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Investigating the evolved tolerance of zooplankton to salt pollution: a microcosm study

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Abstract

Salt pollution caused by excessive deicer application on impervious surfaces is polluting freshwater ecosystems and negatively affecting freshwater organisms in temperate regions. Freshwater environments near oceans experience natural salt intrusion due to tidal cycles and storm events. Therefore, populations of freshwater organisms near marine environments might have evolved a tolerance to increasing salinities over millennia. This contrasts with freshwater organisms far from coasts that have experienced high baseline salt concentrations for the last few decades. The evolutionary effects of increasing salinities on freshwater organisms near and far from marine environments are not fully understood. Understanding differences in evolutionary responses to salt pollution in zooplankton is crucial for maintaining the diversity of freshwater ecosystems. To investigate this, I studied the abiotic and biotic responses of four ponds with different baseline salinities and sources of increasing salinity to understand the resilience of zooplankton populations to survive excessive exposure to salt pollution. The results indicate that total chlorophyll, phycocyanin, dissolved oxygen, total abundance, species richness, and species diversity were all negatively affected by increasing salt pollution. The results also suggest that the response of zooplankton depends on previous exposure, where zooplankton populations residing in environments near marine systems demonstrated a positive response to higher salt concentrations, as opposed to populations situated farther away from marine habitats, which showed a negative response to increasing salt concentrations. These results highlight the importance of considering historical salinities when determining the ecological consequences of human-induced pollution, such as freshwater salinization.

MONTCLAIR STATE UNIVERSITY

Investigating the evolved tolerance of zooplankton to salt pollution — a microcosm study

by

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Introduction

Freshwater ecosystems in temperate regions of the world are being polluted by salts applied to impervious surfaces (e.g., roads, parking lots, and sidewalks) as deicers (Novotny et al. 2008, Schuler et al. 2019). The most common deicer applied is sodium chloride (NaCl), although other types of deicers are applied in some regions (Hintz and Relyea 2019). Salts can enter freshwater environments due to improper storage or excess application on impervious surfaces, and then the salts dissolve in melting snow or stormwater runoff and flow into nearby rivers, lakes, and streams (Hintz and Relyea 2019). The increased salt concentrations in freshwater systems can negatively affect the abundance and diversity of freshwater organisms (Hébert et al. 2023, Castello et al. 2018, James et al. 2003). The salinization of freshwater environments due to human activities such as application of high amounts of road deicers is an increasingly pressing global issue that impacts the availability of safe drinking water, the well-being of ecosystems and biodiversity, infrastructure corrosion, and agricultural productivity (Cañedo-Argüelles et al. 2019).

Salt can also enter freshwater ecosystems through natural means (Cañedo-Argüelles et al. 2019, Melles et al. 2023). In marine-adjacent environments, seawater can intrude into fresh waters through flooding during intense storms, flowing up riverine systems, or through groundwater encroachment (Werner et al. 2013, Venâncio et al. 2022, Alfarrah et al. 2018). However, with global climate change and increasing rates of sea-level rise, the frequency and intensity of natural saltwater intrusion are increasing, especially in low-lying coastal communities (Gong and Shen 2011). The increased intensity and frequency of seawater intrusion into freshwater environments might negatively affect populations of freshwater organisms (Velasco et al. 2019). Populations might be able to persist with low-frequency intrusion events

because the influx of fresh water from rain events provides intermittent relief from salts (Werner et al. 2013). Therefore, populations have time to rebound and reproduce before the next intrusion event. However, if salt intrusion happens more frequently or the baseline concentration of salts steadily increases, even populations adapted to tolerate some salt pollution might be eradicated (Venâncio et al. 2022).

While there are extensive results from lab-based studies exploring the effects of salt pollution on freshwater organisms (Delaune et al. 2021, Schuler and Relyea 2018, Arnott et al. 2020, Prosser et al. 2017, Hintz et al. 2019), little is known about how populations that have and have not experienced natural or artificial salt intrusion respond to increasing salt concentrations. Organisms in freshwater environments near marine ecosystems have likely experienced natural salt intrusion in recent history due to an influx of salt water from storm events (Tully et al. 2019, Venâncio et al. 2022). Climate change is increasing the frequency and intensity of seawater intrusion events due to increased storm intensity and sea-level rise (Michener 1997). The increasing frequency of these events will negatively affect freshwater organisms not adapted to tolerate higher baseline salinities. Populations of freshwater organisms that reside in coastal areas in New Jersey have likely evolved a tolerance to salt water over thousands of years (Jones et al. 2018). This should contrast with freshwater organisms far from coasts that have not experienced increased baseline salt concentrations for millennia.

Freshwater organisms in habitats away from oceans are highly sensitive to added salt pollution, which can result in various harmful effects such as death, delayed growth, reduced feeding efficiency, malformations, and oxidative stress (Cañedo-Argüelles et al. 2019, Hintz and Relyea 2019, Velasco et al. 2019, Lind et al. 2018). Aquatic organisms vary in their tolerance to salt pollution, and most aquatic organisms can osmoregulate to withstand low concentrations of

salt for short periods of time (Jones et al. 2017). However, due to pollution, some freshwater ecosystems have reached 25% of the concentration of seawater (35 g L^{-1}), which is deadly to most freshwater invertebrates (Di Poi et al. 2018). Some populations of freshwater organisms (e.g., Daphnia) that live away from oceans have been shown to evolve a tolerance to salt pollution in lab-induced conditions within a few generations (Hintz et al. 2019), although there are potentially detrimental effects, such as a loss of expression of genes coding for circadian rhythms (Coldsnow et al. 2017).

A complete understanding of how freshwater organisms near and far from marine environments respond to increasing salinities remains unclear. Some zooplankton populations far from marine environments have experienced salt pollution for decades. Populations of freshwater zooplankton away from marine environments that have experienced high, continuous concentrations of salt caused by excess road salt pollution might have evolved a tolerance to high salinities similar to zooplankton near marine environments. Alternatively, the evolutionary responses to natural (i.e., seawater intrusion) might be stronger compared to responses to artificial (i.e., deicer pollution) pollution (Figure 1). Understanding whether natural populations of zooplankton have the potential for rapid evolutionary responses to salt pollution is of vital importance due to the importance of zooplankton for the functioning of freshwater ecosystems (Allan 1976, Bettinetti and Mana 2013).

In this study, I examined the different population and community-level responses of freshwater zooplankton from ponds near and far from oceans that differ in their baseline salt concentrations. I investigated whether the evolution of salt tolerance in zooplankton matches the expected pattern of evolution in response to salt pollution exposure (Figure 1). I hypothesized that coastal populations of zooplankton naturally exposed to continuous, high salt conditions for

millennia have evolved constitutive (i.e., fixed) tolerances to high salinities, and populations from recently (artificially) polluted ponds have yet to evolve tolerances to high salinities.

Methods

Zooplankton

I refer to zooplankton (>20µm) as microcrustaceans (cladocerans, copepods, and ostracods) and Rotifera (Table 1). All rotifers, cladocerans, and copepods were identified to the lowest taxonomic unit (genus or species) using standard taxonomic keys (Balcer et al. 1984). Because of insufficient keys, I could only include two taxa of ostracods in our analysis. I noted the unique identity of taxa that were not identified to species by identifying the family or genus and denoting the numerical order of their identity (e.g., Daphnia 1). Daphnia 1 was morphologically distinct from Daphnia 2, but I could not determine the species epithet.

Site selection

I selected four water bodies throughout New Jersey based on preliminary abiotic data that I collected to ensure the sites fit into pre-defined low and high salinity categories. I then determined whether those differences were naturally or artificially derived. Natural refers to waterbodies located near tidal influences that have either low or high baseline salinities. Polluted or anthropogenic refers to sites far from oceanic influences and not near tidal sources, indicating that the high salt content must have come from anthropogenic sources such as road deicers.

