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INTERACTIONS BETWEEN FRESHWATER MUSSELS, MERCURY CONTAMINATION, AND GLOBAL CLIMATE CHANGE IN FRESHWATER SYSTEMS

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BRENT NORRIS TWEEDY
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INTERACTIONS BETWEEN FRESHWATER MUSSELS, MERCURY CONTAMINATION, AND GLOBAL CLIMATE CHANGE IN FRESHWATER SYSTEMS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

BY

_____
Dr. Caryn C. Vaughn, Chair

_____
Dr. Matthew M. Chumchal

_____
Dr. Michael E. Kaspari

_____
Dr. Robert W. Nairn

_____
Dr. Lara A. Souza

To Ward Tweedy

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ABSTRACT

Humans are impacting the environment at an unprecedented scale, with anthropogenic activities altering environmental processes and cycles planet-wide. Centuries of gold mining and coal burning has more than tripled the amount of inorganic mercury in the global atmospheric pool. As this mercury is deposited across the landscape it is washed into aquatic ecosystems. Anaerobic bacteria in aquatic sediments convert the mercury into methyl mercury which readily assimilates into food webs. Methyl mercury biomagnifies in food webs to high concentrations that pose a threat to both humans and wildlife. The burning of coal, along with other fossil fuels, has also altered the global climate, which further impacts aquatic ecosystems already stressed by mercury contamination. Anthropogenic global climate change (GCC) has warmed the planet 0.85℃ since 1880 and is poised to raise average global temperatures another 0.3–4.8℃ by the end of the century. Freshwater systems will be especially hard hit by these changes due to the limited ability of many organisms to relocate and human demands on limited freshwater resources.

North American rivers are a global biodiversity hotspot for freshwater mussels. These long-lived invertebrates are ecosystem engineers that perform critical ecosystem function680s in many rivers, and they are highly imperiled due to multiple factors including land use change, habitat fragmentation, and pollution. In the southeastern U.S., rivers are experiencing more frequent and more severe droughts as a result of GCC, which have led to declines in mussel populations. These rivers are also experiencing elevated levels of mercury contamination. These two critical, emerging

stressors, GCC and mercury contamination, may interact to impact freshwater systems beyond the effects of each stressor independently. My dissertation investigates the dynamics of mercury contamination alone and mercury contamination plus GCC on freshwater mussels and the ecosystem functions they provide. I asked three questions: 1) What is the extent of mercury contamination in a globally significant river in the southern U.S.? 2) How do freshwater mussels affect the movement of mercury in the ecosystem? and 3) Does toxic stress resulting from the combination of mercury contamination and GCC thermal stress interact to affect freshwater mussels and the ecosystem processes they influence?

To address the first question, I measured the mercury content of multiple species of fish and invertebrates collected from the Kiamichi River, Oklahoma, a well-studied river with high mussel and fish biodiversity. I found that concentrations of mercury in the tissue of multiple fish species in the river exceeded both the Oklahoma and federal (EPA) consumption advisory limits and that the most abundant invertebrate taxa also had the highest mercury concentrations. To address the second question, I conducted a mesocosm experiment examining the effects of mussels on mercury concentrations of benthic consumers. I collected mussels and mercury-contaminated sediments from the Kiamichi River. I added sediments to 32 recirculating mesocosms and constructed mussel communities composed of two common species across four, natural mussel densities, with each treatment replicated 8 times. Snails were added to the mesocosms to serve as primary benthic consumers. After allowing the experiment to run for four months, I measured the biomass and mercury concentration of the snail consumers. Snails had both higher overall mercury concentrations and higher mercury burdens

(concentration of mercury per g snail dry weight) in the presence of higher densities of mussels. To address the third question, I performed a combination of laboratory and mesocosm experiments to examine how thermal stress from GCC combined with toxic mercury stress affects both individual mussel physiological functions and mussel community-influenced ecosystem functions. In the laboratory, I measured the respiration rates and filtering rates of two common mussel species exposed to increased temperature, increased mercury, and these stressors combined. I observed reduced respiration rates and filtration rates in both species in response to these stressors, and increased mortality in one of the species. I conducted a mesocosm experiment where I constructed three-species mussel communities, exposed them to two temperature treatments and two mercury treatments in a factorial design, and measured their influence on nutrient cycling. Mussel mortality was higher in the combined stressor treatments. Ammonia (NH3) concentrations spiked in the double stress treatments and then declined while total phosphorus (TP) showed the opposite trend. Combined we observed a declining NH3:TP in double stress treatments.

My findings have relevance both locally and broadly. Elevated concentrations of mercury in the Kiamichi River indicate that reservoirs in Oklahoma are not the only waterbodies at risk in the state for high levels of mercury even though they currently are the only ones with fish consumption advisories. Additionally, the fact that concentrations were the same throughout the river suggest that methyl mercury is being produced within the river rather than being imported from off-channel impoundments. My results also indicate that mussel-influenced ecosystems, like the Kiamichi River and similar rivers across North America, are more sensitive to mercury contamination than

previously thought. These systems are at risk for both higher concentrations of mercury in consumers as well as larger burdens of mercury present in the systems. Finally, I show that two anthropogenic stressors occurring at a global scale, toxic mercury stress and thermal stress, interact negatively to affect freshwater systems from the organismal to ecosystem level. Unfortunately, both stressors are predicted to worsen over the coming decades and, given the global scope of mercury and GCC, their negative impacts on organisms and ecosystems are an additional emerging threat to imperiled freshwater systems around the world.

CHAPTER 1: MERCURY CONTAMINATION ACROSS A RIVERINE FOOD WEB

Brent N. Tweedy, Brandon J. Sansom, & Caryn C. Vaughn

Keywords:

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INTRODUCTION

Mercury is an environmental contaminant negatively affecting the health of both humans and wildlife (Scheulhammer et al. 2007). Mercury released into the atmosphere from human activities, predominantly coal fired power plants and artisanal gold mining, makes its way into aquatic environments where it can be converted into toxic methyl mercury (MeHg) by anaerobic bacteria and subsequently biomagnify in aquatic food webs (Selin 2009). Because of these non-point, atmospheric sources of mercury, we now face a global mercury crisis. The southeastern US, including parts of east Texas and southeastern Oklahoma, contains numerous mercury contamination hotspots (Drenner et al. 2013; Drenner et al. 2011).

Many mercury monitoring programs focus on sportfish, such as bass or catfish, in lakes and reservoirs. Thus, consumption advisories are issued more often for these waterbodies compared to rivers and streams, which can also can also have levels of mercury above human health risk thresholds (Balogh et al. 2002; Bergeron et al. 2007; Tsui et al. 2009). For example, in the southeastern US, all 36 mercury advisories in the state of Oklahoma are for lakes and reservoirs (Mercury in Fish: A Guide to Healthy Fish Consumption in Oklahoma 2013) and only two out of 14 advisories in the state of Texas are for rivers or streams (Fish Consumption Bans and Advisories 2017). Given the prevalence of elevated mercury in fish in southeastern US reservoirs (Drenner et al. 2013) it is likely that a significant number of streams and rivers in this region also have elevated concentrations.

Rivers and streams are also home to many endemic non-sport fish, such as darters and minnows. These fish are an important part of many stream and riparian food webs, serving as food sources for both aquatic and terrestrial predators (Schlosser 1987; Thomas and Taylor 2013). Because these fish are rarely consumed by humans they are infrequently included in mercury surveys (but see (Riva-Murray et al. 2011)). However, these fish are an important part of the food web and elevated body-burden of mercury in these populations will be transferred to higher trophic levels, including sportfish that may be consumed by humans.

Mercury cycling in streams and rivers is often different from lakes and reservoirs. Anoxic conditions in the hypolimnion of reservoirs are often favorable to the production of MeHg that can be exported downstream to other waterbodies (Green et al. 2016). However, in streams, MeHg can also be produced *in situ* in periphyton mats and in sediment if conditions are right (Tsui et al. 2010). Understanding whether MeHg in streams originates from endogenous or exogenous sources has important implications for management responses, especially in streams that are heavily impacted by impoundments.

To investigate the patterns and extent of mercury contamination in river ecosystems, we sampled food web compartments in a southern U.S. river located in a predicted mercury contamination hotspot. Our sampling focused on macroinvertebrates, non-sport fish and sport fish.

We asked 1) Does the sportfish body burden of MeHg exceed toxic thresholds for human health? 2) Do macroinvertebrates and other non-sportfish species have elevated or dangerous concentrations of mercury? 3) Is there evidence that the mercury is being produced in-stream rather than in a reservoir?

METHODS

Our study site was a 5th-order tributary of the Red River, the Kiamichi River in southeastern Oklahoma. The Kiamichi River drains a largely forested watershed of 4,500 km^2, and is influenced by both a mainstem impoundment (Hugo Lake) and a tributary impoundment (Sardis Lake). Both lakes have consumption advisories issued by the Oklahoma Department of Environmental Quality for multiple fish species (although it should be noted that the minimum Oklahoma advisory limit of 500 ng/g is higher than the U.S. federal advisory (EPA) limit of 300 ng/g) (Oklahoma Mercury in Fish). We sampled ten sites along the Kiamichi River in summer of 2013. Each site was a 100-m long river reach and sites were separated by at least 500 m, although most sites were separated by a kilometer or more. Five of the sites contained dense assemblages of freshwater mussels (Bivalvia: Unionidae), a keystone group of consumers known to influence ecosystem function in the river (Allen et al. 2012; Atkinson and Vaughn 2015; Vaughn and Hakenkamp 2001). Additionally, four of the sites were upstream of the outflow of Sardis Lake, which provides roughly 25% of the downstream flows

where the remaining six sites were located downstream of the tributary (Figure 1) (Vaughn et al. 2015). We sampled fish communities, macroinvertebrate communities, and a suite of abiotic parameters at each site.

Abiotic Sampling

We divided each site into five evenly-spaced transects. Current velocity (Hach FH950.0) and substrate heterogeneity were measured at all five transects. Dissolved oxygen, temperature, pH, and conductivity were measured the at the downstream, 50%, and upstream transects at the left bank, river center, and right bank positions. Dissolved oxygen and temperature were measured using a Hach meter (Hach, HQ36d) and pH and conductivity were measured using a PCSTestr Multi-Parameter (Oakton Instruments, PCSTestr 35 model WD-35425-10). We conducted pebble counts at every transect and measured 20 pebbles per transect for a total of 100 at each site (Kondolf et al. 2003).

Macroinvertebrate Sampling

Macroinvertebrates were collected with a Surber sampler at five randomly selected locations at each site. Water depth and sampling time (effort) were recorded for each sample and all substrate within the sampling area was thoroughly scoured to a sediment depth of 5 cm. The contents of each sample were preserved in 80% ethanol and macroinvertebrates were sorted and identified to family in the laboratory.

At sites with mussel beds, mussels were sampled with 10 haphazardly placed 0.25 m^2 quadrats (Vaughn et al. 1997). Quadrats were excavated to a depth of 15cm. Mussels were removed to shore, identified to species, and measured for length, height, and width

of shell. Ten randomly selected individuals of the 2-3 most dominant species at each site were sacrificed and preserved in 80% ethanol for subsequent mercury analyses and all other individuals were returned to the river alive.

Fish Sampling

Fish were collected by electrofishing. We used a backpack electroshocker (Smith-Root, Inc. Model 12-B) and moved from downstream to upstream going from bank-to-bank in a zig-zag pattern using a pulsed direct current. Captured fish were kept in 20-liter buckets until the entire site had been sampled. Immediately after electroshocking, all fish were euthanized in MS222 and preserved in 80% ethanol for later identification to species and subsequent mercury analyses.

Mercury Analyses

Mercury analyses were conducted on a direct mercury analyzer (Milestone DMA-80). Macroinvertebrates were run as whole body, composite samples by taxa. Mussel whole bodies were homogenized into a fine powder using a mortar and pestle and a Wig-L-Bug (Fisher Scientific). A fillet was removed from fish larger than 8cm. For smaller fish the head and organs were removed, and the remainder of the body was dried and homogenized into a fine powder for analyses.

Statistical Analyses

All statistical tests were conducted using IBM SPSS Statistics Version 19. Mean total mercury concentrations for all taxa and invertebrate families were compared using a one-way ANOVA with a Tukey post-hoc test. Differences in mean mercury concentration above and below Sardis lake were examined with a t-test. All tests used a significance threshold of $p \leq 0.05$.

RESULTS AND DISCUSSION

Physicochemical and geomorphological parameters were similar among sites (Table 1). No pairwise comparison of the sites (mussel/non-mussel or above/below Sardis) had significantly different mean values of these parameters, except depth which was slightly greater at mussel than non-mussel sites (Table 1). However, the 0.07 m mean difference in depth observed between sites is unlikely to be biologically meaningful.

Elevated mercury was found in macroinvertebrate consumers that comprise the base of the food web (Table 2). Riffle beetle (Elmidae) and stonefly (*Eccoptura*) larvae had relatively low mercury concentrations while mayfly (Ephemeroptera) and caddisfly (Trichoptera) larvae had higher mercury concentrations (Figure 2). There were interesting differences within the latter two groups as well. Ryacophilidae, a caddisfly family of mainly predators, had the highest overall mercury concentration. *Tortopus* (Polymitarcyidae), a genus of burrowing mayflies and *Stenoema* (Heptageniidae), a genus of grazing mayflies, also had high concentrations. While Polymytarcidae's close

association with aquatic sediments, where mercury is methylated, might explain the higher concentrations in these larvae, it is less clear why Heptageniidae were also elevated.

In addition to serving as a food source for aquatic predators, larval forms of aquatic insects complete their lifecycle by emerging from aquatic ecosystems into terrestrial adult forms. When these emergent insects leave the water, they transport the mercury in their bodies from aquatic to terrestrial food webs (Gerrard and St Louis 2001; Menzie 1980; Raikow et al. 2011; Speir et al. 2014; Tweedy et al. 2013; Walters et al. 2008). Larval insects with a terrestrial adult form found in the Kiamichi River ranged from approximately 30-300 ng/g total mercury. Similar concentrations in spiders were found to pose a health risk to young songbirds (Gann et al. 2015).

We found elevated mercury concentrations in all fish species (Table 3). At the base of the food web, stoneroller minnows (*Campostoma spadiceum*) had the lowest mercury concentrations of the fishes but were still higher than mussels and aquatic insect larvae. Though largely herbivorous, stoneroller minnows also consume invertebrates and detritus (Evans-White et al. 2001), which could account for their elevated mercury concentration relative to mussels, another primary consumer (Figure 3).

We also observed high mercury concentrations in darters relative to other fishes, consistent with the only other study to have examined this group (Riva-Murray et al. 2011). The mercury concentrations of some darter individuals were as high as that of much larger piscivorous fish such as gar and bass, and they were comparable to or higher than centrarchids (Centrarchidae) and catfish (Ictaluridae) of a similar or larger

size (Figure 3). The diet of darters (Percidae) is a possible explanation. A past study of the stomach contents of darters and sunfish stomach found benthic invertebrates, including mayflies, made up a high proportion of darters' diets while sunfish consumed far fewer mayflies and consumed more terrestrial insects (W.J. Matthews, personal communication). In the Kiamichi River mayfly larvae had some of the highest mercury concentrations in our system (Figure 3). Preferential consumption of mercury-rich mayflies may contribute to elevated mercury concentrations in darters. Age may also play a role in this relationship. Many darters live to be up to 5 years, (Paine 1990) while sunfish in the 5cm length range are young of year (Cargnelli and Gross 1996). Many of the darters we sampled likely had several years to accumulate mercury in their bodies. While darters are not consumed by humans, they are an important prey item for aquatic predators such as bass (Schlosser 1987). Additionally, terrestrial predators, such as birds, are important top-level predators in many aquatic systems (Steinmetz et al. 2003) and are likely consuming darters as well. If darters are accumulating more mercury than other prey items and are preferred prey for top level predators, they may be disproportionately responsible for mercury body burden in top level predators. Because darters tend to only occur in river and streams, (Miller and Robinson 2004) this may be a unique factor that contributes to mercury contamination in rivers and not lakes.

At higher trophic levels, we found several fish taxa with individuals at or above the EPA mercury consumption advisory limit. The majority of smallmouth bass over 10 cm in total length were over the EPA consumption advisory limit as were some sunfish and catfish. These results likely represent a conservative estimate of the extent of mercury contamination in the river. We were only able to electroshock wadeable

portions of the river which likely biased our samples toward smaller fish. For instance, the catfish sampled were all under 20 cm standard length and the largest bass was only 22.6 cm. We visually observed larger fish in deeper habitats of the river that we were unable to sample. These larger fish likely have mercury concentrations higher than smaller fish. Thus, our results represent a conservative estimate of the upper limit of mercury concentrations in fish in the river. Our findings strongly suggest that the Kiamichi River, and others like it, represent a risk to human health with regards to the consumption of fish from the river.

No significant difference in mercury concentrations was detected for fishes between sites with and without dense mussel assemblages. We were unable to test for differences in invertebrate taxa due to several sites where the biomass of invertebrates was too low for mercury analyses.

Finally, our data suggest that the mercury contamination observed in the river is driven largely by riverine processes rather than by inputs from the tributary reservoir, Sardis Lake. We found no difference in mercury concentrations in any taxonomic groups or size classes above and below the inflow from Sardis Lake to the river. Because mercury concentrations are not higher below the inflow it seems unlikely that Sardis Lake is contributing mercury to the lower river. The Kiamichi River occurs in a mountainous area with few wetlands (Fry et al. 2011), which can increase riverine mercury concentrations (Ward et al. 2010). Therefore, the majority of MeHg production is likely occurring in the streambed itself. Additionally, the watershed is dominated by coniferous forests which can cause elevated mercury concentrations. Periphyton can also be a site for mercury accumulation and MeHg production within rivers (Tsui et al.

2010), and the Kiamichi is prone to large blooms of attached periphyton during summer low flow periods.

Our data show that a mid-size river in Oklahoma has mercury concentrations comparable to those found in large lakes and reservoirs and that this mercury is likely being converted into MeHg in-stream. However, while these lakes and rivers are monitored for human health threats, very smaller order streams and rivers rarely receive the same attention. The mercury in the Kiamichi River is at a high enough level to pose a health risk to both humans and wildlife, like the two impoundments on the river. While monitoring of all smaller order streams seems unfeasible, further research exploring the relationship between reservoir mercury (which is monitored) and stream mercury could help in the issuing of more comprehensive and effective mercury advisories.

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Conflict of Interest

Brent N. Tweedy declares that he has no conflict of interest. Brandon J. Sansom declares that he has no conflict of interest. Caryn C. Vaughn declares that she has no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed under University of Oklahoma IACUC Institutional Tracking Number R13-002. This article does not contain any studies with human participants performed by any of the authors.

