature effect. A third round of toxicity testing was conducted to extend the duration for 35 days for exposure. Reproduction occurred after 14 days and survival continued throughout the test duration at 10 degrees C, but not at 25 degrees C. The No Observable Effects Concentration based on the full 35 days was computed at a chloride concentration of 782 mg/L at 10 degrees C and at 607 mg/L at 25 degrees C. Caution should be exercised when using these longer chronic exposure tests, as 35 days of elevated chlorides does not reflect what occurs in Illinois streams, and the USEPA protocol noted it was inappropriate to use extended periods for chronic standards.

Dr. Soucek will discuss his results for the fingernail clam, mayfly and amphipod. Key findings from Dr. Soucek’s work include the absence of acute effects on the Fingernail Clam at 10 degrees C up to a chloride concentration of 2,920 mg/L and when the elevated chloride was pulsed for a seven-day exposure, the chronic exposure for the amphipod increased by a factor greater than two at 10 degrees C compared to the prolonged exposure test at 10 degrees C and was more than 10-fold greater than the chronic value at

23/25 degrees C. For the mayfly, at 10 degrees C, the chronic value was reported at greater than 1,458 mg/L chlorides, compared to 326 mg/L at 25 degrees C.

Derivation of Water Quality Standards

Following the procedure outlined in USEPA's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (1985)*, winter chloride water quality standards were calculated. Where 10-degree C toxicity data were available from our work, these results were substituted in the acute value table for the species tested. Adjustments were also made to all three daphnia species based on the results from our work with the *Ceriodaphnia dubia.* Under the USEPA protocol, the four most sensitive species drive the resulting acute water quality criterion, which included three of the four species tested as part of this work, and the fourth being the *Lampsilis* Mussel, where the acute toxicity value was not temperature adjusted.

The result was a Criterion Maximum Concentration (CMC), or acute water quality standard of 1,010 mg/L. Utilizing the standard chronic toxicity test periods at 10 degrees C, no chronic effects were observed for two of the four species tested. Utilizing the results of the 35-day testing was deemed inappropriate because this period of elevated exposure is far longer than exposures experienced in Illinois streams, and the USEPA has the policy of utilizing a four-day period for the chronic criterion. The USEPA utilizes an acute-to-chronic ratio to derive the chronic criterion, and as Mr. Klocek explains in his testimony, a ratio of acute-to-chronic toxicity for chloride of 3.178 was deemed appropriate. The result of this calculation is the proposed chronic water quality standard of 640 mg/L.

Sensitivity Analysis

Under federal regulation, 40CFR131.5(c), one of the requirements for water quality standards is that the criteria be sufficient to protect the designated uses. The derivation of the winter chloride standard was straightforward, using the USEPA protocol. The chronic derivation was not as straightforward, as no chronic effects were observed using the standard USEPA toxicity test protocol for some of the species tested, making derivation of a chronic standard subject to best professional judgment. Using the published acute:chronic ratio of 3.178 was deemed a conservative approach, which yielded a chronic water quality criterion of 640 mg/L.

A number of extended exposure tests were conducted. The *C. dubia* test where the organisms were exposed to elevated chlorides for 35 days yielded a No-observable Effects Concentration of 782 mg/L chlorides at 10 degrees C, above the 640 mg/L computed chronic criterion, so the laboratory testing of *C. dubia* demonstrates that this sensitive species would be protected if the 640 mg/L criterion was adopted as a winter standard for chlorides.

For the Fingernail clam toxicity testing, run for 28 days of exposure to various chloride concentrations, no chronic effects were detected with concentrations of 1,000 mg/L chlorides at 10 degrees C. This result also indicates that a chronic criterion of 640 mg/L chlorides in the winter would be protective of this sensitive species. When the elevated chlorides were decreased after seven days of exposure (pulsed tests), the chronic value was 1,963 mg/L at 10 degrees C, well above the proposed 640 mg/L chronic criterion.

The third sensitive species, the Mayfly *Neocloeon triangulifer*, showed no chronic effects found in chloride concentrations up to 750 mg/L. Again, the computed chronic criterion of 640 mg/l is protective of this sensitive species.

The final species evaluated was the amphipod *Hyalella azteca* (Burlington strain). The chronic value at 10 degrees C with 28 days exposure to elevated chlorides was 1,257 mg/L; however, when the chlorides were lowered after seven days of exposure, the chronic value was 2,740 mg/L. Both results are well above the proposed 4-day chronic criterion of 640 mg/L chlorides.

In summary, using the 3.178 acute-to-chronic ratio, as described in the previous section, results in a conservative chronic water quality criterion, which will be protective of the most sensitive species. The recent temperature effects on four species of mayflies, as described in Roger Klocek's testimony, is consistent with the findings described by Dr. Soucek. As the temperature declines, the acute toxicity concentration of chlorides becomes less toxic by a minimum of 201 mg Cl^-/L per degree C decrease for the mayflies, and further points to the conservative basis for the proposed winter water quality standard.

Finally, I refer to the DuPage River/Salt Creek written comments on the actual biological quality of these streams, despite the elevated winter chloride levels. These field results clearly indicate the 500 mg/L winter chloride water quality standard is overly restrictively.

Proposed Water Quality Standard

The petition includes specific language to amend the General Use Water Quality Standard with the seasonal water quality standards. I inadvertently did not include the chloride water quality standard as applied to the Chicago Area Waterway System and Lower Des Plaines River, as found in Subpart D of Part 302. Specifically, the proposed language presented in the petition was also intended to replace Section 302.407(g)(2) and (g)(3), so that the same chloride seasonal standards would apply to the Chicago Area Waterway System and Lower Des Plaines River.

In addition, Section 303.449 includes unique chloride water quality standards for the Chicago Sanitary & Ship Canal, which are similar to what is proposed in this proceeding. If the Board adopts the seasonal chloride standards as proposed, I would recommend that the Board also harmonize the water quality standard for chlorides as found in 309.449 as found in this proposal.

Conclusion

Chloride water quality exceedances have existed in the urban streams in Illinois since the Board adopted the 500 mg/L General Use Standard in R70-8 in 1972. The chloride levels in our streams have steadily increased since the adoption of this water quality standard, as the network of roadways and parking lots has increased. The adoption of this same standard to the Chicago Area Waterways brought this problem to the forefront. The river basin variances committing the regulated community to work on implementing Best Management Practices is a good start, but it will not result in attaining the current not-to-exceed 500 mg/L chloride water quality standard. Couple this with USEPA pushing for an even more restrictive chloride water quality standard near 200 mg/L without regard to stream temperatures, and Illinois will never achieve the current chloride water quality standards in its urban streams.

The cold temperature toxicity research funded by the consortium demonstrates that the proposed chloride levels are adequate to protect aquatic life. While more research is always desirable, delaying adoption of a winter chloride standard until someone funds this work makes no technical sense. The approach utilized in the derivation of the proposed winter standard was conservative. As additional research becomes available, the winter standards proposed herein can be adjusted accordingly; however, I have no doubt that with additional species tested at 10 degrees C, the limits would be further relaxed from such efforts.

Contained in the proposed language is recognition that the standard can be exceeded not more than once every three years on average. This is consistent with the USEPA 1985 protocol as found in *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms* that notes “most bodies of water could tolerate exceedences once every three years on the average.” (page 6). Illinois has experienced increasingly severe storms, as has the rest of the country, and specifically including this in the language would provide a recognition that extreme events will continue to occur.

A summer chloride water quality standard was also included in our proposal, based strictly on the USEPA’s most recent publication. If the Board elects to adopt this summer standard, it will result in chloride exceedances in the spring months, and during early snow events as we had in November 2018. It is likely that a new spring/November water quality standard will be necessary, when temperatures are less than 15 degrees C; however, at this time the bioassay tests for such a standard have yet to be conducted, except for the mayflies recently reported by the Stroud Water Research Center. Depending on the outcome of the proposed winter standard, I am confident that the same consortium that funded the winter temperature tests would take on this funding.

Thank you, this concludes my pre-filed testimony.

James E. Huff, P.E.

ATTACHMENT 1

Resume of James E. Huff

Education

B.S., 1970, Chemical Engineering, Purdue University, West Lafayette, Indiana
M.S.E., 1971, Environmental Engineering, Purdue University, West Lafayette, Indiana
Graduate School of Business, 1976, University of Chicago

Registrations & Certificates

Professional Engineer
1975, Illinois, #062-032933
Class 2 and Class K Sewage Treatment Works Operator, Illinois

Affiliations

- ACEC-Illinois (past Environmental Committee Member and Past Chairman)
- ACEC-Illinois (past Board of Directors, Vice President, and Secretary/Treasurer)
- Water Environment Federation Member
- Illinois Water Environment Association
- National Water Well Association

Areas of Specialization

- Water Quality Standards
- Sustainable Wastewater Treatment & Green Infrastructure Wet Weather Design
- Stream Surveys/Antidegradation Analysis
- Soil and Groundwater Remedial Design
- Hazardous Waste Management

James E. Huff, P.E.

Senior Consultant

Summary of Experience

From 1980 through 2016, Mr. Huff was an officer of Huff & Huff, Inc., responsible for projects pertaining to water quality studies including watershed-based plans, 319 grants, stream surveys, wet weather studies, antidegradation assessments, sustainable wastewater treatment designs, and regulatory rule changes. Since October 2016, Mr. Huff has served as a Senior Consultant to Huff & Huff. A significant part of Mr. Huff's practice area has been assisting both municipal and industrial clients with implementation of Best Management Practices (BMPs) for de-icing practices to protect water quality.

Relevant Project Experience

Water Quality: In the area of water quality, Mr. Huff is active in the Chicago Area Waterways, the DuPage River/Salt Creek Workgroup and the Hickory Creek Watershed Planning Group. For the DuPage River/Salt Creek Workgroup, Mr. Huff worked on low dissolved oxygen problems including measuring sediment oxygen demand for the QUAL2k model to evaluating alternative in-stream aeration technologies. This included canoeing the study area of Salt Creek and a number of field visits along the East Branch. He was responsible for the final report on the watershed plans for both the East Branch of the DuPage River and Salt Creek and was responsible for reviewing the QAUL2k modeling work. This work lead to the first project by the Workgroup to improve dissolved oxygen, with the design of the Churchill Woods Dam removal, which Mr. Huff was part of the design/permitting team. This work resulted in an *Honor* Award for Engineering Excellence from ACEC-IL and was featured in Watershed Science Bulletin. Currently Mr. Huff is leading a consortium evaluating cold temperature toxicity of chlorides, in anticipation of supporting a new seasonal chloride water quality standard. Mr. Huff chairs the de-icing committee for industrial users as part of the CAWS variance effort.

Mr. Huff was the lead reviewer for NIPC/CMAP on water quality impacts of proposed expansions/new discharges in northeastern Illinois from 2004 to 2008. On behalf of the Village of New Lenox, Mr. Huff assisted in the formation of the Hickory Creek Watershed Planning Group, and this work continues assisting with development green storm water projects within the watershed and the implementation of BMPs for de-icing practices and chloride monitoring.

On the Fox River, Mr. Huff was project manager for a group of municipal dischargers on a project to collect and analyze weekly water quality samples along the river, its tributaries, and outfalls at over 30 locations to establish a better database on un-ionized ammonia levels. Mr. Huff has directed fish, mussel, and benthic surveys for industrial, storm water, and municipal wastewater discharges located on the following waterways: Cedar Creek, Deep Run, Flint Creek, Mississippi River, Thorn Creek, North Kent Creek, Tyler Creek, Kishwaukee River, Hickory Creek, Jackson Branch of Jackson Creek, the Chicago Sanitary & Ship Canal, Kaskaskia River, and Casey Fork Creek, and has completed antidegradation studies as part of many of these studies. Thermal studies, mixing zone studies, thermal studies, and diffuser designs have been completed for a variety of clients on large rivers (Mississippi River, Ohio River, Illinois River, and the Des Plaines River) as well as small waterways, using Cormix.

James E. Huff, P.E.

Senior Consultant

Wastewater Design: Mr. Huff has directed 15 municipal wastewater treatment design projects. In addition, he has designed a number of pumping systems, including the lift stations, controls, and force main designs. These designs included a wide range of features from converting existing facilities to cutting edge P removal systems.

Wet Weather Design: Mr. Huff has also conducted several CSO studies including Long-term Control Plans, Nine Minimum Controls, O&M Plans, and Water Quality Impact Studies. He has completed three CMOM evaluations and two Long-term Control Plans (LTCP) and assisted on a number of other wet weather plans as a sub consultant. Mr. Huff assisted the Galesburg Sanitary District and currently its LTCP is nearing the end of its planned upgrades and was one of the first communities in Illinois to achieve the USEPA presumptive remedy of less than 4 overflow events annually, through the implementation of a number of sustainable projects, including an aggressive foundation drain disconnection program. Mr. Huff also assisted the Village of Hinsdale. A 20-year program was successfully negotiated as part of its LTCP, which includes one 1-million gallon wet weather tank and extensive sewer separation until the presumptive remedy is achieved. For the Village of Barrington, a value engineering project completed recommended there were more cost-effective ways to eliminate excess flow besides a large holding tank. Extensive modeling work has been followed by extensive smoke testing, installation of overhead sewers with foundation drain disconnections and replacement of a number of key interceptor sections.

Sustainable Solutions: Mr. Huff is a leader in sustainable wastewater issues, with an emphasis on decentralized wastewater treatment approaches or cluster wastewater treatment systems with subsurface discharge for nine residential developers/country clubs, and three temples. These systems are typically 10,000 to 20,000 gpd, utilizing two SBRs, computer controlled, followed by a large leach field allowing for groundwater recharge and more open space within developments. Recently Membrane Bioreactors (MBRs) have been used, with water reuse. The first medical marijuana grower in Illinois was permitted with an MBR followed by using the treated effluent for irrigation in the green house, after ozonation.

Mr. Huff was part of the design team for evaluating three alternative porous pavements for the MWRDGC in 2009, which included the ability to measure water quality from runoff and infiltration, as well as flow rates from the three porous pavements plus a control. Rain gardens have been installed at two facilities and for the Tollway. Mr. Huff assisted with the sustainable stormwater practices for the I-90 exit at Route 47. This project was an ACEC-IL HONOR award recipient in 2015. Recently, Mr. Huff completed a Facilities Plan Report for a wastewater expansion that included the PACT process to address concerns over endocrine disrupter chemicals, a wetted prairie, a bioswale, and solar, wind, and a novel geo-thermal element associated with wastewater expansions to reduce the carbon footprint. In 2010, a floating island was installed on Cedar Creek and a novel matting material for stream bank stabilization installed to evaluate both from a water quality perspective. Wastewater expansions on two streams with endangered mussels have been successfully permitted by Mr. Huff, requiring extraordinary efforts to assure the preservation of the protected species.

Two novel in-stream aeration systems, using high-purity oxygen on Cedar Creek were designed by the firm, and have operated successfully for over 30 years, as an alternative to advanced wastewater treatment, based on a stream model developed for Cedar Creek

Past Experience: Mr. Huff served on the Illinois Nutrient Technical Advisory Committee, representing the American Council of Engineering Companies – Illinois (ACEC-IL) from 2001 to 2015. From 1987 through 1990, Mr. Huff was a part-time faculty member, teaching the senior level environmental courses in the Civil Engineering Department at IIT-West in Wheaton, Illinois. From 1976 to 1980, Mr. Huff was Manager of Environmental Affairs for Akzo Nobel Chemicals, a diversified industrial chemical manufacturer. At Akzo, Mr. Huff was responsible for all environmental activities at eight plants located throughout the U.S. Technical work included NPDES permitting, extensive treatability studies as well as designing new facilities.

Previously, Mr. Huff was an Associate Environmental Engineer in the Chemical Engineering Section at IIT Research Institute (IITRI). Much of this work involved advanced wastewater treatment development, including applying a combination of ozone/UV treatment of cyanide, PCB's, RDX, HMX, and TNT and the use of catalytic oxidation of cyanide using powdered activated carbon impregnated with cupric chloride in petroleum refinery activated sludge units. At Mobil Oil's Joliet Refinery Mr. Huff was employed as an Advanced Environmental Engineer during the construction and start-up of the largest grassroots refinery ever constructed, responsible for wastewater permitting, training, start-up, and technical

James E. Huff, P.E.

Senior Consultant

support as well as for wastewater treatment system as well as water supply, solid waste, and noise abatement issues at the refinery from 1971 to 1973.

Honors:

- 2012 Purdue University Civil Engineering Alumni Achievement Award
- Omega Chi Epsilon (Chem. Engr. Honorary)
- President's Academic Award
- Graduated with Distinction
- Fellowship from the Federal Water Quality Admin.

Thesis: "Destabilizing Soluble Oil Emulsions Using Polymers with Activated Carbon," Major Professor, Dr. James E. Etzel

Selected Papers:

- "Ozone-U.V. Treatment of TNT Wastewater," E.G. Fochtman and **J.E. Huff**, International Ozone Institute Conference, Montreal, May 1975.
- "Alternative Cyanide Standards in Illinois, a Cost-Benefit Analysis," L.L. Huff and **J.E. Huff**, 31st Annual Purdue Industrial Waste Conference, Lafayette, IN, May 1976.
- "Cyanide Removal from Refinery Wastewaters Using Powdered Activated Carbon," **J.E. Huff**, J.M. Bigger, and E.G. Fochtman, American Chemical Society Annual Conference, New Orleans, LA, March 1977. Published in Carbon Adsorption Handbook, P.N. Cheremisinoff and F. Ellerbusch, Eds., Ann Arbor Science Publishers, Inc., 1978.
- "Industrial Discharge and/or Pretreatment of Fats, Oils and Grease," **J.E. Huff** and E.F. Harp, Eighth Engineering Foundation Conference on Environmental Engineering, Pacific Grove, CA, February 1978.
- "A Review of Cyanide of Refinery Wastewaters," R.G. Kunz, **J.E. Huff**, and J.P. Casey, Third Annual Conference of Treatment and Disposal of Industrial Wastewater and Residues, Houston, TX, April 1978. Published as: "Refinery Cyanides: A Regulatory Dilemma," Hydrocarbon Processing, pp 98-102, January 1978.
- "Disinfection of Wastewater Effluents in Illinois-A Cost-Benefit Analysis," L.L. Huff and **J.E. Huff**, Illinois Water Pollution Control Association 2nd Annual Conference, Kankakee, IL, May 20, 1981.
- "Treatment of High Strength Fatty Amines Wastewater - A Case History," **J.E. Huff** and C.M. Muchmore, 52nd Conference - Water Pollution Control Federation, Houston, TX, October 1979. Published JWPCF, Vol. 54, No. 1, pp 94-102, January 1982.
- "Measurement of Water Pollution Benefits - Do We Have the Option?" L.L. Huff, **J.E. Huff**, and N.B. Herlevson, IL Water Pollution Control Assn 3rd Annual Conference, Naperville, IL, May 1983.
- "Evaluation of Alternative Methods of Supplementing Oxygen in a Shallow Illinois Stream," **J.E. Huff** and J.P. Browning, IL Water Pollution Control Assn 6th Annual Meeting, Naperville, IL, May 7, 1985.
- "Engineering Aspects of Individual Wastewater System Design," **J.E. Huff**, 22nd Annual Northern Illinois Onsite Wastewater Contractors Workshop, St. Charles, IL, February 27, 1995.
- "Total Maximum Daily Loadings (TMDL) and Ammonia Conditions in the Fox River Waterway," **J. E. Huff** and S. D. LaDieu, Illinois Water '98 Conference, Urbana, IL, Nov. 16, 1998.
- "The Illinois Ammonia Water Quality Standards: Effluent Implications & Strategies for Compliance," L.R. Cunningham & **J. E. Huff**, Illinois Water '98 Conference, Urbana, IL, Nov. 16, 1998.
- "Phase II Storm Water Regulations – Compliance Strategies for the Gas Transmission/Distribution Industry," **J.E. Huff**, American Gas Association 2003 Operations Conference, Orlando, Florida, April 28, 2003.
- "Endocrine Disruptors or Better Living Through Chemistry," **J. E. Huff**, Illinois Association of Wastewater Agencies Fall Meeting, Bloomington, IL, November 14, 2003.
- "Permitting Wastewater Treatment Plant Expansions in Northeast Illinois in the 21st Century", **J.E. Huff**, 28th Annual Illinois Water Environment Association Conference, Bloomington, IL, March 6, 2007.

James E. Huff, P.E.
Senior Consultant

- "Lessons Learned from the New Lenox Decision," R. Harsch, R. Sly, and **J.E. Huff**, Illinois Association of Wastewater Agencies, Annual Meeting, Springfield, IL, March 12, 2009.
- "Implementation of Antidegradation in Illinois," **J.E. Huff**, Indiana ACEC Environmental Business Conference, Indianapolis, IN, September 16, 2009.
- "Removal of Low Head Dams to Improve Water Quality and other DuPage River/Salt Creek Workgroup Watershed Management Efforts", **J.E. Huff** and D. Bounds, IAFSM, Annual Meeting, March 10, 2010.
- "Stream Dissolved Oxygen Improvement Study-Salt Creek and East Branch DuPage River," S. McCracken and **J.E. Huff**, Watershed Science Bulletin, Vol 3, Issue 1, pgs 17-23, February 2012.
- "The Science Behind the Chloride Water Quality Standard", **J E Huff**, Chicago Area Waterway Chloride Workshop, Oct 29, 2015.
- "Municipal Separate Storm Sewer System (MS4) Permit Requirements", **J E Huff**, APWA Chicago Metro Chapter Expo, May 18, 2016.

ATTACHMENT 2

Work Plan Submittal to USEPA and IEPA

August 2, 2016

Huff & Huff incorporated
A Subsidiary of GZA

GEOTECHNICAL
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August 2, 2016

Ms. Candice Bauer
U.S. EPA Region V-Water Quality Branch (WQ-16J)
77 West Jackson Blvd
Chicago, IL 60604-3590

And

Mr. Sanjay Sofat
Bureau of Water
Illinois EPA
1021 North Grand Avenue East
Springfield, IL 62702

Re: Chlorides

Dear Candice and Sanjay:

As you both may recall, I have expressed concerns that the science behind the chloride toxicity data that are being used for deriving winter water quality criteria is questionable. The vast majority of the toxicity tests have been conducted at temperatures above 20°C. There is good reason to believe that the toxicity of chloride diminishes at colder temperature, as the metabolic activity of the aquatic species slows down. However, the literature on cold temperature toxicity is incomplete. As you both may also recall, while I fully support Best Management Practices for chloride reduction, my analyses on several watersheds indicated that BMP implementation alone will never achieve the attainment of a not-to-exceed 500 mg/L chloride standard. As both the U.S. EPA and Illinois EPA have made clear, the current toxicity data on chloride suggests the water quality standard needs to be lowered significantly, which is clearly problematic for urban areas during the winter months. Absent finding any organization that would take on cold temperature toxicity studies on chlorides, I have assembled a consortium of communities, counties, transportation organizations, sanitary districts, watershed organizations, and industries to fund this research.

To that end, enclosed, please find two work plans for conducting both acute and chronic toxicity testing at 10°C on chlorides on four organisms. We would welcome any comments you may offer on these work plans.

My expectation is that this work, along with an extensive cold temperature literature review will provide the basis for proposing winter chloride standards. I anticipate this research will be completed in time to present to the Pollution Control Board in the anticipated chloride water quality docket that will be forthcoming. As there is already a precedent for winter water quality standards in Illinois, I would hope that both of your agencies will welcome this additional science and research to support the development of more scientifically defensible water quality standards.

I look forward to working with you both on future changes to the chloride water quality standards.

Sincerely;

James E. Huff, P.E.

cc Scott Twait, Illinois EPA

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
AMENDMENTS TO THE GENERAL USE	)	R18-32
WATER QUALITY STANDARDS	)	(Rulemaking – Water)
FOR CHLORIDE	)	

Introduction

My name is Roger Klocek and I am a Senior Biologist at Huff and Huff, Inc., a subsidiary of GZA. I have been a biologist for more than 40 years and have conducted biological surveys in fresh and marine waters during my career. I have a special interest in aquatic invertebrates.

Huff & Huff, Inc. had conducted chloride toxicity testing on four sensitive aquatic species: *Ceriodaphnia dubia* (a planktonic crustacean), *Neocloeon triangulifer* (a mayfly aquatic larva), *Hyalella azteca* (an amphipod crustacean) and *Sphaerium simile*, (a fingernail clam) during 2017/18. Testing was done at 10 degrees centigrade (C) using sodium chloride. These taxa were chosen for testing because they are among the most sensitive organisms to chloride toxicity and their laboratory toxicity studies are well documented. A literature survey was also conducted to gather information on the impact temperature has on chloride toxicity. The annotated literature survey is included with the petition.