The first natural site I selected is a pond (40.619, -74.287) situated on the upper reaches of the tidal part of the Rahway River. I selected the site as the low-salinity, natural site (sal. = 100 mg $L^{-}1$). The low baseline salinity in this pond is likely due to the exceptionally low rates of salt intrusion in the area (Kennedy 2006). For the high-salinity natural site, I selected Clay Avenue Wetland (40.802, -74.101). The pond has naturally high salinity (sal. = 3950 mg L^{-1})

due to the moderate rates of saltwater intrusion from the tidal part of the Hackensack River. South of the Pompton River in Wayne, New Jersey, Walker Avenue Wetlands (40.926, -74.285) is a low-salinity, polluted site (sal. = 100 mg L^{-1}). The final wetland I selected is located within the main campus of Montclair State University (40.872, -74.201). The pond is a high-salinity, polluted site (sal. = 180 mg L^{-1}) and was previously an abandoned mining area that remains flooded.

Experimental design and setup

To understand the impacts of salt on natural and artificial systems, I conducted an experiment using 1-L microcosms in a temperature and light-controlled lab setting. I used food-grade sodium chloride (99.9% pure, NaCl) at concentrations of 90 mg L^{-1}, 450 mg $L^{-1,}$ and 900 mg L^{-1} with the control microcosms not receiving salt treatment. I filled each microcosm with 100 g of homogeneous mixed benthic soil collected from the four selected (one low-natural, one high-natural, one low-polluted, and one high-polluted) sites and filled with 900 mL of stock solution of reverse osmosis (RO) at low, medium, high, and no salt concentrations. I used four replicates for each treatment for each of the four ponds, for a total of 64 microcosms.

For the experiment, I used acid-washed 1-L Thermo Scientific™ Nalgene™ (Fisher Scientific, Pittsburgh, Pennsylvania) jars without lids. I haphazardly placed the jars beneath full-spectrum lamps with a 12h-12h on-off light cycle for 49 days. On 9 October 2023, I aggregated and mixed the soil collected from each location and placed the mixed soil into each experimental jar. I allowed the soil to dry completely for 72 hours to ensure no immature or adult zooplankton survived, and only resting zooplankton eggs remained within the soil. On 12 October 2023, I stock solutions for each treatment to each microcosm to initiate the experiment. I maintained the

temperature in the lab between 18.1 ºC and 25.3 ºC for the duration of the experiment, resulting in water temperatures ranging from 19.0 ºC to 24.0 ºC in the microcosms.

I collected abiotic data and zooplankton emergence from resting eggs within the soil weekly, with the first abiotic data collected on 19 October 2023. For pH and conductivity, I used a pH and conductivity probe (Environmental Express, Charleston, South Carolina 29492; Table 2). Dissolved oxygen was measured in the middle and at the end of the experiment using a Hach HQ4300 portable meter with a dissolved oxygen probe (Hach, Loveland Colorado; Table 2). The goal of collecting data throughout the experiment was to track abiotic conditions in each treatment and to ensure that salt concentrations remained stable for the duration of the experiment in each treatment. After 49 days, I measured *in-vivo* (non-acidified) total chlorophyll (algal abundance), phycocyanin (cyanobacteria abundance), turbidity, and color-dissolved organic matter using a calibrated Turner Trilogy Fluorometer (Turner Designs, San Jose, CA 95112). Total nitrogen and total phosphorus were analyzed with a Hach DR6000 UV-Vis Spectrophotometer (Hach, Loveland Colorado), following the procedures from the corresponding test kit from the Hach TNT line (Table 2). I then harvested the zooplankton by collecting and filtering 900 ml of water from each jar and preserving the zooplankton with Lugol's iodine, which also stains for easier identification. I identified and counted zooplankton from each jar using a Zeiss V20 (Carl Zeiss Microscopy, LLC, White Plains, NY) dissection microscope capable of 345x magnification. I considered all rotifers, cladocerans, copepods, and ostracods to be zooplankton. Due to inadequate keys, I only considered two taxa of ostracods, one taxon of nauplii, and unidentified species, which were distinguished as unique taxa but not fully identified. From these counts, I calculated each jar's abundance (total number of individuals), richness (number of species), and diversity (inverse Simpson's index) of

zooplankton.

Analysis

All analyses and plots were completed using *R* (R Core Team, Team, and Others 2022). Normality was tested for each response variable by generating qqplots using the *qqPlot* function in the *car* package (Fox and Weisberg 2019). For response variables that had distributions other than a normal distribution, I transformed the data prior to analysis to meet the assumptions of normality. I used log or square root transformation to normalize the data depending on the prevalence of zeros in the data (e.g., count data). The majority of total phosphorus measurements were below detectable concentrations (10µg L^{-1}) and were therefore excluded from further analysis.

To investigate the effect of each treatment, I first analyzed the full model employing a MANOVA to understand how all of the response variables of interest (total chlorophyll, phycocyanin, dissolved oxygen, total nitrogen, total abundance, species richness, and species diversity) responded to the three treatment variables (source salinity (high or low), natural or anthropogenic pollution, and the treatment total dissolved solids concentration (control, low, medium, or high). Given that the full model for the Wilk's corrected MANOVA was significant, we conducted univariate ANOVAs for each response variable. I analyzed the abiotic and biotic responses by conducting three-factor ANOVAs for each response variable against the treatments using the *aov* function. I created plots using *ggplot2* (Wickham 2016).

Results

In total, 59 taxa emerged from the sediment in the microcosms, including 32 rotifer taxa, 18 cladoceran taxa, 7 copepod taxa, and 2 ostracod taxa. I was able to identify 44 of the 59 taxa to species (Table 1). The full model, including the interactions of the three predictor variables

(the pond salt concentration, pollution source, and treatment), was statistically significant for the MANOVA (Table 3). Therefore, I conducted seven univariate three-way ANOVAs on each response variable. Total chlorophyll was affected by pond salt concentration, pollution source, and treatment and the interaction among all three factors (Table 4, Figure 2). Phycocyanin concentration (i.e., cyanobacteria abundance) responded to treatment and pond salt concentration and the interaction of those two factors (Table 4, Figure 3). The concentration of salt in the pond, pollution source, treatment, and the interaction between treatment with pollution source and treatment with pond salt concentration were found to have an impact on the dissolved oxygen levels (Figure 4), but there were no effects on total nitrogen concentrations (Table 4). Total abundance was affected by pond salt concentration and the interaction between pond salt concentration and salinity source (Table 5, Figure 5). The concentration of salt in the pond and the interaction between pond salt concentration and the source of salinity were found to influence species richness (Table 5, Figure 6). Lastly, species diversity was affected by treatment and the interaction between pond salt concentration, salinity source, and treatment (Table 5, Figure 7).

Discussion

I investigated the impact of salt pollution on ponds that have experienced long-term, natural influxes due to their proximity to the ocean (near and far) and anthropogenically salinized freshwater ponds that have been differentially polluted by road salts (low or high pollution). This study addresses two key questions: (1) whether freshwater organisms exposed to anthropogenic salt pollution are evolving tolerance to increasing salinity in historically low-salinity ponds, and (2) whether the evolution of salt tolerance in populations of freshwater organisms near coastal ponds matches the anticipated pattern of evolution (Figure 1).

Over the past century, New Jersey has experienced a rapid pace of urbanization that has brought about environmental challenges, particularly in the form of pollution that seeps into freshwater systems (Rossi 2020). As pollutants enter freshwater systems, essential organisms such as zooplankton can be negatively affected. Some species of zooplankton are able to tolerate low concentrations of salts from pollution for a brief time in lab-based settings (Hintz et al. 2019), but high concentrations of salt are deadly (Di Poi et al. 2018, Arnott et al. 2023, Hintz et al. 2023). The disruption of zooplankton communities in aquatic systems is detrimental to the health of the ecosystems as zooplankton are essential to aquatic ecosystems as they are consumers of algae, nutrient recyclers, and indicators of water quality because they are sensitive to toxins (Allan 1976).