REFERENCES

Allen DC, Vaughn CC, Kelly JF, Cooper JT, Engel MH (2012) Bottom-up biodiversity effects increase resource subsidy flux between ecosystems *Ecology* 93:2165-2174

Atkinson CL, Vaughn CC (2015) Biogeochemical hotspots: temporal and spatial scaling of the impact of freshwater mussels on ecosystem function *Freshwater Biology* 60:563-574 doi:10.1111/fwb.12498

Balogh SJ, Huang YB, Offerman HJ, Meyer ML, Johnson DK (2002) Episodes of elevated methylmercury concentrations in prairie streams *Environmental Science & Technology* 36:1665-1670 doi:10.1021/es011265w

Bergeron CM, Husak JF, Unrine JM, Romanek CS, Hopkins WA (2007) Influence of feeding ecology on blood mercury concentrations in four species of turtles *Environmental Toxicology and Chemistry* 26:1733-1741 doi:10.1897/06-594r.1

Cargnelli LM, Gross MR (1996) The temporal dimension in fish recruitment: Birth date, body size, and size-dependent survival in a sunfish (bluegill: Lepomis macrochirus) *Canadian Journal of Fisheries and Aquatic Sciences* 53:360-367 doi:10.1139/cjfas-53-2-360

Drenner RW, Chumchal MM, Jones CM, Lehmann CMB, Gay DA, Donato DI (2013) Effects of Mercury Deposition and Coniferous Forests on the Mercury Contamination of Fish in the South Central United States *Environmental Science & Technology* 47:1274-1279 doi:10.1021/es303734n

Drenner RW, Chumchal MM, Wente SP, McGuire M, Drenner SM (2011) Landscape-level patterns of mercury contamination of fish in North Texas, USA *Environmental Toxicology and Chemistry* 30:2041-2045 doi:10.1002/etc.589

Evans-White M, Dodds WK, Gray LJ, Fritz KM (2001) A comparison of the trophic ecology of the crayfishes (Orconectes nais (Faxon) and Orconectes neglectus (Faxon)) and the central stoneroller minnow (Campostoma anomalum (Rafinesque)): omnivory in a tallgrass prairie stream *Hydrobiologia* 462:131-144 doi:10.1023/a:1013182100150

Fish Consumption Bans and Advisories. (2017). https://tpwd.texas.gov/regulations/outdoor-annual/fishing/general-rules-regulations/fish-consumption-bans-and-advisories. 2017

Fry JA et al. (2011) National Land Cover Database for the Conterminous United States *Photogrammetric Engineering and Remote Sensing* 77:859-864

Gann GL, Powell CH, Chumchal MM, Drenner RW (2015) Hg-Contaminated Terrestrial Spiders Pose a Potential Risk to Songbirds at Caddo Lake (Texas/Louisiana, USA) *Environmental Toxicology and Chemistry* 34:303-306 doi:10.1002/etc.2796

Gerrard PM, St Louis VL (2001) The effects of experimental reservoir creation on the bioaccumulation of methylmercury and reproductive success of tree swallows (Tachycineta bicolor) *Environmental Science & Technology* 35:1329-1338 doi:10.1021/es001537f

Green DJ, Duffy M, Janz DM, McCullum K, Carriere G, Jardine TD (2016) Historical and Contemporary Patterns of Mercury in a Hydroelectric Reservoir and Downstream Fishery: Concentration Decline in Water and Fishes Archives of *Environmental Contamination and Toxicology* 71:157-170 doi:10.1007/s00244-016-0287-3

Kondolf GM, Lisle TE, W.G.M. (2003) *Tools in fluvial geomorphology*. In. West Sussex, England, pp 394-395

Menzie CA (1980) Potential Significance of Insects in the Removal of Contaminants from Aquatic Systems *Water Air and Soil Pollution* 13:473-479 doi:10.1007/bf02191848

Miller RJ, Robinson HW (2004) *Fishes of Oklahoma*. The University of Oklahoma Press, Norman

Oklahoma Department of Environmental Quality, *Oklahoma Mercury in Fish*. http://www.deq.state.ok.us/aqdnew/resources/publications/HgPresentation.pdf

Oklahoma Department of Environmental Quality, (2013) Mercury in Fish: A Guide to Healthy Fish Consumption in Oklahoma

Paine MD (1990) Life-History Tactics of Darters (Percidae, Etheostomantiini) and Their Relationship with Body Size, Reproductive Behavior, Latitude and Rarity *Journal of Fish Biology* 37:473-488 doi:10.1111/j.1095-8649.1990.tb05877.x

Raikow DF, Walters DM, Fritz KM, Mills MA (2011) The distance that contaminated aquatic subsidies extend into lake riparian zones *Ecological Applications* 21:983-990 doi:10.1890/09-1504.1

Riva-Murray K, Chasar LC, Bradley PM, Burns DA, Brigham ME, Smith MJ, Abrahamsen TA (2011) Spatial patterns of mercury in macroinvertebrates and fishes from streams of two contrasting forested landscapes in the eastern United States *Ecotoxicology* 20:1530-1542 doi:10.1007/s10646-011-0719-9

Scheulhammer AM, Meyer MW, Sandheinrich MB, Murray MW (2007) Effects of environmental methylmercury on the health of wild birds, mammals, and fish *Ambio* 36:12-18 doi:10.1579/0044-7447(2007)36[12:eoemot]2.0.co;2

Schlosser IJ (1987) The Role of Predaation in Age-Related and Size-Related Habitat Use by Stream Fishes Ecology 68:651-659 doi:10.2307/1938470

Selin NE (2009) Global Biogeochemical Cycling of Mercury: A Review *Annual Review of Environment and Resources* 34:43-63 doi:10.1146/annurev.environ.051308.084314

Speir SL, Chumchal MM, Drenner RW, Cocke WG, Lewis ME, Whitt HJ (2014) Methyl Mercury and Stable Isotopes of Nitrogen Reveal That a Terrestrial Spider has a Diet of Emergent Aquatic Insects *Environmental Toxicology and Chemistry* 33:2506-2509 doi:10.1002/etc.2700

Steinmetz J, Kohler SL, Soluk DA (2003) Birds are overlooked top predators in aquatic food webs *Ecology* 84:1324-1328 doi:10.1890/0012-9658(2003)084[1324:baotpi]2.0.co;2

Thomas CL, Taylor CA (2013) Scavenger or predator? Examining a potential predator-prey relationship between crayfish and benthic fish in stream food webs *Freshwater Science* 32:1309-1317 doi:10.1899/12-169.1

Tsui MTK, Finlay JC, Balogh SJ, Nollet YH (2010) In Situ Production of Methylmercury within a Stream Channel in Northern California *Environmental Science & Technology* 44:6998-7004 doi:10.1021/es101374y

Tsui MTK, Finlay JC, Nater EA (2009) Mercury Bioaccumulation in a Stream Network *Environmental Science & Technology* 43:7016-7022 doi:10.1021/es901525w

Tweedy BN, Drenner RW, Chumchal MM, Kennedy JH (2013) Effects of Fish on Emergent Insect-Mediated Flux of Methyl Mercury across a Gradient of Contamination *Environmental Science & Technology* 47:1614-1619 doi:10.1021/es303330m

Vaughn CC, Atkinson CL, Julian JP (2015) Drought-induced changes in flow regimes lead to long-term losses in mussel-provided ecosystem services *Ecology and Evolution* 5:1291-1305 doi:10.1002/ece3.1442

Vaughn CC, Hakenkamp CC (2001) The functional role of burrowing bivalves in freshwater ecosystems *Freshwater Biology* 46:1431-1446 doi:10.1046/j.1365-2427.2001.00771.x

Vaughn CC, Taylor CM, Eberhard KJ (1997) A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. In: Cummings KS, Buchanan AC, Mayer CA, Naimo CA (eds) *Conservation and Management of Freshwater Mussels II: Initiatives for the Future*, St. Louis, Missouri, 1995. Proceedings of a UMRCC Symposium. Upper Mississippi River Conservation Committee, Rock Island, Illinois, pp 157-162

Walters DM, Fritz KM, Otter RR (2008) The Dark Side of Subsidies: Adult Stream Insects Export Organic Contaminants to Riparian Predators *Ecological Applications* 18:1835-1841 doi:10.1890/08-0354.1

Ward DM, Nislow KH, Folt CL (2010) Bioaccumulation syndrome: identifying factors that make some stream food webs prone to elevated mercury bioaccumulation *Year in Ecology and Conservation Biology* 2010 1195:62-83 doi:10.1111/j.1749-6632.2010.05456.x

Table 1. Mean abiotic parameters measured for each site.

Site Type	Below Sardis Lake	Temp (°C)	pH	Conductivity (uS)	TDS (ppm)	Salinity (ppm)	Dissolved Oxygen (mg/L)	Substrate Heterogeneity	D50 (mm)	Flow (m/s)	Depth (m)
+Mussel	Yes	31	7.2	51.9	37	32.1	7	4.1	80	0.24	0.44
+Mussel	No	27.8	7.3	55.2	39.2	32.8	5.9	7	50	0.06	0.4
+Mussel	No	30	7.4	55.8	39.7	33.5	7	4.5	80	0.04	0.51
+Mussel	Yes	29.5	7.5	53.5	38	32.5	7.6	3.3	70	0.07	0.45
+Mussel	Yes	28	7.5	54.3	38.5	32.4	7.2	4	40	0.03	0.47
-Mussel	No	30.7	7.7	46.4	33	29.8	8.1	4	80	0.29	0.34
-Mussel	No	28.8	7.7	54.2	38.5	32.6	8.4	4	60	0.06	0.45
-Mussel	Yes	30	7.5	53.6	38.1	32.6	7.4	4	70	0.16	0.32
-Mussel	Yes	30.6	7.9	59.7	42.4	35	8.1	7	60	0.06	0.42
-Mussel	Yes	29.5	7.5	54.3	38.6	32.7	7.6	4	80	0.16	0.36
Means[a]	+Mussel	29.3	7.4	54.1	38.5	32.7	6.9	4.6	64	0.08	0.46
	-Mussel	30	7.6	52.8	37.5	32.2	7.9	4.6	70	0.13	0.39
	Below Sardis	29.8	7.5	54.6	38.8	32.9	7.5	4.4	66.7	0.12	0.41
	Above Sardis	31	7.5	52	37	31.8	7.4	4.9	67.5	0.11	0.42

[a]If applicable, total mean values obtained from average of entire data per mussel and non-mussel sites, not the average values per site seen above. Heterogeneity and D50 means were averaged from totals per site.

Table 2. Average Abundance and Mercury Concentration for Kiamichi Fishes

Taxa	Average Abundance	Average Relative Abundance	[Hg] (ng/g)
Stoneroller	9.6 (±12.2)	12.6% (±14.4)	578 (±227)
Darter	24.4 (±14.1)	32.2% (±15.1)	-
Small (2-8cm)	20.5 (±15)	26.3% (±16.7)	884 (±184)
Large (>8cm)	3.9 (±2.2)	5.9% (±3.9)	1133 (±464)
Sunfish	37.4 (±14.5)	50.8% (±13.9)	-
Small (5-7cm)	18.3 (±7.3)	24.4% (±7.7)	526 (±88.4)
Medium (7-10cm)	15.1 (±7.7)	20.6% (±9.1)	779 (±270)
Large (>10cm)	4 (±2.8)	5.8% (±4.3)	959 (±354)
Catfish	0.5 (±1.3)	0.6% (±1.4)	985 (±439)
Gar	0.3 (±0.5)	0.4% (±0.7)	2669 (±1403)
Smallmouth Bass	2.2 (±1.7)	3.4% (±2.9)	-
Small (4-5cm)	0.5 (±0.7)	0.8% (±1.3)	810 (±158)
Large (>10cm)	1.3 (±1.3)	2% (±1.9)	2986 (±1053)

Table 3. Average Abundance, Biomass, and Mercury Concentrations for Kiamichi River Aquatic Insects

Taxa	Average Abundance	Average Relative Abundance	Average Biomass (g)	Average Relative Biomass	[Hg] (ng/g)
Coleoptera-Elmidae	68 (±19)	16.4% (±19.0)	0.0196 (±0.0247)	27.5% (±24.6)	51.1 (±66.5)
larvae	71 (±24)	11.6% (±16.4)	0.0053 (±0.0056)	13.6% (±14.3)	28.2 (±45.0)
adults	65 (±15)	4.8% (±4.2)	0.0143 (±0.0207)	13.7% (±17.1)	27.9 (±31.1)
Ephemeroptera	127 (±53)	38.9% (±21.4)	0.0552 (±0.0683)	32.0% (±22.8)	71.3 (±104.9)
Heptageniidae	146 (±89)	16.6% (±15.9)	0.0089 (±0.0062)	22.3% (±15.9)	29.7 (±32.5)
Polymytarcidae	136 (±59)	11.9% (±24.0)	0.0167 (±0.0287)	2.2% (±4.4)	4.7 (±7.3)
Isonychidae	77 (±37)	10.4% (±20.0)	0.0296 (±0.0595)	7.5% (±14.3)	36.9 (±73.9)
Trichoptera	119 (±72)	20.9% (±21.5)	0.0302 (±0.0488)	31.2% (±22.8)	83.5 (±143.4)
Hydropsychidae	109 (±60)	14.9% (±13.0)	0.0199 (±0.0294)	25.6% (±16.8)	67.0 (±111.6)
Ryacophilidae	148 (±41)	6.0% (±9.7)	0.0102 (±0.0199)	5.6% (±7.4)	16.5 (±32.9)
Plecoptera	74 (±39)	23.8% (±20.9)	0.0216 (±0.0308)	9.3% (±5.9)	21.0 (±43.7)

FIGURE LEGENDS

Figure 1 A map of the study site, the Kiamichi River in southeastern Oklahoma.

Figure 2 Average total mercury concentration in ng\g for families of aquatic insects. Letters denote homogenous groupings from a one-way ANOVA with a Tukey's post hoc test ($p < 0.05$).

Figure 3 Total mercury concentration (ng/g dry weight) for major taxa in the Kiamichi River. Some fish taxa were split according into size groups based on total length: Small Darters (2-8cm), Large Darters (>8cm), Small Sunfish (5-7cm), Medium Sunfish (7-10cm), Large Sunfish (>10cm), Small Bass (4-5cm), and Large Bass (>10cm). Letters denote homogenous groups from a one-way ANOVA with a Tukey's post-hoc test at $p <= 0.05$.

FIGURES

Figure 1. Map of the Kiamichi River

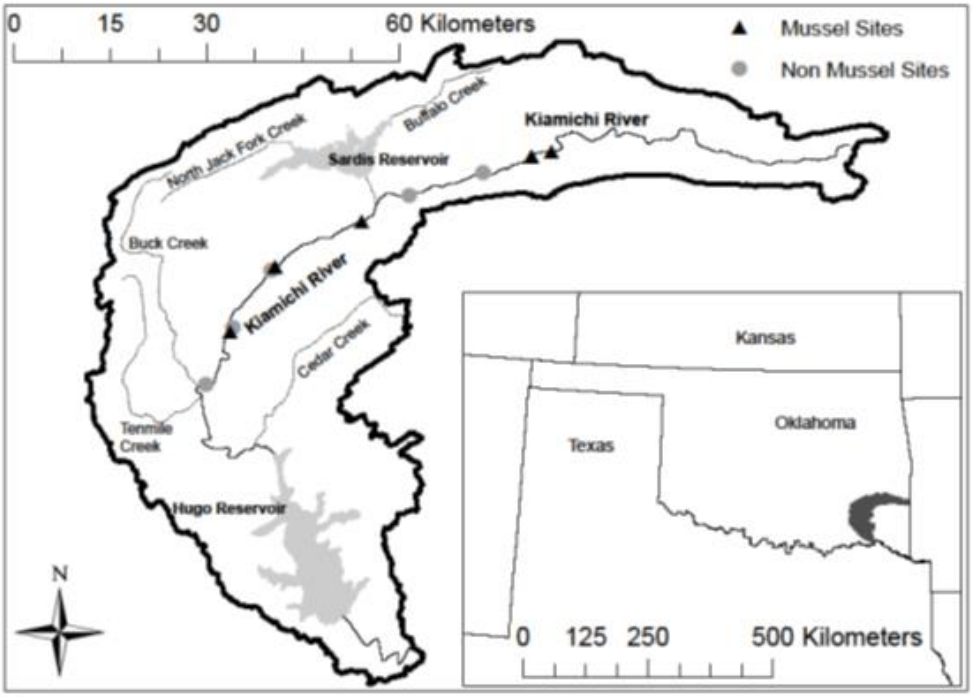


Figure 2. Mercury Concentrations in Aquatic Insects

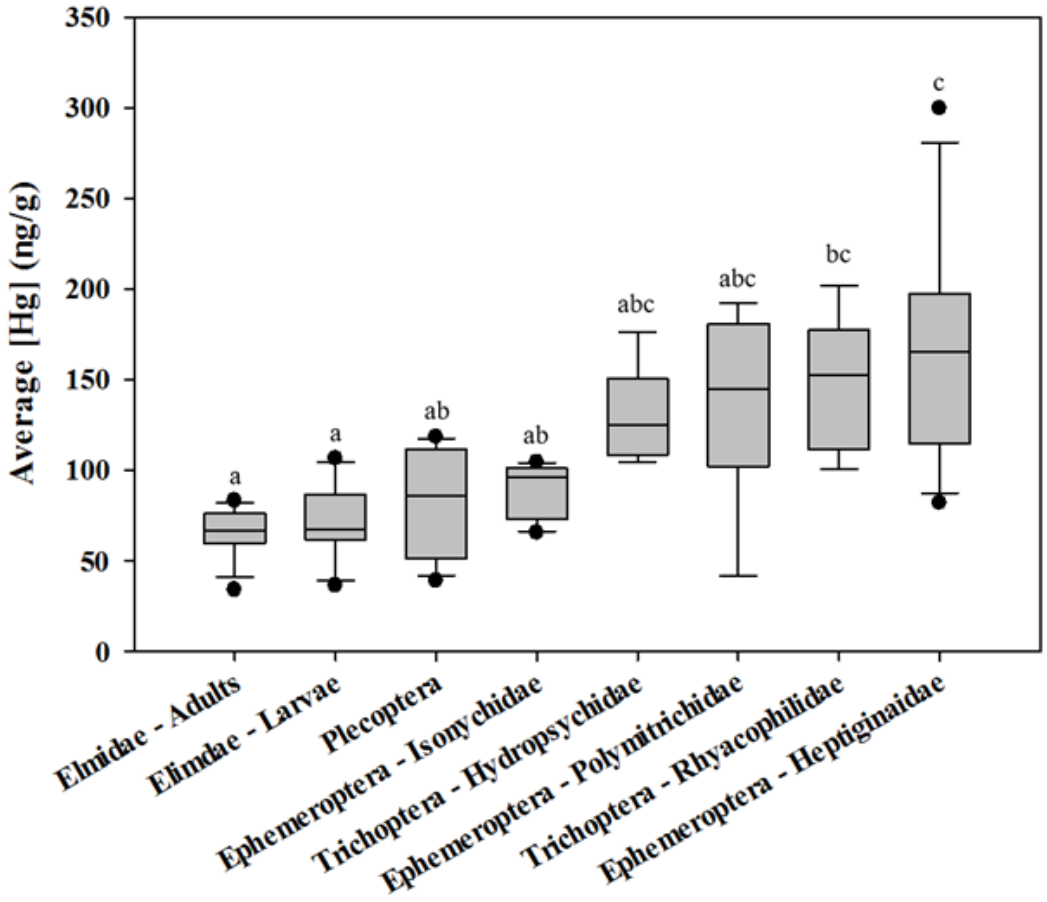
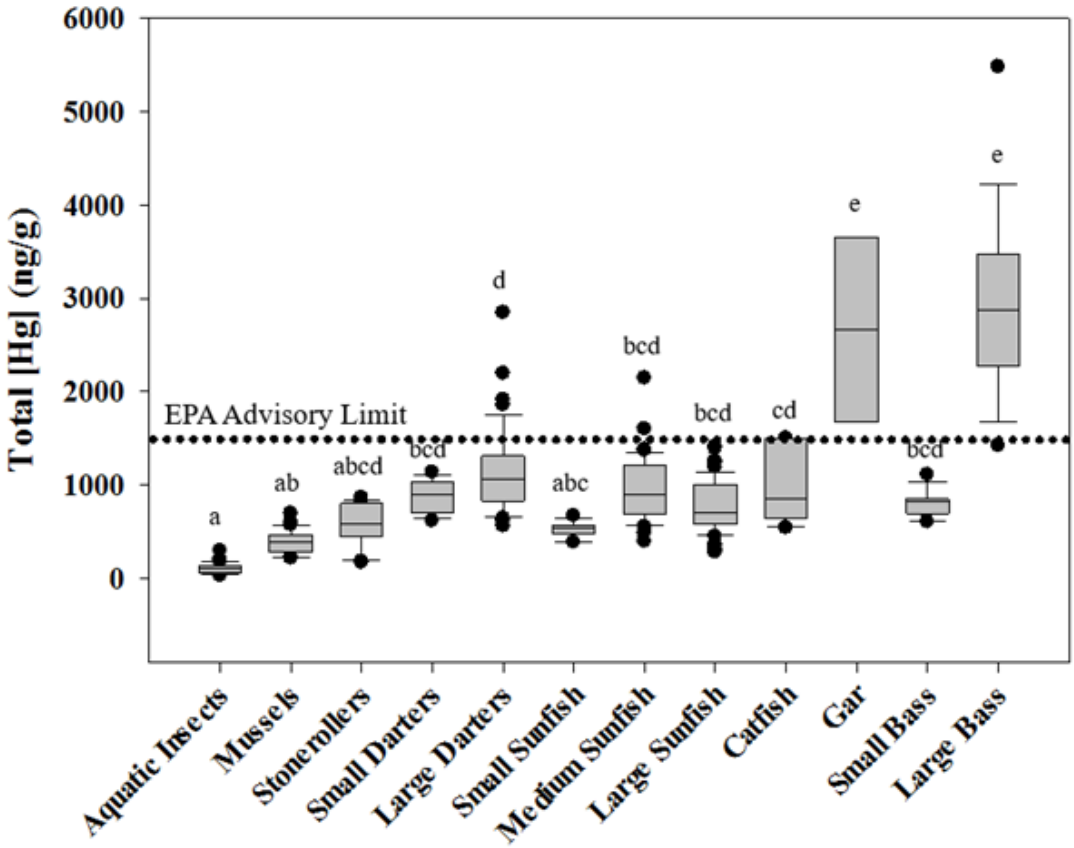


Figure 3. Mercury in Major Kiamichi River Taxa



SUPPLEMENTS

Mercury Analyses QA\QC

For QA/QC, we ran standards (DORM and PACS: Canada National Research Council) approximately every 10 samples while blanks and duplicates were run approximately every 20 samples. Standards had an average calibration factor of 1.0577 with a standard error of 0.0055 (n=40) for DORM and 1.0493 with a standard error of 0.0148 (n=9) for PACS. Blanks (empty sample runs) detected an average 0.1538 ng of mercury with a standard error of 0.0767 (n=43). Duplicates had an average difference of 13.6875% with a standard error of 3.4217% (n=24).