Temperature Effects

When temperatures are low, activity levels and metabolism decrease, which can lead to reductions in chloride uptake compared to under higher temperatures (Cairns et al. 1975). For example, larval dormancy or diapause brought on by low temperatures may be responsible for reduced toxicity in some macroinvertebrates. In a study by Silver et al. (2009) it was found that chironomid larvae (*Chironomus riparius*) at 22° C, experienced 35 percent survival in water with no NaCl, whereas at chloride concentrations of 3,035, 6,070, and 12,140 mg Cl^-/L, survival was less than 10%.

However, at 10° C, survival was greater than 50 percent at 0 and 3,035 mg Cl^-/L and below 20 percent at 10,000 and 20,000 mg Cl^-/L. A subsequent study demonstrated a positive relationship between temperature and toxicity. (Lob & Silver 2012).

The amphipod *Hyalella azteca* belongs to the Amphipod order and Hyalellidae family. It is commonly found in ponds, lakes, and rivers and is the most widespread species complex of amphipod (Pickard and Benke 1996, Soucek et al. 2013). It has a short life cycle and reaches sexual maturity in the laboratory at approximately 25-40 days (Othman and Pascoe 2001). The population density was found to be lowest in winter with a peak biomass in August and September in southern Alberta, Canada (Wen 1992). Reproduction is continuous from spring to fall. In one laboratory experience, under temperatures of less than 15ºC, eggs were not produced (Pickard and Benke 1996).

Neocloeon triangulifer (previously *Centroptilum triangulifer*) belongs to the order Ephemeroptera (mayfly) and Baetidae family. *N. triangulifer* is a parthenogenetic mayfly that is commonly found in slow-flow areas of small to medium sized streams (Funk et al. 2006). The offspring of *N. triangulifer* are female clones (Funk et al. 2006, Soucek and Dickinson 2015). They are nymphs for approximately 3-4 weeks in ideal conditions prior to emerging as adults. *N. triangulifer* larvae are stressed at high temperatures (30ºC) (Chou et al. 2017). *N. triangulifer* were shown to exhibit larval development that took 27 days at 25°C and 179 days at 10°C yet were shown to exhibit inverse relationships between temperature and adult size, and fecundity (Sweeney and Vannote 1984).

Jackson and Funk (2019) of the Stroud Water Research Center presented salinity toxicity data on four species of mayflies representing three different families tested at winter and summer temperatures using sodium chloride during 96-hour static tests at temperatures ranging from 5 to 25ºC. The four mayflies were *Neocloeon*, *Leptophlebia*, *Maccafertium*, and *Procloeon*. The mayflies were tolerant of high sodium chloride values at low temperature and were less tolerant of sodium chloride as temperature increased. Attachment 2 presents a summary table of their findings, which presents results as sodium chloride. Therefore, the equivalent chloride is 60.7 percent of the sodium chloride values presented in the table. For the four species of mayflies, the LC_{50} for chlorides increased with temperature at rates ranging from 201 to 306 mg/L chlorides for every degree C. Or in other words there was reduced toxicity as the temperature declined. The correlation the authors found extended from 5 to 25ºC.

The Stroud Center has provided scientific critiques of the Iowa and Pennsylvania proposed chloride standards to the U.S. Environmental Protection Agency and is respected for its high caliber work.

Calculating Chronic Effects

Due to the slower activity levels and metabolism decrease at colder temperatures, chronic testing is more difficult. Where reproduction is used to measure chronic effects, at colder temperatures, species may die of old age without reproduction. The Acute to Chronic ratio (ACR) is an accepted method of estimating chronic effects. Based on the acute testing completed at 10ºC using the accepted test durations and the ACR of 3.187 suggested by Stephen (2009a) and used in the Iowa

Proposed Water Quality report (2009), chronic toxicities were calculated based on the acute test results.[1]

Stephen (2009a) arrived at the 3.187 ACR by the following method:

> "… there is an alternative approach that is justified on the basis of the "good science" clause in section XII.B of the 1985 Guidelines. This approach is based on the fact that the four low SMACRs for chloride were obtained with invertebrates, whereas the high SMACR was obtained with a vertebrate. This can be interpreted to mean that vertebrates have a higher ACR, on the average, than invertebrates, especially because the qualitative ACR for the fathead minnow is 15.17. Therefore, a vertebrate ACR and an invertebrate ACR can be used with the GMAVs to calculate a predicted Genus Mean Chronic Value for each genus, and then a FCV can be calculated directly from the predicted GMCVs. This approach calculates and uses a predicted chronic value for each genus for which an acute value is available and probably does a better job of taking into account the chronic sensitivities of both vertebrates and invertebrates to chloride…The FACR of 3.187 derived above was derived from all of the acceptable ACRs for invertebrates. The only acceptable ACR for a vertebrate is 7.308. A predicted GMCV can be calculated from each GMAV by using 3.187 as the invertebrate ACR and using 7.308 as the vertebrate ACR."

The five SMACRs (Species Mean Acute Values) of 7.308, 4.148, 1.974, 3.952, and greater than 2.438 were available for use in calculations resulted in three GMACRs (Genus Mean Acute Values) of 7.308 Oncorhynchus, 3.187 Daphnia, greater than 2.470 Ceriodaphnia. Mr. Huff compares the chronic results obtained from our most recent research to the calculated chronic standard proposed, and in all cases the measured chronic values in these tests were at higher

[1] The 3.187 was based on Daphnia, the only invertebrate where Acute/Chronic data were available at the time.

concentrations than derived from using the ACR, which would indicate the proposed chronic winter chloride standard is conservative and will be protective for the aquatic community.

Closure

Thank you, this concludes my pre-filed testimony.

/s/ Roger Klocek

Works Consulted

Chou H, Pathmasiri W, Deese-Spruill J, Sumner S, Buchwalter D B. Metabolomics reveal physiological changes in mayfly larvae (Neocloeon triangulifer) at ecological upper thermal limits. *Journal of Insect Physiology* 2017101107–112. (https://doi.org/10.1016/j.jinsphys.2017.07.008)

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Soucek, D. J., & Kennedy, A. J. (2005). Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. *Environmental Toxicology and Chemistry*, *24*(5), 1204-1210.

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ATTACHMENT 1

Resume of Roger Klocek

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RESUME

Roger Klocek

Senior Biologist

Summary of Experience

Roger Klocek is an aquatic biologist with forty years of experience in both marine and freshwater environments. Roger has conducted fish, macroinvertebrate, plankton and mussel assessments of Midwestern water bodies including headwater stream assessments, lake assessments, and large river assessments. He has experience with surveying threatened and endangered aquatic species, including troglobitic species, and in developing mitigation plans for terrestrial and aquatic species. He has co-authored a field guide and online key for midwestern mussels.

Relevant Project Experience

RECENT AQUATIC ASSESSMENTS

- Mussel survey, Kankakee Co. IL, macroinvertebrate surveys Will and Lake Co. .IL, 2018
- Mussel, macroinvertebrate, fish surveys Rock Island, Lake, Cook, Will Co. mussel survey Butler Co. Ohio, 2017
- Fish, mussel, macroinvertebrate surveys McHenry, Mason, LaSalle, Kane Co. 2016.
- Plankton Assessments, Will Co., IL, 2014-2015
- Assess mussels, fish, macroinvertebrates in Kane, Will, McHenry, LaSalle Co., IL and Stephenson, Douglas, Boone & Porter Co. IN 2014- 2015
- Relocate sensitive species and mussel beds Winnebago Co., IL 2012, 2013
- Prepare update for water intake bio studies, Lake Co., IN, 2012
- Assess mussels, Clinton Iowa and DeKalb Co. IL 2012
- Assess fish (IBI), macroinvertebrates (MIBI), mussels (MCI), Cook Co., LaSalle Co, IL, 2012
- Assess Spring Creek, for 3 years for; mussels, fish (IBI), benthics (MIBI), Will Co., IL 2009-2011.
- Assist with Loggerhead Shrike surveys, Will County, IL 2011.
- Assess mussels Manhattan Creek, Will Co., IL 2011
- Assess mussels, fish, (IBI), macroinvertebrates, (MIBI) Hampshire Creek, Hampshire, IL, 2009, 2011
- Assist with Hines Dragonfly Survey, Will Co., IL
- Assess mussels, fish, (IBI), invertebrates (MBI) in Kishwaukee River at Huntley IL, Kane Co., 2010
- Assess mussels including state listed species in Fox River, Elgin, IL 2010
- Conduct Scuba assessment of mussels at St. Charles, IL, sensitive species survey. 2010

Education

B.S., 1971, Biology/Anthropology,
University of Illinois, Chicago, IL

Registrations & Certificates

Rescue Diver – 1982
Divemaster – 1990

Affiliations

- American Fisheries Society
  - Illinois Chapter
  - Early life history section (larval fish)
- Society for Freshwater Science
- Mussel Conservation Society

Areas of Specialization

- Environmental Assessment
- Sensitive Species Studies
- Fish Assessment
- Mollusk Assessment
- Mussel Relocation
- Water Intake Studies

Roger Klocek
Senior Biologist

- Monitor relocated mussels on a quarter- mile section of the Fox River at Dundee, IL, 2006
- Relocate mussels at the Sullivan Bridge construction site in Aurora, IL in association with the IDNR. 2004
- Manage Lake sturgeon potential restoration project for Illinois Lake Michigan waters, collect eggs, rear sturgeon, survey historic spawning shoals, 1999-2004.
- Relocate mussels inside coffer dams at bank stabilization project, Batavia, IL, 2000
- Assess and relocate all mussels including IL State T&E species, on one mile of the Fox River for Kane Co. 2002
- Assist with Scuba procurement of breeding stock for endangered Higgins Eye mussel, Cordoba, IL 2002-05
- Project Manager for survey and relocation of all mussels on approximately 4000 feet of Eakin Creek, Kane Co. 2000
- Assess mussels in the Fox River Basin at twenty-five stations in partnership with the IDNR. (1999-2001)
- Conduct mussel assessments on 35 stations of Kishwaukee River 2009-2011
- Conduct mussel assessments Midewin Tallgrass Prairie on three watersheds, 2008-09
- Conduct mussels assessments Kankakee River and tributaries for protected species, 2006–2009
- Conduct cave salamander population assessments, multiple caves, Ava, MO. 2001-2005.
- Conduct freshwater spring and cave faunal assessments for Dominican National Parks Service, 2000.
- Conduct juvenile and larval reef fish and queen conch faunal assessments, Dominican Republic, 1995-2000.

Publications :

2011. Klocek, R. and Laura Barghusen. Aquatic Faunal Surveys Before and After Restoration of the Will County Forest Preserve's Spring Creek at Hadley Valley Forest Preserve, Will County, Illinois, 2009 through 2011.

2009. Klocek, Roger. Bland, James, Barghusen, Laura. Key to the mussels of Northern Illinois. Online key posted by the Field Museum, Chicago. http://fm1.fieldmuseum.org/keystonature/mussels/

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ATTACHMENT 2

Jackson and Funk Article

2018

PHILOSOPHICAL TRANSACTIONS B

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Temperature affects acute mayfly responses to elevated salinity: implications for toxicity of road de-icing salts

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Salinity in freshwater ecosystems has increased significantly at numerous locations throughout the world, and this increase often reflects the use or production of salts from road de-icing, mining/oil and gas drilling activities, or agricultural production. When related to de-icing salts, highest salinity often occurs in winter when water temperature is often low relative to mean annual temperature at a site. Our study examined acute (96 h) responses to elevated salinity (NaCl) concentrations at five to seven temperature treatments (5–25°C) for four mayfly species (Baetidae: *Neocloeon triangulifer*, *Procloeon fragile*; Heptageniidae: *Maccaffertium modestum*; Leptophlebiidae: *Leptophlebia cupida*) that are widely distributed across eastern North America. Based on acute LC50s at 20°C, *P. fragile* was most sensitive (LC50 = 767 mg l^{-1}, 1447 µS cm^{-1}), followed by *N. triangulifer* (2755 mg l^{-1}, 5104 µS cm^{-1}), *M. modestum* (2760 mg l^{-1}, 5118 µS cm^{-1}) and *L. cupida* (4588 mg l^{-1}, 8485 µS cm^{-1}). Acute LC50s decreased as temperature increased for all four species ($n = 5$–7, $R^2 = 0.65$–0.88, $p = 0.052$–0.002). Thus, acute salt toxicity is strongly temperature dependent for the mayfly species we tested, which suggests that brief periods of elevated salinity during cold seasons or in colder locations may be ecologically less toxic than predicted by standard 20 or 25°C laboratory bioassays.

This article is part of the theme issue 'Salt in freshwaters: causes, ecological consequences and future prospects'.

1. Introduction

Salinity in fresh waters is naturally variable, primarily reflecting differences in concentrations of dissolved inorganic cations calcium, magnesium and sodium, and anions carbonate, sulfate and chloride [1,2]. The differences in ion concentrations among fresh waters primarily reflect the weathering of soil and bedrock underlying a watershed, atmospheric deposition, and the evaporation–precipitation cycle. Sodium is generally less common than calcium and magnesium, and chloride is generally less common than carbonate or sulfate in natural waters. Elevated Na and Cl concentrations have been observed in effluents from wastewater treatment plants that reflect use of water softeners, table salt in the human diet, and disinfection before discharge [3,4], in wastewaters from some industrial, coal mining, and oil and gas production activities [5–7], in runoff and groundwater associated with various agricultural practices [8], and in road runoff following applications of de-icing products such as rock salt and anti-icing brines [9–13]. Recent analyses of multi-year data have found that sodium and chloride concentrations in surface waters have been increasing over the last two to five decades, at multiple locations (e.g. [14–18], and more recently [19–21]). This increase in sodium and chloride is part of a worldwide trend for increasing salinity along with pH and alkalinity [22–27], which was recently labelled the Freshwater Salinization Syndrome [28,29].

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With these increases in ambient salinity, there has been renewed interest in the toxicity of salt in our aquatic ecosystems. Building on early toxicity tests [30], researchers have again begun examining salt toxicity by focusing on specific ions such as sodium, magnesium, chloride, carbonate, and sulfate (e.g. delivered as NaCl, $MgCl_2$, Na_2SO_4) for a variety of aquatic algae [31], insects and other macroinvertebrates [32–41], mussels [42–46], zooplankton [47,48], amphibians [49,50], and fish [32,51–53]. Other researchers have approached salt toxicity as a function of total salinity (as salt concentration or electrical conductivity), rather than as an ion-specific issue (e.g. [54–57]). The challenge in both cases is general applicability of findings as it is well known that ion composition is important to overall salt toxicity [30,58–62]. Additional references can be found in review articles [63–69]. Salt toxicity has been found to vary greatly among aquatic species, with recent data showing that some mayflies and juvenile mussels are among the most sensitive species tested [34,52,56,70]. The combination of salt sensitivity and elevated ambient salinity suggests that, at least at times, salt may reach levels that may have a negative affect on aquatic organisms [10,31,71–76].

There are two challenges in understanding the potential salinity toxicity under field conditions in colder climates where de-icing salts can increase salinity dramatically during snow/ice storms. First, most salt toxicity studies have been conducted at constant 17–25°C, which are the recommended test temperatures for standard acute and chronic toxicity tests for many species, [77,78]. However, in colder climates where de-icing salts are frequently used, water temperature can vary naturally across seasons, with winter lows of 0–10°C versus summer highs of 20–30°C (e.g. figure 1). In addition, there can be significant differences among years (e.g. an interannual range of 10°C or more; figure 1). It has been found that temperature can affect toxicity of many chemicals [79–86]. For most toxins and species, the relationship between temperature and toxicity is positive—increases in temperature result in increased toxicity (i.e. a lower LC50). Mayer & Ellersieck [80] summarized the relationship as a 10°C increase in temperature results in a two- to fourfold decrease in the LC50. Second, streams and rivers that exhibit a long-term increase in Na and Cl concentrations also often exhibit a strong seasonal cycle that includes frequent, short-term snow and ice events when salinity can be many times greater than at base flow (figure 2) [87,88]. This is a sharp contrast to streams with little urbanization (e.g. figure 1 and [88]). Unfortunately, the magnitude and duration of these events are often not well quantified in the historic data because these data are primarily periodic grab samples while snow and ice events are better described with a continuously recording sensor. While the recent studies of salt toxicity have addressed the range of conditions needed to set regulatory limits [47,89], they have not included seasonal temperature variation as part of their analyses.

This paper describes a series of experiments that examine lethal responses of mayfly (Ephemeroptera) larvae in acute (96 h) exposures to elevated salinity (i.e. NaCl added to moderately hard source water) at five to seven different temperatures. The results show how understanding the experimental relationship between temperature and salt toxicity can provide important insight into the toxicity of ambient salt concentrations, especially those originating from winter de-icing programmes.

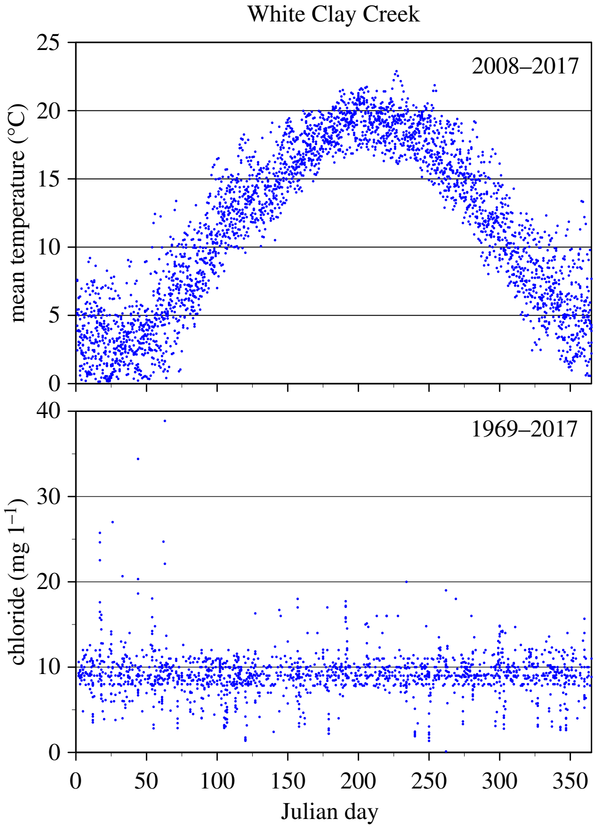

Figure 1. Long-term seasonal variation (date plotted as Julian day) in water temperature (mean daily from various continuous recorders, 2008–2017) and chloride concentration (from grab samples, 1969–2017) for White Clay Creek at the Stroud Water Research Center, 39°51′38.41″ N, 75°47′01.96″ W. Values greater than 20 mg Cl l^{-1} are presumably evidence of local de-icing efforts during winter. (Online version in colour.)

2. Methods

(a) Source water

Water for all tests was collected from White Clay Creek at the Stroud Water Research Center (39°51′38.41″ N, 75°47′01.96″ W), Chester Co. Pennsylvania, a limestone-influenced, headwater stream that drains a 7 km^2, rural (less than 0.5% developed) watershed and is moderately hard (mean 97 mg CO_3^{2-} l^{-1}) with relatively low salinity (143.8 mg l^{-1}, table 1). Seasonal patterns in temperature and chloride (as an indicator of de-icing salts affecting background salinity) from long-term data for White Clay Creek are shown in figure 1. The temperature treatments (see below) are representative of the range of conditions these test mayfly populations have experienced for generations in White Clay Creek. In contrast, the relatively low salinity in the historic data suggests that these wild mayfly populations from White Clay Creek have not been exposed to sodium or chloride concentrations similar to those in our experimental treatments in the last 50 years. Background concentrations on four dates when water was collected for laboratory bioassays averaged 6.6 mg l^{-1} for sodium and 12.3 mg l^{-1} for chloride (table 1).

To provide context for laboratory results, field data were collected every 5 min (30 Mar 2017–1 May 2018) with a Decagon CTD-10 (electrical conductivity or specific conductance corrected to 25°C, temperature, depth) sensor in Rocky Run, First State National Historic Park, New Castle County, Delaware, USA (39°49′00.45″ N and 75°33′02.84″ W), which drains a highly urbanized (60% developed), 2 km^2 watershed about 20 km from the Stroud Water Research Center. Salinity for Rocky Run was estimated from the conductivity : salinity relationship used in our experiments with White Clay Creek water, where salinity =

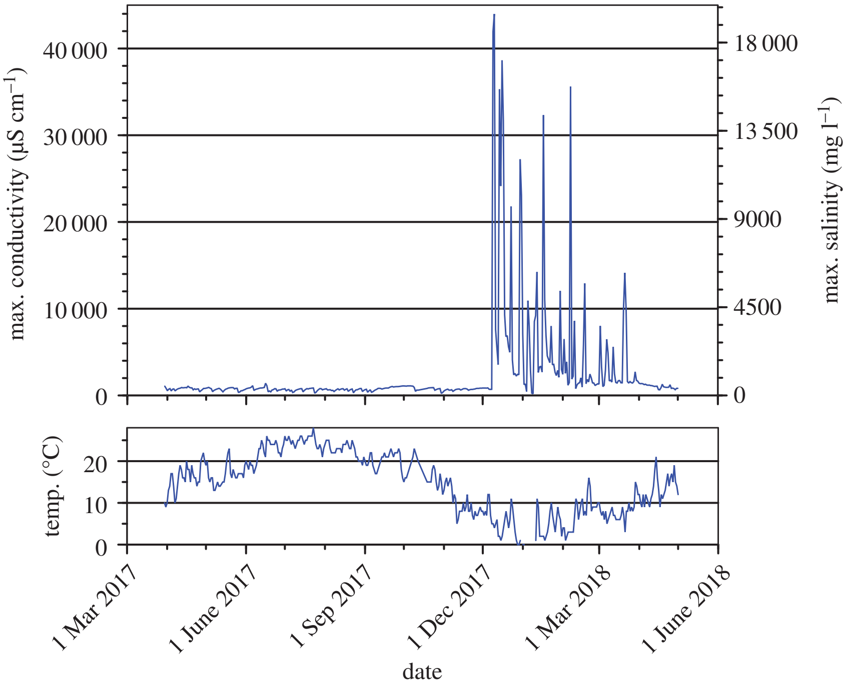

Figure 2. Seasonal variation (from 30 March 2017 to 1 May 2018) in maximum daily salinity as conductivity (μS cm^{-1}) and mg l^{-1}, and maximum daily temperature (°C) for Rocky Run, First State National Historic Park, New Castle County, Delaware. (Online version in colour.)

Table 1. Chemical characteristics of moderately hard water from White Clay Creek, PA used in acute toxicity tests in 2016. TDS, total dissolved salts.

date:	4 Apr 2016	16 Apr 2016	12 May 2016	5 June 2016	
time:	06.30	07.00	08.30	11.45	mean
pH	7.7	8.2	7.7	7.8	7.9
conductivity (μS cm^{-1})	238	232	239	241	238
alkalinity (mg l^{-1})	71.3	66.0	67.8	69.2	68.6
hardness (mg CO_3^{2-} l^{-1})	96	93	97	100	97
Ca^{2+} (mg l^{-1})	23.9	23.9	24.7	26.6	24.8
Mg^{2+} (mg l^{-1})	8.8	8.0	8.6	8.2	8.4
K^{+} (mg l^{-1})	2.0	1.6	1.7	1.9	1.8
Na^{+} (mg l^{-1})	7.1	6.1	6.5	6.5	6.6
Cl^{-} (mg l^{-1})	13.0	12.1	12.0	12.2	12.3
SO_4^{2-} (mg l^{-1})	17.0	17.6	17.5	17.2	17.3
TDS (mg l^{-1})	139	160	152	152	152.5

(electrical conductivity − 23.099)/1.844, where salinity is in mg l^{-1}, and electrical conductivity is in μS cm^{-1} at 25°C.