The data support the hypothesis that coastal zooplankton populations living in natural saline conditions (i.e., close to marine systems) responded positively to increasing concentrations of salts as compared to populations that live slightly farther from marine habitats and experience salt intrusion less frequently, which responded negatively to increasing salinities. Additionally, the response of zooplankton inhabiting anthropogenically polluted areas matched the expectation that their diversity would decrease as salt concentrations increased (Table 5, Figure 7). One possible explanation is that the zooplankton communities in naturally saline environments (i.e., near marine ecosystems) have had millennia to evolve a constitutive tolerance and selection to these conditions and have developed innate mechanisms to handle any alterations (Hintz et al. 2019, Hintz and Relyea 2017).

Examples of such possible mechanisms include producing diapausing eggs that are capable of withstanding extended periods of adverse, high-salinity environmental conditions (Bailey et al. 2004). Alternatively, adult organisms might be able to maintain high osmolyte

content of their hemolymph to reduce the energetic costs of maintaining osmotic balance (Moffett et al. 2023). Results from other mesocosm experiments with coastal communities of *Daphnia* suggest that salt tolerance can evolve in a short amount of time (2 to 3 months) within a few generations (Coldsnow et al. 2017). On the other hand, zooplankton communities from anthropogenically polluted ponds have only experienced increased salt concentrations for a relatively brief period of time, and they lack the adaptive capacity that makes these communities less likely to survive and reproduce in high salt conditions (Hébert et al. 2023, Castello et al. 2018, James et al. 2003). The ability of zooplankton to evolve a tolerance to increased salt pollution appears to be directly tied to previous exposure to salt pollution and the length of time those conditions are experienced.

Total chlorophyll decreased as salt treatments increased (Figure 2). Previous mesocosm research investigating the effects of salt pollution on established plankton communities (i.e., applying salt to adult organisms) indicates that the death of adult zooplankton results in increased eukaryotic algae. However, the results in this study differ likely because this represents the response of algae (emergence and growth) to increasing salt pollution. Several studies show salt reduces algal growth and diminishes the total chlorophyll levels produced by eukaryotic algae (Romanenko et al. 2017, Bartolomé et al. 2009, Affenzeller et al. 2009, Figler et al. 2019). The strongest negative response to salt pollution occurred in the treatments exposing Walker Wetlands (the low-salinity, anthropogenically polluted pond) to increasing salinities (Figure 2). Although I did not investigate the algal species present in the ponds, the species of eukaryotic algae in Walker Avenue Wetlands might be more sensitive due to typically low salt pollution in the region.

The data also suggest that cyanobacteria (phycocyanin) declined in both of the low-source salinity ponds (Figure 3). The ponds with high natural and anthropogenically derived salinity did not experience a drop in cyanobacteria abundance with increasing treatment, but the low-salinity ponds did (Figure 3). The difference in the response suggests that the low-salinity ponds had different species of cyanobacteria that were less able to tolerate increasing salt pollution (Yang et al. 2020, Fatma et al. 2007, Klähn et al. 2021, Singh et al. 2022). Researchers have suggested that cyanobacteria are more tolerant of increasing salinity and should increase or maintain in abundance (Tonk et al. 2007, Pecher et al. 2019). However, results of studies on cyanobacteria, even the same species (*Microcystis arugenosa*), show tolerance to increasing salinity and reductions in abundance due to increasing salinity (Tonk et al. 2007, Bartolomé et al. 2009). More research on the response of cyanobacteria in realistic experimental conditions (e.g., a community-based approach) will provide better information about the response of cyanobacteria to increasing salinities and if salt pollution might be responsible for cyanoblooms (Tonk et al. 2007).

Nitrogen did not statistically change among the treatments or ponds. Previous research on freshwater salinization indicates that nitrate concentrations may be affected as salt increases due to the effects of salts on bacteria responsible for nitrification or denitrification or because these processes are affected by changes in alkalinity, which might be affected by salt pollution (Kaushal et al. 2019). Dissolved oxygen also decreased as salinity increased in each of the treatments from each pond (Figure 4). The high-salinity ponds that have natural or anthropogenically-induced salt concentrations had consistently higher dissolved oxygen concentrations, although the exact mechanism remains unknown, especially given that chlorophyll concentrations were not higher in these ponds. Future research investigating the

biotic and abiotic interactions following salt pollution would help elucidate these likely complex interactions.

The results from this study highlight the negative consequences of salt pollution for freshwater ecosystems. However, the adverse effects on zooplankton might be somewhat temporary as zooplankton evolve higher tolerances of salts given the continued inputs from anthropogenic sources such as deicers. Nonetheless, further exploration is needed to investigate the results of this microcosm study, which has limited capacity to determine pond or lake-level effects of salinization. Microcosm studies can help understand the potential tipping points of zooplankton communities and assess whether the selection of salt tolerance in these communities aligns with the expected tolerance levels in salt-polluted populations (Schuler et al. 2019, Hintz et al. 2019). Additional research is needed to understand the full ecological consequences of road salt pollution entering freshwater ecosystems in temperate regions.

Table 1. A total of 59 taxa were found in the sediment of the microcosms, comprising 32 rotifer taxa, 18 cladoceran taxa, 7 copepod taxa, and 2 ostracod taxa. It was possible to identify 44 of the 59 taxa as species, which accounts for approximately 75% of the total.

Phylum	**Taxa**		
Rotifera	*Monostyla lunaris*	*Monostyla closterocerca*	*Monostyla cornuta*
	Monostyla copies	*Monostyla stenroosi*	*Monostyla closterocerca*
	Lecane tenuiseta	*Lecane tudicola*	*Monostyla cornuta*
	Asplanchna 1	*Keratella earlinae*	*Lecane ohioensis*
	Pompholyx sulcata	*Notholca labis*	*Brachionus ureceolaris*
	Hexarthra mira	*Notholca acuminata*	*Brachionus havanaensis*
	Filinia terminalis	*Keratella hiemalis*	*Lepadella acuminata*
	Keratella testudo	*Keratella valga*	*Platyias patulus*
	Anuraeopsis 1	*Euchlanis triquetra*	*Brachionus angularis*
	Keratella crassa	*Brachionus quadridentatus*	*Lecane luna*
	Lepadella rhomboides	Rotifer 1	
Arthropoda	*Kurzia lattisima*	*Scapholeberis mucronate*	*Ceriodaphnia dubia*
	Alona setulosa	*Daphnia parvula*	*Daphnia lumholtzi*
	Leydigia 1	Eurycercus 1	*Diaphanosoma brachyurum*
	Ceriodaphnia rigaudi	Calenoid copepod 1	*Diacyclops thomasi*
	Alona rustica	*Alona bicolor*	Alonella 1
	Acanthocyclops vernalis	*Polyphemus pediculus*	*Oxyurella brevicaudis*
	Cyclopoid copepod 1	*Chydorus sphaericus*	Daphnia 1
	Daphnia 2	Diaptomidae 1	Eucyclops 1
	Microcyclops 1		
Other	Ostracod spp. 1	Ostracod spp. 2	

Table 2. Summary of methods used to test each water quality parameter.

Parameter	Instrument	Method name	Units
Conductance	Meter	-	µS L^{-1}
Salinity	Meter	-	mg L^{-1}
pH	Meter	-	Standard units
Temp	Meter	-	ºC
Total Dissolved Solids	Meter	-	ppt
Dissolved Oxygen	Meter	-	mg L^{-1}
Phycocyanin	Trilogy	*In-vivo* fluorometric	RFU
Turbidity	Trilogy	Fluorometric	RFU
cDOM	Trilogy	Fluorometric	RFU
Total chlorophyll	Trilogy	*In-vivo* fluorometric	RFU
Nitrogen (total)	DR6000	Persulfate Digestion	mg L^{-1}
Phosphorus (total)	DR6000	Ascorbic Acid Method	mg L^{-1}

Table 3. MANOVA results for a full model that included the response variables chlorophyll, phycocyanin, total abundance, dissolved oxygen, species diversity (ENS_{PIE}), and the Chao-corrected species richness. Wilks lambda is a measure of how much the dependent variables are able to discriminate between levels or groups defined by the independent variables. Eta squared indicates how much of the variation in multiple outcomes can be explained by the predictor variables. Statistically significant ($p<0.05$) predictors are in bold. Salinity source refers to the baseline concentration of the pond's salinity being low or high. Pollution source refers to the source of pollution being natural (seawater) or anthropogenic (e.g., road salt). Treatment refers to the experimental salt concentration (control, low, medium, high).