CHAPTER 2: BIOMASS DEPENDENT INTERACTIONS BETWEEN FRESHWATER CONSUMERS INCREASES MERCURY BURDEN IN BENTHIC FOOD WEBS

Brent N. Tweedy and Caryn C. Vaughn

Keywords:

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INTRODUCTION

Mercury contamination is a global scale environmental problem that negatively affects the health of both wildlife and humans[1]. Centuries of anthropogenic mercury emissions have resulted in elevated deposition of inorganic mercury in ecosystems around the world[2]. Inorganic mercury is not bioavailable and is deposited in low enough concentrations that it typically does not pose a health threat as it is deposited. However, when inorganic mercury is washed into aquatic ecosystems, it can be converted into organic methyl mercury by bacteria in the sediments[3] as well as in periphyton[4]. Methyl mercury is highly bioavailable and can biomagnify in food webs to concentrations high enough to adversely affect the health of wildlife and humans[5].

The movement of mercury through the environment has both biogeochemical and ecological components, thus factors which affect one or both of those aspects of the environment are of research interest. Freshwater mussels (Bivalvia: Unionidae, hereafter "mussels") are ecosystem engineers[6] known to strongly affect both biogeochemistry[7] and ecology[8] when they are present in high densities, and are thus a potentially significant, unexplored factor regulating the movement of mercury through freshwater ecosystems.

Freshwater mussels are a widespread and diverse group of sessile, benthic consumers which often occur at high densities in streams and rivers across eastern North America[8]. In rivers, mussels are often patchily distributed in dense aggregations (mussel beds) where they can comprise over 90% of the benthic biomass[7, 9, 10]. They are powerful filter feeders, feeding on seston from both from the water column and

interstitially from the sediments[11]. Mussels recycle and store nutrients[7, 12, 13], biodeposit feces and rejected food particles (pseudofeces)[14], and mix and oxygenate the sediments through their burrowing activities (bioturbation)[15]. Mussels provide habitat for algae and macroinvertebrates[15, 16] and can increase both primary and secondary production[13, 17, 18]. Because of these processes, mussels have strong effects on both biogeochemical cycling and food webs. Mussels have the potential to affect the fate of mercury in aquatic sediments by influencing the movement of mercury into the sediments, conditions related to methylation, or the release of methyl mercury from sediments. Mussels also have the potential to change the movement through and availability of mercury in aquatic food webs.

Mussels may influence mercury contamination of aquatic food webs by increasing the concentration of mercury in consumers' tissues as well as by increasing the burden, or pool, of mercury in the overall consumer population (Figure 1). Differences in consumer tissue concentration could be driven by mussel activities which affect the transformation of mercury into methyl mercury, the release of mercury from aquatic sediments, or movement of mercury already present in the food web. Changes that make mercury more, or less, available to consumers, such as the bioavailability of its chemical form or its concentration in the environment in relation to a consumer's spatial location, could directly influence the tissue concentrations of mercury in those consumers (Figure 1). The positive, bottom-up effects that mussels have on many consumer populations, namely increased consumer population biomass, could affect the burden of mercury in a population. Mercury burden is the product of biomass times tissue concentration and represents the total, physical amount of mercury in a

population. Increasing tissue concentrations or biomass while the other remains constant would increase mercury burden while increasing both would drastically increase mercury burden. The effects of mussels on both consumer mercury tissue concentrations and consumer biomass could interact to affect consumer mercury burden in several ways (Figure 1). We designed a mesocosm experiment to test the effects of mussels on both consumer mercury tissue concentration and consumer mercury burden. Specifically, we asked: 1) Does the presence of large mussel aggregations affect the mercury tissue concentration of consumers living with the mussels? And 2) Do changes in consumer biomass combine with tissue concentration to affect the mercury burden in consumer populations?

METHODS

Mesocosm Setup

We collected mussels and sediments from the Kiamichi River in southeastern Oklahoma, U.S., a 5th-order tributary of the Red River known for its high freshwater mussel and fish biodiversity[19]. This river is known to have elevated mercury concentrations in fish. The Oklahoma Department of Environmental Quality has issued consumption advisories for some fish species over the minimum Oklahoma advisory limit of 500ng/g in Sardis Lake (an impounded tributary of the river) and Hugo Lake (a mainstem impoundment of the river)[20]. In addition, previous surveys we conducted in the river show that numerous fish species have mercury concentrations that exceed the EPA and Oklahoma mercury consumption advisory limits (Tweedy unpublished data).

Following Allen & Vaughn (2009)[21], we conducted our experiment in recirculating mesocosms (n = 32) housed in a climate controlled greenhouse. Each mesocosm (94 X 44 cm) consisted of a plastic tub inlaid within a larger fiberglass tub. We mixed the sediments collected from the Kiamichi River with clean gravel (10-25mm diameter), and added this to mesocosms to a depth of approximately 12 cm. We filled the mesocosms with water from a nearby pond to depth of 16.5 cm and maintained flow rate of ~ 14 cm/s with a 1/32 horsepower pump in each mesocosm. We allowed the mesocosms to run for four weeks before starting the experiment.

Experimental Design

We used two species of mussels in the experiment, *Actinonaias ligamentina* (Lamark, 1819) and *Amblema plicata* (Say, 1817). These species are abundant in the region and Kiamichi River[22, 23] and have been shown to have strong effects on nutrient cycling and food webs in previous experiments[13, 17, 24]. These two species also encompass the range of differences in physiological tolerance and behavior among species in the river. *Amblema plicata* is a generally sedentary species while *A. ligamentina* is generally more active[25]. *Actinonaias ligamentina* is more sensitive to warm temperatures than *A. plicata,* and this sensitivity results in different, temperature-dependent filtration and excretion rates[26]. We collected mussels from two sites on the Kiamichi River one month before the start of the experiment and transported them to the laboratory in coolers with damp towels. We housed the mussels in two living streams (LS-700 Frigid Units Inc.) at ambient greenhouse temperatures while the mesocosms equilibrated.

We used four mussel density treatments (0, 4, 8, or 16 mussels per mesocosm) that were each replicated 8 times. These treatments correspond to mussel densities of

10, 19, and 38 mussels/m^2 and are within the range of natural mussel densities observed in the Kiamichi River[27]. We measured the length, width, and height, and individually tagged each mussel with prenumbered FLOY® (Floy, Seattle, Washington) shellfish tags. Mussels were randomly assigned to treatments and haphazardly placed in mesocosms. Individuals averaged 97.1cm (±8.5 standard deviation) and ranged 79.7-117.12cm in length for *A. plicata* and averaged 114.6 (±10.9 standard deviation) and ranged from 78.6-155.0cm in length for *A. ligamentina*.

We used snails as the primary response variable for consumer biomass in our study. Snails are important primary consumers in many freshwater systems. They also grow rapidly, and we observed sufficient biomass for mercury analysis in previous mesocosm studies. As benthic feeders, they are also likely to be more sensitive to changes in mercury availability caused by mussels since they live near and on the sediments where mercury is transformed and released. Prior to the experiment, we hand collected snails (*Physella acuta* (Draparnaud, 1805)) from a nearby pond and established a breeding colony in the lab. At the start of the experiment we added an inoculation of 2.5g wet biomass of *P. acuta* to each mesocosm, excluding abnormally large individuals. During the experiment we noticed the mesocosms had been colonized by a second snail species *Gyraulus parvus* (Say, 1817), likely introduced through river sediments or pond water used to fill and maintain the mesocosms. The sediments were taken from a single site on the Kiamichi river and all mesocosms used a common water source, so this effect was homogenous across all mesocosms.

Experimental Procedure

We ran the experiment from July 17th through December 2nd of 2014. During the experiment, all mesocosms were dosed with a natural assemblage of cultured pond algae every three to four days to provide food for the mussels[25]. We also monitored water quality parameters regularly (SI Table 1). At the end of the experiment we sampled snail biomass (both species) by removing all snails found on the surface of a 15x15 cm clay tile placed in the mesocosm. We measured individual snails to the nearest 0.1mm and used published length-weight regressions for *G. parvus* and *Physella integra* (a closely related species to *P. acuta*) to calculate wet biomass[28]. We divided snail biomass by the sample area of the clay tile ($0.0225 m^2$) thus all biomass values are expressed in grams of snail biomass per m^2. In addition, at the end of the experiment we removed all visible snails from each mesocosm to ensure sufficient biomass for mercury analysis. We combined all snails from both the quantitative and the mass sample into a single composite sample for each mesocosm for subsequent mercury analyses.

Mercury Analysis

We conducted mercury analysis for composite snail samples from each mesocosm using a Milestone DMA80 at the Texas Christian University Aquatic Ecology Lab. QA\QC methods and data can be found in the SI. All mercury concentrations are expressed in nanograms of total mercury per gram of dry snail tissue per m^2. We also estimated the mercury burden, or pool, for each mesocosm. Mercury burden represents the physical amount of mercury present in the snails in each mesocosm and was calculated as the product of mercury concentration of each composite sample times the total biomass of

snails collected per unit area in the mesocosm. As such, mercury burden encompasses both the changes in mercury concentration and changes in snail biomass that could be affected by mussels. All mercury burden results are expressed as µg total mercury per m^2.

Statistical Analyses

We natural log transformed all data to ensure normality for all samples as indicated by a Shapiro-Wilk test. Homogeneity of variance could not be achieved for all treatments and response variables through transformation. Therefore, we tested mean differences between mussel density treatments in snail biomass, mercury concentration, and mercury burden with a Welch's t-Test. A Games-Howell post-hoc test was used to determine significance between treatment groups. All analyses were conducted in SPSS version 19.0.0. Statistical values for all tests can be found in SI Table 2.

RESULTS AND DISCUSSION

Snail biomass ranged from 62 to 3573 g snail tissue/m^2. Snail biomass was significantly higher in 8 and 16 mussel treatments than in no and 4 mussel treatments (Figure 2A). This is consistent with our expectations and past literature that mussels increase consumer biomass[29].

Total mercury concentrations in snail tissue ranged from 3 to 63 ng total mercury /g snail tissue. Snail tissue mercury concentrations were positively related to mussel density, with snails in the highest mussel density treatment having the highest

mercury concentration (Figure 2B). We also found no correlation between the percentage that a species contributed to the total biomass of a mesocosm and the composite snail mercury concentration of that mesocosm ($\rho = -0.241$, $p = 0.184$) indicating that the occurrence of one species was not driving the relationships observed. We were not able to measure the percent of mercury in snail tissue that was methyl mercury. However, our total mercury values are within the range for snails found in study in a mercury-contaminated lake, Caddo Lake, Texas[30]. In those snails, approximately 50% of the total mercury was methyl mercury. Assuming percentage of methyl mercury is the same for the snails in our study, the total mercury concentrations we found are also in the range of other mesocosm studies that measured mercury concentrations in snail tissue[31].

Finally, the observed snail biomass and tissue concentration patterns combine to create an amplified mercury burden effect. Snail mercury burdens ranged from 0.48 to 105 μg total mercury / m^2. There was a significant positive relationship between mussel density and snail mercury burden (Figure 2C). We observed very little mercury burden in the absence of mussels, while burdens in in the medium and high-density treatments were 10x higher on average. Snails are an integral part of many aquatic food webs and thus these increases in mercury burden mean that there is more mercury present at the base of the food web that can then be assimilated by higher-level consumers, such as fish or birds. Such increases in overall mercury load to top consumers could pose a health risk to the consumers themselves as well as consumers further along the food chain, including humans.

Freshwater mussels have strong bottom up effects on food webs, usually by stimulating primary production[16]. Stimulation of productivity alone by mussels would likely result in decreased mercury tissue concentrations through the process of bloom dilution. Bloom dilution assumes that the burden of mercury in a population stays constant, so as biomass increases, the mercury is spread out among more biomass resulting in lower mercury concentrations in consumers and recent mesocosm studies have supported this hypothesis[31]. However, in our study we observed the opposite trend; higher mercury concentrations in systems with productivity stimulated by mussels (Figure 2B). This suggests that in addition to bottom up effects on biomass, mussels are also affecting processes that determine the amount of mercury available to the food web. There are several possible mechanisms through which mussels could facilitate this pattern.

First, mussels may alter the conversion of mercury into methyl mercury and/or its subsequent release from aquatic sediments. As they move, mussels mix or 38ioturbated the sediments[15]. Several studies have observed increased mercury methylation in the presence of bioturbators[32, 33] or increased net methyl mercury production, possibly due to the inhibition of bacterial species which demethylate methylmercury[34]. Higher efflux of methyl mercury has also been noted as a likely effect of bioturbators[33, 35]. While no direct connection has been observed between mercury methylation and release and the activity of freshwater mussels, we do know that they can affect microbial denitrification processes[36] in the same manner as marine bivalves[37] and invasive freshwater bivalves[38, 39], which have been studied with respect to mercury. In fact, the factors governing denitrification processes are very similar to those

governing mercury methylation processes and have been used as the basis for understanding marine bioturbators' effects on mercury methylation[33]. Therefore, freshwater mussels are likely affecting mercury cycling in a manner similar to that of marine bioturbators. This mechanism is a potential explanation for why bloom dilution was not observed, and our systems have increased mercury burdens in addition to increased consumer biomass. Focused studies on the role freshwater mussels play in sediment mercury dynamics could confirm this hypothesis.

Alternatively, mussel filter feeding activity can translocate large quantities of phytoplankton and particulate matter from the water column into the benthos, both on the surface of sediments as well as in the sediments themselves, in the form of feces and pseudofeces[15]. It is possible that rather than altering the flux of mercury from the sediments, instead mussels are moving mercury between benthic and pelagic food web compartments. By translocating phytoplankton and other organisms from the water column into the benthic sediments, where snails feed, mussels could be concentrating the available mercury pool in the snails' food web. In this model, rather than increasing the total burden of mercury in the environment, mussels are relocating the mercury burden from the pelagic food web into the benthic food web. If this were the case we would predict higher mercury concentrations and burdens in benthic consumers, such as snails, and lower mercury concentrations and burdens in pelagic feeding consumers, like filtering caddisflies or other dipterans. Unfortunately, we do not have the samples to test this hypothesis but think that it warrants further study.

Regardless of mechanism, many rivers and streams across eastern North America are influenced by mussel activity and according to our results, may be more sensitive to mercury contamination than previously thought. The southeastern U.S. has the highest global diversity and highest overall abundance of freshwater mussels[8] and these mussels play important roles in the functioning of these ecosystems with many other organisms, including humans, depending on ecosystems they provide. Unfortunately, this region also receives highly elevated wet mercury deposition (Figure 3). This region is also predominantly forested, which has been shown to lead to increased mercury concentrations in top level consumers[40]. Our results indicate that mussels may be making mercury even more prevalent in these systems, or at the very least concentrating it in specific food webs. This has significant ramifications for higher level consumers in these systems. Mercury emissions and subsequent pollution are projected to increase even under optimistic scenarios[2, 41]. As mercury emissions, and subsequent deposition, increase more of this mercury may be made available to food webs in mussel dominated rivers and streams leading to higher mercury concentrations in and more dire risks to top level consumers, including humans.

Mercury contamination is not the only stressor impacting eastern North American Rivers. Rivers in this area are also highly impacted by numerous other stressors including impoundment, land-use change, and climate change[42-44], a situation not unique to this region[45]. While the impacts of these individual changes, or stressors, are being studied it is less clear how these stressors will affect aquatic ecosystems in concert. With projected mercury levels on the rise, looming increases in the severity of the effects of climate change, and numerous other longstanding stressors the question

becomes, “How much is too much?” If mussel activity exacerbates mercury contamination in these systems, then understanding the dynamics and impacts of the global mercury crisis on these systems may be an important factor to consider in attempts to manage and conserve our declining freshwater ecosystems.

In summary, our results indicate that the impacts of mussels, likely through effects on the biogeochemical and ecological functioning of ecosystems, increase mercury tissue concentration and mercury burden of benthic consumers. More work is needed to understand the precise mechanism underlying these observations, the implications for the rest of the aquatic, and terrestrial, food web, and the ramifications of elevated mercury levels both alone and in combination with other stressors prevalent in these unique and imperiled ecosystems.

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REFERENCES

1. Scheulhammer, A. M.; Meyer, M. W.; Sandheinrich, M. B.; Murray, M. W., Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 2007, 36, (1), 12-18.

2. Amos, H. M.; Jacob, D. J.; Streets, D. G.; Sunderland, E. M., Legacy impacts of all-time anthropogenic emissions on the global mercury cycle. *Global Biogeochemical Cycles* **2013,** *27*, (2), 410-421.

3. Morel, F. M. M.; Kraepiel, A. M. L.; Amyot, M., The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology and Systematics* **1998,** *29*, 543-566.

4. Tsui, M. T. K.; Finlay, J. C.; Balogh, S. J.; Nollet, Y. H., In Situ Production of Methylmercury within a Stream Channel in Northern California. *Environmental Science & Technology* **2010,** *44*, (18), 6998-7004.

5. Selin, N. E., Global Biogeochemical Cycling of Mercury: A Review. *Annual Review of Environment and Resources* **2009,** *34*, 43-63.

6. Gutierrez, J. L.; Jones, C. G.; Strayer, D. L.; Iribarne, O. O., Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos* **2003,** *101*, (1), 79-90.

7. Atkinson, C. L.; Vaughn, C. C., Biogeochemical hotspots: temporal and spatial scaling of the impact of freshwater mussels on ecosystem function. *Freshwater Biology* **2015,** *60*, (3), 563-574.