(b) Study species

Mayflies were chosen for this study because Ephemeroptera are ecologically significant in most streams and rivers, and they are considered pollution sensitive and have historically played important roles in water quality monitoring programmes [90–92]. We quantified acute responses to short-term (96 h) chloride exposures for four mayfly species that are common in White Clay Creek (where test species were collected) and widely distributed in eastern North America. *Neocloeon triangulifer* (McDunnough 1931) was until recently called *Centroptilum triangulifer* [93] and before that *Cloeon triangulifer* [94]. It is a parthenogenetic (clonal) mayfly species [95,96] that is most abundant during summer, when it has a relatively rapid larval development (egg hatch to adult in 25–30 days at 20°C). We worked with Stroud Water Research Center (SWRC) Clone WCC-2®, which occurs in low larval numbers during the winter, with minimal growth below 9.6°C. This specific clone has also been recently used in a number of experiments examining the toxic effects of cadmium, mercury, selenium and zinc [97–102], and chloride and sulfate salts [34,36,38–40]. *Procloeon fragile* (McDunnough 1923) was for many years called *Centroptilum fragile* [94]. It is a sexual mayfly species that exhibits a life history similar to that of *N. triangulifer* except that it has a winter egg diapause. *Maccaffertium modestum* (Banks, 1910) was long known as *Stenonema modestum*, but was recently reclassified [103]. It is a sexual

Table 2. Acute (96 h) salinity toxicity (LC50; geometric mean with 95% CI) expressed as mg l^{-1} and μS cm^{-1} for four mayfly species exposed to elevated NaCl in five to seven constant temperature (°C) treatments.

species	LC50 per temperature treatment (geometric mean with 95% CI, mg l^{-1} and μS cm^{-1})						
	5°C	7.5°C	10°C	12.5°C	15°C	20°C	25°C
salinity (mg l^{-1})							
N. triangulifer	9655 (8751–10 653)	10 462 (9655–11 337)	6719 (5541–8148)	5101 (4843–5373)	2573 (389–17 017)	2755 (2018–3762)	364 (171–773)
P. fragile			6874 (4595–10 284)	4115 (3111–5443)	3239 (1662–6312)	767 (187–3146)	766 (748–784)
L. cupida	10 086 (9037–11 257)	10 152 (9299–11 083)	10 439 (8551–12 743)	11 908 (11 650–12 172)	8368 (6955–10 067)	4588 (3108–6774)	3216 (1821–5678)
M. modestum		7236 (6005–8719)	5429 (2442–12 067)	7792 (5483–11 075)	7808 (3742–16 293)	2760 (1436–5303)	1656 (680–4035)
electrical cond. (μS cm^{-1})							
N. triangulifer	17 829 (16 161–19 668)	19 317 (17 829–20 929)	12 414 (10 241–15 049)	9430 (8953–9932)	4788 (739–31 042)	5104 (3745–6958)	698 (335–1453)
P. fragile			12 700 (8495–18 986)	7612 (5759–10 060)	5997 (3086–11 656)	1447 (367–5707)	1435 (1402–1469)
L. cupida	18 623 (16 688–20 782)	18 744 (17 172–20 461)	19 273 (15 792–23 522)	21 983 (21 506–22 470)	15 454 (12 849–18 587)	8485 (5754–12 514)	5955 (3381–10 487)
M. modestum		13 366 (11 096–16 102)	10 036 (4525–22 260)	14 393 (10 133–20 444)	14 425 (6923–30 053)	5118 (2676–9788)	3081 (1274–7451)

species that exhibits a bivoltine or multivoltine life history at White Clay Creek, with larval development of about 80 days at 20°C. *Leptophlebia cupida* (Say 1923) is a sexual mayfly species that exhibits a univoltine life history that begins with eggs hatching in mid-June and adult emergence the following April.

(i) Experimental treatments

We quantified acute responses of four mayfly species in short-term (96 h) exposures to elevated NaCl (A.C.S. reagent; J.T. Baker 33624-05). NaCl was chosen for these experiments because it represents 90–98% of the rock salt (halite) used for de-icing roads [104]. We conducted 100 temperature-specific acute tests (each test had one replicate of 20 individuals for each salinity treatment), with 20 newly hatched 1st instar larvae for *N. triangulifer*, *P. fragile*, *L. cupida* or *M. modestum* placed in a 30 ml beaker containing 15 ml of treatment solution. Newly hatched larvae were chosen because younger/smaller individuals are often more sensitive than older/larger individuals of the same species [55,105–107].

Each toxicity test had six treatments: a control (0 mg NaCl l^{-1} added to White Clay water) and five elevated salinity treatments that represented a 50% dilution series (i.e. 412, 824, 1649, 3297, 6594 mg NaCl l^{-1} added to White Clay water for *N. triangulifer* and *P. fragile*, and 824, 1649, 3297, 6594, 13 188 mg NaCl l^{-1} added to White Clay water for *L. cupida* and *M. modestum*). These were static (no renewal) experiments, conducted at five to seven constant (±0.1°C) temperature treatments (i.e. 10, 12.5, 15, 20, 25°C for all species, with the addition of a 7.5°C treatment for *M. modestum* and 5 and 7.5°C treatments for *N. triangulifer* and *L. cupida*). A diatom slurry (i.e. *ca* 20 µl of biofilm scrapings suspended in White Clay water) was provided as food in each test vessel for *N. triangulifer* and *P. fragile*. Food was not provided in *L. cupida* and *M. modestum* tests. Four replicate tests were run for each temperature treatment. Photoperiod (light : dark) was 16 : 8 h during the tests. Temperature in the rearing system was recorded every 5 min, and calibrated with a certified thermometer. Salinity across treatments was monitored with a calibrated conductivity meter.

Mayfly response was reported as survivorship after 96 h, and summarized as the lethal salinity associated with 50% mortality (or LC50) estimated using the nonparametric trimmed-Spearman–Karber method [76,108] of test population at a specific temperature. The relationship between temperature and LC50 for each species was assessed with a simple linear regression of geometric means. Linear regressions were used because it was a simple assessment of the relationship between five to seven temperature treatments and salinity toxicity, and because regression slope was consistent across the temperature range, which facilitates interpretation and incorporation into regulatory standards.

3. Results and discussion

(a) Interspecific differences in mayfly sensitivity to elevated salinity

Control survival was greater than 90% in most of the acute toxicity tests reported for *P. fragile*, *N. triangulifer* and *L. cupida*, and those tests with slightly higher control mortality were still included in these analyses as their dose-responses were similar to other tests. Survival was less than 90% for many tests with *M. modestum* (suggesting this species should be fed during 96 h tests), but the response to temperature was similar to the other mayfly species and is included in this report. However, because of low control survival, the

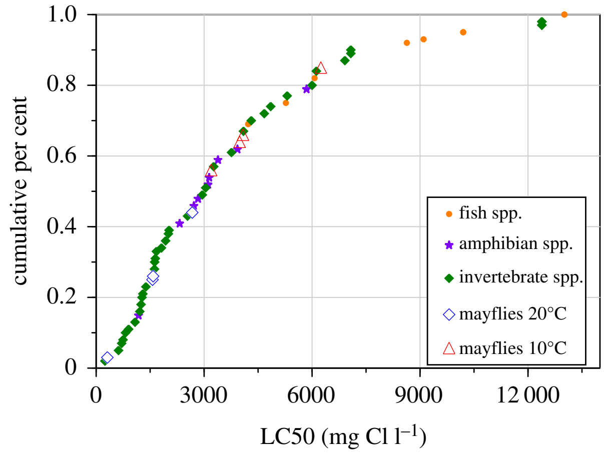

Figure 3. Relative sensitivities for the mayflies *N. triangulifer*, *P. fragile*, *M. modestum*, and *L. cupida* based on LC50s (expressed as mg Cl l^{-1}, electronic supplementary material, table S1) for 10 and 20°C (table 2) plotted with fish, amphibian and invertebrate data included in fig. 3 from [52].

LC50s for *M. modestum* should be used with caution until further verification.

Mean LC50s estimated by the nonparametric trimmed-Spearman–Karber method are expressed as salinity (mg l^{-1}) and electrical conductance (µS cm^{-1}) in table 2. We prefer to compare toxicities among mayflies at 20°C because it appears in some mayfly species we have examined that 25°C is physiologically stressful, independent of the chemical stressor being evaluated. Based on acute LC50s at 20°C, *P. fragile* was most sensitive (LC50 = 767 mg l^{-1}, 1447 µS cm^{-1}), followed by *N. triangulifer* (2755 mg l^{-1}, 5104 µS cm^{-1}) and *M. modestum* (2760 mg l^{-1}, 5118 µS cm^{-1}), and finally *L. cupida* (4588 mg l^{-1}, 8485 µS cm^{-1}) (table 2). NaCl toxicity for *N. triangulifer* has been examined in earlier studies [34,36,39], but all at 25°C. The acute LC50 for *N. triangulifer* at 25°C in our study was markedly lower than LC50 we observed at 20°C as well as the LC50s estimated by Soucek & Dickinson [34], Struewing *et al.* [36], and Soucek *et al.* [39]. Our LC50s for 25°C for all four mayfly species were not out of line with LC50s from colder temperature treatments, and the temperature versus LC50 regressions fitted the data relatively well (see below), so we do not currently have an explanation for differences observed among the studies of *N. triangulifer*. When salinity was expressed as electrical conductivity (µS cm^{-1} or mS cm^{-1}), the LC50s we observed for the baetids *P. fragile* and *N. triangulifer* (1447–5104 µS cm^{-1}) were similar to those observed for the baetid *Centroptilum* sp. (1.8–5.6 mS cm^{-1} in [59], and 10 mS cm^{-1} in [57]), and less than was observed for the baetid *Cloeon* sp. (21 mS cm^{-1} in [57]).

Mayflies are generally considered pollution sensitive, and are important contributors to metrics used to assess pollution impacts [90–92]. When we compared the LC50s for our mayflies at 20°C (expressed as mg Cl l^{-1}, electronic supplementary material, table S1) relative to the acute LC50s included in fig. 3 of [52], *P. fragile* was among the most sensitive species, *M. modestum* and *N. triangulifer* was moderately sensitive (*ca* 25th percentile) and *L. cupida* was average (45th percentile) (figure 3). Relative sensitivity for mayflies in our study would be even higher if we used LC50s from the common test temperature of 25°C (table 2)—*P. fragile*, *N. triangulifer* and *M. modestum* would be among the most sensitive, and *L. cupida* would be moderately sensitive.

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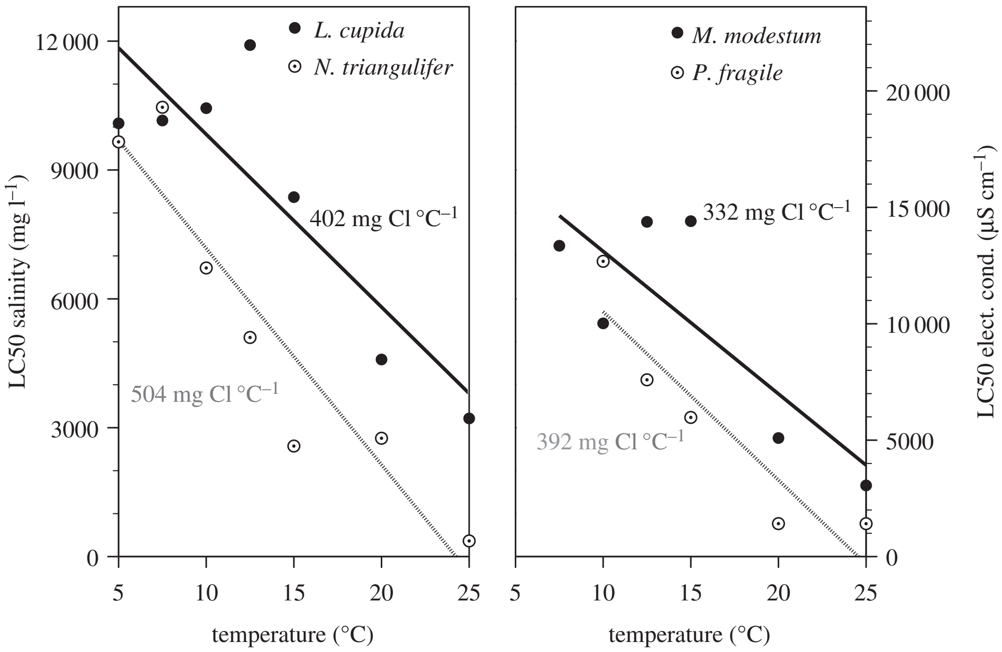

Figure 4. Simple linear regressions describing the relationship between temperature and acute salinity LC50s for the mayflies *L. cupida*, *N. triangulifer*, *M. modestum* and *P. fragile*.

Table 3. Simple linear regression results for figure 4 describing the relationship between temperature and acute salinity toxicity expressed as LC50 (geometric means, mg l^{-1}).

species	N	Pr > F	R^2	intercept	slope
N. triangulifer	7	0.002	0.881	12 211.2	−503.7
P. fragile	5	0.026	0.850	9616.0	−391.8
L. cupida	7	0.011	0.756	13 851.5	−402.2
M. modestum	6	0.052	0.653	10 425.3	−331.9

Conversely, the mayflies in our study would not be considered sensitive if we used LC50s from the 10°C test temperature (figure 3) We saw similar relative sensitivity when our study mayflies were compared to the mayflies and other macroinvertebrates presented in Wang *et al*. [70], and in the broader global survey of salinity sensitivity for mayflies and other macroinvertebrates in Kefford *et al*. [56].

The four mayfly species included in our study were not selected based on presumed or known pollution sensitivity. In fact, it is possible there are mayfly species that are as or more sensitive to elevated salinity than the species we examined. Our data, in combination with other published observations such as Wang *et al*. [70] and Kefford *et al*. [56], support the general belief that mayflies as a group are relatively sensitive to elevated salinity, although the physiological mechanisms surrounding mayfly sensitivity to salt remain to be determined [109]. Cormier *et al*. [110] defined a maximum acute benchmark of 680 $\mu S\ cm^{-1}$ for salinity derived from field observations of occurrence for 142 stream macroinvertebrate genera and annual chemistry data. While this hypothetical benchmark might not be directly comparable with our laboratory studies (Cormier *et al*. [110] eliminated several sites with high chloride), 680 $\mu S\ cm^{-1}$ (=369 $mg\ l^{-1}$ in our study) would appear to be over-protective for all species based on the LC50s at 5–10°C, and protective for *N. triangulifer*, *M. modestum* and *L. cupida*, and possibly *P. fragile*, based on the LC50s at 20°C. The benchmark might not be protective for *P. fragile* and *N. triangulifer* based on the LC50s at 25°C.

It is important to note that salinity toxicity is known to vary among salts and dilution waters tested [30,39,41,47,58–61], so our toxicities for elevated salinity that is predominately NaCl must be used with caution when referring to other de-icing and anti-icing salts such as $MgCl_2$, $CaCl_2$, KCl or calcium magnesium acetate ($CaMg_2(CH_3COO)_6$), to the 'chemical cocktail' that characterizes the Freshwater Salinization Syndrome [29], or to ambient waters with natural salinities that are markedly lower or higher than in White Clay Creek (e.g. a soft-water stream or a limestone stream).

(b) Changes in salinity toxicity in response to temperature

The relationship between salinity toxicity and temperature is important because, in regions where de-icing salts are frequently used, water temperature can change significantly with seasons (figure 1). Moreover, salinity from de-icing efforts peaks following snow and ice events when stream temperature is often nearest its lowest level, and well below the 20 or 25°C temperature used in standard bioassays (figure 2). We observed a significant or nearly significant ($n = 5–7$, $R^2 = 0.65–0.88$, $p = 0.052–0.002$) decrease in toxicity (i.e. acute LC50s increased) as temperature decreased for all four species (figure 4 and table 3). Based on the

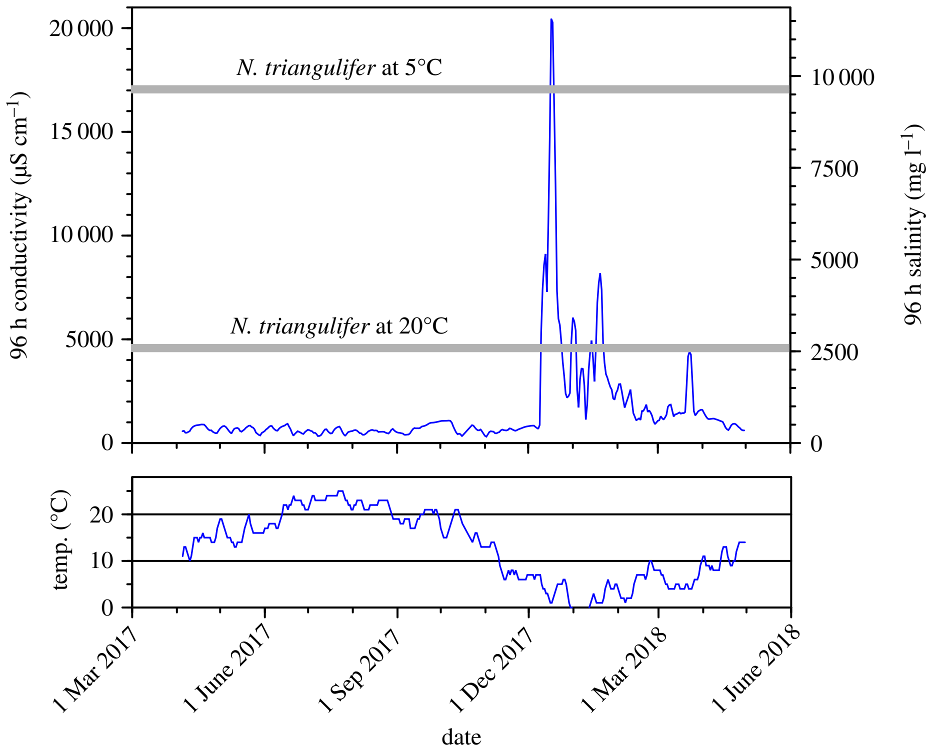

Figure 5. Plot showing mean 96 h LC50 for *N. triangulifer* at 5 and 20°C with seasonal variation (from 30 March 2017 to 1 May 2018) in 96 h running salinity as conductivity (µS cm^{-1}) and mg l^{-1}, and 96 h mean running daily temperature (°C) for Rocky Run, First State National Historic Park, New Castle County, Delaware, USA. (Online version in colour.)

regression slopes, the rate of change was similar for *P. fragile*, *M. modestum* and *L. cupida*. Their LC50s decreased between 332 and 402 mg l^{-1} for each 1°C increase in temperature. The response for *N. triangulifer* was somewhat stronger and its LC50s decreased 504 mg l^{-1} for each 1°C increase in temperature. The LC50s for *L. cupida* and *M. modestum* increased 1.7–1.9-fold for each 10°C decrease in temperature while the LC50s for *N. triangulifer* and *P. fragile* increased 3.5–3.6-fold for each 10°C decrease. This difference between *L. cupida* and *M. modestum* versus *P. fragile* reflects the estimated LC50s relative to the rate of change per °C. The species with lowest LC50 (*P. fragile*) increased proportionally more per °C than species with higher LC50s (*L. cupida* and *M. modestum*). The higher proportional change for *N. triangulifer* reflects a moderately low LC50 with a higher rate of change per °C. Our results almost match the summarization by Mayer & Ellersieck [80] that a 10°C increase in temperature results in a two- to fourfold decrease in the LC50. There are a few studies where reduced salt toxicity has been observed at lower versus higher temperature [73,111,112], but the relationship between acute salt toxicity and temperature has not been quantified in a manner that can be applied to water quality criteria (table 3).

To illustrate how the interaction between temperature and salinity toxicity provides important perspective to understanding aquatic ecosystems receiving de-icing salts, we took the raw data used to generate figure 2 and calculated 96 h (i.e. the duration of the acute toxicity tests) running mean values for conductivity, salinity (from conductivity) and temperature (figure 5). We then added the LC50 for *N. triangulifer* at 5°C and 20°C to figure 5. Based on the LC50 at 20°C, there were 21 dates that were preceded by 96 h with an average salinity that exceeded the LC50 at 20°C. In contrast, based on the LC50 at 5°C (which is more representative of thermal conditions at the time of elevated salinity), there were only two dates that were preceded by 96 h with an average salinity that exceeded the LC50 at 5°C. Thus, accounting for lower salt toxicity for an acute exposure at low temperature can change one's perspective on the apparent toxicity of ambient conditions during winter. However, it is important to note that, even after accounting for lower toxicity at 5–10°C, salinity in Rocky Run still appears to have been acutely toxic (i.e. ≥50% mortality in a 96 h period) for all four mayflies we examined. This suggests that elevated salinity (e.g. averaging 9500–11 500 mg l^{-1} for 96 h) during winter when snow and ice management programmes are being implemented may contribute to the overall impairment of the macroinvertebrate assemblage in Rocky Run, and probably other small urban streams that receive salt-laden runoff from roads, car parks and pavements. However, this is not to suggest that elevated (but not peak) salt concentrations during winter are not contributing to overall impairment. These non-peak exposures are more frequent (i.e. exposure time can be longer), and based on results for polar marine invertebrates, exposure time must be considered in the evaluation and interpretation of potential impact of toxicants at cold temperature [113,114].

(c) Regulatory and management implications of the relationship between salinity toxicity and temperature

As salinization of freshwater ecosystems resulting from de-icing and anti-icing salts continues, the regulatory and management challenge for winter road maintenance programmes will be to balance the need to protect public safety and reduce the economic costs of winter storms with the need to protect environmental health and infrastructure integrity related to excess salt, and to address potential drinking water/public health related to increased dietary intake of sodium [4,21,115,116]. Our study found low temperature can reduce the frequency or intensity of salt-related toxic events expected based on winter de-icing activities that increase NaCl concentrations. But it also shows that NaCl concentrations during winter can be so high that NaCl-related toxic events may still occur even after accounting for low temperature. Our results can also be applied to other activities that result in acute exposure to elevated salt. For example, spills or discharges of high salinity wastewaters such as oil and gas brine [6,7] may have more of an impact in summer, when both the stored wastewater and receiving stream water are seasonally warmer, than in winter, when both are cool. The negative relationship between temperature and salt toxicity we observed highlights the potential importance in considering water temperature when interpreting current environmental conditions or events, or setting regulatory standards for salinity or NaCl.

Data accessibility. Data are available as electronic supplementary material.

Authors' contributions. Overall project and experimental design: J.K.J. and D.H.F.; experimental set-up and data collection: D.H.F.; data analyses and interpretation: J.K.J. and D.H.F.; drafted manuscript: J.K.J.; edited manuscript: J.K.J. and D.H.F.

Competing interests. We declare we have no competing interests.

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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
AMENDMENTS TO THE GENERAL USE	)	R18-32
WATER QUALITY STANDARDS	)	Rulemaking – Water
FOR CHLORIDE	)	

PRE-FILED TESTIMONY OF DAVID J. SOUCEK, Ph.D.

Introduction

My name is David J. Soucek, and I am a Principal Ecotoxicologist at the Illinois Natural History Survey, a division of the Prairie Research Institute at the University of Illinois at Urbana-Champaign. I conducted acute, chronic, and "pulsed" sodium chloride toxicity testing with three invertebrate species (detailed below) at summer and lower temperatures for this proposed rule change. I have been involved with generating data for use in the development or updating of water quality standards in Illinois since 2003, when my laboratory started work to determine how different water quality characteristics (specifically, water hardness and chloride concentration) influence the acute toxicity of sodium sulfate to aquatic organisms. This work lead to development of the current Illinois water quality standard for sulfate. Since Illinois established this sulfate WQS, Iowa and Indiana have adopted it as well. I have since collaborated with the IL Environmental Protection Agency (EPA) and US EPA Region 5 on a number of other projects to generate data used to update WQS for boron, manganese and fluoride in Illinois, chloride in Iowa, and nitrate in Minnesota. A copy of my full CV is included as Attachment 1.

Selection of Test Species and Test Temperatures

Specific details about all aspects of these experiments can be found in a report included as Attachment 2.

Our objective was to compare the acute and chronic sensitivity of freshwater invertebrates to sodium chloride at warmer temperatures (23 to 25 °C) and at a colder temperature, in this case 10 °C. The test organisms we selected represented three major invertebrate groups: an insect (the mayfly *Neocloeon triangulifer*), a crustacean (the amphipod *Hyalella azteca*, Burlington strain), and a bivalve mollusk (the fingernail clam, *Sphaerium simile*). Two of these species (the mayfly and the fingernail clam) have previously been shown in studies from my laboratory to be sensitive to sodium chloride (Soucek et al. 2011; Soucek and Dickinson 2015; Soucek et al. 2018). The third species, the amphipod, is a commonly used organism for both water-only and sediment toxicity testing. The strain of *Hyalella azteca* used for this study was genetically identified as the "Burlington" strain by Major et al. (2013). We used this strain rather than the more commonly tested "US Lab" strain (Major et al. 2013) because the Burlington strain is found in the wild in the Great Lakes region, whereas the US Lab strain appears to be distributed across the southern United States (Hrycyshyn 2015). Furthermore, previous studies in my laboratory showed that the Burlington strain is more sensitive to acute sodium chloride exposure than the US Lab strain (Soucek et al. 2013).