Predictor variable(s)	df	Wilks	Eta sq.	F (approx.)	P-value
Salinity source	1	0.243	0.760	22.280	**<0.001**
Pollution source	1	0.383	0.621	11.552	**<0.001**
Treatment	3	0.335	0.321	2.779	**<0.001**
Salt source * pollution source	1	0.652	0.348	3.828	**0.004**
Salt source * treatment	3	0.565	0.188	1.422	0.096
Pollution source * treatment	3	0.554	0.217	1.549	0.076
Salt source * pollution source * treatment	3	0.504	0.216	1.700	**0.026**
Residuals	48				

Table 4. Univariate ANOVA results for total chlorophyll, phycocyanin, and dissolved oxygen measured at the end of the experiment. Salinity source refers to the baseline concentration of the pond's salinity being low or high. Pollution source refers to the source of pollution being natural (seawater) or anthropogenic (e.g., road salt). Treatment refers to the experimental salt concentration (control, low, medium, high).

Total chlorophyll (log RFU)	df	Sum Sq.	Mean Sq.	F	P
Salinity source	1	0.048	0.048	0.542	0.465
Pollution source	1	3.478	3.478	39.378	**<0.001**
Treatment	3	2.202	0.734	8.311	**<0.001**
Salt source * pollution source	1	0.525	0.525	5.941	**0.019**
Salt source * treatment	3	0.881	0.294	3.324	**0.027**
Pollution source * treatment	3	0.867	0.289	3.272	**0.029**
Salt source * pollution source * treatment	3	0.765	0.255	2.888	**0.045**
Residuals	48	4.239	0.088		
Phycocyanin (log RFU)	df	Sum Sq.	Mean Sq.	F	P
Salinity source	1	0.008	0.008	0.720	0.400
Pollution source	1	0.001	0.001	0.064	0.802
Treatment	3	0.160	0.053	4.564	**0.007**
Salt source * pollution source	1	0.016	0.016	1.332	0.254
Salt source * treatment	3	0.098	0.033	2.795	**0.050**
Pollution source * treatment	3	0.018	0.006	0.502	0.682
Salt source * pollution source * treatment	3	0.005	0.002	0.130	0.942
Residuals	48	0.563	0.012		
Dissolved oxygen (log mg L^{-1})	df	Sum Sq.	Mean Sq.	F	P
Salinity source	1	200.152	200.152	129.356	**<0.001**
Pollution source	1	37.454	37.454	24.206	**<0.001**
Treatment	3	57.498	19.166	12.387	**<0.001**
Salt source * pollution source	1	0.406	0.406	0.263	0.611
Salt source * treatment	3	14.710	4.903	3.169	**0.033**
Pollution source * treatment	3	16.899	5.633	3.641	**0.019**
Salt source * pollution source * treatment	3	11.359	3.786	2.447	0.075
Residuals	48	74.270	1.547		
Total nitrogen (log mg L-1)	df	Sum Sq.	Mean Sq.	F	P
Salinity source	1	0.863	0.863	0.486	0.489
Pollution source	1	2.269	2.269	1.277	0.264
Treatment	3	6.983	2.328	1.311	0.282
Salt source * pollution source	1	0.059	0.059	0.033	0.856
Salt source * treatment	3	6.752	2.251	1.267	0.296
Pollution source * treatment	3	7.213	2.404	1.354	0.268
Salt source * pollution source * treatment	3	2.037	0.679	0.382	0.766
Residuals	48	85.247	1.776		

Table 5. Univariate ANOVA results for total abundance, Chao-corrected species richness, and species diversity (ENS_{PIE}). Salinity source refers to the baseline concentration of the pond's salinity being low or high. Pollution source refers to the source of pollution being natural (seawater) or anthropogenic (e.g., road salt). Treatment refers to the experimental salt concentration (control, low, medium, high).

Total abundance (log)	df	Sum Sq.	Mean Sq.	F	P
Salinity source	1	1.834	1.834	6.889	**0.012**
Pollution source	1	0.784	0.784	2.946	0.093
Treatment	3	0.181	0.060	0.227	0.877
Pond salt * salinity source	1	2.365	2.365	8.881	**0.005**
Pond salt * treatment	3	0.090	0.030	0.113	0.952
Salinity source * treatment	3	0.686	0.229	0.859	0.469
Pond salt * salinity source * treatment	3	0.726	0.242	0.909	0.444
Residuals	48	12.781	0.266		

Species richness (Sq. rt. Chao corrected)	df	Sum Sq.	Mean Sq.	F	P
Salinity source	1	0.677	0.677	3.549	**0.066**
Pollution source	1	0.298	0.298	1.564	0.217
Treatment	3	1.004	0.335	1.755	0.168
Pond salt * salinity source	1	0.951	0.951	4.989	**0.030**
Pond salt * treatment	3	0.614	0.205	1.074	0.369
Salinity source * treatment	3	1.476	0.492	2.581	0.064
Pond salt * salinity source * treatment	3	1.087	0.362	1.899	0.142
Residuals	48	9.153	0.191		

Species diversity (Sq. rt. ENS_{PIE})	df	Sum Sq.	Mean Sq.	F	P
Salinity source	1	0.058	0.058	0.750	0.391
Pollution source	1	0.142	0.142	1.841	0.181
Treatment	3	0.830	0.277	3.589	**0.020**
Pond salt * salinity source	1	0.259	0.259	3.356	0.073
Pond salt * treatment	3	0.082	0.027	0.354	0.786
Salinity source * treatment	3	0.531	0.177	2.295	0.090
Pond salt * salinity source * treatment	3	0.997	0.332	4.310	**0.009**
Residuals	48	3.701	0.077		

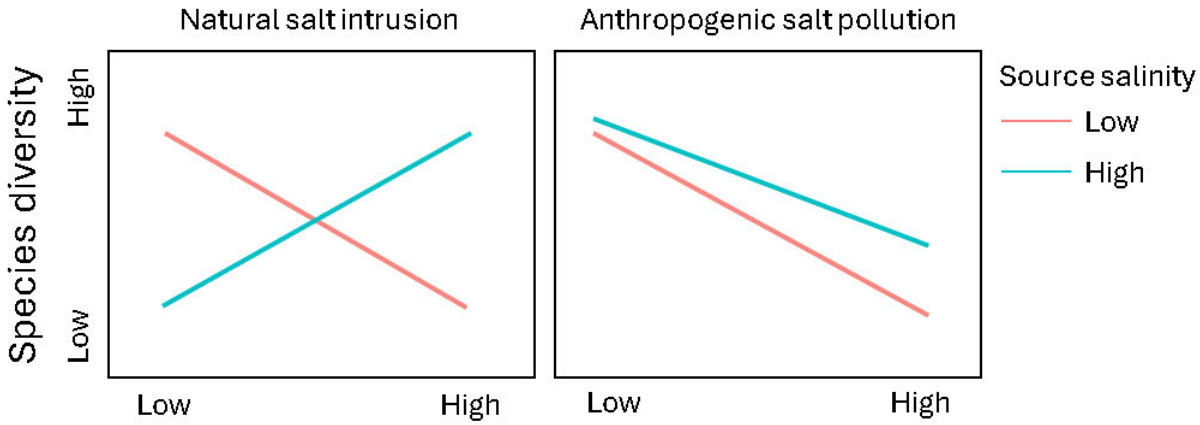

Figure 1. Expected results for the effects of increasing salt treatments (x-axis) on the species diversity (y-axis) given the source pond salinity (low or high) and whether the pond experiences natural or anthropogenic salt pollution.