8. Haag, W. R., *North American Freshwater Mussels*. Cambridge University Press: Cambridge, 2012.

9. Ökland, J., Notes on population density, age distribution, growth, and habitat of Anodonta piscinalis Nilss. (Moll., Lamellibr.) in a eutrophic Norwegian lake. *Nytt Magasin for Zoologi* **1963,** *11*, 19-43.

10. Negus, C. L., A quantitative study of growth and production of unionid mussels in the River Thames at Reading. *Journal of Animal Ecology* **1966,** *35*, 513-532.

11. Vaughn, C. C.; Nichols, S. J.; Spooner, D. E., Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society* **2008,** *27*, (2), 409-423.

12. Vaughn, C. C., Biodiversity Losses and Ecosystem Function in Freshwaters: Emerging Conclusions and Research Directions. *Bioscience* **2010,** *60*, (1), 25-35.

13. Atkinson, C. L.; Vaughn, C. C.; Forshay, K. J.; Cooper, J. T., Aggregated filter-feeding consumers alter nutrient limitation: consequences for ecosystem and community dynamics. *Ecology* **2013,** *94*, (6), 1359-1369.

14. Strayer, D. L., Understanding how nutrient cycles and freshwater mussels (Unionoida) affect one another. *Hydrobiologia* **2014,** *735*, (1), 277-292.

15. Vaughn, C. C.; Hakenkamp, C. C., The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology* **2001,** *46*, (11), 1431-1446.

16. Spooner, D. E.; Vaughn, C. C., Species' traits and environmental gradients interact to govern primary production in freshwater mussel communities. *Oikos* **2012,** *121*, (3), 403-416.

17. Allen, D. C.; Vaughn, C. C.; Kelly, J. F.; Cooper, J. T.; Engel, M. H., Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. *Ecology* **2012,** *93*, (10), 2165-2174.

18. Howard, J. K.; Cuffey, K. M., The functional role of native freshwater mussels in the fluvial benthic environment. *Freshwater Biology* **2006,** *51*, (3), 460-474.

19. Matthews, W. J.; Vaughn, C. C.; Gido, K. B.; Marsh-Matthews, E., Southern Plains rivers. In *Rivers of North America*, Benke, A. C.; Cushing, C. E., Eds. Burlington, MA, 2005; pp 283-325.

20. Mercury in Fish: A Guide to Healthy Fish Consumption in Oklahoma. In Quality, O. D. o. E., Ed. 2013.

21. Allen, D. C.; Vaughn, C. C., Burrowing behavior of freshwater mussels in experimentally manipulated communities. *Journal of the North American Benthological Society* **2009,** *28*, (1).

22. Spooner, D. E.; Vaughn, C. C., Species richness and temperature influence mussel biomass: A partitioning approach applied to natural communities. *Ecology* **2009,** *90*, 781-790.

23. Galbraith, H. S.; Spooner, D. E.; Vaughn, C. C., Synergistic effects of regional climate patterns and local water management on freshwater mussel communities. *Biological Conservation* **2010,** *143*, 1175-1183. .

24. Vaughn, C. C.; Spooner, D. E.; Galbraith, H. S., Context-dependent species identity effects within a functional group of filter-feeding bivalves. *Ecology* **2007,** *88*, (7), 1654-1662.

25. Allen, D. C.; Vaughn, C. C., Burrowing behavior of freshwater mussels in experimentally manipulated communities. *Journal of the North American Benthological Society* **2009,** *28*, 93-100.

26. Spooner, D. E.; Vaughn, C. C., A trait-based approach to species' roles in stream ecosystems: climate change, community structure, and material cycling. *Oecologia* **2008,** *158*, (2), 307-317.

27. Galbraith, H. S.; Spooner, D. E.; Vaughn, C. C., Synergistic effects of regional climate patterns and local water management on freshwater mussel communities. *Biological Conservation* **2010,** *143*, (5), 1175-1183.

28. Eckblad, J. W., Weight-Length Regression Models for Three Aquatic Gastropod Populations. *American Midland Naturalist* **1971,** *85*, (1), 271-274.

29. Spooner, D. E.; Vaughn, C. C., Context-dependent effects of freshwater mussels on stream benthic communities. *Freshwater Biology* **2006,** *51*, (6), 1016-1024.

30. Chumchal, M. M.; Rainwater, T. R.; Osborn, S. C.; Roberts, A. P.; Abel, M. T.; Cobb, G. P.; Smith, P. N.; Bailey, F. C., Mercury Speciation and Biomagnification in the Food Web of Caddo Lake, Texas and Louisiana, USA, a Subtropical Freshwater Ecosystem. *Environmental Toxicology and Chemistry* **2011,** *30*, (5), 1153-1162.

31. Walters, D. M.; Raikow, D. F.; Hammerschmidt, C. R.; Mehling, M. G.; Kovach, A.; Oris, J. T., Methylmercury Bioaccumulation in Stream Food Webs Declines with Increasing Primary Production. *Environmental Science & Technology* **2015,** *49*, (13), 7762-7769.

32. Correia, R. R. S.; Guimaraes, J. R. D., Impacts of crab bioturbation and local pollution on sulfate reduction, Hg distribution and methylation in mangrove sediments, Rio de Janeiro, Brazil. *Marine Pollution Bulletin* **2016,** *109*, (1), 453-460.

33. Benoit, J. M.; Shull, D. H.; Robinson, P.; Ucran, L. R., Infaunal burrow densities and sediment monomethyl mercury distributions in Boston Harbor, Massachusetts. *Marine Chemistry* **2006,** *102*, (1-2), 124-133.

34. Nogaro, G.; Hammerschmidt, C. R., Influence of benthic macrofauna on microbial production of methylmercury in sediments on the New England continental shelf. *Hydrobiologia* **2013,** *701*, (1), 289-299.

35. Guimaraes, J. R. D.; Ikingura, J.; Akagi, H., Methyl mercury production and distribution in river water-sediment systems investigated through radiochemical techniques. *Water Air and Soil Pollution* **2000,** *124*, (1-2), 113-124.

36. Benelli, S.; Bartoli, M.; Racchetti, E.; Moraes, P. C.; Zilius, M.; Lubiene, I.; Fano, E. A., Rare but large bivalves alter benthic respiration and nutrient recycling in riverine sediments. *Aquatic Ecology* **2017,** *51*, (1), 1-16.

37. Smyth, A. R.; Geraldi, N. R.; Piehler, M. F., Oyster-mediated benthic-pelagic coupling modifies nitrogen pools and processes. *Marine Ecology Progress Series* **2013,** *493*, 23-30.

38. Bruesewitz, D. A.; Tank, J. L.; Bernot, M. J., Delineating the effects of zebra mussels (Dreissena polymorpha) on N transformation rates using laboratory mesocosms. *Journal of the North American Benthological Society* **2008,** *27*, (2), 236-251.

39. Turek, K. A.; Hoellein, T. J., The invasive Asian clam (Corbicula fluminea) increases sediment denitrification and ammonium flux in 2 streams in the midwestern USA. *Freshwater Science* **2015,** *34*, (2), 472-484.

40. Drenner, R. W.; Chumchal, M. M.; Jones, C. M.; Lehmann, C. M. B.; Gay, D. A.; Donato, D. I., Effects of Mercury Deposition and Coniferous Forests on the Mercury Contamination of Fish in the South Central United States. *Environmental Science & Technology* **2013,** *47*, (3), 1274-1279.

41. Pacyna, E. G.; Pacyna, J. M.; Sundseth, K.; Munthe, J.; Kindbom, K.; Wilson, S.; Steenhuisen, F.; Maxson, P., Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. *Atmospheric Environment* **2010,** *44*, (20), 2487-2499.

42. Magilligan, F. J.; Nislow, K. H., Changes in hydrologic regime by dams. *Geomorphology* **2005,** *71*, (1-2), 61-78.

43. Kaushal, S. S.; Likens, G. E.; Jaworski, N. A.; Pace, M. L.; Sides, A. M.; Seekell, D.; Belt, K. T.; Secor, D. H.; Wingate, R. L., Rising stream and river temperatures in the United States. *Frontiers in Ecology and the Environment* **2010,** *8*, (9), 461-466.

44. Sudduth, E. B.; Meyer, J. L.; Bernhardt, E. S., Stream restoration practices in the southeastern United States. *Restoration Ecology* **2007,** *15*, (3), 573-583.

45. Strayer, D. L.; Dudgeon, D., Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society* **2010,** *29*, (1), 344-358.

46. Watters, G. T., Essay 4: Freshwater Mussels: A Complicated Resource. In *Freshwater Ecoregions of North America: A Conservation Assessment*, Abell, R. A.; Olson, D. M.; Einerstein, E.; Hurley, P. T.; Diggs, J. T.; Eichbaum, W.; Walters, S.; Wettengel, W.; Allnutt, T.; J., L. C.; Hedao, P., Eds. Island Press: Washington, D.C. Covelo, CA, 2000; pp 37-41.

Table 1. Average water quality parameters for mesocosm treatments

Month	Mussel Treatment	Temperature (°C)	pH	Conductivity (µS)	TDS (ppm)	Chlorophyll-*α* (ug\L)	Dissolved Oxygen (mg/L)
July	0	19.8	8.87	619	439	0.0074	9.24
	4	19.7	8.83	595	422	0.0069	9.24
	8	19.9	8.86	594	421	0.0114	9.24
	16	19.7	8.78	594	422	0.0109	9.25
August	0	24.2	8.98	677	480	0.0105	11.37
	4	24.5	8.87	664	471	0.0044	11
	8	25	9.07	657	466	0.0049	11.5
	16	24.6	8.97	672	477	0.0081	12.41
September	0	23.4	8.92	721	512	0.0088	10.61
	4	23.5	9	709	503	0.0044	10.7
	8	23.6	9.01	724	514	0.0039	10.42
	16	23.4	8.94	732	519	0.0048	10.67
October	0	22.2	8.91	659	472	0.0323	10.22
	4	21.7	8.95	643	456	0.0028	10.25
	8	22.1	9.01	654	464	0.0032	10.52
	16	21.8	8.88	670	475	0.01	9.7

Table 2. Statistical values for Welch's T-test

Value	Statistic	df1	df2	p-value
Snail Biomass	3.686	3	15.335	0.035
Total [Hg]	10.536	3	14.796	0.001
Mercury Burden	13.673	3	15.08	>0.001

FIGURE LEGENDS

Figure 1. Predicted effects of mussels on tissue concentrations and burden (concentration X biomass). The blue line (H_{1A}) shows a positive effect of mussels on tissue concentrations, the orange line (H_{1B}) a negative effect, and the black line (H_{1C}) assumes no change in tissue concentration. The bottom panel multiplies the lines in the first panel by an assumed increase in biomass due to increased mussel density.

Figure 2. Snail biomass (g/m^2) (A), tissue mercury concentrations (ng/g) (B), and mercury burdens ($\mu g/m^2$) (C) for each mussel treatment. Letter groupings indicate significant differences between groups as indicated by Welch's t-Test with a Games-Howell post-hoc test at $p < 0.05$.

Figure 3. A) Map of wet mercury deposition across North America in 2015 from the National Atmospheric Deposition Program (NRSP-3) 2017. B) Distribution of freshwater mussel species in North America from *Freshwater Ecoregions of North America: A Conservation Assessment,* by Robin A. Abell, David M. Olson, Eric Dinerstein, and Patrick T. Hurley et al. Copyright © 2000 World Wildlife Fund. Reproduced by permission of Island Press, Washington, D.C(Watters 2000).

FIGURES

Figure 1. Conceptual Figure of Effects of Mussels on Mercury Contamination

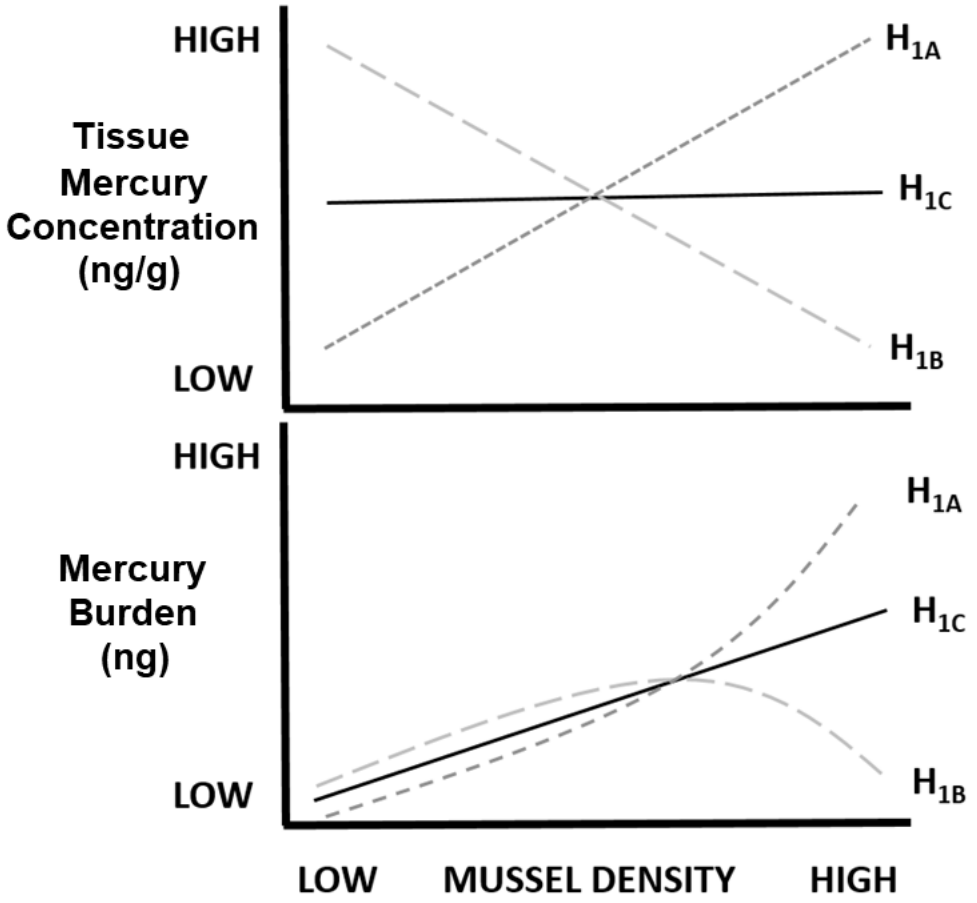


Figure 2. Snail Biomass, Mercury Concentration, and Mercury Burden

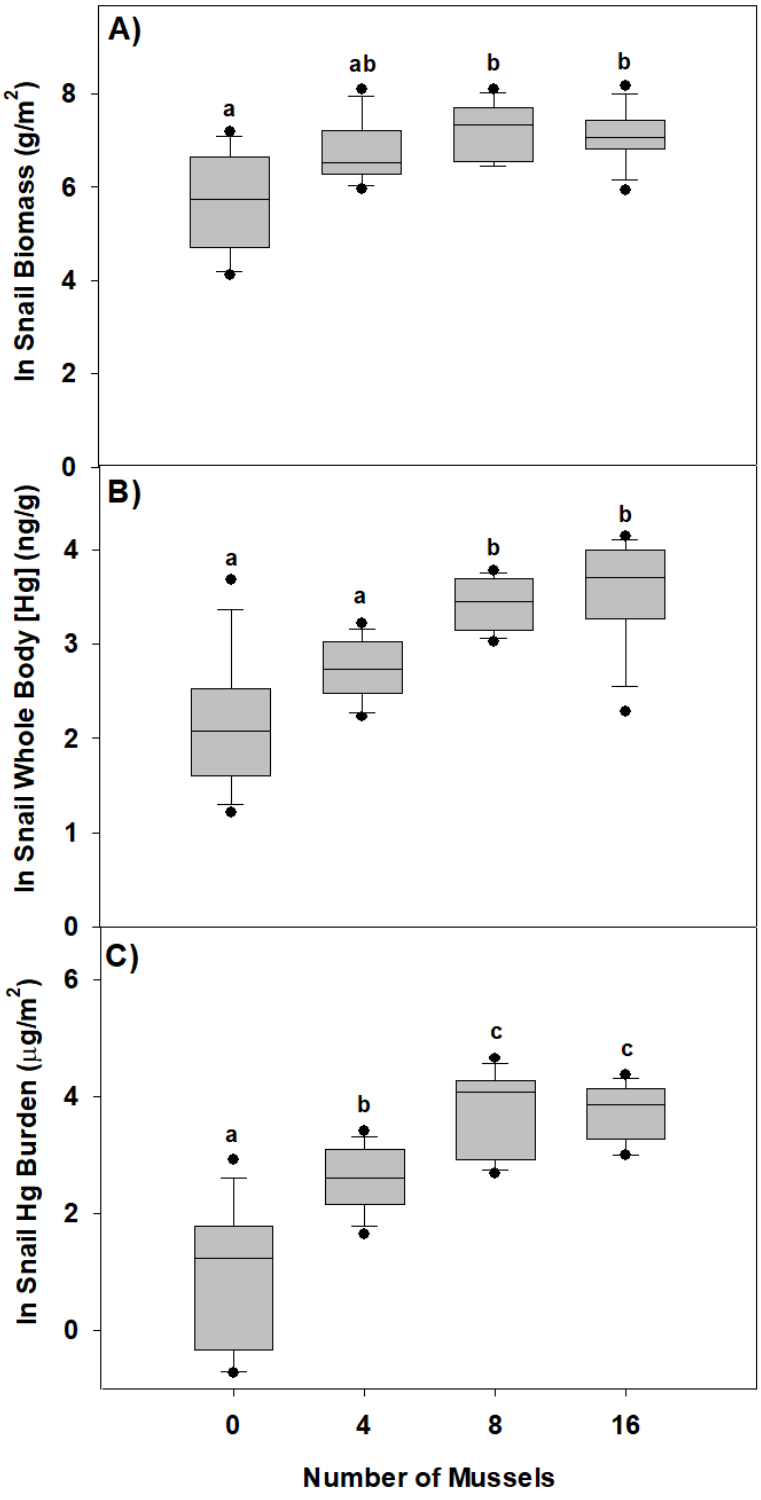
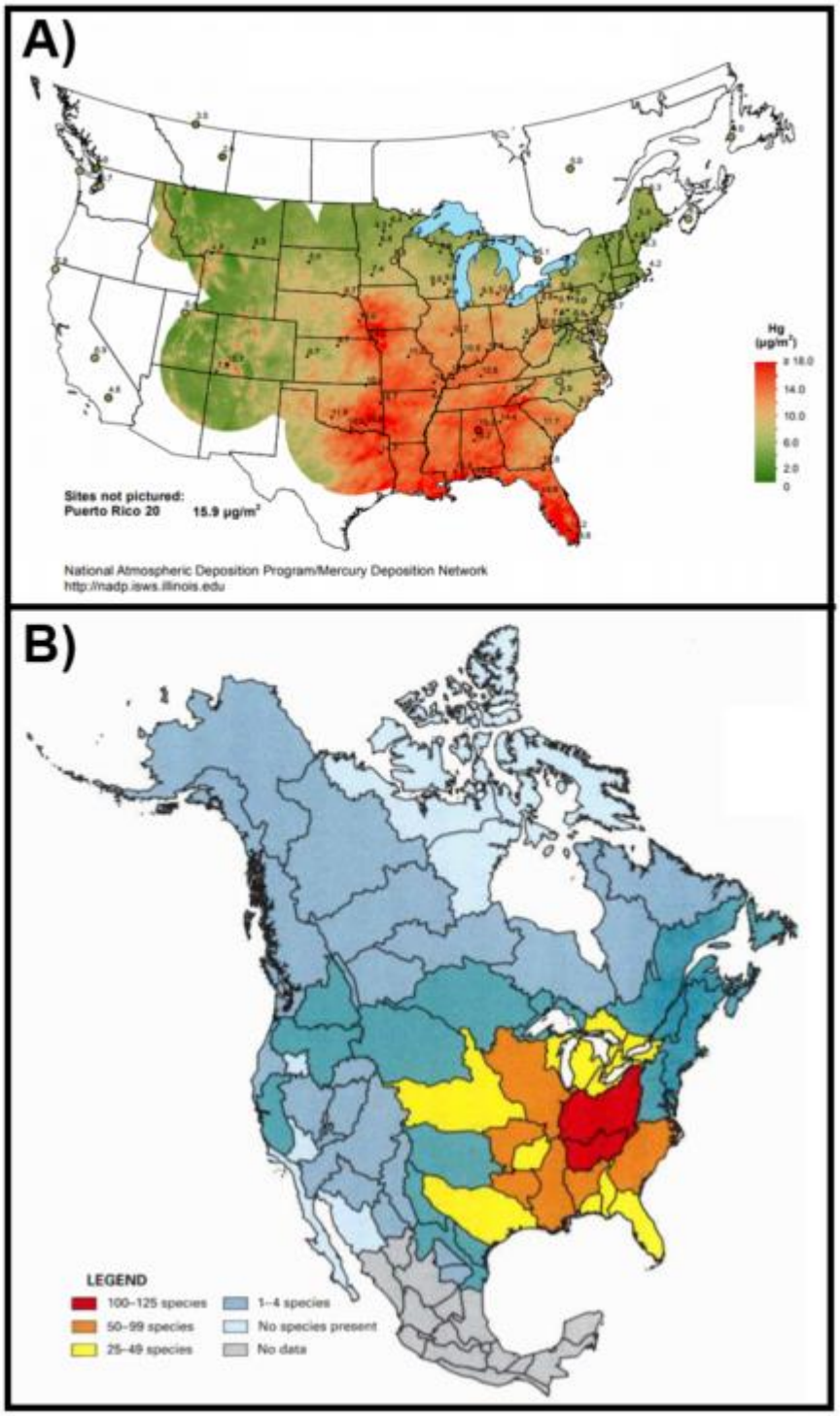


Figure 3. Map of Annual Mercury Deposition and Freshwater Mussel Diversity in North America



SUPPLEMENTS

Mercury Analysis QA\QC

Thirty-two samples were run. Blanks (empty sample containers) were run approximately every 10 samples and yielded an average [Hg] of 0.19 ± 0.001 ng/g (n=3). DORM 4 (National Research Council Canada) was used as a standard and run approximately every 10 samples and had an average calibration factor of 1.005 ± 0.014 (n = 4). Entire snail composite samples were run for each tank, so no duplicate samples were used.