We conducted warmer temperature tests for the mayfly and the clam at 25 °C and for the amphipod at 23 °C. In the case of the amphipod, we selected 23 °C because that is the recommended test temperature for chronic sediment toxicity testing with this species according to US EPA (2000). For the mayfly, all of our previous work with this species has been conducted at 25 °C, in part because development time for this organism is strongly tied to temperature (Sweeny and Vannote 1984) and lower temperatures result in longer chronic tests if using emergence as an endpoint. We have continuously cultured this species in the laboratory at 25 °C for approximately 6 years. For the fingernail clam, while previous testing with this species was conducted at 22 °C (Soucek et al. 2011), lacking specific recommendations from USEPA or ASTM, we decided to conduct the warmer temperature studies for the present work at 25 °C to reduce the number of different warm water temperatures used. The 25 °C temperature used is likely to be frequently encountered in the wild by this species, which we collected from a stream in Iroquois County, Illinois. The rationale for selection of 10 °C for the colder temperature testing is provided in the testimony of James Huff.

The three sub-objectives of this research were as follows:

1. Conduct 96-hour acute sodium chloride toxicity tests at 10 °C and 23 or 25 °C with the mayfly, the amphipod, and the fingernail clam.
2. Conduct 14- to 28-day chronic sodium chloride toxicity tests at 10 °C and 23 or 25 °C with the mayfly, the amphipod, and the fingernail clam.
3. Conduct 7-day continuous sodium chloride exposures at 10 °C with the mayfly, the amphipod, and the fingernail clam, followed by gradual returns to background/control sodium chloride concentrations over the course of 7 days. We will refer to this study as the "pulsed" exposure.

For two species in the chronic toxicity testing, we chose to conduct somewhat shortened tests. The standard chronic test duration for *Hyalella* is 42 days (USEPA 2000), and in our laboratory (Soucek and Dickinson 2015), we have conducted chronic toxicity testing with the mayfly until emergence (approximately 30 days). However, development time for both species would be substantially delayed at 10 °C; for example, Sweeny and Vannote (1984) showed that *Neocloeon (Cloeon) triangulifer* larval development to the adult stage took 179 days at 10 °C compared to 27 days at 25 °C. In the case of this mayfly and the amphipod, evaluating the reproductive endpoints these longer tests capture would not be practical at 10 °C. Furthermore, recent testing in our laboratory (DJS unpublished data) indicated that for the mayfly, a dry mass endpoint at 14-d was as sensitive to nickel and zinc toxicity as the most sensitive endpoints in full-life tests (incorporating emergence and reproduction). Therefore, we conducted tests of identical duration at both temperatures for all three species: 14-day tests with the mayfly, and 28-day tests for the amphipod and the fingernail clam.

<u>Results</u>

Detailed findings are provided in a report included as Attachment 2. For each species, the most sensitive endpoints for each test are summarized in Table 19 of the attached report (pasted below for convenience).

For all three species, there was a clear ameliorative effect of decreased temperature in the acute exposures, with fold-differences between the two temperatures ranging from 1.26 (amphipod) to more than 1.7 (clam). Comparing the acute values produced in the present study at warm temperatures to previously published data from my lab, the mayfly median lethal value (LC50) of 1359 mg Cl^-/L was nominally higher than the mean of 1062 mg Cl^-/L from Soucek and Dickinson (2015), but reasonably similar. For the amphipod, the value of 1733 mg Cl^-/L from the present study was very similar to the fed LC50 of 1741 mg Cl^-/L for the same strain in Soucek et al. (2013). For the fingernail clam, test conditions in the Soucek et al. (2011) study were different for temperature (22 vs 25 °C for the present study), water hardness (192 vs 100mg/L), and feeding (no food, versus a fed test). The latter point likely contributes most strongly to the disparity between the LC50 in the present test (1673 mg Cl^-/L) and that in the previous study (1100 mg Cl^-/L).

Table 19. Summary of acute and chronic values generated in the present study at standard temperature (25 or 23 °C) and at 10 °C. All values in mg Cl^-/L.

Species	96-hour LC50		chronic value*		chronic value
	25/23 °C	10 °C	25/23 °C	10 °C	10 °C, pulsed
N. triangulifer	1359	1960	326	>1458	1313
H. azteca	1733	2185	226	1257	2740
S. simile	1673	>2920	1672	1664	1963

* chronic values shown are lowest effect levels observed in a given test, either EC50 or EC20.

In the chronic tests, two of the three species (mayfly and amphipod) saw at least 4-fold increases in chronic values at 10 °C compared to the warmer test temperature. Chronic sodium chloride toxicity to the clam was not reduced at 10 °C relative to its response at 25 °C. Comparing the chronic values at 10 °C to the values obtained in the 7-day sustained followed by 7 days dilution (referred to as "pulsed" exposures in Table 19), two of the three species (amphipod and clam) demonstrated substantial increases in effect levels in the pulsed exposures. These increases ranged from approximately 1.2 to 2.2 fold. In the case of the mayfly, an increased effect level was not observed in the pulsed test, relative to the chronic test. This may have been because the chronic test for the mayfly was relatively shorter than that for the other two species. Although the pulsed effect level for the mayfly was nominally lower than the value in the full chronic test, the upper 95% confidence limit for the pulsed test was 1621 mg/L so it could be concluded that the two values were similar.

To summarize observations by species:

-The mayfly had consistent relief (acute and chronic) from low temperature, but the pulsed exposure did not lessen the chronic effect.

-The amphipod had consistent relief (acute and chronic) from low temperature, and the pulsed exposure very much reduced the chronic effect (it is important to remember the amphipod acute tests were conducted with juveniles and the chronic tests were conducted with adults; see attachment 2 for more details).

-The clams had relief from low temperature in the acute tests, but not in the chronic tests; and the pulsed exposure somewhat lessened the effect observed in the chronic test.

Cited references are listed below.

Thank you, this concludes my pre-filed testimony.

David J. Soucek, Ph.D.

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ATTACHMENT 1

Resume of David J. Soucek, Ph.D.

David J. Soucek, Ph.D.

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Work: (217) 265-5489
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Education:
- Ph.D. in Biology (2001), Virginia Tech, Blacksburg, VA
- M.S. in Zoology (1997), Clemson University, Clemson, SC
- B.A. in Zoology (1993), Miami University, Oxford, OH

Employment:
- Interim Director, Illinois Natural History Survey, Champaign, IL (Jan. 1 – June 30, 2018)
    - Directly responsible for administration of INHS, a research institute of the University of Illinois, Champaign-Urbana, with ~200 full time employees and an annual budget of ~$4M
- Principal Ecotoxicologist, Illinois Natural History Survey, Champaign, IL (Jan. 2015 – present)
- Senior Ecotoxicologist, Illinois Natural History Survey, Champaign, IL (2007 – Jan. 2015)
- Lead Ecotoxicologist, Illinois Natural History Survey, Champaign, IL (2001 – 2007)

Responsibilities of current position (since 2001):
- Develop, plan, and direct original, independent research program of significance on a broad range of questions in aquatic ecotoxicology including:
    - exposure to, and fate and effects of contaminants in aquatic systems in laboratory and field studies
    - development and improvement of toxicity testing methods for aquatic organisms
    - research in support of updating water quality criteria (WQC), and interpreting laboratory or field collected data relative to existing WQC
    - factors that influence the toxicity of water and sediment contaminants
- Supervise and manage a research team including graduate students and/or full-time or hourly research staff
- Publish the results of original aquatic ecotoxicology research in high-quality peer-reviewed scientific journals
- Seek external funding to support independent research in aquatic ecotoxicology
- Provide service to major scientific societies, journals, and/or to the scientific community in general
- Present research results at scientific meetings and at meetings with sponsoring agencies

Adjunct Appointments:
- Adjunct faculty member, Department of Forest Resources and Environmental Conservation, Virginia Tech, Blacksburg, VA (2014 – Present)
- Adjunct faculty member, Department of Entomology, University of Illinois at Urbana-Champaign, IL (2007 – Present)
- Adjunct faculty member, Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, IL (2004 – Present)

Professional Service/Scientific Advisory Activities:

- Member of Society of Environmental Toxicology and Chemistry (1998 – present)
- Member of Board, Society of Environmental Toxicology and Chemistry's Freshwater Salinization Advisory Group (2016 – present)
- Member of steering/organizing committee for virtual workshop addressing the State of Science for Mayfly Ecotoxicity Testing. September 25, 2018
- Member of steering/organizing committee for US Environmental Protection Agency sponsored "Workshop on Evaluating the Effects of Major Ions on Aquatic Organisms". Chicago, IL (2012)
- Member of workgroup tasked with updating the US Environmental Protection Agency's (2000) "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates" (2010 – present)
- Member of Scientific Advisory Panel to US Environmental Protection Agency on Ecological Impacts Associated with Mountaintop Mining and Valley Fills (2010 – present)
- Member of steering/organizing committee for US Environmental Protection Agency sponsored "Workshop on Advancing Methods for Culture and Toxicity Testing with the Amphipod *Hyalella azteca"*. Chicago, IL (2010)
- Member of Board of Directors, Ozark-Prairie Regional Chapter of the Society of Environmental Toxicology and Chemistry (2008 – present)
- President of Ozark-Prairie Regional Chapter of the Society of Environmental Toxicology and Chemistry; organized 2007 Annual Regional Chapter Meeting (2007)
- Vice President of Ozark-Prairie Regional Chapter of the Society of Environmental Toxicology and Chemistry (2006)

References: Available upon request.

INHS/PRI Committee Service:

- INHS Center for Ecological Entomology (CEE) W.H. Luckmann Award Committee, '01-'02, '02-'03 (Chair), '08-present
- CEE Social Committee, '01-'06
- CEE Space Committee, '03-'04, '06-'07
- INHS ad hoc Field Station Committee '02-'03
- INHS Research Committee '03-'04
- INHS Seminar Committee '04-'10 (Chair '07 – '10)
- CEE Search Committee for Weed Biological Control Scientist '04
- CEE Search Committee for Center Director '05
- INHS Search Committee for Stream Ecologist '07.
- INHS Publications Committee '10 – '12
- INHS Search Committee for Forbes Biological Station Director (Chair) '17
- Prairie Research Institute Search Committee for Safety Coordinator '18
- INHS Professional Advancement Committee '11 – present
- INHS Budget Committee '12 – '18
- INHS Safety Committee (Chair) '12 – present
- INHS Facilities Committee (Chair) '14 – present
- Prairie Research Institute (PRI) Safety Committee '12 – present
- INHS Director's Cabinet '14– present (Facilities and Safety focus)

Grant proposal reviewer for:
- Illinois/Indiana Sea Grant
- Maryland Sea Grant
- U.S. Army Corps of Engineers Engineer Research and Development Center
- Great Lakes Fishery Commission
- Oklahoma Water Research Resources Institute
- University of Southern California Sea Grant

Manuscript Reviewer for:
- Annales de Limnologie
- Archives of Environmental Contamination and Toxicology
- Biological Invasions
- BMC Physiology
- Bulletin of Environmental Contamination and Toxicology
- Chemosphere
- Ecotoxicology
- Ecotoxicology and Environmental Safety
- Environmental Monitoring and Assessment
- Environmental Pollution
- Environmental Toxicology
- Environmental Toxicology and Chemistry
- Freshwater Mollusk Biology and Conservation
- Hydrobiologia
- Journal of the American Water Resources Association
- Journal of Heredity
- Journal of Inorganic Biochemistry
- Journal of the North American Benthological Society (Freshwater Science)
- Philosophical Transactions of the Royal Society B
- Polar Biology
- Science of the Total Environment
- Walkerana

Program Affiliates:
- Amy Dickinson-Research Assistant, July 2006 – present
- Caroline Caton – undergraduate researcher
- Rachel Pence – M.S. student at Virginia Tech (advisory committee member)
- Thomas Cianciolo – M.S. student at Virginia Tech (advisory committee member)
- Keridwen Whitmore – M.S. student at Virginia Tech (advisory committee member)
- Dallas Glazik – undergraduate researcher
- Maggie Hung – undergraduate researcher
- Anthony Timpano – Ph.D. student at Virginia Tech (advisory committee member, graduated 2017)
- Abigail McEwen – undergraduate researcher (thesis advisor, graduated with Distinction 2013)
- Damion Drover –Ph.D. student at Virginia Tech (advisory committee member)
- Beth Boehme – M.S. student at Virginia Tech (advisory committee member, graduated 2013)
- Kaley Major- M.S. student (major advisor, graduated 2012)
- Stephanie Kilburn – M.S. student (advisory committee member, graduated 2012)
- Sue Gallo- M.S. student (major advisor, graduated 2011)

- Anthony Timpano – M.S. student at Virginia Tech (advisory committee member, graduated 2011)
- Claire Thomas – M.S. Student (advisory committee member, graduated 2011)
- Cindi Jablonski- M.S. student (advisory committee member, graduated 2008)
- Sandra Yi- Ph.D. student (advisory committee member, graduated 2008)
- Laura Roberts - M.S. student (advisory committee member, graduated 2006)

Peer Reviewed Journal Articles:
Total Google Scholar citations = 1210; H-index = 19 (as of December 2018)

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21. Rogowski DL, Soucek DJ, Levengood JM, Johnson S, Chick JH, Dettmers JM, Pegg MA, Epifanio JM. 2009. Contaminant concentrations in Asian carps, invasive species in the Mississippi and Illinois Rivers. Environmental Monitoring and Assessment 157:211-222

20. Esarey JC, Levengood JM, Soucek DJ, Hudson R, Halbrook R. 2008. Contaminants in unionid mussels from the Mississippi and Illinois Rivers confluence area. IL Natural History Survey Bulletin 39(5):197-214

19. Cropek DM, Esarey JC, Conner CL, Goran JM, Smith T, Soucek DJ. 2008. Toxicological effects of fog oil obscurant on *Daphnia magna* and *Ceriodaphnia dubia* in field and laboratory exposures. Ecotoxicology 17:517-525

18. Soucek DJ. 2007. Sodium sulfate impacts feeding, specific dynamic action, and growth rate in the freshwater bivalve *Corbicula fluminea*. Aquatic Toxicology 83:315-322

17. Soucek DJ. 2007. Bioenergetic effects of sodium sulfate on the freshwater crustacean *Ceriodaphnia dubia*. Ecotoxicology 16:317-325

16. Soucek DJ. 2007. Comparison of hardness- and chloride-regulated acute effects of sodium sulfate on two freshwater crustaceans. Environmental Toxicology and Chemistry 26(4): 773-779

15. Taylor CA, Soucek DJ, Organ EL. 2006. A new crayfish of the genus *Cambarus* Erichson, 1846, from a potentially under-sampled habitat type in central Tennessee, USA (Decapoda: Cambaridae). Zootaxa 1200:29-41

14. Soucek DJ. 2006. Effects of freshly neutralized aluminum on oxygen consumption by freshwater invertebrates. Archives of Environmental Contamination and Toxicology 50:353-360

13. Soucek DJ, Kennedy AJ. 2005. Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. Environmental Toxicology and Chemistry 24:1204-1210

12. Soucek DJ, Cherry DS, Zipper CE. 2003. Impacts of mine drainage and other nonpoint source pollutants on aquatic biota in the upper Powell River system, Virginia. Human and Ecological Risk Assessment 9:1059-1073

11. Hull MS, Cherry DS, Soucek DJ, Currie RJ, Neves RJ. 2002. Comparison of Asian clam field bioassays and benthic community surveys in quantifying effects of a coal-fired power plant effluent on Clinch River biota. Journal of Aquatic Ecosystem Stress and Recovery 9:271-283

10. Schmidt TS, Soucek DJ, Cherry DS. 2002. Integrative assessment of benthic macroinvertebrate community impairment from metal-contaminated waters in tributaries of the North Fork Powell River, Virginia. Environmental Toxicology and Chemistry 21:2233-2241

9. Cherry DS, Van Hassel JH, Farris JL, Soucek DJ, Neves RJ. 2002. Site-specific derivation of the acute copper criteria for the Clinch River, Virginia. Human and Ecological Risk Assessment 8:591-601

8. Soucek DJ, Denson BC, Schmidt TS, Cherry DS, Zipper CE. 2002. Impaired *Acroneuria* sp. (Plecoptera, Perlidae) populations associated with aluminum contamination in neutral pH surface waters. Archives of Environmental Contamination and Toxicology 42:416-422

7. Schmidt TS, Soucek DJ, Cherry DS. 2002. Modification of an ecotoxicological rating to bioassess small acid mine drainage impacted watersheds exclusive of benthic macroinvertebrate analysis. Environmental Toxicology and Chemistry 21:1091-1097

6. Soucek DJ, Cherry DS, Zipper CE. 2001. Aluminum dominated acute toxicity to the cladoceran *Ceriodaphnia dubia* in neutral waters downstream of an acid mine drainage discharge. Canadian Journal of Fisheries and Aquatic Sciences 58: 2396-2404

5. Soucek DJ, Schmidt TS, Cherry DS. 2001. In situ studies with Asian clams (*Corbicula fluminea*) detect acid mine drainage and nutrient inputs in low order streams. Canadian Journal of Fisheries and Aquatic Sciences 58:602-608

4. Cherry DS, Currie RJ, Soucek DJ, Latimer HA, Trent GC. 2001. An integrative assessment of a watershed impacted by abandoned mined land discharges. Environmental Pollution 111:377-388

3. Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 2000. Laboratory to field validation in an integrative assessment of an acid mine drainage impacted watershed. Environmental Toxicology and Chemistry 19:1036-1043

2. Soucek DJ, Cherry DS, Trent GC. 2000. Relative acute toxicity of acid-mine drainage water column and sediments to *Daphnia magna* in the Puckett's Creek Watershed, VA. Archives of Environmental Contamination and Toxicology 38:305-310

1. Soucek DJ, Noblet GP. 1998. Copper toxicity to the endoparasitic trematode *Posthodiplostomum minimum* relative to physid snail and bluegill intermediate hosts. Environmental Toxicology and Chemistry 17:2512-2516

Book Chapters:

Cherry DS, Soucek DJ. 2007. Case Study: Comparison of Asian clam (*Corbicula fluminea*) in situ testing to several nontarget test organism responses to biocidal dosing at a nuclear power plant. In: Van Hassel JH, Farris JL, (Eds), Freshwater Bivalve Ecotoxicology, SETAC Press, Pensacola FL. pp 285-305

Westphal LM, Levengood JM, Wali A, Soucek DJ, Stotz DF. 2005. Brownfield redevelopment: a hidden opportunity for conservation biology. In: Bengston D. (Ed) 2005. Policies for Managing Urban Growth and Landscape Change: a Key to Conservation in the 21st Century. General Technical report NC-265. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 52 pp

Conference Proceedings and Non-Refereed Publications:

Levengood JM, Soucek DJ, Dickinson A, Sass GG, Epifanio JM. 2012. Environmental contaminants in Asian Carp from the Illinois River. Illinois Natural History Survey Reports

Timpano AJ, Schoenholtz S, Soucek DJ, Zipper CE. 2011. Levels of dissolved solids associated with aquatic life effects in Virginia's central Appalachian coalfield streams. p. 85-86. In: Proceedings, 2011 West Virginia Surface Mine Drainage Task Force Symposium

Zipper CE, Schoenholtz S, Soucek DJ, Timpano AJ, Boehme EA. 2011. Total dissolved solids in Appalachian coalfield streams: Current research approaches. Virginia Mining Journal 24(3) 21-26

Timpano AJ, Zipper CE, Schoenholtz S, Soucek DJ. 2011. Comparing total dissolved solids, conductivity, and major ions as potential aquatic life stressors in Appalachian coalfield streams. p. 22-25. In: Reclamation Matters (publication of the American Society of Mining and Reclamation)

Timpano AJ, Schoenholtz S, Soucek DJ, Zipper CE. 2010. Determining water quality criteria for total dissolved solids in streams of southwestern Virginia. p. 71-79. In: 2010 Powell River Project Research and Education Program Reports

Timpano AJ, Schoenholtz S, Soucek DJ, Zipper CE. 2010. Isolating effects of total dissolved solids on aquatic life in central Appalachian coalfield streams. p. 80-93. In: 2010 Powell River Project Research and Education Program Reports

Timpano AJ, Schoenholtz S, Zipper CE, Soucek DJ. 2010. Isolating effects of total dissolved solids on aquatic life in central Appalachian coalfield streams. p. 1284 -1302. In: Proceedings, National Meeting of the American Society of Mining and Reclamation

Gallo SE, Soucek DJ, Levengood JM. 2007. Contaminants in tree swallows. Illinois Natural History Survey Reports

Taylor CA, Soucek DJ. 2005. Possible displacement mechanisms in non-native crayfishes. Illinois Natural History Survey Reports 384: 2

Esarey JC, Soucek DJ, Cropek DM, Smith T. 2004. Toxicological effects of military smokes and obscurants on aquatic threatened and endangered species. Proceedings of the 24th Army Science Conference

Soucek DJ, Esarey JC. 2004. From air to water contamination: steps to reduce high sulfur coal pollution. Illinois Natural History Survey Reports

Cropek DM, Smith TS, Williams R, Soucek DJ. 2003. Military smokes and obscurants formulations. White Paper submitted to Dept of Defense's Strategic Environmental Research and Development Program. 18 pp

Soucek DJ, Cherry DS, Zipper CE. 2001. Impacts of mine drainage and other nonpoint source pollutants on aquatic biota in the upper Powell River watershed, Virginia. Powell River Project Research and Education Program Report

Schmidt TS, Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 2000. Integrative bioassessment techniques to predict ecotoxicological impairment of acid mine drainage. In: Daniels WL, Richardson SG (Eds) Proceedings of the American Society for Surface Mining and Reclamation

Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 2000. Ecotoxicological impacts of acid mine drainage in streams of increasing order in the Powell River watershed, Virginia. In: Daniels WL, Richardson SG (Eds) Proceedings of the American Society for Surface Mining and Reclamation

Soucek DJ, Cherry DS, Zipper CE. 2000. Influence of acid mine drainage from abandoned mines on aquatic biota in the Powell River system. Powell River Project Research and Education Program Report

Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 1998. Benthic macroinvertebrate assemblages and sediment toxicity testing in the Ely Creek watershed restoration project. Proceedings of the American Society for Surface Mining and Reclamation

<u>Grants and Contracts Received</u>:

Soucek DJ. 2018. Extension with supplemental funds and additional tasks. Validation of biotic ligand model for nickel and zinc by toxicity testing with natural waters. 8/1/17 – 7/31/18. US Geological Survey. $20K additional funds added to $96,871original amount.

Czesny SJ (PI), Soucek DJ (Co-investigator). 2017. Survey of plankton community and water toxicity levels in the Waukegan Harbor and North Point Marina. IL DNR Coastal Management Program 3/1/17 – 6/30/18. $102,191.

Soucek DJ. 2017. Extension with supplemental funds and additional tasks. Acute and chronic toxicity of Ni and Zn to the mayfly *Neocloeon triangulifer*. 8/1/17 – 7/31/18. US Geological Survey. $34,997 additional funds added to $93,916 original amount.

Soucek DJ. 2016. Validation of biotic ligand model for nickel and zinc by toxicity testing with natural waters. 12/1/2016 – 12/07/19. US. Geological Survey. $96,871.

Soucek DJ. 2016. Influence of temperature on acute and chronic toxicity of sodium chloride to selected freshwater invertebrates. 10/4/16 – 12/31/19. IL Toll Highway Authority, $42,136.

Soucek DJ. 2016. Technical review from INHS. 9/29/16 – 7/30/18. IL Environmental Protection Agency, Bureau of Land. $30,000.

Machesky M, Slowikowski J, Levengood JM, Soucek DJ, Taylor CA (all Co-PIs). 2016. Pond 6 site assessment study. 4/1/16 – 4/1/17. IL Department of Natural Resources, $98,326.