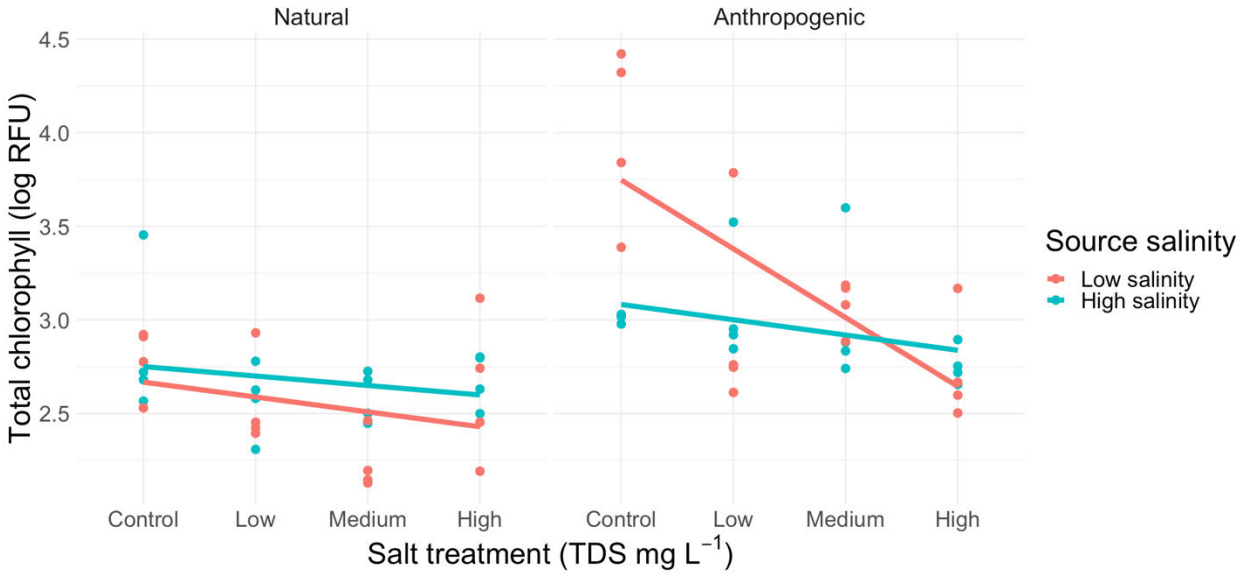

Figure 2. Measured total chlorophyll (Table 4) measured across increasing salt treatments (no salt (0 mg L $^{-1)}$, low (90 mg L $^{-1}$), medium (450 mg L $^{-1}$), and high (900 mg L $^{-1}$)) from ponds that have low baseline and high baseline salinity. Ponds were categorized as being naturally saline (near the ocean) and anthropogenic salt pollution (far from the ocean).

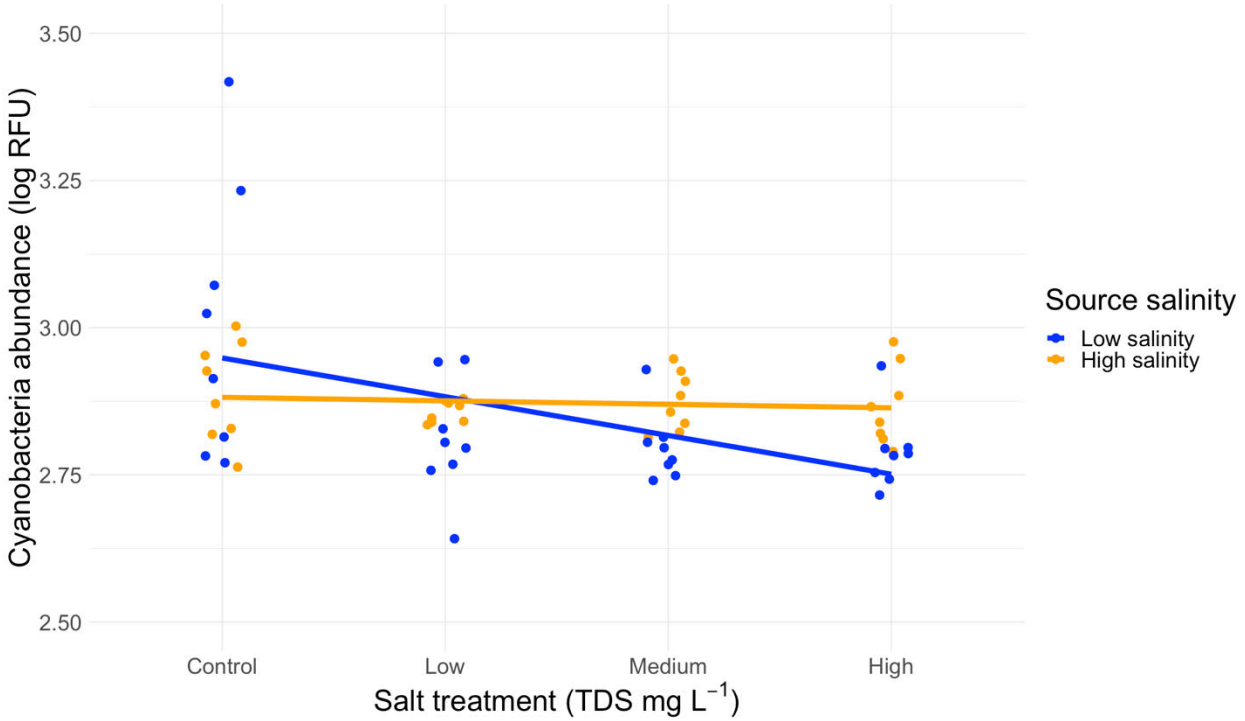

Figure 3. Phycocyanin (i.e., cyanobacteria abundance) (Table 4) across increasing salt treatments (no salt (0 mg L $^{-1)}$, low (90 mg L $^{-1}$), medium (450 mg L $^{-1}$), and high (900 mg L $^{-1}$)) from ponds that have low baseline and high baseline salinity. Ponds were categorized as being naturally saline (near the ocean) and anthropogenic salt pollution (far from the ocean).