CHAPTER 3: COMBINED EFFECTS OF MERCURY AND TEMPERATURE ON FRESHWATER MUSSELS AND ECOSYSTEM FUNCTION

Brent N. Tweedy and Caryn C. Vaughn

Keywords:

mercury, multiple stressors, climate change, freshwater ecosystems, mussels, ecosystem function

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INTRODUCTION

Throughout history two processes have been a consistent theme in human survival and technological advancement: using metals and burning fuels. In prehistoric times, humans burned wood and made crude copper tools to carve out a competitive edge. Over the millennia, advancement of these skills was often related. For example, learning to burn fuels at higher temperatures allowed humans to forge tools from iron as early as 1200 BC (Waldbaum, 1978). Newly acquired tools allowed people to obtain and use more metals, such as silver and gold and eventually alloys, and ultimately led to access to fossil fuels and increasingly rapid technological advancement (Smil, 2004). However, these advancements have come at a cost to the environment. Human extraction and manipulation of metals and burning of fossil fuels are now altering cycles and processes at a global scale (Broecker *et al.*, 1979, Thornton, 1996, Vitousek *et al.*, 1997, Wright & Schindler, 1995).

Anthropogenic releases of metals have increased steadily over the last few centuries and they are now a common environmental stressor (Nriagu, 1996). The most severe environmental effects of these metals are generally limited to local areas where mining occurs (Förstner & Wittmann, 2012). Mercury, a highly volatile metal, is an exception to this trend. The earliest large-scale use of mercury was as an amalgam to aid in gold mining; more recently it has been introduced into the environment from industrial applications and as a byproduct of burning coal (Streets *et al.*, 2011). Humans now contribute two-thirds of all mercury to the planet's global mercury pool, primarily through burning coal and the use of mercury in small-scale gold mining. Emissions

from these sources enter a global atmospheric mercury pool, often travelling vast distances from their points of origin over the course of months or years to be deposited across the landscape (Selin, 2009). When this inorganic mercury is washed into waterbodies, microbes in anoxic sediments, primarily sulfate-reducing bacteria, can transform the less toxic inorganic mercury into methyl mercury (MeHg), a highly toxic form (Morel *et al.*, 1998). Methyl mercury readily assimilates in aquatic food webs and in top-level predators can biomagnify to concentrations high enough to pose a major health risk for both wildlife and humans (Scheulhammer *et al.*, 2007). Even under optimistic emissions scenarios, there is already enough mercury in the environment to result in increasing contamination for decades to come (Amos *et al.*, 2013).

Emissions from burning coal, and other fossil fuels, have also altered the global climate. While the impacts of GCC on freshwater ecosystems are diverse (Ficke *et al.*, 2007), perhaps the most obvious impact is increased water temperatures. Average global temperatures have already increased by 0.85℃ from 1880 to 2012 and are projected to increase an additional 0.3-4.8℃ by the end of the century (IPCC, 2014). The resulting changes to thermal regimes in river systems is one of the most significant impacts that GCC will have on freshwater systems (Heino *et al.*, 2009, Malmqvist & Rundle, 2002). Furthermore, human demands on freshwater resources will place further strain on warming systems (Kundzewicz *et al.*, 2008). Many fish and aquatic invertebrate species have narrow thermal tolerances and will be faced with receding habitat because of these changes (Poff *et al.*, 2010, Wenger *et al.*, 2011).

There is already evidence that toxic stress from mercury can interact with thermal stress to negatively impact organisms. Concentrations of mercury sublethal to fiddler crabs (*Uca pugilator*) have been shown to reduce respiration and survival when thermal and salinity stress were applied in addition to mercury stress (Vernberg & Vernberg, 1971), while in terrestrial systems mercury exposure was shown to reduce heat tolerance and heat hardening ability of soil dwelling springtails (Slotsbo *et al.*, 2009). However, combinations of stress caused by mercury and GCC is particularly interesting for freshwater systems because of the unique dynamics both stressors have in those systems. Mercury is converted into its toxic form almost exclusively in aquatic systems and thus species living in or feeding in those systems are most heavily exposed and impacted. Additionally, riverine species often have a very limited ability to respond to rising temperatures associated with GCC because of barriers to movement unique to aquatic environments such as the dendritic arrangement of river systems, the relative rarity of dispersal-enhancing events like floods, and longitudinal variation of range controlling factors, such as temperature, pH, or habitat availability, along lengths of rivers (Olden *et al.*, 2010, Ostfeld *et al.*, 2012, Spooner *et al.*, 2011). Furthermore, droughts in many regions will become increasingly frequent and severe and, combined with poor water management policies in the face of dwindling freshwater resources, this will likely compound the thermal stress experienced by aquatic organisms (Xenopoulos *et al.*, 2005). In light of these unique factors, it is critical that we understand how multiple anthropogenic stressors, such as mercury contamination and increased temperature associated with GCC, will affect freshwater systems across multiple levels of organization (Woodward *et al.*, 2010).

The interactive effects of stressors resulting from GCC and chemical contaminants extend beyond the organismal level (Hooper *et al.*, 2013) to affect ecosystem function (Moe *et al.*, 2013). At the scale of individual organisms, survival is the most frequently used endpoint to assess the effects of stress. However, survival is a coarse and final endpoint. Finer-grained sublethal endpoints, such as physiological condition and function, often allow assessment of pre-mortality effects of stressors (Ankley & Villeneuve, 2006). Scaling up, lethal and sublethal effects of stressors on individual organisms can have effects at the ecosystem level. For instance, the loss of native freshwater mussels in a severe drought resulted in losses in carbon, nitrogen, and phosphorus cycling and storage and filtration capacity (Atkinson *et al.*, 2014a, Vaughn *et al.*, 2015). In a similar event, mass mortality of an invasive freshwater clam also resulted in drastically reduced filtration capacity and changes in phosphorus dynamics (McDowell *et al.*, 2017). However, even if stress is not severe enough to kill organisms, stress-related changes to their activity and physiology can have significant ramifications for ecosystem processes. For example, thermal stress induced changes in freshwater mussel individual physiological functions have been demonstrated to alter population level ecosystem processes and services (Spooner & Vaughn, 2008, Vaughn *et al.*, 2015). Therefore, it is important to not only consider the effects of stressors on individual organisms, but to also consider the ramifications of those stressors at ecosystem level processes.

An ideal system in which to study the combined effects of these stressors are North American rivers. These rivers, particularly in the southeastern U.S., have high mercury deposition levels (Drenner *et al.*, 2013) and are already experiencing warmer

water temperatures and increased drought associated with GCC (Melillo *et al.*, 2014). These rivers are also home to the highest global diversity of freshwater mussels (Bivalvia: Unionidae; hereafter "mussels") (Lopes-Lima *et al*., 2014). Mussels are large, long-lived, sedentary invertebrates that occur as dense, species-rich aggregations, mussel beds, in rivers (Strayer *et al*., 2004). Mussels can filter immense volumes of water (Chowdhury *et al.*, 2016, Welker & Walz, 1998) and influence nutrient cycling by excreting nitrogen and phosphorus, biodepositing feces and pseudofeces, and storing nutrients in their tissue (Atkinson *et al.*, 2017a, Atkinson & Vaughn, 2015, Strayer, 2014). Mussels also have strong interactions with other species by providing and modifying habitat (Beckett *et al.*, 1996, Spooner & Vaughn, 2006) and supporting aquatic (Atkinson *et al.*, 2014b, Howard & Cuffey, 2006, Limm & Power, 2011, Spooner *et al.*, 2012, Vaughn *et al.*, 2007) and terrestrial (Allen *et al.*, 2012) food webs. Previous studies have shown that mussels are sensitive to temperature (Archambault *et al.*, 2014, Ferreira-Rodriguez & Pardo, 2017, Ganser *et al.*, 2015, Spooner & Vaughn, 2008) and mercury (Valenti *et al.*, 2005), and while the effects of both stressors on mussels have not been examined in freshwater bivalves, interactive effects of these two stressors have been shown to impact marine bivalves (Nelson *et al.*, 1977, Verlecar *et al.*, 2007). Further, we know that freshwater mussels tend to have atypically high concentrations of methyl mercury in their tissues for a primary consumer (Chumchal *et al.*, 2011) and that the presence of mussels can enhance the flux of mercury into aquatic food webs (Tweedy, 2017).

In this paper, we explore the effects of combined mercury and temperature stress on mussels and the ecosystem functions that they provide (Fig. 1). We present the results of two experiments and a geographic information system (GIS) exercise. We used a small-scale laboratory experiment to investigate the effects of toxic mercury stress and thermal stress on the physiological performance of two common mussel species. We conducted a mesocosm experiment to examine the effects of both stressors on a three-species community of mussels and the ecosystem processes they influence. Finally, we performed a GIS analysis using global mercury deposition, projected GCC temperature increases, and freshwater fish biodiversity to explore which global regions are most threatened by future GCC and mercury contamination.

METHODS

Mussel Source and Collection

Rivers in southeastern Oklahoma, U.S., are biodiversity hotspots for mussels (Master *et al.*, 1998, Matthews *et al.*, 2005), and are known to be impacted by both mercury contamination (ODWC, 2016, Tweedy, 2017) and climate warming. To ensure a consistent history of mercury exposure, we collected the mussels used in both experiments described below from a single site in the Kiamichi River, a well-studied river in this region where mercury contamination in mussels and other organisms has been quantified (Tweedy 2017). We transported mussels back from the field in coolers with a damp towel and held mussels in 500-L, recirculating tanks (Frigid Units Inc.

Living Stream model 700) inside a climate controlled greenhouse until the start of each experiment. Mussels were fed cultured pond algae every 2-3 days and we conducted regular water changes until the start of each experiment.

Individual Level Impacts

Mussels are thermo-conformers whose physiological performance and subsequent ecosystem functions vary along ecological gradients (Vaughn, 2010). We performed a laboratory experiment to assess two essential components of mussel performance, resource acquisition and resource assimilation, under different combined stressor levels. We modified the design of Spooner & Vaughn (2008) previously used to examine thermal tolerance in mussels. We used two common species in our experiment: *Actinonaias ligamentina* (Lamark, 1819), a thermally sensitive species with decreased feeding rates and higher nutrient excretion rates at warm temperatures, and *Amblema plicata* (Say, 1817), a thermally tolerant species whose feeding and excretion rates increase with temperature (Spooner & Vaughn, 2008). *Actinonaias ligamentina* individuals averaged 109.7mm (± 9.0 sd) in length while *A. plicata* individuals averaged 85.1mm (± 8.9 sd).

We established four exposure treatments: control (20°C, no added mercury), elevated temperature (30°C, no added mercury), elevated mercury (20°C, 0.7 ng/L MeHg added to food), or elevated temperature and elevated mercury (30°C, 0.7ng/L MeHg added to food). Mussels (N = 15 per treatment) were exposed to these conditions in four separate 500-L recirculating tanks for two months. We fed each group of mussels 2L of a mixed assemblage of cultured pond algae (Vaughn *et al.*, 2008) every

other day. For mussels in the elevated mercury treatment, 1.4ng of MeHg (Brooks and Rand 1ppm MeHgCl Standard) was added to the algae a day prior to each feeding. We used the chillers in the recirculating tanks in combination with aquarium heaters to maintain our temperature treatments. After two months, we randomly selected 10 surviving individuals from each treatment to measure filtration and respiration rates.

We used mussel filter feeding rates as a measure of resource acquisition. We measured filtration rates as algal clearance rates, the ability of mussels to remove algae from the water column (Kryger and Riisgard 1988). We placed individual mussels in glass containers whose volume varied from 500 ml to 1,500 ml, depending on mussel size. Containers were placed on stir plates in environmental chambers set to the experiment treatment temperature. We added a standard 100mL aliquot of cultured algae and a stir bar to each container. We let the mussels feed for ~90 minutes while gently stirring the containers to maintain algae in suspension, and then took a water sample for the analysis of chlorophyll-*a*, a measure of algae concentration. Chlorophyll *a* was extracted and quantified from frozen glass fiber filters after overnight extraction in $MgCO_3$ buffered acetone (APHA, 1996). We calculated soft tissue dry mass of mussels using previously established length-weight regressions for each species (Vaughn unpublished data).

We calculated mass-specific clearance rates following (Horgan & Mills, 1997) as:

$CR = V \ln(conc_i/conc_f) (M\ t)^{-1}$

Where CR is clearance rate (volume of water filtered g^{-1} dry weight h^{-1}), V is water volume (L), $conc_i$ is the initial algal concentration (mg chl-α L^{-1}), $conc_f$ is the final algal concentration (mg chl-α L^{-1}), M is dry mass (g), and t is time (h).

We used mussel respiration rates as a measure of resource assimilation. After measuring clearance rates, we measured respiration rates of the same individual mussels. We placed mussels in containers (volumes as above) with filtered well water and a stir bar, took an initial measure of dissolved oxygen (DO), and sealed the containers with Parafilm so that they were airtight. Containers were placed on stir plates in environmental chambers set to the experiment treatment temperature. Containers were gently stirred (to distribute oxygen equally) and left for ~90 minutes when we took a final DO reading. We calculated mass-specific O_2 consumption as the change in O2 concentration over time corrected for mussel dry mass.

Ecosystem Level Impacts

We conducted a mesocosm experiment to measure the effects of combinations of increased mercury and temperature on mussel survival and their effects on an important ecosystem process known to be influenced by mussels, nutrient cycling. We measured mussel nitrogen and phosphorus excretion rates as their contribution to nutrient remineralization and the nutrient pools (Atkinson et al., 2013, Atkinson and Vaughn 2015, Vaughn et al. 2015). Following Allen & Vaughn (2009), we used 94 x 44 cm recirculating mesocosms (n = 32) that consisted of a fiberglass tub with an inlaid plastic

tub and housed in a climate controlled greenhouse. To establish a low mercury control, we collected sediments from a local pond in central Oklahoma where atmospheric mercury deposition is lower than in the southeastern part of the state where we collected our mussels (Drenner *et al.*, 2013). We mixed these sediments with clean gravel (10-25mm diameter) to a depth of approximately 12cm in each mesocosm. We filled the mesocosms with water from a nearby pond to depth of 16.5 cm and used an electric pump in each unit to maintain a flow rate of approximately 13.7 cm/s (Allen & Vaughn, 2009). We allowed the mesocosms to equilibrate for four weeks before starting the experiment. We conducted 10% water changes every week to prevent other stressors from interfering with the intended treatments (e.g. ammonia and conductivity).

We used a factorial design with two mussel and four stressor treatments, each replicated five times. Mussel treatments were no mussel controls and a three-species mussel community. We use three species of mussels to construct a community that mirrored the abundance we found at our collection site with four individuals of *A. plicata*, four individuals of *Cyclonaias pustulosa* (Lea, 1831), and a single individual of *Fusconaia flava* (Rafinesque, 1820) for a total of nine mussels in each mussel treatment. Stressor treatments were a control (20℃ and no added mercury), temperature stress treatment (25℃ and no added mercury), mercury stress treatment (20℃ and 200ug/kg mercury in sediment), and combined stress treatment (25℃ and 200ug/kg mercury in sediment). Mesocosms were housed in a greenhouse maintained at 20℃. We maintained 25℃ in the elevated temperature treatments with aquarium heaters. Low

mercury treatments had no mercury added to the sediments. For high mercury treatments, the sediments were spiked with approximately 200ug HgCl per kilogram of sediment.

We added mussels to the mesocosms on September 21, 2015 and ran the experiment until December 21, 2015. We collected water samples for ammonia (NH3) and total phosphorus (TP) from the water column and porewater every three weeks. We refer to ammonia generally as both the ionized (NH3) and unionized (NH_4^+) forms). We also recorded water quality parameters (Table 1) at this time. We took 10ml samples from the water column with a pipette for NH_4^+ and TP analysis and placed the samples in 30ml scintillation vials. For porewater samples, we used a 14in PushPoint 1/8in diameter Sediment Research Sampler (MHE Products). After inserting the sampler into the sediments, we used a 50ml syringe to extract approximately 30ml of water. The sample was placed in a separate 30ml scintillation vial. All water samples were frozen until analysis. We used the phenate method for NH_4^+ and the ascorbic acid method with persulfate digestion for TP (APHA, 1996). Mesocosms were visually inspected every few days and dead individuals were removed. During the experiment, some mussels burrowed under the sediment and died, but were not visible and thus not removed until the end of the experiment. We estimated the shell-free dry mussel biomass in each mesocosm with previously established length-weight regressions for each species (*A. plicata*, *F. flava*, Vaughn unpublished data; *C. pustulosa*, Atkinson unpublished data). In mussel treatments, we scaled NH_4^+, TP, and NH_4^+:TP by live mussel biomass at each

sample date by dividing each value by the calculated dry weight of all mussels in the mesocosm subtracting the weight individuals confirmed dead in that mesocosm on or before each sample date.

At the end of the experiment we noticed differences in submerged aquatic macrophytes between mesocosms, so we opportunistically sampled above ground plant matter from all mesocosms. We dried the plant matter in a paper sack in a drying oven for four days and then weighed the contents of each bag.