Soucek DJ (PI). 2015. Aquatic toxicity testing with chemicals and effluents of concern in Illinois. 9/1/15 – 8/31/17. IL Environmental Protection Agency. $11,480

Soucek DJ (PI). 2015. Acute and chronic toxicity of Ni and Zn to the mayfly *Neocloeon triangulifer*. 8/1/15 – 7/31/16. US Geological Survey. $93,916

Timpano AJ, Schoenholtz SH, Zipper CE, Soucek DJ (all Co-PIs). 2015. Stream Ecosystem Response to Mining-Induced Salinization in Appalachia. 7/1/14/15 – 6/30/17. US Office of Surface Mining. $200,000 (submitted through Virginia Tech, no funds to INHS)

Soucek DJ (PI), Levengood JM. 2015. Contaminant concentrations and toxicity of Indian Creek (Chicago, IL) sediments to aquatic organisms. 4/1/15 – 3/31/16. IL Department of Natural Resources, Coastal Management Program. $10,086

Levengood JM (PI), Soucek DJ. 2014. Risks to biota from selected elements in Newton Lake. 7/1/14 – 6/30/14. Prairie Rivers Network. $7,790

Levengood JM (PI), Soucek DJ. 2013. Vermillion Power Station Ash Pond: Risks to the aquatic resources of the Middle Fork of the Vermillion River. 9/1/13 – 8/31/14. Prairie Rivers Network. $20,380

Soucek DJ (PI). 2013. Influence of ionic composition on toxicity of major ions to *Centroptilum triangulifer*. 9/20/13 – 9/19/14. US Geological Survey. $92,911

Soucek DJ (PI). 2012. Generation of acute and chronic nitrate toxicity data for the cladoceran *Ceriodaphnia dubia* and for the amphipod *Hyalella azteca*. 8/1/12 – 7/31/13. US Geological Survey. $87,154

Schoenholtz SH (PI), Benfield EF, Daniels WL, McGuire K, Soucek DJ, Timpano AJ, Webster J, Zipper CE. 2012. Headwater stream water quality responses to mountaintop removal mining. 7/1/12 – 6/30/13. Institute for Critical Technology and Applied Science, Virginia Tech. $32,000 (No funds to INHS)

Epifanio JM (PI), Soucek DJ. 2012. Ecosystem responses to a large-scale reduction of Asian carp in the lower Illinois River (Years 2 & 3). 4/1/12 – 12/31/13. IL Department of Natural Resources. $280,764

Soucek DJ (PI). 2012. An evaluation of methods used to culture and conduct laboratory toxicity tests with the parthenogenetic mayfly, *Centroptilum triangulifer*. 1/15/12 – 1/14/13. US Geological Survey. $64,183

Soucek DJ (PI). 2011. Toxicity of boron, fluoride, and manganese to freshwater organisms, and whole effluent toxicity testing of permitted Illinois discharges. 10/1/11 – 9/30/13. IL Environmental Protection Agency. $29,965

Soucek DJ (PI). 2010. Improving methods for laboratory culture and laboratory toxicity testing of the amphipod *Hyalella azteca*. 8/15/10 – 8/14/11. US Geological Survey. $29,375

Levengood JM (PI), Soucek DJ, Sass G, Epifanio JM. 2010. Potential contaminants in Asian carps of the Illinois River. IL Department of Natural Resources. $150,791

Soucek DJ (PI). 2010. Effects of land use on freshwater mussel communities in Illinois-continuation. 10/01/10 – 9/30/11. USDA-CSREES Hatch funds. $4,000

Soucek DJ (PI). 2009. Toxicity of nitrate, nitrite, manganese, boron, fluoride, and chloride to freshwater invertebrates. Great Lakes Environmental Center. $14,532

Soucek DJ (PI). 2009. Toxicity of boron, fluoride, and manganese to freshwater organisms, and whole effluent toxicity testing of permitted Illinois discharges. 3/1/09 – 9/30/11. IL Environmental Protection Agency. $75,000

Soucek DJ (PI). 2009. Effects of land use on freshwater mussel communities in Illinois-continuation. 10/01/09 – 9/30/10. USDA-CSREES Hatch funds. $4,000

Soucek DJ (PI). 2008. Effect of starvation and acclimation on the results of toxicity test using *Hyalella azteca*. US Environmental Protection Agency. $2,900

Soucek DJ (PI). 2008. Effects of land use on freshwater mussel communities in Illinois-continuation. 10/01/08 – 9/30/09. USDA-CSREES Hatch funds. $4,000

Soucek DJ (PI) 2008. Determining water quality criteria for total dissolved solids in streams of Southwestern Virginia: benthic macroinvertebrate community sampling and physical habitat assessment. 6/6/08 – 9/30/10. Subcontract from Virginia Tech. $16,000

Soucek DJ (PI). 2008. Effects of hardness and sulfate on chloride toxicity to freshwater invertebrates. Great Lakes Environmental Center. $7,500

Soucek DJ (PI). 2007. Effects of land use on freshwater mussel communities in Illinois-continuation. 10/01/07 – 9/30/08. USDA-CSREES Hatch funds. $4,000

Soucek DJ (PI). 2007. Assessment of potential for dietary uptake of millimeter wave carbon fibers by freshwater organisms: Continuation. 5/1/07 – 4/30/08. US Army Corps of Engineers, Engineer Research & Development Center, Construction Engineering Research Laboratory, Champaign, IL. $29,923

Soucek DJ (PI). 2006. Effects of land use on freshwater mussel communities in Illinois-continuation. 10/01/06 – 9/30/07. USDA-CSREES Hatch funds. $4,500

Soucek DJ (PI). 2006. Assessment of potential for dietary uptake of millimeter wave carbon fibers by freshwater organisms. 5/1/06 – 4/30/07. US Army Corps of Engineers, Engineer Research & Development Center, Construction Engineering Research Laboratory, Champaign, IL. $29,954

Soucek DJ (PI). 2005. Effects of land use on freshwater mussel communities in Illinois. 10/01/05 – 9/30/06. USDA-CSREES Hatch funds. $5,000

Soucek DJ (PI). 2004. Effects of water quality on acute and chronic toxicity of sulfate to freshwater bivalves, *Ceriodaphnia dubia,* and *Hyalella azteca*. 9/1/04 – 8/31/05. US Environmental Protection Agency CFDA 66.463—Water Quality Cooperative Agreements. $30,000

Soucek DJ (Co-PI), Epifanio J (Co-PI). 2004. A preliminary ecotoxicological assessment of Asian carp species in the Chicago Waterway and Illinois River. 7/1/04 – 6/30/05. IL Department of Natural Resources, Environmental Protection Trust Fund. $10,000

Soucek DJ (PI). 2003. Acute toxicity of sulfate to freshwater invertebrates, with studies on the influence of multiple cations and acclimation: continuation. 10/1/03 – 1/31/04. Illinois Coal Association. $3,700

Levengood JM (PI), Soucek DJ, Hudson R, Halbrook R. 2003. Contaminants of concern in Illinois large river mussel populations. 9/1/03 – 8/31/04. The National Great Rivers Research and Education Center. $29,368

Soucek DJ (PI), Levengood JM, Hill WH, Talbott JL, Bordson GO. 2003. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. 9/1/03 – 6/30/06. Illinois Waste Management and Research Center. $221,189

Soucek DJ (PI). 2003. Acute toxicity of sulfate to freshwater invertebrates, with studies on the influence of multiple cations and acclimation. 7/1/03 – 10/1/03. IL Environmental Protection Agency. $4,900

Soucek DJ (PI), Shaw JT. 2002. Toxicological effects of smokes and obscurants on aquatic threatened and endangered species. 3/12/03 – 2/21/07. US Army Corps of Engineers, Engineer Research & Development Center, Construction Engineering Research Laboratory, Champaign, IL. $182,038

Soucek DJ (PI). 2002. Mechanisms of aluminum toxicity to aquatic invertebrates in circum-neutral surface waters. 5/9/02 – 5/31/03. UIUC Campus Research Board. $9,800

Soucek DJ (PI), Cummings KS. 2002. Impacts of coalmine discharges on Illinois unionid mussels. 4/1/02 – 6/30/03. IL Department of Natural Resources, Wildlife Preservation Fund. $44,112

Cherry DS (PI), Zipper CE, Soucek DJ. 2000. Influence of acid mine drainage from abandoned mines on unionid mussels in the Powell River receiving system: Request for continued support. Powell River Project. $20,000

Cherry DS (PI), Soucek DJ, Schmidt TS, Currie RJ. 2000. Continuation of the integrative bioassessment of the Straight, Reeds, Jones, and Cox sub-basins impacted by abandoned mined land and acid mine discharges in the Powell River watershed. Virginia Department of Mines Minerals and Energy, Division of Mined Land Reclamation, and David Miller and Associates, Inc, Vienna, VA. $59,478

Cherry DS (PI), Zipper CE, Currie RJ, Soucek DS, Latimer HA. 1999. Streamlined feasibility study for ecosystem restoration: interim feasibility report for Ely and Puckett’s Creeks in the Powell River watershed, VA. US Army Corps of Engineers and Virginia Department of Mines Minerals and Energy. $8,500

Cherry DS (PI), Zipper CE, Soucek DJ. 1999. Influence of acid mine drainage from abandoned mines on unionid mussels in the Powell River receiving system. Powell River Project. $23,000

Invited Seminars/Presentations:

Soucek DJ, Dickinson A, Mount DR, Erickson RJ, Hockett JR. 2016. Exploring drivers of sodium salt toxicity to the mayfly *Neocloeon triangulifer*. Invited poster presented at Aquatic Toxicology Symposium, June 7-9, 2016, Bar Harbor, ME.

Soucek DJ. 2016. Developing tests methods, supporting regulatory needs, and investigating toxic mechanisms with the mayfly *Neocloeon triangulifer*. US Geological Survey, Columbia Environmental Research Center. April 13, 2016.

Soucek DJ, Dickinson A. 2016. Updates on major ion toxicity to *Neocloeon triangulifer* and *Hyalella azteca*. USEPA webinar on major ion toxicity. February 16, 2016.

Wang N, Ingersoll CG, Besser JM, Ivey CD, Kunz J, Brumbaugh W, Augspurger T, Hammer E, Bauer C, Raimondo S, Mebane C, Soucek DJ. Minimum data requirement for developing water quality criteria: use of toxicity data from under-represented organisms. Platform presentation at invited expert meeting on revising USEPA's guidelines for deriving aquatic life criteria. Arlington, VA. September 14-16, 2015

Soucek DJ. Aquatic ecotoxicology at INHS: collaborations with Columbia Environmental Research Center, US Geological Survey. USGS, Columbia Environmental Research Center, Columbia, MO. August 5, 2015

Soucek DJ. Putting freshwater bugs in salty water: Developing better laboratory models to understand salinization impacts on stream communities. Virginia Tech, Department of Forest Resources and Environmental Conservation seminar series. Blacksburg, VA. April 27, 2015

Soucek DJ. Influence of hardness and chloride on the acute toxicity of sodium sulfate: Development of the Illinois sulfate standard. Water Resources Advisory Committee; Molybdenum/Sulfates Ad Hoc Workgroup. Pennsylvania Department of Environmental Protection. Harrisburg, PA. August 27, 2012

Soucek DJ, Dickinson A, Wang N, Ingersoll CG. Influence of water quality on acute toxicity of sodium sulfate and sodium chloride to freshwater invertebrates. State Water Quality Standards Workshop, Federal Endangered Species Consultation. US Fish and Wildlife Service. Moline, IL. August 22, 2012

Soucek DJ. Putting freshwater animals in salty water: the ecotoxicology of major ions. Water Quality Seminar Series, Institute of Natural Resource Sustainability, and Department of Natural Resources and Environmental Sciences, University of Illinois. Champaign, IL. December 15, 2009

Soucek DJ, Gallo S, Levengood JM, Hill WR, Bordson GO. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Illinois Sustainable Technology Center's Sponsored Research Symposium, ISTC. Champaign, IL. September 9, 2009

Taylor CA, Soucek DJ. Resource use by *Orconectes* crayfishes and its potential impact on displacement mechanisms. Illinois Natural History Survey's Seminar Series. Champaign, IL. October 16, 2007

Taylor CA, Soucek DJ. Resource use by *Orconectes* crayfishes and its potential impact on displacement mechanisms. Illinois State University, Department of Biological Sciences. Bloomington, IL. October 4, 2007

Soucek DJ. Interactions between energy acquisition and anthropogenic stressors in freshwater mollusks and crustaceans. Auburn University, Department of Fisheries and Allied Aquacultures. Auburn, AL. April 2, 2007

Soucek DJ. Bioenergetic effects of coal related contaminants on freshwater invertebrates. University of Illinois, Department of Entomology. Urbana, IL. January 22, 2007

Soucek DJ, Gallo S, Levengood JM, Hill W, Talbott J, Bordson G. Tree swallows as monitors of contaminant risks to insectivorous birds nesting in Calumet wetlands. Calumet Research Summit, Hammond, IN. January 10, 2006

Soucek DJ. Effects of traditionally “non-toxic”, coal-related contaminants on freshwater invertebrates. University of Arkansas, Department of Entomology. Fayetteville, AR. October 21, 2005

Soucek DJ. Effects of traditionally “non-toxic”, coal-related contaminants on freshwater invertebrates. Oklahoma State University, Department of Zoology. Stillwater, OK. October 7, 2005

Soucek DJ, Ivey C, Ingersoll CG, Wang N. Relative sensitivity of juvenile fatmuckets (*Lampsilis siliquoidea*) and four other invertebrates to sulfate. Mussel Toxicity Testing Procedures Workshop. Chicago, IL. August 23-24, 2005

Soucek DJ, Esarey JC, Levengood JM, Gallo SE. Potential sources of variability in unionid mussel metal burdens in the great rivers of Illinois. Mussel Toxicity Testing Procedures Workshop. Chicago, IL. August 23-24, 2005

Soucek DJ. Pyrite oxidation as a source of multiple stressors: impacts beyond the zone of pH depression. Joint meeting of Ozark-Prairie and Mid-South Chapters of the Society of Environmental Toxicology and Chemistry. Carbondale, IL. May 16-18, 2005

Cropek DM, Soucek DJ, Esarey JC, Smith T, Rush T, Williams R, Conway B, Lembi C, Furnari D. Toxicological Effects of Military Smokes and Obscurants on Aquatic Threatened and Endangered Species. Strategic Environmental Defense Program and Environmental Security and Technology Certification Program combined Symposium. Washington, DC. November 29-December 2, 2004

Smith T, Soucek DJ, Cropek DM. Toxicological effects of military smokes and obscurants on selected species. USFWS training workshop, Monitoring and Adaptive Management for Endangered Species Conservation. Shepherdstown, WV. September 13-17, 2004

Soucek DJ. Emerging issues in coal industry-related ecotoxicology. University of Illinois, Interdisciplinary Environmental Toxicology Seminar Series. Champaign, IL. September 3, 2004

Esarey JC, Levengood JM, Soucek DJ, Hudson RJ, Wimer W, Halbrook RS. Contaminants of concern in Illinois large river mussel populations. US Geological Survey NAWQA Office. Urbana, IL. June 16, 2004

Esarey JC, Levengood JM, Soucek DJ, Hudson RJ, Wimer W, Halbrook RS. Contaminants of concern in Illinois large river mussel populations. Illinois State Museum Brownbag Lecture Series. March 31, 2004

Soucek DJ. The influence of water hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. US Environmental Protection Agency’s 3rd annual Surface Water Monitoring and Standards Meeting. Chicago, IL. February 4, 2004

Soucek DJ. Impacts of acid mine drainage-associated aluminum on aquatic invertebrates in circumneutral pH surface waters. Purdue University, Department of Entomology. West Lafayette, IN. October 24, 2002

Soucek DJ, Cherry DS, Zipper CE. Impacts of mine drainage-associated aluminum in circum-neutral pH surface waters of the North Fork Powell River. Regional Coalfield Water Resource Symposium, University of Virginia’s College at Wise. Wise, Virginia. September 4, 2002

Soucek DJ. Stressor identification in the Powell River, Virginia: Individual and community level impacts of nonpoint-source pollution. Clemson University Department of Biological Sciences. March 29, 2002

Soucek DJ. Impacts of acid mine drainage on the benthic macroinvertebrate communities of the Powell River watershed. Virginia Tech Chapter of the Wildlife Society. Blacksburg, VA. February 7, 2001

Contributed Presentations at Scientific Conferences:

Soucek DJ, Dickinson A, Norberg-King TJ. 2018. Method development for conducting effluent tests with the mayfly *Neocloeon triangulifer*. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Sacramento, CA. November 4-8, 2018.

Soucek DJ, Dickinson A, Schlekat CE, Van Genderen E, Besser JM, Ivey CD, Cleveland D. 2018. Influence of water chemistry on the acute and chronic toxicity of nickel and zinc to the mayfly *Neocloeon triangulifer* in natural waters. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Sacramento, CA. November 4-8, 2018.

Ivey CD, Besser JM, Cleveland D, Steevens JA, Schlekat CE, Van Genderen E, Soucek DJ. 2018. Toxicity of nickel and zinc to *Ceriodaphnia dubia* in natural waters with extreme levels of hardness and dissolved organic carbon. Poster presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Sacramento, CA. November 4-8, 2018.

Wirtz JR, McCoole M, Sibley PK, Lagadic LL, Soucek DJ, Norberg-King TJ, Roessink I. 2018. State of the science of mayfly ecotoxicity testing. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Sacramento, CA. November 4-8, 2018.

Sibley PK, Raby M, Wirtz JR, McCoole M, Lagadic LL, Soucek DJ, Norberg-King TJ, Roessink I. 2018. Evaluating the relative sensitivity of the "P&T" in EPT: implications for standardized toxicity testing. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Sacramento, CA. November 4-8, 2018.

Bubnyte R, Goodrich-Mahoney J, Adams WJ, Brix KV, Delos CG, Farley K, Paquin PR, Soucek DJ. 2018. Predicting the aquatic toxicity of mixtures of major ions: chronic exposures and additional test species. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Sacramento, CA. November 4-8, 2018.

Buchwalter DB, Chou H, Scheibener S, Westrud S, Hoon M, Elphick J, Soucek DJ. 2018. Sulfate toxicity in the mayfly *N. triangulifer*: pumping ions is energetically taxing. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Sacramento, CA. November 4-8, 2018.

Soucek DJ, Dickinson A, Mount DR, Erickson RJ, Hockett JR. 2017. Using binary mixtures of major ion salts to assess toxicity drivers with the mayfly *Neocloeon triangulifer*. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Minneapolis, MN. November 12-16, 2017.

Mount DR, Erickson RJ, Forsman BB, Highland TL, Hockett JR, Jenson CT, Norberg-King TJ, Soucek DJ. 2017 Similarities and differences in acute response to major ions among several aquatic species: Implications for guideline development. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Minneapolis, MN. November 12-16, 2017.

Bubnyte R, Goodrich-Mahoney J, Adams WJ, Brix KV, Delos CG, Farley K, Paquin PR, Soucek DJ. 2017 Predicting the aquatic toxicity of mixtures of major ions: Application to additional test species. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Minneapolis, MN. November 12-16, 2017.

Mount DR, Erickson RJ, Forsman B, Highland TL, Hockett JR, Hoff DJ, Jenson CT, Norberg-King TJ, Soucek DJ. 2016. Thoughts on applying existing toxicological understanding to risk assessment for major ions in fresh waters. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Orlando, FL. November 6-10, 2016.

Soucek DJ, Dickinson A, Mount DR, Erickson RJ, Hockett JR. 2016. Comparing responses and dose metrics for mayflies and daphnids exposed to major ion salts. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Orlando, FL. November 6-10, 2016.

Delos CG, Paquin PR, Bubnyte R, Brix KV, Soucek DJ, Farley K, Goodrich-Mahoney J, Adams W. 2016. Progress in predicting the aquatic toxicity of mixtures of the major ions using mechanistically based models. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Orlando, FL. November 6-10, 2016.

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Soucek DJ, Dickinson A, Mount DR, Erickson RJ, Hockett JR. 2015. Influence of dilution water composition on acute major ion toxicity to the mayfly *Neocloeon triangulifer*. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Salt Lake City, UT. November 1-6, 2015

Lazorchak J, Haring H, Wyatt K, Thoeny W, DeCelles S, Miller J, Dickinson A, Schmidt T, Soucek D, Walters D. 2015. Evaluation and improvements of a mayfly *Neocloeon* (*Centroptilum*) *triangulifer* (Ephemeroptera: Baetidae) toxicity test method. Platform presentation at Society of Environmental Toxicology and Chemistry North America Annual Meeting. Salt Lake City, UT. November 1-6, 2015

Drover D, Schoenholtz SH, Zipper CE, Soucek DJ. 2015. Benthic macroinvertebrate communities and habitat characteristics in mining-influenced streams with elevated conductivity. Platform presentation at Environmental Considerations in Energy Production Conference. Pittsburgh, PA. September 20-23, 2015

Levengood JM, Soucek DJ, Sass GG, Epifanio JM. 2015. Spatial and inter-specific patterns of contaminant burdens in Bighead and Silver carp from the Illinois River. Presented Asian Carp Symposium, 75th Midwest Fish and Wildlife Conference, Indianapolis, IN. February 8-11, 2015

Norberg-King TJ, Hockett JR, Mount DR, Soucek DJ, Ingersoll CG, Ivey CD, Kemble N, Brumbaugh WG, Taylor L. 2014. Inter-lab testing of *Hyalella azteca* water and sediment methods: 1. Background and overview of the 42-d survival, growth and reproduction test. Platform presentation made at the 35th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Vancouver, BC. November 9-13, 2014

Ivey CD, Ingersoll CG, Brumbaugh WG, Hammer E, Mount DR, Hockett JR, Norberg-King TJ, Soucek DJ, W, Taylor L. 2014. Inter-lab testing of *Hyalella azteca* water and sediment methods: 2. Results from 10- to 42-d tests conducted with sand and sediment substrates. Platform presentation made at the 35th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Vancouver, BC. November 9-13, 2014

Norberg-King TJ, Hockett JR, Mount DR, Ivey CD, Ingersoll CG, Kemble N, Brumbaugh WG, Hammer E Soucek DJ, Taylor L. 2014. Inter-lab testing of *Hyalella azteca* water and sediment methods: 3. Results from 10- to 42-d tests conducted with the new water-only method. Poster presentation made at the 35th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Vancouver, BC. November 9-13, 2014

Ivey CD, Ingersoll CG, Brumbaugh WG, Hammer E, Mount DR, Hockett JR, Norberg-King TJ, Soucek DJ, W, Taylor L. 2014. Inter-lab testing of *Hyalella azteca* water and sediment methods: 4. Results from 10- to 42-d tests conducted with sediment substrates. Poster presentation made at the 35th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Vancouver, BC. November 9-13, 2014

Drover D, Schoenholtz SH, Zipper CE, Timpano AJ, Soucek DJ. 2014. Detection of invertebrate community change in mine-influenced streams using quantitative sampling. Poster presentation at Joint Aquatic Sciences Meeting 2014. Portland, OR. May 18-23, 2014

Timpano AJ, Schoenholtz SH, Soucek DJ, Zipper CE. 2014. Effects of long-term salinization: a multi-year study of invertebrate community structure in coal mine-influenced streams. Joint Aquatic Sciences Meeting 2014. Portland, OR. May 19, 2014

Soucek DJ, Dickinson A. Assessment of whole-life toxicity of major ions to *Centroptilum triangulifer* using a laboratory cultured diet. Platform presentation at 34th annual meeting of the Society of Environmental Toxicology and Chemistry. Nashville, TN. November 18-21, 2013

Timpano A, Schoenholtz SH, Soucek DJ, Zipper CE. 2013. Continuous conductivity data for field-based exposure-response modeling. Platform presentation at 34th annual meeting of the Society of Environmental Toxicology and Chemistry. Nashville, TN. November 18-21, 2013

Ingersoll CG, Mount DR, Soucek DJ, Hockett JR, Norberg-King TJ, Kemble NE, Ivey CD, Kunz JL, Valenti T, Taylor L. 2013. USEPA and ASTM sediment and water toxicity methods for *Hyalella azteca* and *Chironomus dilutus*: Draft guidance for control test acceptability and historic performance. Platform presentation at 34th annual meeting of the Society of Environmental Toxicology and Chemistry. Nashville, TN. November 18-21, 2013

Boehme EA, Schoenholtz SH, Zipper CE, Timpano AJ, Soucek DJ. 2013. Temporal dynamics of benthic macroinvertebrate communities in Appalachian coalfield streams with elevated specific conductance. Poster presentation at Society for Freshwater Science 61st Annual Meeting. Jacksonville, FL. May 19-23, 2013

Timpano A, Soucek DJ, Schoenholtz SH, Zipper CE. 2013. Continuous conductivity monitoring for predicting macroinvertebrate community structure in coal mining influenced streams. Platform presentation at Society for Freshwater Science 61st Annual Meeting. Jacksonville, FL. May 19-23, 2013