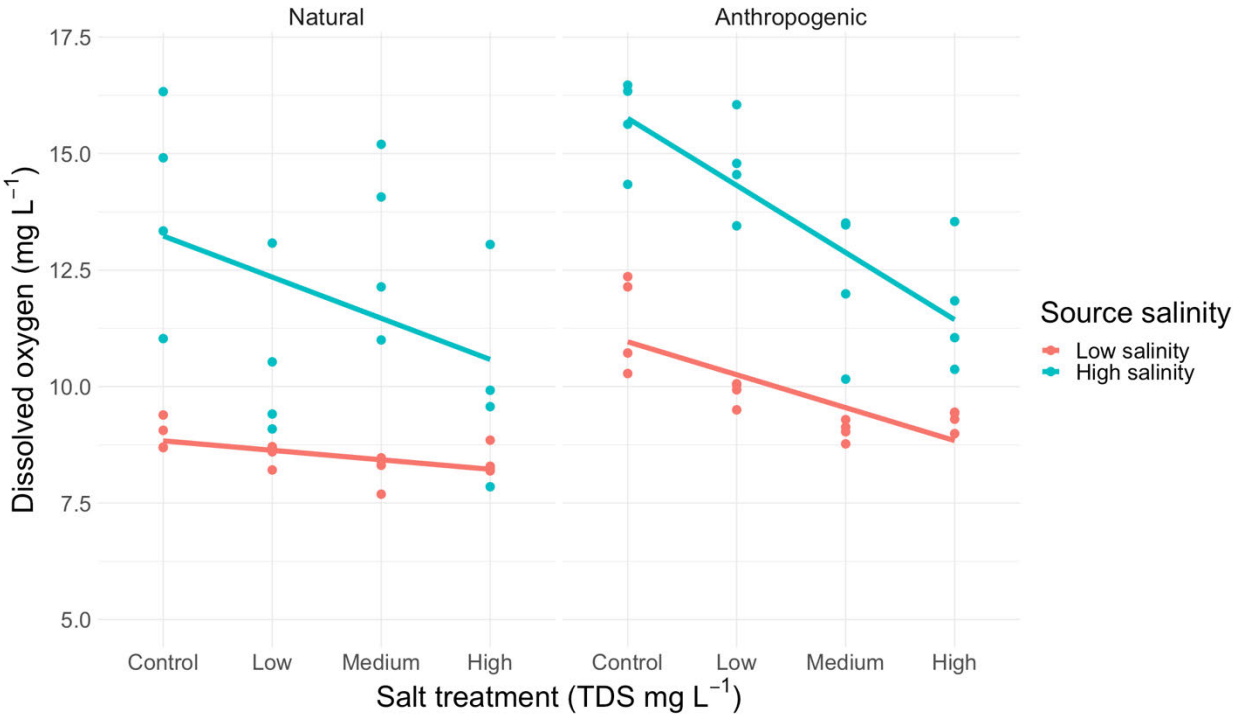

Figure 4. Dissolved oxygen (Table 4) across increasing salt treatments (no salt (0 mg L $^{-1)}$, low (90 mg L $^{-1}$), medium (450 mg L $^{-1}$), and high (900 mg L $^{-1}$)) from ponds that have low baseline and high baseline salinity. Ponds were categorized as being naturally saline (near the ocean) and anthropogenic salt pollution (far from the ocean).

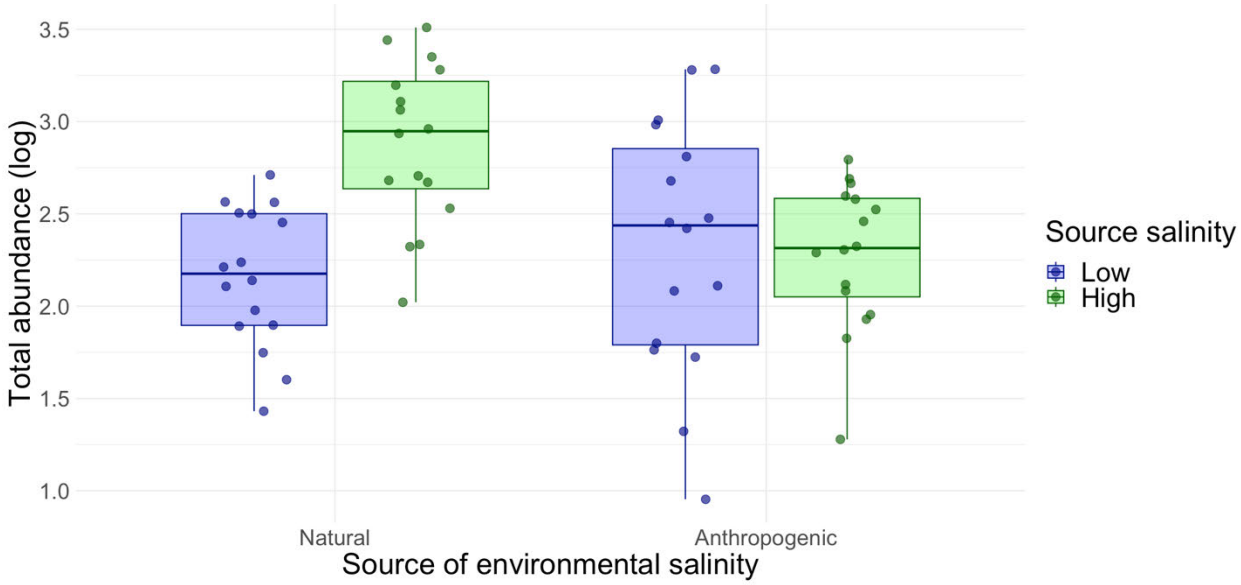

Figure 5. Total abundance (Table 5) differences in natural compared to anthropogenically influenced communities given their respective low and high salinities across a range of salt treatments (no salt (0 mg L^{-1}), low (90 mg L^{-1}), medium (450 mg L^{-1}), and high (900 mg L^{-1}). Ponds were categorized as being naturally saline (near the ocean) and anthropogenic salt pollution (far from the ocean). Ponds were categorized as being naturally saline (near the ocean) and anthropogenic salt pollution (far from the ocean).

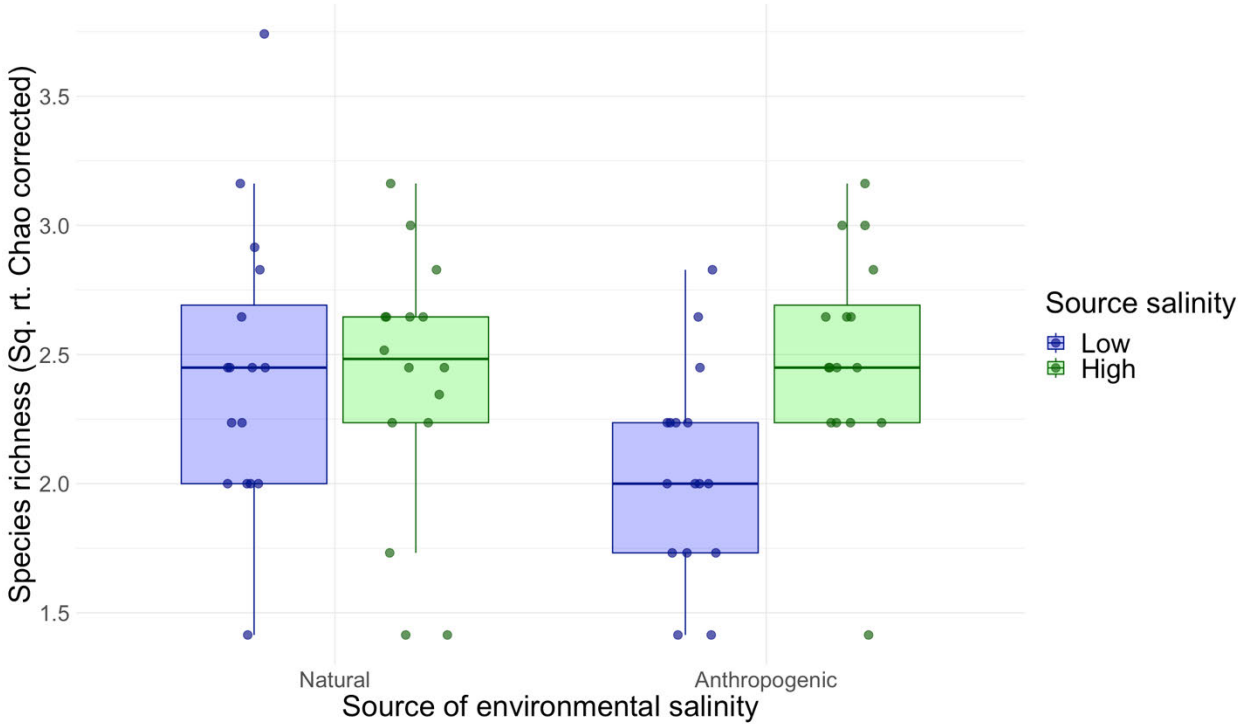

Figure 6. Species richness (Table 5) differences in natural compared to anthropogenically influenced communities given their respective low and high salinities across a range of salt treatments (no salt (0 mg L^{-1}), low (90 mg L^{-1}), medium (450 mg L^{-1}), and high (900 mg L^{-1}). Ponds were categorized as being naturally saline (near the ocean) and anthropogenic salt pollution (far from the ocean). Ponds were categorized as being naturally saline (near the ocean) and anthropogenic salt pollution (far from the ocean).