Regional Level Impacts

To assess the global risk to freshwater ecosystems posed by mercury contamination and GCC, we used GIS to identify biodiverse locations that receive the highest annual mercury deposition and that are projected to have the highest mean annual temperature increase by 2050. We used ArcMap (version 10.5) to create a map identifying regions where freshwater ecosystems are at greatest risk for thermal and toxic mercury stress. We used average annual mercury deposition modelling results published by the Arctic Monitoring and Assessment Programme (AMAP/UNEP, 2015) and average annual temperature change projections in 2050 based on the CCSM4 climate model under Scenario 6.0 in a dataset available from the Environmental Systems Research Institute (ESRI) to assess potential exposure to mercury stress and GCC related thermal stress. To identify freshwater systems of greatest conservation concern we used a global dataset of freshwater fish species occurrence at the basin level (Tedesco *et al.*, 2017) as a proxy for overall freshwater biodiversity. While global data on freshwater mussel biodiversity do exist (Lopes-Lima *et al.*, 2014) they are predominantly delineated by

country, state or province; boundaries that are not biologically significant. In addition, some areas with high mussel biodiversity are greatly under explored and sampled, such as the Amazon basin and southeast Asia, which would have underweighted the importance of these areas in our analysis (Graf & Cummings, 2007). We totaled the number of freshwater fish species occurring in each basin in the dataset to measure the freshwater fish richness in that region. We converted all data layers to raster format and then rescaled the mercury deposition data and projected temperature increase data by dividing all values in each dataset by their maximum value. Next, we used raster calculations to multiply fish biodiversity for each grid unit by the product of the rescaled mercury deposition and projected temperature increase. Regions where there were no fish biodiversity data were set to zero and the final product was rescaled to range from one to zero. The final product was a risk map where regions with the greatest values represent those where the most species are predicted to be exposed to the highest levels of mercury and temperature stress.

Statistical Analyses

We conducted all statistical tests in IBM SPSS version 19.0.0 with a significance threshold of $p = 0.05$. All statistical values not listed in the text are available in the supplemental materials (Tables 2-5).

Individual Level Impacts

We tested respiration and clearance rate results for normality using a Shapiro-Wilks test. No transformation could make all cases conform to a normal distribution. Therefore, we used an Independent Samples Kruskal-Wallis test with a Dunn-Bonferroni post hoc test to test for mean differences between treatment groups.

Ecosystem Level Impacts

We conducted a χ^2 goodness of fit test to test the null hypothesis that mussel deaths occurred with equal frequency between stress treatments for three groupings: 1) all mussels in a mesocosm combined, only *A. plicata* individuals, and only *C. pustulosa* individuals (Whitlock & Schluter, 2009). We also conducted post-hoc pairwise comparisons between stress treatments for each group. The mortality data for *F. flava* alone did not meet the assumptions of a χ^2 goodness of fit test and were not statistically analyzed. We did not conduct a temporal analysis of mussel mortality because the exact time of death was uncertain for mussel individuals that had burrowed into the sediment and were recovered at the end of the study.

Because we were primarily interested in relative rather than absolute differences between nutrient values, we converted water column and porewater NH_3, TP, and NH_3:TP data to Z-scores for each response variable for each of the four sample dates. We then used a doubly multivariate design profile analysis to test for differences in water column and porewater nutrients and interactions between mussel treatments and stress treatments across all sample dates (Tabachnick & Fidell, 2013). We used the doubly multivariate because we measured multiple noncommensurate dependent variables (NH_3, TP, and NH_3:TP for the water column and porewater) at multiple times

(our four sample dates). Our model used two between-subject independent variables: mussel treatment (with or without a mussel community) and stress treatment (control, elevated temperature, elevated mercury, and both stressors). Sample dates served as our within-subject independent variable. The model tested the effects of all independent variables alone as well as for all pair-wise interactions. We conducted post-hoc one-way ANOVA tests on effects found to be significant in the model as indicated by Wilks' λ to determine specific differences between factors. While we used the Z-score transformed data in statistical calculations to determine significant trends, we visually present the data as untransformed values for ease of interpretation.

Macrophyte data were not normally distributed, so we also tested for mean differences between treatment groups with an Independent Samples Kruskal-Wallis test with a Dunn-Bonferroni post hoc test. To ensure that the differences observed in macrophytes did not affect the results of our profile analysis we conducted a MANCOVA on the data from the last sampling point, using vegetation biomass as a covariate.

RESULTS

Individual Level Impacts

Physiological performance of mussels varied between the two species and among treatments. Respiration rates of the thermally tolerant species, *A. plicata*, were significantly lower in the elevated mercury treatment, but did not otherwise differ among treatments (Fig. 2). Respiration rates of the thermally sensitive species, *A.*

ligamentina, were significantly elevated at higher temperatures and significantly reduced at elevated mercury concentrations (Fig. 2). Algae clearance rates, also varied between species and among treatments for *A. ligamentina*. There were no significant effects of treatment on the clearance rates of the thermally tolerant species, *A. plicata* (Fig. 2). However, clearance rates were significantly reduced in *A. ligamentina* exposed to the elevated mercury treatment. No *A. ligamentina* individuals survived in the combined stress treatment (Fig. 2).

Ecosystem Level Impacts

Mussel mortality was significantly higher in the presence of stressors, and the highest mortality rates occurred when mussels were exposed to both stressors simultaneously (Fig. 3A). *Amblema plicata* had a mortality rate of over 50% in the elevated mercury and combined stressor treatments (Fig. 3B). *Cyclonaias pustulosa* also experienced its highest mortality, over 25%, in the combined stressor treatment (Fig. 3C). We were unable to statistically analyze mortality in *F. flava* because of insufficient power given the low number of individuals in each mesocosm. However, it is notable that no *F. flava* deaths were observed in the control treatment and that the highest number of deaths were individuals in the combined stress treatment, a pattern consistent with the mortality trends in *A. plicata* and *C. pustulosa* (Fig. 3D).

We observed significantly higher above ground macrophyte biomass in the absence of mercury (Fig. 4). Only one mesocosm had macrophyte growth in the no mussel, both stressor treatment (0.0647g). Plants were predominantly of the genera *Vallisneria*, *Najas*, *Ruppia*, *Potamogeton*, and *Ceratophyllum*. There was no significant

effect of macrophyte biomass on the model (Wilks' $\lambda = 0.887$, $F = 0.755$, $df = 5,27$, $p = 0.590$) and we found no differences in the significance results for effects of treatments on nutrient responses on the final sampling date when compared to the profile analysis. We found significant effects between subjects (mesocosms) for the stress treatment and an interaction effect between the mussel treatment and the stress treatment. We also found a significant interaction effect between sample date and the stress treatment. Within mussel treatments, we observed a general trend of decreasing NH_3 over time. NH_3 was highest in the elevated mercury and combined stressors treatment before decreasing over the course of the experiment (Fig. 5). We observed the opposite trend with TP, with concentrations starting low and then increasing over time. The largest increase was observed in the combined stressors treatment (Fig. 5). Finally, we observed a decrease in NH_3:TP ratios over time. The elevated mercury and combined stressors treatments had the highest initial NH_3:TP but all treatments decreased to a low NH3:TP by the end of the experiment (Fig. 5).

Regional Level Impacts

The graphic produced by our analyses has a scale from 0-1 (Fig. 6). The higher the value the higher the combination of biodiversity, mercury deposition and projected temperature increase for that region. Using this analysis, the areas most threatened by combined GCC and mercury contamination in the future are in South America, particularly the Amazon basin. Other areas of concern include central Africa, Southeast Asia, China, India, and central North America.

DISCUSSION

Our results demonstrate that toxic stress from increased mercury contamination and thermal stress associated with GCC interact to lethally and sub-lethally impact freshwater mussels and the ecosystem functions that they influence. Combined effects of these two stressors led to higher mussel mortality, reduced resource acquisition and assimilation, and depressed nutrient recycling rates over time. Unionid freshwater mussels are already among the most imperiled faunal groups in the world (Haag & Williams, 2014, Lopes-Lima *et al.*, 2014) and even common species, like the ones we used in our study, are declining (Atkinson *et al.*, 2014a, Haag, 2012). Mercury contamination of the environment and GCC are projected to increase in the coming decades (Amos *et al.*, 2013, IPCC, 2014, Xenopoulos *et al.*, 2005) increasing toxic mercury stress and thermal stress on freshwater mussels, which could put further strain on a faunal group already struggling to survive. Our results indicate that these multiple stressors have consequences for the conservation of mussels and the ecosystem services that they provide.

Multiple stressors affected the physiological performance of mussels, reducing both filtration and respiration rates. In the short term, this can decrease mussel condition and growth (Jokela & Mutikainen, 1995, Payton *et al.*, 2016). Under conditions of food limitation, like that caused by reduced filtration rates, mussels have been shown to devote more energy to maintenance rather than growth (Roznere *et al.*, 2014) and long-term starvation is derogative for the health of the mussels (Mahapatra *et al.*, 2017). In the long term, extended exposure to these stressors could depress the resources

allocated to reproduction, which could have long term impacts on recruitment and negatively affect mussel populations (Jokela & Mutikainen, 1995). Further, decreased physiological performance will also lead to altered or decreased ecosystem services provided by mussels (Spooner and Vaughn 2008). As mussels filter water, they remove impurities and their filtration capacity can be very high (Vaughn, 2017). Reduced filtration rates will lead to reduced provisioning of this service. In addition, mussels are important in stimulating primary and secondary production in food webs through the excretion of N and P (Atkinson & Vaughn, 2015). Mussels that are less metabolically active are excreting fewer nutrients. Thus, the reduced respiration we observed in mussels exposed to mercury contamination would result in fewer nutrients available in stream ecosystems with elevated mercury contamination. Even when the effects of stressors are not lethal to mussels, the effects of those stressors can be profound at both the individual and organismal level. Our findings suggest that these detriments are likely to occur in the presence of mercury contamination and increased temperatures.

The combined effects of mercury and temperature will likely impact recruitment of mussel populations, which will affect future population growth and their ability to continue to contribute to ecosystem processes. In the mesocosm experiment, we observed elevated NH_3 concentrations in both the high mercury and combined stressor treatments, that was likely due to decomposition of deceased mussels. Juvenile unionid mussels are highly sensitive to elevated NH_3 concentrations (Newton *et al.*, 2003, Wang *et al.*, 2007a, Wang *et al.*, 2007b). While the peak NH_3 concentrations observed in our study were below the 2013 revised EPA aquatic life ambient water quality criteria for ammonia (USEPA, 2013), the levels were close to the low end of chronic values

reported to affect survival and growth in some juvenile mussels (Wang *et al.*, 2007a). In addition, the mussel densities used in our mesocosm experiment were below some of the maximum densities observed in many natural mussel beds (Galbraith *et al.*, 2010).

At higher mussel densities, NH_3 concentrations could be even higher and reach toxic levels in both the water column and porewater. This phenomenon was observed in a die off event of the invasive Asian clam, *Corbicula fluminea* (Cherry *et al.*, 2005, Cooper *et al.*, 2005). These studies associated mass die-offs with elevated concentrations of ammonia in both the water column and porewater at concentrations dangerous to unionid mussels. These findings are supported by an assessment of the recruitment failure of freshwater mussels (Strayer & Malcom, 2012). This study found a strong association between recruitment failure in unionids and ammonia concentrations far lower than previous toxicity thresholds. Peak concentrations of ammonia far exceeded their threshold of 0.02 µg N/L in both the porewater and water column. Therefore, it seems likely that ammonia excreted into the water column and porewater by dying mussels would likely add yet another layer of toxic stress on maturing juveniles, potentially reducing recruitment and the ability of the mussel population to recover from the deaths of adult mussels and rebuild populations.

Losses of mussels from multiple stressors could lead to changes in nutrient dynamics. A primary reason that mussel populations have strong effects on many ecosystem processes is because mussel biomass is often an order of magnitude higher than other benthic macroinvertebrates (Atkinson & Vaughn, 2015, Negus, 1966, Ökland, 1963). Because of this, when mussel populations wane so do their ecosystem effects. Mass mortality events in mussel populations have been directly linked to

decreases in ecosystem services such as nutrient storage (Vaughn *et al.*, 2015). In the mesocosm experiment we observed the highest mussel mortality in the elevated mercury and combined stressor treatments and saw a simultaneous increase in total phosphorus in those treatments. This is consistent with a loss of nutrient storage for P; as the mussels died, the P sequestered in their tissues was released into the water column. These results mirror effects observed after a mass mortality events for both invasive and native bivalves. The death of dominant invasive bivalves in a river in southeastern North America resulted in elevated concentrations of phosphorus in the river similar to our study (McDowell *et al.*, 2017) while multiple mass mortality events in European rivers were noted as resource pulses to terrestrial environments (Bodis *et al.*, 2014, Sousa *et al.*, 2012). Nutrient pulses, like the pulse of P we observed, might have short-term benefits to ecosystem nutrient cycling, especially in P limited systems, however the loss of stored nutrients could have negative repercussions over the long-term. Mussels aggregations often excrete more P than they sequester in their tissues (Atkinson *et al.*, 2017b), which could make the loss of mussels in mass mortality events a net loss of P for the system despite the initial pulse resulting from their deaths, especially if other factors in the river do not promote uptake of the P made available in the nutrient pulse (Withers & Jarvie, 2008).

Large scale mussel die-offs might also have effects on community composition of primary producers, and subsequently the rest of river food webs. We observed a decline in NH_3:TP across all mesocosm treatments, however this decrease was most significant in the combined stressor treatments. Alterations to nutrient stoichiometry in freshwater systems can affect food resources and consumers (Cross *et al.*, 2005). Algae

communities often vary considerably in their nutrient stoichiometry either through plasticity in the nutrient ratios within individual algae (Frost *et al.*, 2002, Frost *et al.*, 2005) or algal community shifts based on the available nutrients (Bowman *et al.*, 2005, Frost & Elser, 2002, Hillebrand & Kahlert, 2001, Hillebrand & Kahlert, 2002). Both have implications for consumers. Shifts in the stoichiometry of algae may alter their quality as a food source which in turn will impact which herbivores are most successful. Shifts in primary producer communities could favor certain consumers more than others, or consumers may alter their feeding behaviors and/or preferences (Hillebrand *et al.*, 2009). Both responses could have other consumers throughout the food web.

Considering impacts of these two global stressors at both the individual and ecosystem level, it is informative to explore where these interactions might be poised to have the greatest impact. Our mapping exercise provides a good starting point to explore this question by highlighting several locations with overlaps between freshwater biodiversity hotspots, mercury deposition, and projected temperature increase, including our study system in southeastern Oklahoma. While the brightest locations on the map are naturally the locations with the highest inherent freshwater fish diversity, our analysis highlights regions within basins that are most at risk. For example, regions of interior China have a higher risk than the rest of the country, while more peripheral regions of the Amazon basin have the highest risk. These results could guide future field research efforts to examine these effects *in situ*. Additionally, it is notable that many of the at-risk locations are in developing countries. Sustainable development of freshwater resources in these regions will face significant challenges (Capps *et al.*, 2016, Cohen, 2006) and our results indicate the impacts of GCC and mercury could

further complicate these efforts. Finally, the presence of a high threat in Asia, South America, and North America is significant because these are centers of freshwater mussel diversity (Lopes-Lima *et al.*, 2014). This reinforces our assumption that freshwater fish diversity is a good proxy for overall freshwater biodiversity. It also highlights the significance of our findings to the conservation efforts for freshwater mussels. Future research efforts investigating these stressors may be particularly important in the areas highlighted on our map.

We found that mercury depressed the growth of aquatic vegetation. While we lack the data to make robust commentary on the sensitivity of aquatic macrophytes to combined mercury and temperature stress, this result is still interesting. First, it highlights the fact that different taxa will be impacted by mercury and increased temperature differently. Where mussels survived in the mercury stress treatment, macrophytes did not. Further research investigating the effects of these stressors on disparate taxa are needed. Additionally, it would be interesting to investigate effects of the stressors on macrophyte community composition and growth over time in subsequent studies to determine what mechanisms may have led to the final snapshot we captured at the end of the experiment.

Our study has limitations that should be addressed by future research. First, there are different potential mercury exposure pathways among the various mussel species. In the laboratory study of physiological performance, *A. ligamentina*, a species known to be sensitive to warmer temperatures (Spooner & Vaughn, 2008) was exposed by filtering methyl mercury contaminated food. However, the species in the mesocosm experiment, *A. plicata*, *Q. pustulosa*, and *F. flava*, died in response to the addition of

inorganic mercury to the sediments at a relatively high dose. Undoubtedly some of the inorganic mercury in the mesocosms was converted to methyl mercury by sediment microbes, which could have entered the mesocosm food web, exposing the mussels in the same pathway as in the laboratory experiment. However, these mussels may have also been exposed to toxic stress from inorganic mercury in the sediments. Our data don't allow us to discern how much mortality is attributable to each form of mercury. Regardless, we show there are interactive effects of both stressors that lead to higher mortality in these mussel species. Additionally, we used only two treatment levels of each stressor. While this allowed us to detect broad level patterns across multiple species, future studies investigating the sensitivity thresholds for both stressors are needed. Finally, our GIS map has some notable limitations. The resolution of the data is coarse and will need to be expanded in finer scale studies. Additionally, our analysis may be overly-sensitive to regions that are particularly high in one of the three values but low in the others. The analysis is also not sensitive to relative differences between stressors. Future studies utilizing in-depth modelling could address these issues.

In conclusion, we show that toxic mercury stress and thermal stress combine in both lethal and sublethal ways to negatively impact multiple species of freshwater unionid mussels. Interactive effects between these stressors have consequences for the conservation of mussels as a taxon as well was for understanding how the ecosystem processes they influence will change. As both mercury contamination and GCC affect the entire planet, these findings have far reaching implications, especially considering the seemingly ever-increasing list of anthropogenic impacts resulting in environmental damage. Further research is needed to better understand the effects of mercury and

temperature, including finer scale studies to determine what levels of stress cause these effects, studies to examine these effects in the field, and inclusion of additional stressors that might interact with or compound the effects of mercury and GCC. As we continue to make further advances in our use of fuels and metals the need to understand the environmental impacts of these advancements will become increasingly important.

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REFERENCES

Allen, D. C., & Vaughn, C. C. (2009). Burrowing behavior of freshwater mussels in experimentally manipulated communities. *Journal of the North American Benthological Society*, *28*(1), 93-100.

Allen, D. C., Vaughn, C. C., Kelly, J. F., Cooper, J. T., & Engel, M. H. (2012). Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. *Ecology, 93*(10), 2165-2174.

AMAP/UNEP. (2015*). Global mercury modelling: Update of modelling results in the global mercury assessment 2013*. Retrieved from Olso, Norway/UNEP Chemicals Branch, Geneva, Switzerland:

Amos, H. M., Jacob, D. J., Streets, D. G., & Sunderland, E. M. (2013). Legacy impacts of all-time anthropogenic emissions on the global mercury cycle. *Global Biogeochemical Cycles, 27*(2), 410-421.

Ankley, G. T., & Villeneuve, D. L. (2006). The fathead minnow in aquatic toxicology: Past, present and future. *Aquatic Toxicology, 78*(1), 91-102.

APHA. (1996). Standard methods for the examination of water and wastewater: Including bottom sediments and sludges. In (18th ed.). Washington: American Public Health Association, American Water Works Association and Water Environment Federation.

Archambault, J. M., Cope, W. G., & Kwak, T. J. (2014). Survival and behaviour of juvenile unionid mussels exposed to thermal stress and dewatering in the presence of a sediment temperature gradient. *Freshwater Biology, 59*(3), 601-613.

Atkinson, C. L., Capps, K. A., Rugenski, A. T., & Vanni, M. J. (2017). Consumer-driven nutrient dynamics in freshwater ecosystems: From individuals to ecosystems. *Biological Reviews, 92*(4), 2003-2023.

Atkinson, C. L., Julian, J. P., & Vaughn, C. C. (2014). Species and function lost: Role of drought in structuring stream communities. *Biological Conservation, 176*, 30-38.