Timpano A, Boehme B, Drover, D, Schoenholtz S, Zipper CE, Soucek DJ. 2013. Benthic macroinvertebrate response to total dissolved solids in coal mine streams. Platform presentation at Symposium on Environmental Considerations in Energy Production. Charleston, WV. April 14-18, 2013

Soucek DJ. 2012. Influence of water quality on toxicity of sodium salts to crustaceans, with notes on acclimation. Platform presentation at Workshop on Evaluating the Effects of Major Ions on Aquatic Organisms. Sponsored by USEPA. Chicago, IL. April 2-4, 2012

Timpano AJ, Schoenholtz SH, Soucek DJ, Zipper CE, Drover D. 2012. Field-based approaches for evaluating aquatic life effects of salinization in central Appalachian coalfield streams. Platform presentation at: Workshop on Evaluating the Effects of Major Ions on Aquatic Organisms. Sponsored by USEPA. Chicago, IL. April 2-4, 2012

Soucek DJ, Dickinson A. 2012. Toxicity of nitrate and nitrite to freshwater invertebrates. Platform presentation at Annual Meeting of the Ozark-Prairie Regional Chapter of the Society of Environmental Toxicology and Chemistry. Ankeny, IA. May 22, 2012

Major KM, Soucek DJ, Giordano R. 2012. Differences in life history characteristics among different clades of *Hyalella azteca* from laboratory and field populations. Platform presentation at Annual Meeting of the Ozark-Prairie Regional Chapter of the Society of Environmental Toxicology and Chemistry. Ankeny, IA. May 22, 2012

Major K, Soucek DJ, Giordano R. 2012. Differences in life history characteristics among different clades of *Hyalella azteca* from laboratory and field populations. Poster presentation at the 33rd Annual Meeting, Society for Environmental Toxicology and Chemistry. Long Beach, CA. November 11-15, 2012

Soucek DJ, Dickinson A, Major K. 2012. Benefits of biofilm in optimizing survival, growth, and reproduction of the amphipod *Hyalella azteca* in static-renewal, water only exposures Platform presentation at the 33rd Annual Meeting, Society for Environmental Toxicology and Chemistry. Long Beach, CA. November 11-15, 2012

Major K, Soucek DJ, Giordano R. 2011. Use of water-only chronic toxicity test methods to compare life history characteristics of four genetically distinct populations of *Hyalella azteca*. Poster presentation at Society of Environmental Toxicology and Chemistry North America 32nd Annual Meeting. Boston, MA. November 2011

Major K, Soucek DJ, Giordano R. 2011. Comparing genetic diversity and life history characteristics among laboratory and wild populations of *Hyalella azteca*. Presentation for the Interdisciplinary Toxicology Seminar Series and the Aquatic Ecology Seminar Series. Urbana, IL. March 2011

Timpano AJ, Schoenholtz SH, Soucek DJ, Zipper CE. 2011. Levels of dissolved solids associated with aquatic life effects in Virginia's central Appalachian coalfield streams. Presentation at Virginia Coal Interagency Task Force Meeting. Abingdon, VA. May 18, 2011

Timpano AJ, Schoenholtz SH, Soucek DJ, Zipper CE. 2011. Levels of dissolved solids associated with aquatic life effects in Virginia's central Appalachian coalfield streams. Presentation at: Virginia Mining Association Professional Engineers' Recertification Seminar. Abingdon, VA. March 9, 2011

Ingersoll CG, Hammer E, Mount DR, Norwood WP, Soucek DJ. 2010. Summary Discussion and Findings from an Expert Workshop on Use of *Hyalella azteca* in Water and Sediment Toxicity Testing. Platform presentation at 31st Annual Meeting, Society for Environmental Toxicology and Chemistry. Portland OR. November 7-11, 2010

Soucek DJ, Linton TK, Ingersoll CG, Wang N, Dickinson A, Tarr C. 2010. Acute toxicity of sodium sulfate and sodium chloride: comparison of ionic composition effects and species sensitivity. Platform presentation at 31st Annual Meeting, Society for Environmental Toxicology and Chemistry. Portland OR. November 7-11, 2010

Timpano AJ, Schoenholtz SH, Zipper CE, Soucek DJ. 2010. Determination of dissolved solids limits for aquatic life protection in central Appalachian coalfield streams. Platform presentation at, 31st Annual Meeting, Society for Environmental Toxicology and Chemistry. Portland OR. November 7-11, 2010

Soucek DJ, Dickinson A, Major K. 2009. Acute and chronic toxicity of boron to freshwater organisms. Platform presentation at the Annual Meeting of the Ozark-Prairie Chapter of the Society of Environmental Toxicology and Chemistry, Gray Summit, MO. July 14-15, 2009

Levengood JM, Soucek DJ, Taylor CA. 2009. Mercury in Illinois fishes: historical perspectives and current issues. Platform presentation at the Annual Meeting of the Ozark-Prairie Chapter of the Society of Environmental Toxicology and Chemistry, Gray Summit, MO. July 14-15, 2009

Soucek DJ, Dickinson A, Cropek DM. 2008. Effects of millimeter wave carbon fibers on feeding and reproductive rates of filter-feeding freshwater invertebrates. Platform presentation at the Annual Meeting of the Ozark-Prairie Chapter of the Society of Environmental Toxicology and Chemistry, Gray Summit, MO. July 14-15, 2008

Smith T, Cropek DM, Soucek DJ, Curtin D, Williams R. 2007. Military Smoke and Obscurant Use In Training - Support for Continued Use and in NEPA and ESA Analysis" Presented at 2007 US Army Sustainable Range Program Conference, Hampton, VA, 15 May 2007

Soucek DJ. 2007. Mechanism of reduced metabolic rates in filter feeding invertebrates exposed to sodium sulfate. Platform presentation at the Annual Meeting of the Ozark-Prairie Chapter of the Society of Environmental Toxicology and Chemistry, St Louis, MO. May 21, 2007

Gallo S, Soucek DJ, Levengood JM, Hill WR, Bordson GO. 2007. Biological transport and organic contaminant profiles in a food web in three Chicago, Illinois wetlands. Platform presentation at the Annual Meeting of the Ozark-Prairie Chapter of the Society of Environmental Toxicology and Chemistry, St Louis, MO. May 21, 2007

Taylor CA, Soucek DJ. 2006. Resource competition as a potential displacement mechanism in the genus *Orconectes*. The 16th Biennial Symposium of the International Association of Astacology, Gold Coast, Queensland, Australia, July 30 – August 4, 2006

Soucek DJ. 2006. Bioenergetic effects of sodium sulfate on *Ceriodaphnia dubia*. Platform presentation at the Annual Meeting of the Ozark-Prairie Chapter of the Society of Environmental Toxicology and Chemistry, Columbia, MO. May 22-24, 2006

Gallo SE, Soucek DJ, Levengood JM, Hill WR, Talbott J, Bordson GO. 2006. Using stable N and C isotope analysis to understand the origin of contaminants in tree swallow nestlings in Calumet, Illinois. Platform presentation at the Annual Meeting of the Ozark-Prairie Chapter of the Society of Environmental Toxicology and Chemistry, Columbia, MO. May 22-24, 2006

Gallo SE, Soucek DJ, Levengood JM, Hill WR, TalbottJ, Bordson GO. 2006. Sediment contaminant uptake in tree swallows in Calumet, IL: the contribution of stable 15N and 13C isotope analysis in determining origin. Platform presentation at the 98th annual meeting of the Illinois State Academy of Science, Chicago, IL, April 21, 2006

Mosher R, Soucek DJ. 2006. Derivation of a water quality standard for sulfate. 5th annual Surface Water Monitoring and Standards Meeting (SWiMS), Chicago, IL. February 2, 2006

Cropek DM, Soucek DJ, Esarey JC, Smith T, Conner C. 2005. Toxicity of the military obscurant, fog oil, to aquatic prey species , Poster presentation at the Strategic Environmental Defense Program and Environmental Security and Technology Certification Program combined Symposium, Washington, DC. December 2005

Soucek DJ. 2005. Chloride and hardness influence the acute and chronic toxicity of sodium sulfate to freshwater crustaceans. Platform presentation at the 26th annual meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 13-17, 2005

Gallo SE, Levengood JM, Soucek DJ, Hill WR, Talbot J, Bordson G. 2005. Tree swallows nesting in Calumet, IL as an indicator of contaminant transfer from aquatic to terrestrial systems. Platform presentation at the 26th annual meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 13-17, 2005

Esarey JC, Soucek DJ, Cropek DM, Smith T. 2005. Toxicity of the military obscurant, fog oil, to *Daphnia magna*. Poster presentation at the 26th annual meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 13-17, 2005

Ivey C, Ingersoll CG, Soucek DJ, Wang N. 2005. Influence of water hardness and chloride on the acute toxicity of sulfate to fatmuckets (*Lampsilis siliquoidea*) and fingernail clams (*Sphaerium simile*). Poster presentation at the 26th annual meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 13-17, 2005

Esarey JC, Soucek DJ, Cropek DM, Smith T. 2005. Toxicological effects of military fog oil obscurant to *Daphnia magna* in field exposures. Platform presentation at the Technical Symposium & Workshop on Threatened, Endangered, and At-Risk Species on DoD and Adjacent Lands, Baltimore, MD. June 7-9, 2005

Esarey JC, Levengood JM, Soucek DJ, Gallo SE. 2005. Potential Sources of variability in unionid mussel metal burdens in the great rivers confluence area. Platform presentation at the 9th Annual Meeting of the Illinois State Academy of Science, Galesburg, IL. April 7-9, 2005

Gallo SE, Levengood JM, Soucek DJ, Hill WR, Talbot J, Bordson G. 2005. Nest site selection and maternal contaminant transfer in tree swallows nesting in Calumet, IL. Platform presentation at the 9th Annual Meeting of the Illinois State Academy of Science, Galesburg, IL. April 7-9, 2005

Esarey JC, Levengood JM, Soucek DJ, Gallo SE. 2005. Potential Sources of variability in unionid mussel metal burdens in the great rivers confluence area. Platform presentation at the 13th Annual Meeting of the Midwest Regional Chapter of the Society of Environmental Toxicology and Chemistry, Madison, WI. April 5-6, 2005

Gallo SE, Levengood JM, Soucek DJ, Hill WR, Talbot J, Bordson G. 2005. Nest site selection and maternal contaminant transfer in Tree Swallows nesting in Calumet, IL. Platform presentation at the 13th Annual Meeting of the Midwest Regional Chapter of the Society of Environmental Toxicology and Chemistry, Madison, WI. April 5-6, 2005

Cropek DM, Soucek DJ, Esarey JC, Smith T, Rush T, Williams R, Conway B, Lembi C, Furnari D. 2004. Toxicological effects of military smokes and obscurants on aquatic threatened and endangered species. Poster presentation at the Strategic Environmental Defense Program and Environmental Security and Technology Certification Program combined Symposium, Washington, DC. 29 Nov - 2 Dec 2004

Cropek DM, Soucek DJ, Esarey JC, Smith T, Rush T, Williams R, Conway B, Lembi C, Furnari D. 2004. Toxicological effects of military smokes and obscurants on aquatic threatened and endangered species, Poster presentation at the 24th Army Science Conference, Orlando, FL. 29 Nov - 2 Dec 2004

Esarey JC, Levengood JM, Soucek DJ, Hudson RJ, Wimer W, Halbrook RS. 2004. Contaminants of concern in Illinois large river mussel populations. Poster presentation at the 25nd Annual Meeting and 4th World Congress of the Society of Environmental Toxicology and Chemistry, Portland, Oregon. November 14-18, 2004

Soucek DJ. 2004. The influence of water hardness and chloride on the acute toxicity of sulfate to freshwater invertebrates. Poster presentation at the 25nd Annual Meeting and 4th World Congress of the Society of Environmental Toxicology and Chemistry, Portland, Oregon. November 14-18, 2004

Smith T, Cropek DM, Soucek DJ, Brandt T, Kerns H, Nolde C. 2004. Toxicological effects of military smokes and obscurants on selected fish species. Platform presentation at the Annual Meeting of the American Fisheries Society, Madison, WI. August 22-26, 2004

Smith T, Cropek DM, Soucek DJ. 2004. Fog oil and other smoke and obscurant effects on threatened and endangered species. Platform presentation at the U.S. Army National Guard Sustainable Range Conference, Camp Blanding, FL. 29 Mar - 2 Apr 2004

Esarey JC, Levengood JM, Soucek DJ, Hudson RJ, Wimer W, Halbrook RS. 2004. Contaminants of concern in Illinois large river mussel populations. Platform presentation at the 42nd Annual Meeting of the Illinois Chapter of the American Fisheries Society, Champaign, IL. March 2-4, 2004

Soucek DJ. 2004. The influence of water hardness and chloride on the acute toxicity of sulfate to freshwater invertebrates. Platform presentation at the joint meeting of the Ozark-Prairie and Midwest Regional Chapters of the Society of Environmental Toxicology and Chemistry, LaCrosse, WI. March 3-5, 2004

Cropek DM, Soucek DJ, Smith TS, Williams R, Lembi C, Furnari D. 2003. Toxicological effects of smokes and obscurants on aquatic threatened and endangered species. Poster presented at the Partners in Environmental Technology Technical Symposium & Workshop, Washington, DC. December 2-4, 2003

Cherry DS, Soucek DJ (presented by DJS). 2003. Detoxified molluscicide impacts on target and non-target organisms in situ and in laboratory exposures. Platform presentation at the 24th Annual National Meeting of the Society of Environmental Toxicology and Chemistry, Austin, Texas. November 9-13, 2003

Soucek DJ. 2003. Impacts of acid mine drainage-associated aluminum on invertebrate oxygen consumption in circumneutral pH waters. Poster presented at the 24th Annual National Meeting of the Society of Environmental Toxicology and Chemistry, Austin, Texas. November 9-13, 2003

Currie RJ, Cherry DS, Soucek DJ, Schmidt TS, Trent GC. 2002. An integrative assessment of AMD influences in the Powell River: A sub-watershed based approach. Interactive poster presented at the 23rd Annual National Meeting of the Society of Environmental Toxicology and Chemistry, Salt Lake City, Utah. November 16-20, 2002

Soucek DJ, Cherry DS. 2002. Transplanted Asian clam growth reflects indigenous benthic macroinvertebrate community responses to dilute organic pollution. Platform presentation at the 50th Annual National Meeting of the North American Benthological Society, Pittsburgh, PA. May 28-June 1, 2002

Soucek DJ. 2002. Use of in situ experiments to eliminate confounding variables in benthic macroinvertebrate community impairment studies. Poster presented at the 2002 Environmental Horizons Program, University of Illinois Environmental Council, Urbana, IL. April 1, 2002

Hull MS, Cherry DS, Soucek DJ, Currie RJ. 2001. Recovery assessment of a power plant influenced section of the Clinch River. Poster presented at the 22nd Annual National Meeting of the Society of Environmental Toxicology and Chemistry. Baltimore, MD. November 11-15, 2001

Schmidt TS, Soucek DJ, Cherry DS. 2001. Delineating coal mine drainage impacted watersheds: importance of reference site selection. Platform presentation made at the 22nd Annual National Meeting of the Society of Environmental Toxicology and Chemistry. Baltimore, MD. November 11-15, 2001

Soucek DJ, Schmidt TS, Cherry DS, Zipper CE. 2001. Use of in situ experiments to eliminate confounding variables in community impairment studies. Poster presented at the 22nd Annual National Meeting of the Society of Environmental Toxicology and Chemistry. Baltimore, MD. November 11-15, 2001

Schmidt TS, Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 2000. A modified ecotoxicological rating to delineate acid mine drainage impacted sub-watersheds. Platform presentation at the 21st Annual National Meeting of the Society of Environmental Toxicology and Chemistry. Nashville, TN. November 12-16, 2000

Soucek DJ, Schmidt TS, Cherry DS. 2000. Transplanted Asian clams (*Corbicula fluminea*) as dual-purpose bioassessment tools in low order streams. Poster presented at the 21st Annual National Meeting of the Society of Environmental Toxicology and Chemistry. Nashville, TN. November 12-16, 2000

Schmidt TS, Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 2000. Integrative bioassessment techniques to predict ecotoxicological impairment of acid mine drainage. Poster presented at the 17th National Meeting of the American Society for Surface Mining and Reclamation, Tampa, FL. June 11-15, 2000

Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 2000. Ecotoxicological impacts of acid mine drainage in streams of increasing order in the Powell River watershed, Virginia. Platform presentation made at the 17th National Meeting of the American Society for Surface Mining and Reclamation, Tampa, FL. June 11-15, 2000

Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 1999. Laboratory to field validation in an integrative assessment of an acid mine drainage impacted watershed. Platform presentation at the 1999 National Meeting of the Society of Environmental Toxicology and Chemistry, Philadelphia, PA. November 15-18, 1999

Soucek DJ, Cherry DS, Trent GC. 1998. Relative toxicity of acid-mine drainage water column and sediments to Daphnia magna. Poster presented at the National Meeting of the Society of Environmental Toxicology and Chemistry, Charlotte, NC, Nov. 14-18, 1998

Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 1998. Benthic macroinvertebrate assemblages and sediment toxicity testing in the Ely Creek watershed restoration project. Poster presented at the 1998 National Meeting of the American Society of Surface Mining and Reclamation, St. Louis, MO, May 16-21, 1998

Soucek DJ, Noblet GP. 1997. Effects of copper contamination on recruitment of *Posthodiplostomum minimum* (Trematoda) by bluegill sunfish (*Lepomis macrochirus*). Platform presentation at a joint meeting of the Association of Southeastern Biologists and the Southeastern Society of Parasitologists, Furman University, Greenville, SC, April 1997

Technical Reports:

Machesky ML, Levengood JM, Soucek DJ, Slowikowski JA, Taylor CA. 2017. Draft Pond 6 site Assessment Study Report. Submitted to IL Department of Natural Resources.

Soucek DJ, Levengood JM, Dickinson A. 2016. Contaminant concentrations and toxicity of Indian Creek (Chicago, IL) sediments to aquatic organisms. Final Report to IL DNR Coastal Management Program, June 30, 2016.

Soucek DJ, Levengood JM, Dickinson A. 2015. Interim Report. Contaminant concentrations and toxicity of Indian Creek (Chicago, IL) sediments to aquatic organisms. Submitted to IL DNR, Coastal Management Program. September 15, 2015

Soucek DJ, Dickinson A, 2014. Final Report. Influence of dilution water ionic composition on toxicity of major ions to the mayfly *Neocloeon triangulifer* (Baetidae). Submitted to Geological Survey (USGS-Columbia) and U.S. Environmental Protection Agency, Region 5. December 22, 2015

Soucek DJ, Dickinson A, 2014. Final Report. Influence of chloride concentration in dilution water on acute and chronic nitrate toxicity to the cladoceran *Ceriodaphnia dubia* and the amphipod *Hyalella azteca*. Submitted to Geological Survey (USGS-Columbia) and U.S. Environmental Protection Agency, Region 5. May 30, 2014

Soucek DJ, Dickinson A, 2013. Final Report. Progress on development of a long-term chronic toxicity test method for *Centroptilum triangulifer*. Submitted to Geological Survey (USGS-Columbia) and U.S. Environmental Protection Agency, Region 5. October 18, 2013

Soucek DJ, Dickinson A, Major KM. 2012. Final Report. Improving methods for laboratory culture and laboratory toxicity testing of the amphipod *Hyalella azteca*. Submitted to Geological Survey (USGS-Columbia) and U.S. Environmental Protection Agency, Region 5. January 13, 2012

Levengood, JM, Soucek DJ, Dickinson A, Sass G, Epifanio J. 2011. Studies of Potential Environmental Contaminants in Asian Carp from the Illinois River: Final Report. Submitted to Southern Illinois University and IL Department of Natural Resources. December 20, 2011

Timpano AJ, Schoenholtz SH, Zipper CE, Soucek DJ. 2011. Levels of Dissolved Solids Associated with Aquatic Life Effects in Headwater Streams of Virginia's Central Appalachian Coalfield Region. Final report prepared for Virginia Department of Environmental Quality; Virginia Department of Mines, Minerals, and Energy; and Powell River Project. April 2011

Soucek DJ. 2009. Analysis of current Illinois ambient water quality criteria for nickel and zinc. Report prepared for Illinois Association of Wastewater Agencies.

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2007. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Fifteenth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. July 19, 2007

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2006. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Fourteenth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. March 31, 2007

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2006. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Thirteenth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. December 31, 2006

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2006. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Twelfth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. September 30, 2006

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2006. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Eleventh Quarterly Progress Report submitted to Illinois Waste Management and Research Center. June 30, 2006

Soucek DJ. Effects of water quality on acute and chronic toxicity of sulfate to freshwater bivalves, *Ceriodaphnia dubia*, and *Hyalella azteca*. Final Report to the United States Environmental Protection Agency, Region 5, Chicago, IL. June 20, 2006

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2006. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Tenth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. April 4, 2006

Rogowski D, Soucek DJ, Chick J, Dettmers J, Pegg M, Johnson S, Epifanio J. 2005. A preliminary ecotoxicological assessment of Asian Carp species in the Mississippi and Illinois Rivers. Project Completion Report to Illinois-Indiana Sea Grant Program. Center for Aquatic Ecology and Conservation Technical Report

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2005. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Ninth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. December 30, 2005

Soucek DJ. Effects of water quality on acute and chronic toxicity of sulfate to freshwater bivalves, *Ceriodaphnia dubia*, and *Hyalella azteca*. Third Quarterly Report to the United States Environmental Protection Agency, Region 5, Chicago, IL. October 20, 2005

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2005. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Eighth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. September 30, 2005

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO 2005. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Seventh Quarterly Progress Report submitted to Illinois Waste Management and Research Center. June 30, 2005

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2005. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Sixth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. April 13, 2005

Soucek DJ. Effects of water quality on acute and chronic toxicity of sulfate to freshwater bivalves, *Ceriodaphnia dubia*, and *Hyalella azteca*. Second Quarterly Report to the United States Environmental Protection Agency, Region 5, Chicago, IL. April 10, 2005

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2004. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Fifth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. December 31, 2004

Soucek DJ. Effects of water quality on acute and chronic toxicity of sulfate to freshwater bivalves, *Ceriodaphnia dubia*, and *Hyalella azteca*. First Quarterly Report to the United States Environmental Protection Agency, Region 5, Chicago, IL. December 21, 2004

Levengood JM, Soucek DJ, Esarey JC, Hudson RJ, Wimer W, Halbrook RS. Contaminants in mussels from the Mississippi and Illinois Rivers confluence area: Final Report to The National Great Rivers Research and Education Center, Godfrey, IL. November 30, 2004

Soucek DJ, Levengood JM, Gallo SE, Hill WR, Talbott JL, Bordson GO. 2004. Risks to insectivorous birds in the Calumet Region from transfer of contaminants from sediments to emergent aquatic insects. Fourth Quarterly Progress Report submitted to Illinois Waste Management and Research Center. September 30, 2004

Soucek DJ. 2004. Impacts of coalmine discharges on Illinois unionid mussels. Final Report submitted to Illinois Department of Natural Resources, Springfield, IL. June 28, 2004

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ATTACHMENT 2

Report by David J. Soucek, Ph.D., and Amy Dickinson

December 13, 2018

Influence of temperature on acute and chronic toxicity of sodium chloride to selected freshwater invertebrates

REPORT

by

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Introduction

A recent assessment of surface and groundwater major ion concentrations indicated that road salt runoff is increasing the concentrations of sodium and chloride in the Chicago, IL, region (Kelly et al. 2012). For example, comparing the time-periods of 1981-1985 and 2001-2005, the median chloride concentration in the North Branch of the Chicago River increased ~100% from 97 to 197 mg/L. Likewise, median concentrations in the Chicago Sanitary and Ship Canal increased from 70.5 to 122 mg/L over the same time-period (Kelly et al. 2012). The United States Environmental Protection Agency (USEPA) has set acute and chronic water quality criteria for chloride at 860 and 230 mg/L, respectively, while the Illinois Environmental Protection Agency (IEPA) has a single-value standard of 500 mg/L. While most of the reported median chloride concentrations at various Chicago region monitoring stations still met both the IEPA and USEPA standards, distinct seasonal variability was observed in the Kelly et al. (2012) study, with peak winter-time concentrations frequently reaching 600 to 800 mg/L (see figure below from Kelly et al. 2012).