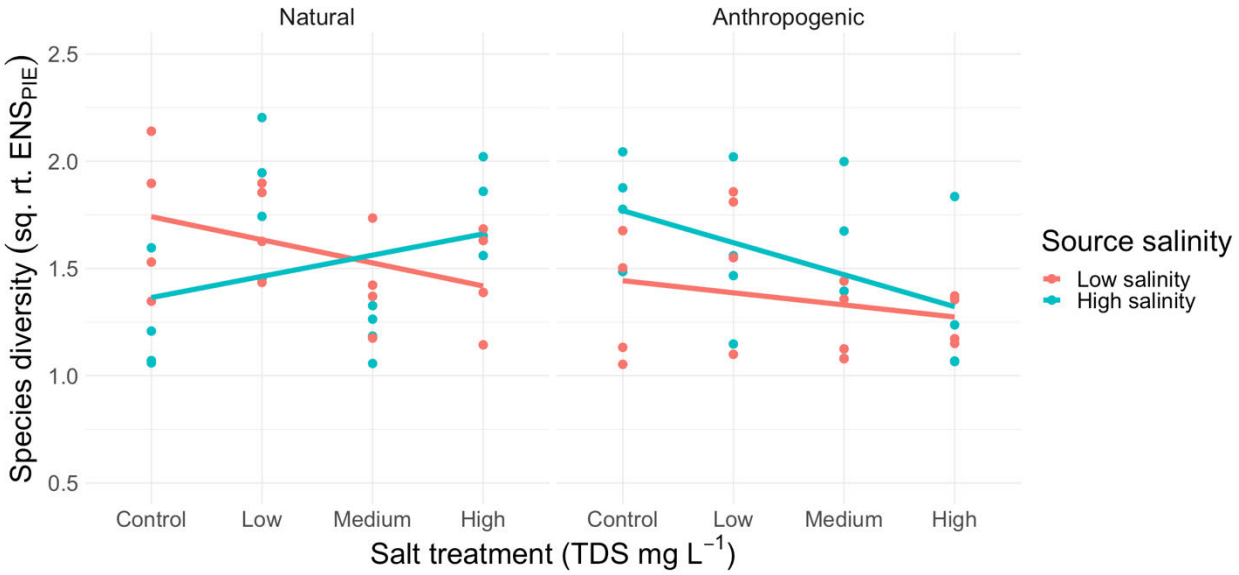

Figure 7. Species diversity (Table 5) differences in natural compared to anthropogenically influenced communities given their respective low and high salinities across a range of salt treatments (no salt (0 mg L $^{-1)}$, low (90 mg L $^{-1}$), medium (450 mg L $^{-1}$), and high (900 mg L $^{-1}$). Ponds were categorized as being naturally saline (near the ocean) and anthropogenic salt pollution (far from the ocean).

References

Acosta-Motos, J. R., Ortuño, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J., & Hernandez, J. A. (2017). Plant responses to salt stress: adaptive mechanisms. Agronomy, 7(1), 18.

Affenzeller, M. J., Darehshouri, A., Andosch, A., Lütz, C., & Lütz-Meindl, U. (2009). Salt stress-induced cell death in the unicellular green alga Micrasterias denticulata. Journal of experimental botany, 60(3), 939-954.

Alfarrah, N., & Walraevens, K. (2018). Groundwater overexploitation and seawater intrusion in coastal areas of arid and semi-arid regions. Water, 10(2), 143.

Allan, J.D., 1976. Life history patterns in zooplankton. The American Naturalist, 110(971), pp.165-180.

Araújo, L. R., Lopes, P. M., Santangelo, J. M., de Assis Esteves, F., & Bozelli, R. L. (2014). Long-term dynamics of the zooplankton community during large salinity fluctuations in a coastal lagoon. Marine and Freshwater Research, 66(4), 352-359.

Arnott, S. E., Celis-Salgado, M. P., Valleau, R. E., DeSellas, A. M., Paterson, A. M., Yan, N. D., ... & Rusak, J. A. (2020). Road salt impacts freshwater zooplankton at concentrations below current water quality guidelines. Environmental Science & Technology, 54(15), 9398-9407.

Arnott, S. E., Fugère, V., Symons, C. C., Melles, S. J., Beisner, B. E., Cañedo-Argüelles, M., ... & Derry, A. M. (2023). Widespread variation in salt tolerance within freshwater zooplankton species reduces the predictability of community-level salt tolerance. Limnology and Oceanography Letters, 8(1), 8-18.

Bailey, S. A., Duggan, I. C., van Overdijk, C. D., Johengen, T. H., Reid, D. F., & MacIsaac, H. J. (2004). Salinity tolerance of diapausing eggs of freshwater zooplankton. Freshwater Biology, 49(3), 286-295.

Balcer, M. D., Korda, N. L., & Dodson, S. I. (1984). Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. Univ of Wisconsin Press.

Bartolomé, M. C., D'ors, A., & Sánchez-Fortún, S. (2009). Toxic effects induced by salt stress on selected freshwater prokaryotic and eukaryotic microalgal species. Ecotoxicology, 18, 174-179.

Bartolomé, M. C., D'ors, A., & Sánchez-Fortún, S. (2009). Toxic effects induced by salt stress on selected freshwater prokaryotic and eukaryotic microalgal species. Ecotoxicology, 18, 174-179.

Bettinetti, R., & Manca, M. (2013). Understanding the role of zooplankton in transfer of pollutants through trophic food webs. Zooplankton: Species Diversity, Distribution and Seasonal Dynamics; Kehayias, G., Ed, 1-18.

Castillo, A. M., Sharpe, D. M., Ghalambor, C. K., & De León, L. F. (2018). Exploring the effects of salinization on trophic diversity in freshwater ecosystems: a quantitative review. Hydrobiologia, 807, 1-17.

Cañedo-Argüelles, M., Kefford, B., & Schäfer, R. (2019). Salt in freshwaters: causes, effects and prospects-introduction to the theme issue. Philosophical Transactions of the Royal Society B, 374(1764), 20180002.

Coldsnow, K.D., Relyea, R.A. and Hurley, J.M., 2017. Evolution to environmental contamination ablates the circadian clock of an aquatic sentinel species. Ecology and Evolution, 7(23), pp.10339-10349.

Cole, J. J., Carpenter, S. R., Kitchell, J., Pace, M. L., Solomon, C. T., & Weidel, B. (2011). Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proceedings of the National Academy of Sciences, 108(5), 1975-1980.

Delaune, K. D., Nesich, D., Goos, J. M., & Relyea, R. A. (2021). Impacts of salinization on aquatic communities: Abrupt vs. gradual exposures. Environmental Pollution, 285, 117636.

Di Poi, C., Costil, K., Bouchart, V. and Halm-Lemeille, M.P., 2018. Toxicity assessment of five emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic organisms. Environmental Science and Pollution Research, 25, pp.6122-6134.

Fatma, T., Khan, M. A., & Choudhary, M. (2007). Impact of environmental pollution on cyanobacterial proline content. Journal of Applied Phycology, 19, 625-629.

Figler, A., B-Béres, V., Dobronoki, D., Márton, K., Nagy, S. A., & Bácsi, I. (2019). Salt tolerance and desalination abilities of nine common green microalgae isolates. Water, 11(12), 2527.

Fox, J., and S. Weisberg. 2019. "An ${R}$ Companion to Applied Regression 3rd Ed Sage Thousand Oaks." CA.

Gong, W., & Shen, J. (2011). The response of salt intrusion to changes in river discharge and tidal mixing during the dry season in the Modaomen Estuary, China. Continental Shelf Research, 31(7-8), 769-788.

Hintz, W. D., & Relyea, R. A. (2017). A salty landscape of fear: responses of fish and zooplankton to freshwater salinization and predatory stress. Oecologia, 185(1), 147-156.