Atkinson, C. L., Kelly, J. F., & Vaughn, C. C. (2014). Tracing consumer-derived nitrogen in riverine food webs. *Ecosystems, 17*(3), 485-496.

Atkinson, C. L., Sansom, B. J., Vaughn, C. C., & Forshay, K. J. (2017). Consumer aggregations drive nutrient dynamics and ecosystem metabolism in nutrient-limited systems. *Ecosystems*.

Atkinson, C. L., & Vaughn, C. C. (2015). Biogeochemical hotspots: Temporal and spatial scaling of the impact of freshwater mussels on ecosystem function. *Freshwater Biology, 60*(3), 563-574.

Atkinson, C. L., C. C. Vaughn, K. J. Forshay & J. T. Cooper (2013) Aggregated filter-feeding consumers alter nutrient limitation: consequences for ecosystem and community dynamics. *Ecology*, 94, 1359-1369.

Beckett, D. C., Green, B. W., Thomas, S. A., & Miller, A. C. (1996). Epizoic invertebrate communities on upper mississippi river unionid bivalves. *American Midland Naturalist, 135*(1), 102-114.

Bodis, E., Toth, B., & Sousa, R. (2014). Massive mortality of invasive bivalves as a potential resource subsidy for the adjacent terrestrial food web. *Hydrobiologia, 735*(1), 253-262

Bowman, M. F., Chambers, P. A., & Schindler, D. W. (2005). Changes in stoichiometric constraints on epilithon and benthic macroinvertebrates in response to slight nutrient enrichment of mountain rivers. *Freshwater Biology, 50*(11), 1836-1852.

Broecker, W. S., Takahashi, T., Simpson, H., & Peng, T.-H. (1979). Fate of fossil fuel carbon dioxide and the global carbon budget. *Science*, 409-418.

Capps, K. A., Bentsen, C. N., & Ramirez, A. (2016). Poverty, urbanization, and environmental degradation: Urban streams in the developing world. *Freshwater Science, 35*(1), 429-435.

Cherry, D. S., Scheller, J. L., Cooper, N. L., & Bidwell, J. R. (2005). Potential effects of asian clam (corbicula fluminea) die-offs on native freshwater mussels (unionidae) i: Water-column ammonia levels and ammonia toxicity. *Journal of the North American Benthological Society, 24*(2), 369-380.

Chowdhury, G. W., Zieritz, A., & Aldridge, D. C. (2016). Ecosystem engineering by mussels supports biodiversity and water clarity in a heavily polluted lake in dhaka, bangladesh. *Freshwater Science, 35*(1), 188-199.

Chumchal, M. M., Rainwater, T. R., Osborn, S. C., Roberts, A. P., Abel, M. T., Cobb, G. P., . . . Bailey, F. C. (2011). Mercury speciation and biomagnification in the food web of caddo lake, texas and louisiana, usa, a subtropical freshwater ecosystem. *Environmental Toxicology and Chemistry, 30*(5), 1153-1162.

Cohen, B. (2006). Urbanization in developing countries: Current trends, future projections, and key challenges for sustainability. *Technology in Society, 28*(1), 63-80.

Cooper, N. L., Bidwell, J. R., & Cherry, D. S. (2005). Potential effects of asian clam (*Corbicula fluminea*) die-offs on native freshwater mussels (Unionidae) ii: Porewater ammonia. *Journal of the North American Benthological Society, 24*(2), 381-394.

Cross, W. F., Benstead, J. P., Frost, P. C., & Thomas, S. A. (2005). Ecological stoichiometry in freshwater benthic systems: Recent progress and perspectives. *Freshwater Biology, 50*(11), 1895-1912.

Drenner, R. W., Chumchal, M. M., Jones, C. M., Lehmann, C. M. B., Gay, D. A., & Donato, D. I. (2013). Effects of mercury deposition and coniferous forests on the mercury contamination of fish in the south central united states. *Environmental Science & Technology, 47*(3), 1274-1279.

Ferreira-Rodríguez, N., & Pardo, I. (2017). The interactive effects of temperature, trophic status, and the presence of an exotic clam on the performance of a native freshwater mussel. *Hydrobiologia, 797*(1), 171-182.

Ficke, A. D., Myrick, C. A., & Hansen, L. J. (2007). Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries, 17*(4), 581-613.

Frost, P. C., & Elser, J. J. (2002). Growth responses of littoral mayflies to the phosphorus content of their food. *Ecology Letters, 5*(2), 232-240.

Frost, P. C., Elser, J. J., & Turner, M. A. (2002). Effects of caddisfly grazers on the elemental composition of epilithon in a boreal lake. *Journal of the North American Benthological Society, 21*(1), 54-63.

Frost, P. C., Hillebrand, H., & Kahlert, M. (2005). Low algal carbon content and its effect on the c : P stoichiometry of periphyton. *Freshwater Biology, 50*(11), 1800-1807.

Förstner, U., & Wittmann, G. T. (2012). *Metal pollution in the aquatic environment*: Springer Science & Business Media.

Galbraith, H. S., Spooner, D. E., & Vaughn, C. C. (2010). Synergistic effects of regional climate patterns and local water management on freshwater mussel communities. *Biological Conservation, 143*(5), 1175-1183.

Ganser, A. M., Newton, T. J., & Haro, R. J. (2015). Effects of elevated water temperature on physiological responses in adult freshwater mussels. *Freshwater Biology, 60*(8), 1705-1716.

Graf, D. L., & Cummings, K. S. (2007). Review of the systematics and global diversity of freshwater mussel species (bivalvia: Unionoida). *Journal of Molluscan Studies, 73*(4), 291-314.

Haag, W. R. (2012). *North American Freshwater Mussels*. Cambridge: Cambridge University Press.

Haag, W. R., & Williams, J. D. (2014). Biodiversity on the brink: An assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia, 735*(1), 45-60.

Heino, J., Virkkala, R., & Toivonen, H. (2009). Climate change and freshwater biodiversity: Detected patterns, future trends and adaptations in northern regions. *Biological Reviews, 84*(1), 39-54.

Hillebrand, H., Borer, E. T., Bracken, M. E. S., Cardinale, B. J., Cebrian, J., Cleland, E. E., . . . Smith, M. D. (2009). Herbivore metabolism and stoichiometry each constrain herbivory at different organizational scales across ecosystems. *Ecology Letters, 12*(6), 516-527.

Hillebrand, H., & Kahlert, M. (2001). Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. *Limnology and Oceanography, 46*(8), 1881-1898.

Hillebrand, H., & Kahlert, M. (2002). Effect of grazing and water column nutrient supply on biomass and nutrient content of sediment microalgae. *Aquatic Botany, 72*(2), 143-159.

Hooper, M. J., Ankley, G. T., Cristol, D. A., Maryoung, L. A., Noyes, P. D., & Pinkerton, K. E. (2013). Interactions between chemical and climate stressors: A role for mechanistic toxicology in assessing climate change risks. *Environmental Toxicology and Chemistry, 32*(1), 32-48.

Horgan, M. J., & Mills, E. L. (1997). Clearance rates and filtering activity of zebra mussel (*Dreissena polymorpha*): Implications for freshwater lakes. *Canadian Journal of Fisheries and Aquatic Sciences, 54*(2), 249-255.

Howard, J. K., & Cuffey, K. M. (2006). The functional role of native freshwater mussels in the fluvial benthic environment. *Freshwater Biology, 51*(3), 460-474.

IPCC. (2014). *Climate change 2014: Synthesis report. Contribution of working groups i, ii and iii to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Retrieved from Geneva, Switzerland:

Jokela, J., & Mutikainen, P. (1995). Phenotypic plasticity and priority rules for energy allocation in a fresh-water clam - a field experiment. *Oecologia, 104*(1), 122-132.

Kundzewicz, Z., Mata, L., Arnell, N. W., Döll, P., Jimenez, B., Miller, K., . . . Shiklomanov, I. (2008). The implications of projected climate change for freshwater resources and their management.

Kryger, J. & H. U. Riisgård (1988) Filtration rate capacities in 6 species of European freshwater bivalves. *Oecologia*, 77, 34-38.

Limm, M. P., & Power, M. E. (2011). Effect of the western pearlshell mussel *Margaritifera falcata* on pacific lamprey *Lampetra tridentata* and ecosystem processes. *Oikos, 120*(7), 1076-1082.

Lopes-Lima, M., Teixeira, A., Froufe, E., Lopes, A., Varandas, S., & Sousa, R. (2014). Biology and conservation of freshwater bivalves: Past, present and future perspectives. *Hydrobiologia, 735*(1), 1-13.

Mahapatra, E., Dasgupta, D., Bhattacharya, N., Mitra, S., Banerjee, D., Goswami, S., Chakraborty, S. (2017). Sustaining immunity during starvation in bivalve mollusc: A costly affair. *Tissue & Cell, 49*(2), 239-248.

Malmqvist, B., & Rundle, S. (2002). Threats to the running water ecosystems of the world. *Environmental Conservation, 29*(2), 134-153.

Master, L. L., Flack, S. R., & Stein, B. A. (1998). *Rivers of life: Critical watersheds for protecting freshwater biodiversity*: Nature Conservancy Arlington, Virginia.

Matthews, W. J., Vaughn, C. C., Gido, K. B., & Marsh-Matthews, E. (2005). Southern plains rivers. In A. C. Benke & C. E. Cushing (Eds.), *Rivers of North America* (pp. 283-325). Burlington, MA.

McDowell, W., McDowell, W. H., & Byers, J. E. (2017). Mass mortality of a dominant invasive species in response to an extreme climate event: Implications for ecosystem function. *Limnology and Oceanography, 62*(1), 177-188.

Melillo, J. M., Richmond, T., & Yohe, G. (2014). Climate Change Impacts in the United States. *Third National Climate Assessment*.

Moe, S. J., De Schamphelaere, K., Clements, W. H., Sorensen, M. T., Van den Brink, P. J., & Liess, M. (2013). Combined and interactive effects of global climate change and toxicants on populations and communities. *Environmental Toxicology and Chemistry*, *32*(1), 49-61.

Morel, F. M. M., Kraepiel, A. M. L., & Amyot, M. (1998). The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology and Systematics, 29*, 543-566.

Negus, C. L. (1966). A quantitative study of growth and production of unionid mussels in the River Thames at Reading. *Journal of Animal Ecology, 35*, 513-532.

Nelson, D. A., Calabrese, A., & Macinnes, J. R. (1977). Mercury stress on juvenile bay scallops, *Argopecten irradians*, under various salinity-temperature regimes. *Marine Biology*, *43*(4), 293-297.

Newton, T. J., Allran, J. W., O'Donnell, J. A., Bartsch, M. R., & Richardson, W. B. (2003). Effects of ammonia on juvenile unionid mussels (*Lampsilis cardium*) in laboratory sediment toxicity tests. *Environmental Toxicology and Chemistry*, *22*(11), 2554-2560.

Nriagu, J. O. (1996). A history of global metal pollution. *Science, 272*(5259), 223.

ODWC. (2016). *Oklahoma Mercury in Fish*. Retrieved from http://www.deq.state.ok.us/aqdnew/resources/publications/HgPresentation.pdf.

Ökland, J. (1963). Notes on population density, age distribution, growth, and habitat of *Anodonta piscinalis* nilss. (moll., lamellibr.) in a eutrophic Norwegian lake. *Nytt Magasin for Zoologi, 11*, 19-43.

Olden, J. D., Kennard, M. J., Leprieur, F., Tedesco, P. A., Winemiller, K. O., & Garcia-Berthou, E. (2010). Conservation biogeography of freshwater fishes: Recent progress and future challenges. *Diversity and Distributions*, *16*(3), 496-513.

Ostfeld, A., Barchiesi, S., Bonte, M., Collier, C. R., Cross, K., Darch, G., . . . Wright, J. (2012). Climate change impacts on river basin and freshwater ecosystems: Some observations on challenges and emerging solutions. *Journal of Water and Climate Change, 3*(3), 171-184.

Payton, S. L., Johnson, P. D., & Jenny, M. J. (2016). Comparative physiological, biochemical and molecular thermal stress response profiles for two unionid freshwater mussel species. *Journal of Experimental Biology*, *219*(22), 3562-3574.

Poff, N. L., Pyne, M. I., Bledsoe, B. P., Cuhaciyan, C. C., & Carlisle, D. M. (2010). Developing linkages between species traits and multiscaled environmental variation to explore vulnerability of stream benthic communities to climate change. *Journal of the North American Benthological Society, 29*(4), 1441-1458.

Roznere, I., Watters, G. T., Wolfe, B. A., & Daly, M. (2014). Nontargeted metabolomics reveals biochemical pathways altered in response to captivity and food limitation in the freshwater mussel *Amblema plicata*. *Comparative Biochemistry and Physiology D-Genomics & Proteomics, 12*, 53-60.

Scheulhammer, A. M., Meyer, M. W., Sandheinrich, M. B., & Murray, M. W. (2007). Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio, 36*(1), 12-18.

Selin, N. E. (2009). Global biogeochemical cycling of mercury: A review. *Annual Review of Environment and Resources, 34*, 43-63.

Slotsbo, S., Heckmann, L.-H., Damgaard, C., Roelofs, D., de Boer, T., & Holmstrup, M. (2009). Exposure to mercury reduces heat tolerance and heat hardening ability of the springtail *Folsomia candida*. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology, 150*(1), 118-123.

Smil, V. (2004). World history and energy. *Encyclopedia of Energy, 6*, 549-561.

Sousa, R., Varandas, S., Cortes, R., Teixeira, A., Lopes-Lima, M., Machado, J., & Guilhermino, L. (2012). Massive die-offs of freshwater bivalves as resource pulses. *Annales De Limnologie-International Journal of Limnology, 48*(1), 105-112.

Spooner, D. E., & Vaughn, C. C. (2006). Context-dependent effects of freshwater mussels on stream benthic communities. *Freshwater Biology, 51*(6), 1188-1188.

Spooner, D. E., & Vaughn, C. C. (2008). A trait-based approach to species' roles in stream ecosystems: Climate change, community structure, and material cycling. *Oecologia, 158*(2), 307-317.

Spooner, D. E., Vaughn, C. C., & Galbraith, H. S. (2012). Species traits and environmental conditions govern the relationship between biodiversity effects across trophic levels. *Oecologia, 168*(2), 533-548.

Spooner, D. E., Xenopoulos, M. A., Schneider, C., & Woolnough, D. A. (2011). Coextirpation of host-affiliate relationships in rivers: The role of climate change, water withdrawal, and host-specificity. *Global Change Biology, 17*(4), 1720-1732.

Strayer, D. L. (2014). Understanding how nutrient cycles and freshwater mussels (Unionoida) affect one another. *Hydrobiologia, 735*(1), 277-292.

Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton & S. J. Nichols (2004) Changing perspectives on pearly mussels, North America's most imperiled animals. *Bioscience*, 54, 429-439.

Strayer, D. L., & Malcom, H. M. (2012). Causes of recruitment failure in freshwater mussel populations in southeastern New York. *Ecological Applications, 22*(6), 1780-1790.

Streets, D. G., Devane, M. K., Lu, Z. F., Bond, T. C., Sunderland, E. M., & Jacob, D. J. (2011). All-time releases of mercury to the atmosphere from human activities. *Environmental Science & Technology, 45*(24), 10485-10491.

Tabachnick, B. G., & Fidell, L. S. (2013). *Using Multivariate Statistics* (6th ed.). Boston: Pearson.

Tedesco, P. A., Beauchard, O., Bigorne, R., Blanchet, S., Buisson, L., Conti, L., . . . Oberdorff, T. (2017). A global database on freshwater fish species occurrence in drainage basins. *Scientific Data, 4*.

Thornton, I. (1996). Impacts of mining on the environment; some local, regional and global issues. *Applied Geochemistry, 11*(1-2), 355-361.

Tweedy, B. N. (2017). *Interactions between freshwater mussels, mercury contamination, and global climate change in freshwater systems.* (Ph.D. Biology), University of Oklahoma, Norman, Oklahoma.

USEPA. (2013). *Aquatic life ambient water quality criteria for ammonia - freshwater 2013*. (EPA-822-R-13-001). Washington, DC: USEPA.

Valenti, T. W., Cherry, D. S., Neves, R. J., & Schmerfeld, J. (2005). Acute and chronic toxicity of mercury to early life stages of the rainbow mussel, *Villosa iris* (Bivalvia : Unionidae). *Environmental Toxicology and Chemistry*, *24*(5), 1242-1246.

Vaughn, C. C. (2010). Biodiversity losses and ecosystem function in freshwaters: Emerging conclusions and research directions. *Bioscience*, *60*(1), 25-35.

Vaughn, C. C. (2017). Ecosystem services provided by freshwater mussels. *Hydrobiologia*. doi:10.1007/s10750-017-3139-x

Vaughn, C. C., Atkinson, C. L., & Julian, J. P. (2015). Drought-induced changes in flow regimes lead to long-term losses in mussel-provided ecosystem services. *Ecology and Evolution, 5*(6), 1291-1305.

Vaughn, C. C., Nichols, S. J., & Spooner, D. E. (2008). Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society, 27*(2), 409-423.

Vaughn, C. C., Spooner, D. E., & Galbraith, H. S. (2007). Context-dependent species identity effects within a functional group of filter-feeding bivalves. *Ecology, 88*(7), 1654-1662.

Verlecar, X. N., Jena, K. B., & Chainy, G. B. N. (2007). Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature. *Chemico-Biological Interactions, 167*(3), 219-226.

Vernberg, W. B., & Vernberg, J. (1971). The synergistic effects of temperature, salinity, and mercury on survival and metabolism of the adult fiddler crab, *Uca pugilator*. *Fishery Bulletin, 70*(2), 415-420.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., . . . Tilman, D. G. (1997). Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications, 7*(3), 737-750.

Waldbaum, J. C. (1978). From bronze to iron. The transition from the bronze age to the iron age in the eastern mediterranean. In *Studies in Mediterranean Archaeology* (Vol. 54, pp. 106): Paul Åströms förlag.

Wang, N., Ingersoll, C. G., Greer, I. E., Hardesty, D. K., Ivey, C. D., Kunz, J. L., . . . Barnhart, M. C. (2007). Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry, 26*(10), 2048-2056.

Wang, N., Ingersoll, C. G., Hardesty, D. K., Ivey, C. D., Kunz, J. L., May, T. W., . . . Barnhart, C. (2007). Acute toxicity of copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry, 26*(10), 2036-2047.

Welker, M., & Walz, N. (1998). Can mussels control the plankton in rivers? - a planktological approach applying a lagrangian sampling strategy. *Limnology and Oceanography, 43*(5), 753-762.

Wenger, S. J., Isaak, D. J., Luce, C. H., Neville, H. M., Fausch, K. D., Dunham, J. B., . . . Rieman, B. E. (2011). Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. *Proceedings of the National Academy of Sciences, 108*(34), 14175-14180.

Whitlock, M. C., & Schluter, D. (2009). *The Analysis of Biological Data*. Greenwood Village, Colorado: Roberts and Company Publishers.

Withers, P. J. A., & Jarvie, H. P. (2008). Delivery and cycling of phosphorus in rivers: A review. *Science of the Total Environment, 400*(1-3), 379-395.

Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: Impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B-Biological Sciences, 365*(1549), 2093-2106.

Wright, R., & Schindler, D. (1995). Interaction of acid rain and global changes: Effects on terrestrial and aquatic ecosystems. *Water, Air, and Soil Pollution, 85*(1), 89-99.

Xenopoulos, M. A., Lodge, D. M., Alcamo, J., Marker, M., Schulze, K., & Van Vuuren, D. P. (2005). Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biology, 11*(10), 1557-1564.