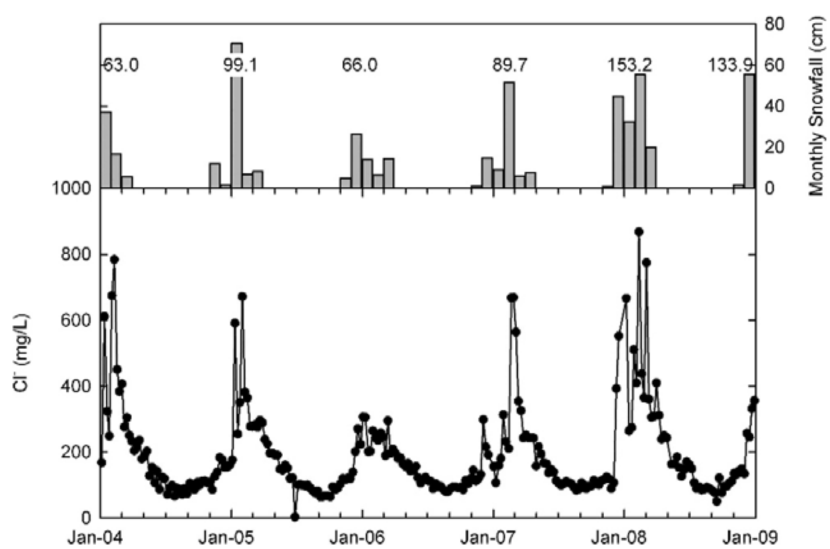

Figure 9. Chloride concentrations at a MWRDGC station on the CSSC at Lockport, IL, and monthly snowfall totals in Chicago (winter totals shown above bar). Snowfall data are from NOAA (2010).

Water quality criteria/standards are usually developed using toxicity tests conducted at temperatures indicative of spring or summer conditions (20 to 25 °C). If chloride toxicity to freshwater species is lower at winter temperatures, chloride standards may be overprotective during the time when concentrations peak. Therefore, our goal was to compare the acute and chronic sensitivity of freshwater invertebrates to sodium chloride at warm test temperature (23 to 25 °C) and at a colder temperature, in this case 10 °C. The test organisms we selected represented three major invertebrate groups: an insect, a crustacean, and a bivalve mollusk. The three sub-objectives of this research were as follows:

1. Conduct 96-hour acute sodium chloride toxicity tests at 10 °C and 23 or 25 °C with the mayfly *Neocloeon triangulifer*, the amphipod *Hyalella azteca*, and the fingernail clam *Sphaerium simile.*

2. Conduct 14- to 28-day chronic sodium chloride toxicity tests at 10 °C and 23 or 25 °C with the mayfly *Neocloeon triangulifer*, the amphipod *Hyalella azteca*, and the fingernail clam *Sphaerium simile*.

3. Conduct 7-day chronic sodium chloride toxicity tests at 10 °C with the mayfly *Neocloeon triangulifer*, the amphipod *Hyalella azteca*, and the fingernail clam *Sphaerium simile*, followed by a gradual return to background/control sodium chloride concentrations over the course of 7 days.

In two cases for the chronic toxicity testing, we chose to conduct somewhat shortened tests. The standard chronic test duration for *Hyalella* is 42-days (USEPA 2000), and in our laboratory (Soucek and Dickinson 2015), we have conducted chronic toxicity testing with *Neocloeon* until emergence (approximately 30 days). However, development time for both species would be delayed at 10 °C; for example, Sweeny and Vannote (1984) showed that *Neocloeon* (*Cloeon*) *triangulifer* larval development to the adult stage took 179 days at 10 °C compared to 27 days at 25 °C. In the case of this mayfly and the amphipod, evaluating the reproductive endpoints these longer tests capture would not be practical at 10 °C. Furthermore, recent testing in our laboratory (DJS unpublished data) indicated that for the mayfly, a dry mass endpoint at 14-d was as sensitive to nickel and zinc toxicity as the most sensitive endpoints in full-life tests (incorporating emergence and reproduction). Therefore, we conducted tests of identical duration at both temperatures for all three species: 14-day tests with *Neocloeon* and 28-day tests for *Hyalella* and *Sphaerium*.

Materials and Methods

Mayfly culturing methods

The parthenogenetic mayfly *Neocloeon triangulifer* (family Baetidae; McDunnough 1931) was originally described as *Cloeon triangulifer*, later transferred to the genus *Centroptilum* (McCafferty and Waltz 1990), and most recently assigned to *Neocloeon* (Jacobus and Wiersema 2014). The genetic strain we used was Stroud Water Research Center Clone #WCC-2. It is reared in the laboratory on a diatom diet; the mayfly and diatom biofilm diet culturing methods were similar to those reported in Soucek and Dickinson (2015) which were based on those reported by Weaver et al. (2015). Diatoms used to feed mayflies included *Mayamaea* sp., and *Nitzschia* sp. Both diatoms were obtained from Carolina Biological Supply, sold as *Navicula* sp. and *Synedra* sp., respectively. We had the genus-level identities taxonomically confirmed by an expert (S. Decelles) at the US Environmental Protection Agency (USEPA), Office of Research and Development (Cincinnati, OH).

Mixed diatom stocks

To culture each diatom, we autoclaved (30 min at 121 °C, liquid cycle) a 1-L flask containing 0.8 L of filtered (Whatman™ 934-AH) dechlorinated tap water and a 2-inch long Teflon-coated stir bar. After allowing to cool, sterile technique was used to add 0.455 mL of Proline F/2 algal food A, 0.455 mL of Proline F/2 algal food B, 53 mg of sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$), and 200 mL of fresh diatom stock solution (just removed from the stir plate). Diatom species were grown in separate stock cultures. The flasks were placed on stir plates with moderate to fast stirring (a large vortex was visible) in an environmental chamber set for a 16:8-hour light:dark photoperiod and 23 °C. Light intensity in the chamber varied between 800 and 1200 lux depending on position in the chamber. Diatom stocks were allowed to grow for 7 days, then 200mL of stock was used to seed the next flask and cages for mixed diatom slides (see "Mixed diatom slides" section). Stocks were not refrigerated prior to seeding subsequent flasks or mixed diatom slide cages.

Mixed diatom slides

To culture mixed diatom slides, which are used as the mayfly diet either as whole slides (in culture) or as scrapings (in toxicity tests), 15 fully frosted microscope slides (catalog no. 12-544-5CY, Fisher Scientific) were placed in a single layer (with frosted side facing up) on the bottom of a 7.2-L (189 x 297 x128 mm) autoclavable polysulfone mouse cage (no. PC7115HT, Allentown, Inc., Allentown, PA) filled with 2.5 L of filtered (Whatman™ 934-AH) dechlorinated (carbon-filtered and aged) tap water. The container with the slides was autoclaved (40 minutes at 121 °C, liquid cycle) and allowed to cool. Sterile technique was used to add 1.3 mL of Proline F/2 algal food A, 1.3 mL of Proline F/2 algal food B, 150 mg of sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$, dissolved in a small amount of deionized water prior to addition), and 200 mL of fresh (never refrigerated) mixed diatom stock. The container with slides was covered with clear plastic wrap and placed in an environmental chamber set for a 16:8-h light: dark photoperiod and 23 °C. Light intensity at the level of the slides varied between 300 and 100 lux, depending on position in the chamber. Growth was allowed for 6 to 10 days. Soucek and Dickinson (2015) provided further details on assessing biofilm quality prior to feeding to mayflies.

Mayfly nymph rearing method

Mayflies were reared in an environmental chamber at 25 °C under a 16:8-hour light:dark photoperiod, and light intensity of approximately 200 lux. Culture water was a reconstituted water (hereafter referred to as Duluth 100) with a nominal hardness of 100mg/L as $CaCO_3$, prepared according to a formula developed at the US EPA laboratory in Duluth, Minnesota (Table 1). This water recipe was designed with the goal of better mimicking the chemistry of "typical" North American freshwaters relative to other commonly used reconstituted waters. When eggs hatched, approximately 250 mL of culture water were added to a 300-mL clear glass jar. All water was filtered using Whatman 934-AH glass microfiber filters. One mixed diatom slide was added to the jar. Newly hatched mayfly larvae (hundreds to thousands) were then added to the jar, the lid was loosely replaced, and the jar was covered with aluminum foil to block direct overhead lighting. When mayflies were 4 to 8 days old (usually 6 or 7 days), 40 individuals were placed in a 1-L glass beaker containing 400 mL Duluth 100 reconstituted water and fed as described for the 300-mL glass jar. The diatom slide was placed in the beaker prior to adding mayflies to avoid injury. Again, the container was covered with aluminum foil to block direct overhead lighting. When mayflies were 11 to 12 days old, 20 individuals were transferred to a 19 x 24 x 6.5–cm Pyrex casserole dish containing 1.5 L of Duluth 100 water and 5 mixed diatom slides. Slides were replaced when diatom biofilms were depleted, and water was changed twice per week or more if water appeared to be littered with loose diatoms and waste products. The container was covered loosely with foil. Using this method, aeration was not necessary at any point during mayfly culturing. When pre-emergent nymph (PEN) stages (determined by the presence of black wing pads) appeared (typically days 20–23), they were placed in a 300-mL glass jar containing culture water and a mixed diatom slide. A screened cover was placed on the jar to allow for emergence of sub-imagoes and molting to imago stage (within 24 h after pre-emergent nymph stage). To induce the imago to release its eggs, we held it by the wings with forceps and touched its abdomen to water held in a depression slide. This procedure was conducted with the aid of a dissecting microscope. Eggs were then pipetted from the depression slide into a scintillation vial; when possible, eggs of two females were combined in each vial. Eggs were either allowed to hatch (approximately 6 days) or placed in an environmental chamber at 10 °C for later use.

Amphipod culturing methods:

A host of recent studies with wild and laboratory-reared populations indicated that *Hyalella azteca* is in reality a complex of many cryptic species (Duan et al., 1997; Hogg et al., 1998; Major et al. 2013; McPeek and Wellborn 1998; Witt et al., 2006), and Duan et al. (1997) cautioned that laboratories should identify the particular genetic strain they are using to generate toxicity data. The strain of *Hyalella azteca* used for this study was genetically identified as the "Burlington" strain by Major et al. (2013); a complete cytochrome oxidase subunit 1 (COI) sequence for this strain can be found at Genbank accession # JX446364. This strain is cultured in the INHS laboratory, with specimens originally obtained from Environment Canada's Centre for Inland Waters laboratory in Burlington, Ontario, Canada. We used this strain rather than the more commonly tested "US Lab" strain (Major et al. 2013) because the Burlington strain is found in the wild in the Great Lakes region whereas the US Lab strain appears to be distributed across the southern United States (Hrycyshyn 2015).

Organisms were cultured in Duluth 100 water at ambient room temperature (~23 ºC), with a 16:8 hour (L:D) photoperiod. Approximately 250 adults were held in 2.5 gallon glass fish tanks with 5-L of water provided with aeration. Two or three 4" x 4"- 12 ply cotton gauze pads (Dukal Corp., Ronkonkoma, NY.) were provided as substrate. At any given time, one or two culture tanks were maintained for each water type. Organisms were fed approximately 50 mg of dry, ground and sieved (<500 µm) Tetramin® (TetraWerke, Melle, Germany) and diatom suspension (prepared as described in Soucek et al. (2013)) at a rate of 0.5 mg solid/L three times per week. Water was changed weekly; young were counted and collected at that time. Upon collection, young were transferred to a 23 °C environmental chamber for acclimation to test temperature and experimental conditions. Adults were obtained from mass cultures by collecting organisms retained in a USA Standard Test Sieve #18 (1.00 mm equivalent). Adults were held in an environmental chamber in groups of 30 in 1-L of water and acclimated to test temperature and experimental conditions.

Fingernail clam collection/holding methods
Fingernail clams (*Sphaerium simile*) were field-collected from Spring Creek, near Loda, IL, in Iroquois County. Clams were collected as adults, returned to the laboratory (at INHS, Champaign, IL) in site water, and allowed to release juveniles from their brood chambers in the laboratory. Testing was conducted with juveniles that were gradually acclimated to laboratory conditions (water and temperature) for approximately two weeks. An exception to this was that for the 7-day sustained/7-day dilution test (detailed below), juveniles were collected in the field rather than being released from adults in the lab. Juveniles were held in aquaria containing Duluth 100 water with quartz sand as a substrate. Prior to testing, clams were fed an algal mixture diet (Wang et al. 2007) prepared by adding 1 ml of *Nannochloropsis* concentrate and 2 ml of Shellfish diet® (both purchased from Reed Mariculture, Campbell, CA) to 1.8 L of Duluth 100.

Acute toxicity testing procedures
Two acute tests were conducted for each species, one at warmer temperature (25 ºC for the mayfly and the clam; 23 ºC for the amphipod) and one at 10 ºC. Static, non-renewal, 96-hour acute toxicity tests were conducted generally according to guidelines detailed in ASTM E729-96 (2014) and ASTM E2455-06 (2014). All tests were conducted using Duluth 100 water (Table 1) as both the control and dilution water. Reagent grade sodium chloride (CAS # 7647-14-5) was used as the toxicant source. Treatments were comprised of a 50% dilution series, with the highest test concentration determined based on literature values and range finding tests if necessary. Previous acute sodium chloride tests in our laboratory with these three species produced median lethal concentrations (LC50s) of 1062, 1741, 1100 mg Cl^-/L for *N. triangulifer*, *H. azteca* (Burlington strain), and *S. simile*, respectively, although the *S. simile* LC50 was produced at a hardness of approximately 200 mg/L (Soucek and Dickinson 2015; Soucek et al., 2013; Soucek et al., 2011). Five concentrations were tested in addition to controls. Further details on test conditions are provided in Table 2.

For all three species, acute tests were fed. In the case of the mayfly tests, food was added to test chambers by grasping a diatom slide (cultured as described above) with a forceps, and scraping off a ~6-mm to 7.2-mm by 25-mm area of biofilm with another clean microscope slide, and releasing the biofilm into each replicate test chamber by submerging the end of the slide containing the scraping into the test water. Mayfly tests were fed because in a previous study (Soucek and Dickinson, 2015), early instar mayflies had only 22% survival after 48 hours with no food. This has been observed by others as well (Struewing et al. 2015; SWRC unpublished data). Mayfly test chambers were fed on day zero only, because one biofilm scraping was more than enough for the 96-hour test duration. For the amphipod, the acute tests were fed because Soucek et al. (2013) observed that for the Burlington clade of *Hyalella azteca*, acute sensitivity to contaminants was strongly influenced by the presence of food. In the case of the clams, the acute results were taken from the first four days of the chronic test so the test was fed. Because the tests in the present study were conducted with sodium chloride, food was not expected to impact bioavailability of the contaminant, and analytical chemistry confirmed this (detailed below).

Mayflies used for testing were 6 days old, rather than the usual <24 hours old because we did not expect sufficient hatch at 10 ºC to start a test; therefore, organisms were hatched at 25 ºC, and then acclimated to test temperature over 6 days. The 25 ºC test was conducted with 6-day-old organisms as well to allow for comparability between test temperatures. For all three species, mortality was assessed daily using a dissecting microscope. Individuals were considered dead if they did not respond to gentle prodding with a blunt instrument. In the case of the fingernail clams, we watched for foot or siphon movement and at times were able to observe the heart beating in live individuals.

Chronic toxicity testing procedures

As with acute tests, two chronic (or sub-chronic) tests were conducted for each species, one at warmer temperature (25 ºC for the mayfly and the clam; 23 ºC for the amphipod) and one at 10 ºC.

Neocloeon

For static-renewal chronic toxicity tests, six treatments, including a control (Duluth 100 water) were tested, with the highest sodium chloride concentration being approximately the LC50 determined in the acute test at the corresponding temperature. Test conditions are summarized in Table 3. Prior to the start of testing at both temperatures, a vial containing eggs from 2 to 3 females was moved from a 10 ºC environmental chamber (which we use for long-term egg storage) to the 25 ºC chamber to encourage hatching of the eggs. The test began when sufficient numbers hatched to conduct the test. As described above for acute testing, organisms were 6 days old at the start to allow for acclimation of hatched larvae to test temperature. Tests were conducted under a 16:8 hour (L:D) photoperiod at both temperatures. All test chambers were covered with plastic wrap to minimize evaporation, and with aluminum foil to eliminate direct overhead light. Light intensity in tests chambers was approximately 100 – 300 lux. The test chambers were 30 ml glass beakers with 20 ml of test water (or control) for the first 7 days, and then 150 ml beakers with 100 ml water for the second week. No substrate was used, but a 6 mm scraping from a diatom biofilm slide was added to each beaker for food as described above for acute testing. Chambers were fed on day 0, then on every water change day thereafter. Complete water changes were conducted with organisms being transferred to new beakers. Survival was evaluated in each treatment daily, and at the end of the test (day 14) all surviving organisms were transferred to aluminum weigh pans and dried in an oven (60 to 70 °C) for at least 48 hours before they were weighed to the nearest 0.001 mg using a Cahn C-35 microbalance.

Hyalella

Tests were conducted generally according to recommendations detailed in the USEPA sediment toxicity testing methods (2000), but with modifications to suit the particular experimental conditions of these tests (i.e., test temperatures, shorter test duration, and modifications to feeding regimes as detailed in Soucek et al. (2016)). Details on test conditions are provided in Table 4. As described above for the mayfly, six treatments, including a control (Duluth 100 water) were tested, with the highest sodium chloride concentration being approximately the

LC50 as determined in the acute test at the corresponding temperature. Test chambers were 300-ml, high-form glass beakers with 200 ml of test or control water added to each beaker. Approximately 3.5 X 4 cm pieces of nitex screen were provided to each beaker as substrate. Tests were performed with a photoperiod of 16:8 hours (L:D; approximately 100 - 300 lux light intensity) for both temperatures (23 and 10 ºC). Test solutions were not aerated. Organisms were adults at the beginning of the tests, with one individual added to each test chamber. Adults were used because repeated attempts to use 7- to 9-day-old organisms resulted in control failures at 10 ºC. It was surmised that adults, which are more robust than juveniles, might be the life-stage that over-winters in this species. Ten replicates were used per treatment in each test. Complete water renewals were performed for these static tests, with test organisms being transferred to clean weigh-boats containing test water while test chambers were cleaned, every Monday, Wednesday, and Friday for each test. Test duration was 28 days.

Survival was evaluated with every water renewal on Monday, Wednesday, and Friday. At the end of 28 days, surviving organisms were transferred to aluminum weigh pans and dried in an oven (60 to 70 °C) for at least 48 hours before they were weighed to the nearest 0.001 mg using a Cahn C-35 microbalance. Endpoints included survival and mean dry weight (per individual).

Sphaerium

As with the other two species, six treatments, including a control (Duluth 100 water) were tested, with the highest sodium chloride concentration being approximately the LC50 determined in the acute test at the corresponding temperature. Test conditions are summarized in Table 5. Five juvenile clams were held in each of five replicate 300 ml beakers (200 ml Duluth 100 water) per treatment. Each beaker contained 50 ml of quartz sand as substrate, and test solutions were renewed twice daily (1 full volume addition per renewal) using a Zumwalt et al. (1994) type water renewal system (Fig. 1). This system is designed to allow the addition of new water to test chambers without disturbing the substrate or organisms. This renewal system was used because of the potentially higher feeding requirements of this species, which under less frequent water renewal conditions may result in insufficient dissolved oxygen or elevated ammonia concentrations. As recommended for the freshwater mussel *Lampsilis siliquoidea* under static-renewal conditions (N. Wang, USGS, pers. comm.), juvenile clams were fed 2 to 4 ml of an algal mixture diet (described above) twice daily, depending on algal build-up rates. Tests were performed with a photoperiod of 16:8 hours (L:D; approximately 100 - 300 lux light intensity) for both temperatures. Test solutions were not aerated. Organisms were transferred to clean beakers with clean quartz sand every seven days, and survival was evaluated at that time. At the end of 28 days, surviving organisms were transferred to aluminum weigh pans and dried in an oven (60 to 70 °C) for at least 48 hours before they were weighed to the nearest 0.001 mg using a Cahn C-35 microbalance. Endpoints included survival and mean dry weight (per individual).

7-day exposure followed by dilution tests (“pulsed” tests)

For these tests, conducted at 10 °C, we exposed organisms to sustained concentrations of sodium chloride for the first 7 days of the test. Then, beginning on day 8, each treatment was serially diluted by 50% to mimic a natural dilution of road salt. For all three species, the same dilution series was used, and nominal concentrations for days 0 through 7, and then 8 through 14 are shown in Table 6. Aside from the test duration and the changing treatment concentrations, tests were otherwise conducted in a similar fashion to chronic tests for each species as detailed above.

In this set of tests, rather than directly measuring weights, mayflies were measured for length at the end of the test using Lumenera Corporation Infinity2-1RC microscope camera with Capture and Analyze® software, and then lengths were used to calculate dry weights according to the formula: log dry weight (mg) = (2.7155X log length(mm))-2.7368. This formula was developed from specimens in our laboratory and those from the USEPA laboratory in Cincinnati, OH.

Water quality and analytical chemistry for acute and chronic tests

For acute toxicity tests, standard water chemistry parameters were measured at both the beginning and the end of each test, including temperature, pH, conductivity, and dissolved oxygen. Alkalinity and hardness were measured at the beginning and end of the test only in controls and the highest test treatment. For the static-renewal chronic tests with Monday, Wednesday, and Friday water renewals (*Neocloeon* and *Hyalella*), temperature, pH, conductivity, and dissolved oxygen were measured in both new and old solutions on each water renewal. For the fingernail clam test, with more frequent water renewal, temperature was measured daily, and pH, conductivity, and dissolved oxygen measurements were made every Monday, Wednesday and Friday in old solutions. For chronic tests, alkalinity and hardness were measured (in control and highest test treatment) in new test solutions each water renewal and in composite samples of old test solutions weekly.

The pH measurements were made using an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet® gel-filled combination electrode (accuracy < ± 0.05 pH at 25 °C). Dissolved oxygen was measured using an air-calibrated Yellow Springs Instruments (RDP, Dayton, OH, USA) model 55 meter. Conductivity measurements were made using a Mettler Toledo® (Fisher Scientific, Pittsburgh, PA, USA) model MC226 conductivity/TDS meter. Alkalinity and hardness were measured by titration as described by American Public Health Association (2005). For each acute test, water samples from each treatment were collected at the beginning and end of tests and submitted to the IL State Water Survey analytical laboratory for verification of chloride concentrations by ion chromatography. In addition, because the ionic composition of a water can have a large effect of major ion toxicity (Mount et al. 1997), the four major cations (sodium, potassium, magnesium and calcium) were measured by inductively coupled plasma (ICP) spectrometry for each acute and chronic test in the dilution water only.

For chronic tests, sampling for chloride analysis varied slightly for each organism. For 14-day mayfly chronic tests samples from each treatment were collected from new water on day zero and day 7, and from composite samples of old water on days 7 and 14. For 28-day amphipod chronic tests samples from each treatment were collected from new water on days zero and 21, and from composite samples of old water on days 7, 14, 21 and 28. For 28-day clam chronic tests, samples from each treatment were collected from new water on day zero and 18, and from composite samples of old water on days 18 and 25.

For the 7-day pulsed exposures, temperature was measured daily, while pH, and dissolved oxygen were measured three times per week. Alkalinity and hardness were measured in all treatments at test day 0, day 7 and at the end of the tests. Conductivity was measured three times per week during the chronic portion of the tests and measured daily during the dilution portion. At the beginning of each test, the four major cations (sodium, potassium, magnesium and calcium) were sampled from dilution water only and test solutions were sampled for chloride analysis. Composite samples were collected by treatment on days 7 and 14 for chloride analysis.

Statistical analysis
For acute toxicity tests, LC50s were calculated using the trimmed Spearman-Karber method (Hamilton et al. 1977). For chronic toxicity tests, survival EC50s were calculated using the trimmed Spearman-Karber method, and for sub-lethal endpoints, the 20% effective concentrations (EC20s) were calculated using TRAP® software version 1.30a (R. Erickson, USEPA Duluth).