Table 1. Mesocosm Water Quality Parameters

Time	pH	Conductivity	TDS (ppm)	Temperature	DO (mg/L)
Experiment Start					
Treatment					
-M Control	8.89 (± 0.04)	744 (± 60)	531 (± 39)	22.1 (± 2)	7.99 (± 0.27)
-M Elevated Temp	8.64 (± 0.42)	805 (± 25)	571 (± 18)	25.8 (± 1.7)	7.96 (± 1.27)
-M Elevated Hg	8.93 (± 0.16)	797 (± 11)	568 (± 11)	21.1 (± 0.9)	8.56 (± 0.21)
-M Both Stressors	8.87 (± 0.11)	834 (± 60)	562 (± 42)	24.2 (± 1.9)	7.99 (± 0.35)
+M Control	8.88 (± 0.17)	734 (± 18)	524 (± 15)	21.1 (± 0.4)	7.96 (± 0.44)
+M Elevated Temp	8.78 (± 0.14)	755 (± 29)	535 (± 20)	24.7 (± 2.5)	7.75 (± 0.43)
+M Elevated Hg	8.98 (± 0.06)	758 (± 63)	599 (± 161	21.1 (± 0.9)	8.42 (± 0.17)
+M Both Stressors	8.74 (± 0.1)	875 (± 21)	619 (± 15)	25.6 (± 0.5)	7.55 (± 0.44)
Experiment End					
Treatment					
-M Control	9.18 (± 0.05)	659 (± 39)	469 (± 26)	22 (± 2.6)	8.37 (± 0.46)
-M Elevated Temp	9.13 (± 0.05)	723 (± 24)	532 (± 35)	25.8 (± 1.1)	7.79 (± 0.24)
-M Elevated Hg	9.11 (± 0.06)	681 (± 5)	484 (± 3)	20.9 (± 0.7)	8.08 (± 0.17)
-M Both Stressors	9.09 (± 0.04)	721 (± 27)	511 (± 19)	24.7 (± 2.2)	7.61 (± 0.46)
+M Control	9.12 (± 0.11)	668 (± 27)	475 (± 19)	21.2 (± 0.7)	8.17 (± 0.38)
+M Elevated Temp	9.15 (± 0.13)	734 (± 56)	520 (± 39)	23.9 (± 3.7)	7.92 (± 0.88)
+M Elevated Hg	9.14 (± 0.04)	683 (± 17)	505 (± 53)	20.7 (± 1.1)	8.28 (± 0.21)
+M Both Stressors	9.09 (± 0.06)	736 (± 24)	522 (± 17)	26.2 (± 0.9)	7.59 (± 0.42)

Table 2. Statistical Values for Mussel Respiration and Water Clearance Rates

Measure	H	Df	p-value
A. plicata respiration	17.703	3	0.001
A. plicata water clearance	3.956	3	0.266
A. ligamentina respiration	18.719	2	< 0.001
A. ligamentina water clearance	12.436	2	0.002

Table 3. Statistical Values for Mesocosm Mussel Death Rates

Group	$\chi 2$	df	p-value
All Species	13.85	3	0.001
A. plicata	35.33	3	>0.001
A. pustulosa	10.43	3	0.006

Table 4. Statistical Values for Profile Analysis

Effect	Wilks' Lambda	F value	Hypothesis df	Error df	p value
Between Subjects					
Intercept	0.23	14.93	6	27.0	<0.001
Mussel Treatment	0.91	0.43	6	27.0	0.853
Stress Treatment	0.18	3.54	18	76.9	<0.001
Mussel Treatment X Stress Treatment	0.28	2.16	18	76.9	0.003
Within Subjects					
Sample Date	0.28	2.16	18	15.0	0.068
Sample Date X Mussel Treatment	0.97	0.23	18	15.0	1
Sample Date X Stress Treatment	0.16	2.56	54	45.5	0.001
Sample Date X Mussel Treatment X Stress Treatment	0.07	1.24	54	45.5	0.226

FIGURE LEGENDS

Fig 1. Proposed effects of multiple stressors from anthropogenic activity and global change on freshwater mussels, their ecosystem response, and ultimately human and environmental well-being.

Figure 2. Clearance rates (top) and respiration rates (bottom) for *Amblema plicata* (left) and *Actinonaias ligamentina* (right) exposed to three stressor treatments and a control. Boxes represent the 25th and 75th interquartile range with the bar denoting the median. Error bars denote the 5th and 95th interquartile ranges with dots marking outliers. Letters on each graph denote significant pairwise differences as indicated by an Independent Samples Kruskal-Wallis test with a Dunn-Bonferroni post hoc test a $p = 0.05$.

Fig 3. Proportions of mussels dead and alive at the end of the mesocosm experiment combined (A) and by each species (B-D) for three stress treatments and a control. The horizontal line denotes the expected frequency calculated from a $\chi 2$ goodness of fit test and letters denote homogenous subgroups as indicated by pairwise comparisons.

Fig 4. Dry mass of aquatic vegetation sampled from mesocosms for three stressors and one control with (+M) and without (-M) mussel communities. Boxes represent the 25th and 75th interquartile range with the bar denoting the median. Error bars denote the 5th and 95th interquartile ranges. Letters on each graph denote significant pairwise differences as indicated by an Independent Samples Kruskal-Wallis test with a Dunn-Bonferroni post hoc test a $p = 0.05$.

Fig 5. Concentration of ammonia (top), total phosphorus (middle), and ammonia:total phosphorus (bottom) for all mussel treatments on each sampling date. Each symbol represents the mean for the stress treatment on that sample date and the bars denote one standard deviation.

Fig 6. A global map of the areas where biodiversity is most likely to be threatened by mercury deposition and increased temperatures resulting from global climate change.

FIGURES

Figure 1. Conceptual Figure of Impacts of Environmental Stressors on Mussels and Ecosystem Processes

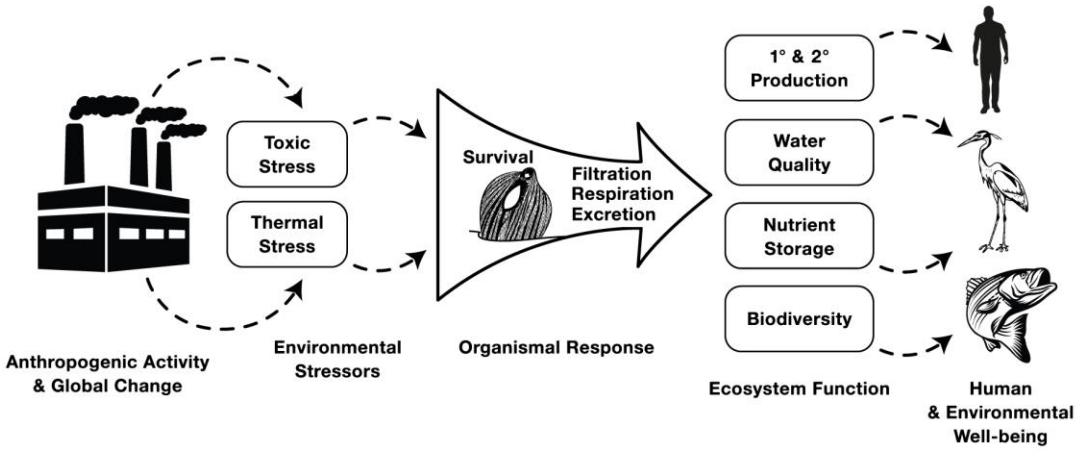

Figure 2. Physiological Responses of Mussels to Multiple Stressors

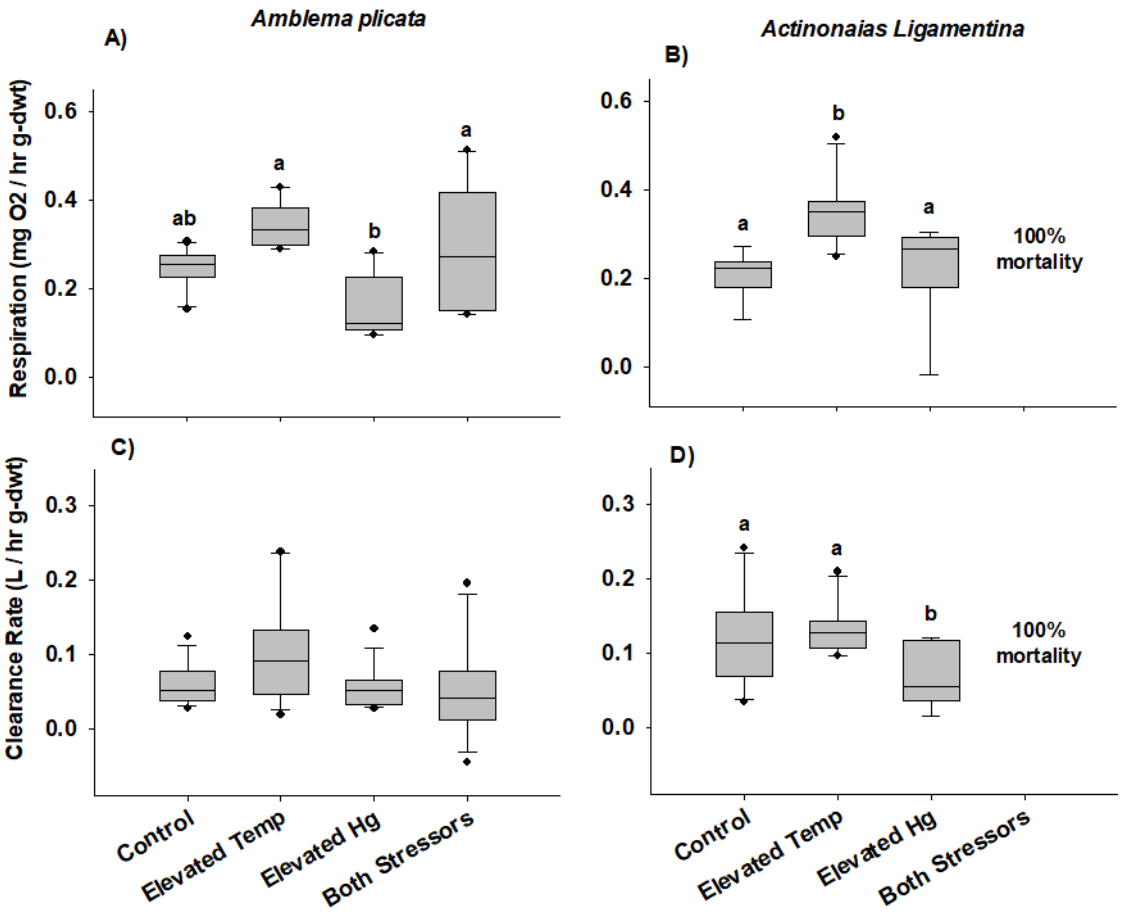

Figure 3. Mussel Death Combined and by Species for Stressor Treatments

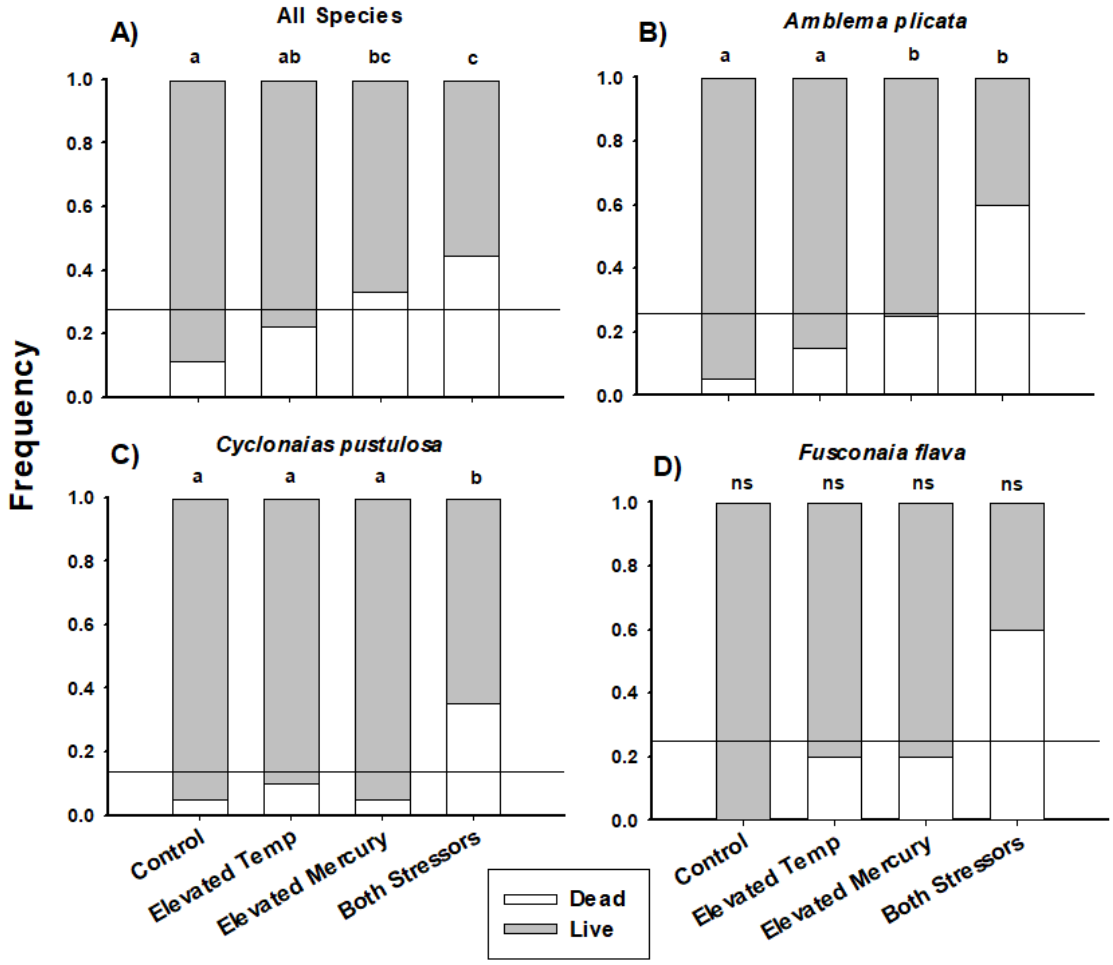

Figure 4. Macrophyte Dry Biomass

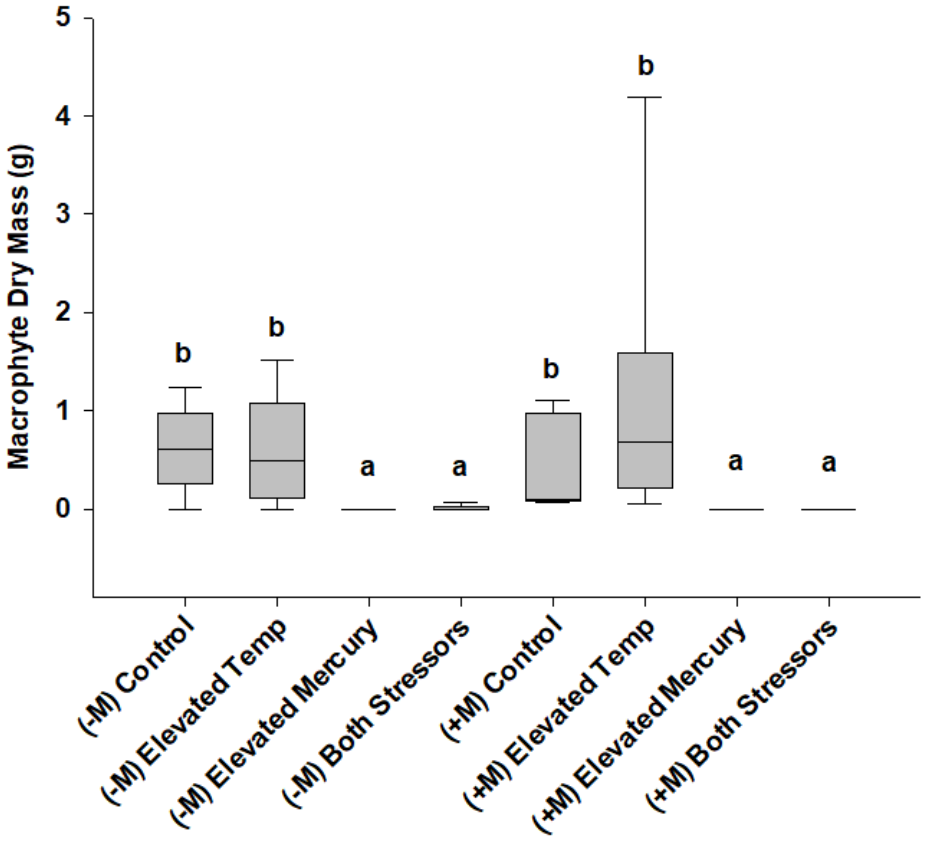

Figure 5. Nutrient Responses in Mussel Treatment Mesocosms

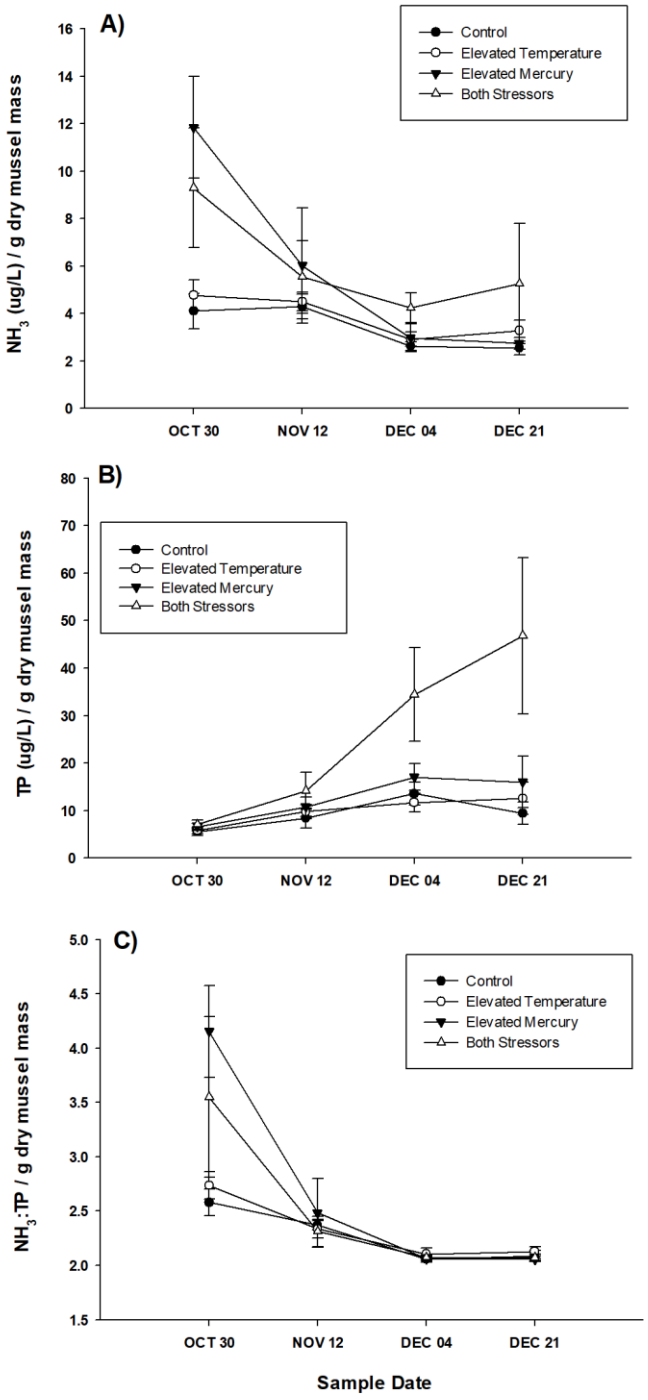

Figure 6. Map of Areas of Most Concern for Exposure to Mercury Contamination and Temperature Rise