RESULTS

Acute toxicity tests
Water chemistry – All general water chemistry parameters met expected targets with mean (± standard deviation) temperature, pH, dissolved oxygen, alkalinity, and hardness values as follows: mayfly 25 °C – 25.2 ± 0.1 °C, 8.3 ± 0.1, 6.8 ± 0.5 mg/L, 81 ± 1 mg/L as $CaCO_3$, and 97 ± 3 mg/L as $CaCO_3$; mayfly 10 °C – 10.3 ± 0.1 °C, 8.2 ± 0.1, 10.8 ± 0.2 mg/L, 77 ± 1 mg/L as $CaCO_3$, and 94 ± 3 mg/L as $CaCO_3$; amphipod 23 °C – 23.2 ± 0.2 °C, 8.1 ± 0.1, 7.5 ± 0.2 mg/L, 84 ± 7 mg/L as $CaCO_3$, and 103 ± 8 mg/L as $CaCO_3$; amphipod 10 °C – 10.3 ± 0.3 °C, 8.1 ± 0.1, 10.2 ± 0.5 mg/L, 79 ± 1 mg/L as $CaCO_3$, and 98 ± 2 mg/L as $CaCO_3$; clam 25 °C – 24.8 ± 0.1 °C, 8.2 ± 0.1, 7.1 ± 0.2 mg/L, 79 ± 1 mg/L as $CaCO_3$, and 99 ± 2 mg/L as $CaCO_3$; clam 10 °C – 10.4 ± 0.1 °C, 8.2 ± 0.2, 9.9 ± 0.2 mg/L, 79 ± 1 mg/L as $CaCO_3$, and 98 ± 2 mg/L as $CaCO_3$. Conductivity values changed with chloride concentration as expected (data not shown).

Toxicity data – In the 96-hour acute NaCl toxicity tests with the mayfly, amphipod, and clam, measured chloride concentrations ranged from 92 to 106% of nominal concentrations (calculated as the geometric mean of all measured chloride concentrations for a treatment divided by the nominal concentration times 100 percent; Tables 7- 9). All reported LC50s are in terms of measured chloride concentrations. For all three species, 96-hour LC50s at 10 °C were at least a factor of 1.26 fold higher than those in the warmer temperature test (Tables 7-9). The amphipod had the lowest relative increase from warmer temperature to 10 °C, but even in that case the 95% confidence intervals around the LC50s did not overlap. We were unable to generate an actual LC50 value for the clam at 10 °C, because there was no mortality in the highest test concentration at 96 hours, and the acute test consisted of the first four days of the chronic test due to low organism availability. Therefore, we had to consider the 10 °C LC50 as >2920 mg Cl^-/L for this species.

Chronic toxicity tests
Water chemistry – All general water chemistry parameters met expected targets with mean (± standard deviation) temperature, pH, dissolved oxygen, alkalinity, and hardness values as follows: mayfly 25 °C – 25.2 ± 0.2 °C, 8.3 ± 0.1, 7.9 ± 0.4 mg/L, 79 ± 1 mg/L as $CaCO_3$, and 97 ± 1 mg/L as $CaCO_3$; mayfly 10 °C – 10.5 ± 0.3 °C, 8.2 ± 0.1, 8.9 ± 0.6 mg/L, 79 ± 1 mg/L as $CaCO_3$, and 97 ± 1 mg/L as $CaCO_3$; amphipod 23 °C – 23.2 ± 0.7 °C, 7.7 ± 0.3, 7.7 ± 0.3 mg/L, 78 ± 2 mg/L as $CaCO_3$, and 99 ± 1 mg/L as $CaCO_3$; amphipod 10 °C – 10.3 ± 0.3 °C, 7.8 ± 0.2, 10.1 ± 0.5 mg/L, 79 ± 1 mg/L as $CaCO_3$, and 97 ± 1 mg/L as $CaCO_3$; clam 25 °C – 25.1 ± 0.2

°C, 8.1 ± 0.1, 6.9 ± 0.5 mg/L, 79 ± 1 mg/L as $CaCO_3$, and 98 ± 1 mg/L as $CaCO_3$; clam 10 °C – 10.3 ± 0.3 °C, 8.0 ± 0.2, 9.8 ± 0.4 mg/L, 79 ± 1 mg/L as $CaCO_3$, and 98 ± 1 mg/L as $CaCO_3$. Conductivity values changed with chloride concentration as expected (data not shown).

Toxicity data – In the 14- to 28-day (sub-)chronic NaCl toxicity tests with the mayfly, amphipod, and clam, measured chloride concentrations ranged from 94 to 101% of nominal concentrations (Tables 10-15). All reported EC50s and EC20s are in terms of measured chloride concentrations.

In the mayfly test conducted at 25 °C, dose-related responses were observed for both survival and dry weight, with the weight endpoint being more sensitive than survival (Table 10). In contrast, weight was not a sensitive endpoint in the 10 °C test. In fact, weight increased nominally with increasing chloride concentration at this temperature. Survival was not strongly affected at the tested chloride concentrations either, with 55% survival observed in the highest test concentration, resulting in a survival EC50 of >1458 mg Cl^-/L (Table 11).

In the amphipod test conducted at 23 °C, survival was variable in the lower concentrations, but sufficient mortality occurred in the highest test concentration to generate a survival EC50 of 1640 mg Cl^-/L. Dry weight was much more sensitive to chloride with a strong dose-related response, and an EC20 of 226 mg Cl^-/L (Table 12). At 10 °C, a similar pattern was observed with variable survival in the lower chloride concentrations, but sufficient mortality in the highest test concentration to generate a survival EC50 of 1257 mg Cl^-/L. Again, with dry weight, a dose-related response was observed, but the change in weights were not sufficient to generate an EC20 value for this endpoint (Table 13).

The fingernail clam chronic results did not appear to be influenced by temperature (Tables 14, 15). In both cases, organism dry weight was not sufficiently sensitive to generate EC20 values, and for survival, the patterns were identical at both temperatures with the EC50 values differing only due to slight variation in measured chloride concentrations.

7-day exposure followed by dilution tests

Water chemistry – All general water chemistry parameters met expected targets with mean (± standard deviation) temperature, pH, dissolved oxygen, alkalinity, and hardness values as follows: mayfly – 10.5 ± 0.1 °C, 8.0 ± 0.1, 10.0 ± 0.5 mg/L, 80 ± 0 mg/L as $CaCO_3$, and 100 ± 1 mg/L as $CaCO_3$; amphipod – 10.4 ± 0.2 °C, 7.8 ± 0.1, 9.8 ± 0.6 mg/L, 80 ± 1 mg/L as $CaCO_3$, and 100 ± 1 mg/L as $CaCO_3$; clam – 10.2 ± 0.2 °C, 7.6 ± 0.3, 11.0 ± 0.9 mg/L, 80 ± 1 mg/L as $CaCO_3$, and 100 ± 1 mg/L as $CaCO_3$. Conductivity values changed with chloride concentration as expected (data not shown).

Toxicity data – In the tests with 7-day sustained exposure followed by 7 days of 50% daily dilution, measured chloride concentrations at the end of the first 7 days ranged from 89 to 99% of nominal concentrations (Tables 16-18), and measurements on day 14 after 7 days of 50% daily dilution were on target with expectations shown in Table 6. For all three species, the reported EC50s and EC20s are in terms of measured chloride concentrations over the first 7 days.

In the mayfly test, survival and weight both responded to increasing chloride concentration, but only survival decreased sufficiently to produce an effect value (EC50 = 1313 mg Cl^-/L; Table 16). For the amphipod, both survival and weight resulted in effect levels (3108 mg Cl^-/L for survival and 2740 mg Cl^-/L for weight; Table 17). Like the mayfly, there was only a survival effect for the fingernail clam, with an EC50 of 1963 m Cl^-/L (Table 18).

Summary of most sensitive endpoints

For each species, the most sensitive endpoints for each test are summarized in Table 19. For all three species, there was a clear ameliorative effect of decreased temperature in the acute exposures, with fold-differences between the two temperatures ranging from 1.26 (amphipod) to more than 1.7 (clam). In the chronic tests, two of the three species (mayfly and amphipod) saw at least 4-fold increases in chronic values at 10 °C compared to the warmer test temperature. Chronic sodium chloride toxicity to the clam was not reduced at 10 °C relative to its response at 25 °C. Comparing the chronic values at 10 °C to the values obtained in the 7-day sustained followed by 7 days dilution (referred to as “pulsed” exposures in Table 19), two of the three species (amphipod and clam) saw substantial increase in effect levels in the pulsed exposures. These increases ranged from ~1.2 to 2.2 fold. In the case of the mayfly, an increased effect level was not observed in the pulsed test, relative to the chronic test. This may have been because the chronic test for the mayfly was relatively shorter than that for the other two species. Although the pulsed effect level for the mayfly was nominally lower than the value in the full chronic test, the upper 95% confidence limit for the pulsed test was 1621 mg/L so it could be concluded that the two values were similar.

To summarize observations by species:

-The mayfly had consistent relief from low temperature, but the pulsed exposure did not lessen the chronic effect

-The amphipod had consistent relief from low temperature, and the pulsed exposure very much reduced the chronic effect (it is important to remember the amphipod acutes were conducted with juveniles and the chronics were conducted with adults)

-The clams had relief from low temperature in the acute tests, but not in the chronic tests; and the pulsed exposure somewhat lessened the effect observed in the chronic test

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Table 1. Salt concentrations (mg/L) added to deionized water for generation of culture water for mayflies and amphipods, and dilution/control water for all testing. an = anhydrous salt used.

Water name	$KHCO_3$	$NaHCO_3$	$MgSO_4$(an)	$CaSO_4$(an)	$CaCl_2$	NaBr
Duluth 100[1]	10	125	38	40	43	0.05

[1] Recipe received via personal communication (DR Mount and JR Hockett, US EPA)

Table 2. Test conditions for acute sodium chloride toxicity tests with *Neocloeon triangulifer* (NT), *Hyalella azteca* (HA), and *Sphaerium simile* (SS).

1. Standard temperature (˚C)	NT, SS 25 ± 1; HA 23 ± 1
2. Low temperature (˚C)	10 ± 1
2. Photoperiod (L:D)	16:8
3. Light intensity	100 to 300 lux
4. Test chamber size	NT 30 ml; HA 50 ml; SS 300 ml
5. Test solution volume	NT 20 ml; HA 40 ml; SS 200 ml
6. Age of organisms	NT 6 d; HA 7-9 d; SS ~2 weeks
7. Dilution water	Duluth 100
8. Substrate	NT none; HA nitex screen; SS 50 ml quartz sand
9. # organisms per chamber	5
10. # chambers/treatment	4
11. Food	NT 6 mm scraping of live diatom biofilm; HA 0.25 mg diatom suspension on day 0 and 2; SS 4 ml algal diet mixture daily
12. Aeration	none
13. Test type	static
14. Renewal frequency	none
15. Test duration	96 h
16. Control survival	≥ 90%
17. Endpoint	survival

Table 3. Test conditions for 14-d chronic, static-renewal, water only sodium chloride toxicity tests with the mayfly *Neocloeon triangulifer*.

1. Temperature (˚C)	10 ± 1 and 25 ± 1
2. Photoperiod (L:D)	16:8
3. Light intensity	100 to 300 lux
4. Test chamber size	30 ml d 0-7, 150 ml d 8-14
5. Test solution volume	20 ml d 0-7, 100 ml d 8-14
6. Age of organisms at start of test	6 d
7. Dilution water	Duluth 100
8. Substrate	none except food
9. # organisms per chamber	2
10. # chambers/treatment	10
11. Food	6 mm scraping of live diatom biofilm on water renewal days
12. Aeration	none
13. Test type	static, renewal
14. Renewal frequency	MWF
15. Test duration	14 days
16. Test acceptance criteria	≥ 80% control survival
17. Endpoints	survival, dry weight

Table 4. Test conditions for 28-d chronic, static-renewal, water only sodium chloride toxicity tests with the amphipod *Hyalella azteca* (Burlington strain).

1. Temperature (˚C)	10 ± 1 and 23 ± 1
2. Photoperiod (L:D)	16:8
3. Light intensity	100 to 300 lux
4. Test chamber size	300 ml
5. Test solution volume	200 ml
6. Age of organisms at start of test	Adults
7. Dilution water	Duluth 100
8. Substrate	nitex screen
9. # organisms per chamber	1
10. # chambers/treatment	10
11. Food	23 ˚C: 2.5 mg Tetramin (<500 µm): MWF; 2.0 mg diatom suspension MWG 10 ˚C: 2.0 mg Tetramin (<500 µm): MWF; 2.0 mg diatom suspension MWG
12. Aeration	none
13. Test type	static, renewal
14. Renewal frequency	MWF
15. Test duration	28 days
16. Test acceptance criteria	≥ 80% control survival
17. Endpoints	survival, dry weight

Table 5. Test conditions for 28-d chronic, static-renewal, water only sodium chloride toxicity tests with the fingernail clam *Sphaerium simile*.

1. Temperature (˚C)	10 ± 1 and 25 ± 1
2. Photoperiod (L:D)	16:8
3. Light intensity	100 to 300 lux
4. Test chamber size	300 ml
5. Test solution volume	200 ml
6. Age of organisms at start of test	<1 month
7. Dilution water	Duluth 100
8. Substrate	50 ml quartz sand
9. # organisms per chamber	5
10. # chambers/treatment	5
11. Food	4 ml algal mixture diet daily
12. Aeration	none
13. Test type	static, renewal
14. Renewal frequency	1 full volume addition twice daily; organisms transferred to clean beakers and sand weekly
15. Test duration	28 days
16. Test acceptance criteria	≥ 80% control survival
17. Endpoints	survival, dry weight

Table 6. Estimated daily nominal chloride concentrations for each treatment in 14-d pulsed exposures with 7-d at sustained concentrations, and 7-d at 50% daily dilution.

Day	Trt F [Cl^-]	Trt E [Cl^-]	Trt D [Cl^-]	Trt C [Cl^-]	Trt B [Cl^-]	Trt A [Cl^-]
0-7	5000	2500	1250	625	313	28
8	2514	1264	639	327	171	28
9	1271	646	334	177	99	28
10	650	337	181	103	64	28
11	339	183	104	65	46	28
12	183	105	66	47	37	28
13	106	67	47	37	32	28
14	67	47	38	33	30	28

Table 7. 96-h acute sodium chloride toxicity test results for the mayfly *Neocloeon triangulifer*.

Test	nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L)	% measured/ nominal	%survival
Test at 25 °C	28	28	100	100
	530	543	102	100
	745	783	105	100
	1053	1063	101	90
	1492	1503	101	30
	2120	2140	101	0
Measured 96-h LC50 (95% confidence interval) = 1359 (1249-1478) mg Cl^-/L				
Test at 10 °C	28	26	92	95
	276	268	97	100
	524	533	102	95
	1021	1029	101	95
	2014	2003	99	55
	4000	4071	102	0
Measured 96-h LC50 (95% confidence interval) = 1960 (1640-2343) mg Cl^-/L				

Table 8. 96-h acute sodium chloride toxicity test results for the amphipod *Hyalella azteca*.

Test	nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L)	% measured/ nominal	%survival
Test at 23 °C	28	29	104	95
	741	785	106	95
	1047	1098	105	100
	1484	1550	104	75
	2108	2173	103	10
	3000	3084	103	0
Measured 96-h LC50 (95% confidence interval) = 1733 (1592-1887) mg Cl^-/L				
Test at 10 °C	28	27	96	100
	741	745	101	100
	1047	1055	101	100
	1484	1492	101	95
	2108	2100	100	65
	3000	3008	100	0
Measured 96-h LC50 (95% confidence interval) = 2185 (2013-2372) mg Cl^-/L				

Table 9. 96-h acute sodium chloride toxicity test results for the fingernail clam *Sphaerium simile*.

Test	nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L)	% measured/ nominal	%survival
Test at 25 °C	28	27	98	100
	100	98	98	100
	300	292	97	100
	600	573	95	100
	1000	989	99	100
	3000	2831	94	0
Measured 96-h LC50 (95% confidence interval) = 1673 (unreliable) mg Cl^-/L				
Test at 10 °C	28	27	96	100
	100	99	99	100
	300	289	96	100
	600	568	95	100
	1000	978	98	100
	3000	2920	97	100
Measured 96-h LC50 (95% confidence interval) = >2920 mg Cl^-/L				

Table 10. Results of 14-d sub-chronic sodium chloride toxicity test for the mayfly *Neocloeon triangulifer* conducted at 25 °C.

Nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L)	% survival	dry weight (mg)	% nominal/ measured [Cl^-]
28	27	95	0.069 ± 0.037	98
100	100	100	0.090 ± 0.034	100
200	203	100	0.095 ± 0.039	101
400	394	95	0.049 ± 0.022	98
750	727	95	0.049 ± 0.017	97
1500	1478	0	na	99
Effect levels (95% confidence intervals) based on measured dissolved Cl (mg/L)				
Survival EC50		971 (888 – 1062)		
Weight EC50		529 (393 – 712)		
Weight EC20		326 (202 – 527)		

Table 11. Results of 14-d sub-chronic sodium chloride toxicity test for the mayfly *Neocloeon triangulifer* conducted at 10 °C.

Nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L)	% survival	dry weight (mg)	% nominal/ measured [Cl^-]
28	27	100	0.004 ± 0.0	96
100	98	89	0.006 ± 0.0	98
200	198	83	0.008 ± 0.0	99
400	388	95	0.010 ± 0.0	97
750	716	90	0.010 ± 0.0	95
1500	1458	55	0.046 ± 0.0	97
Effect levels (95% confidence intervals) based on measured dissolved Cl (mg/L)				
Survival EC50		>1458		
Weight EC50		no trend, not calculated		
Weight EC20		no trend, not calculated		

Table 12. Results of 28-d chronic sodium chloride toxicity test for the amphipod *Hyalella azteca* conducted at 23 °C.

Nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L)	% survival	dry weight (mg)	% nominal/ measured [Cl^-]
28	26	80	1.287 ± 0.0	96
200	191	70	1.100 ± 0.0	95
400	385	100	0.907 ± 0.0	96
750	725	90	0.697 ± 0.0	97
1500	1428	70	0.784 ± 0.0	95
2000	1883	30	0.574 ± 0.0	94
Effect levels (95% confidence intervals) based on measured dissolved Cl (mg/L)				
Survival EC50		1640 (1423 – 1889)		
Weight EC50		1548 (750 – 3195)		
Weight EC20		226 (74 – 698)		

Table 13. Results of 28-d chronic sodium chloride toxicity test for the amphipod *Hyalella azteca* conducted at 10 °C.

Nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L)	% survival	dry weight (mg)	% nominal/ measured [Cl^-]
28	26	90	0.911 ± 0.0	94
200	193	70	0.871 ± 0.0	97
400	387	100	0.896 ± 0.0	97
750	704	67	0.803 ± 0.0	94
1500	1451	70	0.727 ± 0.0	97
2000	1926	10	nm*	96
Effect levels (95% confidence intervals) based on measured dissolved Cl (mg/L)				
Survival EC50		1257 (864 – 1828)		
Weight EC50		insufficient effect, not calculated		
Weight EC20		insufficient effect, not calculated		

*nm=not measured, insufficient mass to obtain accurate weight

Table 14. Results of 28-d chronic sodium chloride toxicity test for the fingernail clam *Sphaerium simile* conducted at 25 °C.

Nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L)	% survival	dry weight (mg)	% nominal/ measured [Cl^-]
28	26	100	10.048 ± 0.830	95
100	98	100	9.928 ± 0.514	98
300	290	100	9.480 ± 0.639	97
600	581	100	8.700 ± 0.956	97
1000	988	100	8.692 ± 0.733	95
3000	2831	0	na	94

Effect levels (95% confidence intervals) based on measured dissolved Cl (mg/L)

Survival EC50	1672 (unreliable)
Weight EC50	insufficient effect, not calculated
Weight EC20	insufficient effect, not calculated

Table 15. Results of 28-d chronic sodium chloride toxicity test for the fingernail clam *Sphaerium simile* conducted at 10 °C.

Nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L)	% survival	dry weight (mg)	% nominal/ measured [Cl^-]
28	26	100	9.576 ± 0.669	95
100	97	100	9.160 ± 0.924	97
300	290	100	8.476 ± 0.936	97
600	572	100	9.656 ± 0.559	95
1000	970	100	9.488 ± 0.619	97
3000	2855	0	na	95

Effect levels (95% confidence intervals) based on measured dissolved Cl (mg/L)

Survival EC50	1664 (unreliable)
Weight EC50	insufficient effect, not calculated
Weight EC20	insufficient effect, not calculated

Table 16. Toxicity of sodium chloride to the mayfly, *Neocloeon triangulifer,* in 7-d sustained exposure followed by 7 days of 50% daily dilution, conducted at 10°C.

Nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L) days 1-7	measured [Cl^-] (mg/L) day 14	% survival	dry weight (mg)
28	26	26	100	0.0017 ± 0.0003
313	303	28	100	0.0014 ± 0.0002
625	617	31	100	0.0012 ± 0.0003
1250	1234	34	60	0.0013 ± 0.0004
2500	2406	na*	0	na
5000	4844	na	0	na

Effect levels (95% confidence intervals) based on measured dissolved Cl (mg/L)

Survival EC50	1313 (1063 – 1621)
Weight EC50	insufficient effect, not calculated
Weight EC20	insufficient effect, not calculated

*all organisms in these treatments were dead by day 7 so no dilution was necessary

Table 17. Toxicity of sodium chloride to the amphipod, *Hyalella azteca,* in 7-d sustained exposure followed by 7 days of 50% daily dilution, conducted at 10°C.

Nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L) days 1-7	measured [Cl^-] (mg/L) day 14	% survival	dry weight (mg)
28	25	25	100	1.22 ± 0.00
313	298	28	100	1.25 ± 0.00
625	603	30	100	1.42 ± 0.00
1250	1210	37	100	1.30 ± 0.00
2500	2340	47	70	1.13 ± 0.00
5000	4833	79	20	0.55 ± 0.00

Effect levels (95% confidence intervals) based on measured dissolved Cl (mg/L)

Survival EC50	3108 (2016 – 4793)
Weight EC50	4370 (3571 – 5349)
Weight EC20	2740 (1982 – 3788)

Table 18. Toxicity of sodium chloride to the fingernail clam, *Sphaerium simile*, in 7-d sustained exposure followed by 7 days of 50% daily dilution, conducted at 10°C.

Nominal [Cl^-] (mg/L)	measured [Cl^-] (mg/L) days 1-7	measured [Cl^-] (mg/L) day 14	% survival	dry weight (mg)
28	26	26	100	52 ± 0.00
313	302	28	100	53 ± 0.00
625	609	29	100	59 ± 0.00
1250	1221	32	100	59 ± 0.00
2500	2399	47	20	57 ± 0.00
5000	4804	72	0	na
Effect levels (95% confidence intervals) based on measured dissolved Cl (mg/L)				
Survival EC50	1963 (1736 – 2219)			
Weight EC50	insufficient effect, not calculated			
Weight EC20	insufficient effect, not calculated			

Table 19. Summary of acute and chronic values generated in the present study at standard temperature (25 or 23 °C) and at 10 °C. All values in mg Cl^-/L.

Species	96-hour LC50		chronic value*		chronic value
	25/23 °C	10 °C	25/23 °C	10 °C	10 °C, pulsed
N. triangulifer	1359	1960	326	>1458	1313
H. azteca	1733	2185	226	1257	2740
S. simile	1673	>2920	1672	1664	1963

* chronic values shown are lowest effect levels observed in a given test, either EC50 or EC20.

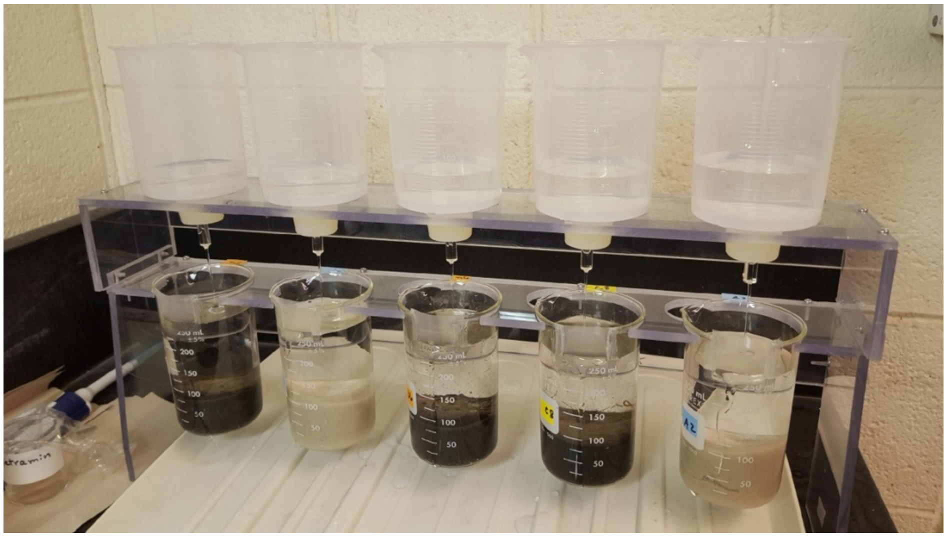

Figure 1. Zumwalt et al. (1994) type water addition apparatus.