Hintz, W. D., & Relyea, R. A. (2019). A review of the species, community, and ecosystem impacts of road salt salinisation in fresh waters. Freshwater biology, 64(6), 1081-1097.

Hintz, W.D., Arnott, S.E., Symons, C.C., Greco, D.A., McClymont, A., Brentrup, J.A., Cañedo-Argüelles, M., Derry, A.M., Downing, A.L., Gray, D.K. and Melles, S.J., 2022. Current water quality guidelines across North America and Europe do not protect lakes from salinization. Proceedings of the National Academy of Sciences, 119(9), p.e2115033119.

Hintz, W.D., Jones, D.K. and Relyea, R.A., 2019. Evolved tolerance to freshwater salinization in zooplankton: life-history trade-offs, cross-tolerance and reducing cascading effects. Philosophical Transactions of the Royal Society B, 374(1764), p.20180012.

Howard, K.W. and Haynes, J. 1993. Groundwater contamination due to road de-icing chemicals—salt balance implications. Geoscience Canada.

Hébert, M.P., Symons, C.C., Cañedo-Argüelles, M., Arnott, S.E., Derry, A.M., Fugère, V., Hintz, W.D., Melles, S.J., Astorg, L., Baker, H.K. and Brentrup, J.A., 2023. Lake salinization drives consistent losses of zooplankton abundance and diversity across coordinated mesocosm experiments. Limnology and oceanography Letters, 8(1), pp.19-29.

Hébert, M.P., Symons, C.C., Cañedo-Argüelles, M., Arnott, S.E., Derry, A.M., Fugère, V., Hintz, W.D., Melles, S.J., Astorg, L., Baker, H.K. and Brentrup, J.A., 2023. Lake salinization drives consistent losses of zooplankton abundance and diversity across coordinated mesocosm experiments. Limnology and oceanography Letters, 8(1), pp.19-29.

James, K. R., Cant, B., & Ryan, T. (2003). Responses of freshwater biota to rising salinity levels and implications for saline water management: a review. Australian Journal of Botany, 51(6), 703-713.

Jones, D.K., Mattes, B.M., Hintz, W.D., Schuler, M.S., Stoler, A.B., Lind, L.A., Cooper, R.O. and Relyea, R.A., 2017. Investigation of road salts and biotic stressors on freshwater wetland communities. Environmental Pollution, 221, pp.159-167.

Jones, D.K., Mattes, B.M., Hintz, W.D., Schuler, M.S., Stoler, A.B., Lind, L.A., Cooper, R.O. and Relyea, R.A., 2017. Investigation of road salts and biotic stressors on freshwater wetland communities. Environmental Pollution, 221, pp.159-167. Di Poi, C., Costil, K., Bouchart, V. and Halm-Lemeille, M.P., 2018. Toxicity assessment of five emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic organisms. Environmental Science and Pollution Research, 25, pp.6122-6134.

Kaushal, S. S., Likens, G. E., Pace, M. L., Haq, S., Wood, K. L., Galella, J. G., ... & Jaworski, N. (2019). Novel 'chemical cocktails' in inland waters are a consequence of the freshwater salinization syndrome. Philosophical Transactions of the Royal Society B, 374(1764), 20180017.

Kennedy, M. J. J. (2006). Rahway, New Jersey. Population, 1.

Klähn, Stephan, Stefan Mikkat, Matthias Riediger, Jens Georg, Wolfgang R. Hess, and Martin Hagemann. "Integrative analysis of the salt stress response in cyanobacteria." Biology Direct 16, no. 1 (2021): 26.

Lind, L., Schuler, M. S., Hintz, W. D., Stoler, A. B., Jones, D. K., Mattes, B. M., & Relyea, R. A. (2018). Salty fertile lakes: How salinization and eutrophication alter the structure of freshwater communities. Ecosphere, 9(9), e02383.

Melles, S. J., Cañedo-Argüelles, M., & Derry, A. M. (2023). Documenting the impacts of increasing salinity in freshwater and coastal ecosystems: Introduction to the special issue. Limnology and Oceanography Letters.

Michener, W. K., Blood, E. R., Bildstein, K. L., Brinson, M. M., & Gardner, L. R. (1997). Climate change, hurricanes and tropical storms, and rising sea level in coastal wetlands. Ecological applications, 7(3), 770-801.

Moffett, E. R., Baker, H. K., Bonadonna, C. C., Shurin, J. B., & Symons, C. C. (2023). Cascading effects of freshwater salinization on plankton communities in the Sierra Nevada. Limnology and Oceanography Letters, 8(1), 30-37.

Novotny, E.V., Murphy, D. and Stefan, H.G., 2008. Increase of urban lake salinity by road deicing salt. Science of the Total Environment, 406(1-2), pp.131-144.

Pecher, W. T., Al Madadha, M. E., DasSarma, P., Ekulona, F., Schott, E. J., Crowe, K., ... & DasSarma, S. (2019). Effects of road salt on microbial communities: Halophiles as biomarkers of road salt pollution. PLoS One, 14(9), e0221355.

Prosser, R. S., Rochfort, Q., McInnis, R., Exall, K., & Gillis, P. L. (2017). Assessing the toxicity and risk of salt-impacted winter road runoff to the early life stages of freshwater mussels in the Canadian province of Ontario. Environmental Pollution, 230, 589-597.

R Core Team, A., R. Core Team, and Others. 2022. "R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 2012."

Romanenko, E. A., Romanenko, P. A., Babenko, L. M., & Kosakovskaya, I. V. (2017). Salt stress effects on growth and photosynthetic pigments' content in algoculture of Acutodesmus dimorphus (Chlorophyta). International Journal on Algae, 19(3).

Rossi, A. (2020). Water Quality and Management Challenges in Urban New Jersey. Montclair State University.

Schuler, M.S. and Relyea, R.A., 2018. A review of the combined threats of road salts and heavy metals to freshwater systems. BioScience, 68(5), pp.327-335. Coldsnow, K.D., Relyea, R.A. and Hurley, J.M., 2017. Evolution to environmental contamination ablates the circadian clock of an aquatic sentinel species. Ecology and Evolution, 7(23), pp.10339-10349.

Schuler, M.S., Canedo-Argüelles, M., Hintz, W.D., Dyack, B., Birk, S. and Relyea, R.A., 2019. Regulations are needed to protect freshwater ecosystems from salinization. Philosophical Transactions of the Royal Society B, 374(1764), p.20180019.

Signorell, A., K. Aho, A. Alfons, N. Anderegg, and T. Aragon. 2019. "DescTools: Tools for Descriptive Statistics." R Package Version 0.99.

Singh, R. P., Yadav, P., Kujur, R., Pandey, K. D., & Gupta, R. K. (2022). Cyanobacteria and salinity stress tolerance. In Cyanobacterial lifestyle and its applications in biotechnology (pp. 253-280). Academic Press.

Tonk, L., Bosch, K., Visser, P. M., & Huisman, J. (2007). Salt tolerance of the harmful cyanobacterium Microcystis aeruginosa. Aquatic microbial ecology, 46(2), 117-123.

Tully, K., Gedan, K., Epanchin-Niell, R., Strong, A., Bernhardt, E. S., BenDor, T., ... & Weston, N. B. (2019). The invisible flood: The chemistry, ecology, and social implications of coastal saltwater intrusion. BioScience, 69(5), 368-378.

Velasco, J., Gutiérrez-Cánovas, C., Botella-Cruz, M., Sánchez-Fernández, D., Arribas, P., Carbonell, J. A., ... & Pallarés, S. (2019). Effects of salinity changes on aquatic organisms in a multiple stressor context. Philosophical Transactions of the Royal Society B, 374(1764), 20180011.

Venâncio, C., Ribeiro, R., & Lopes, I. (2022). Seawater intrusion: An appraisal of taxa at most risk and safe salinity levels. Biological Reviews, 97(1), 361-382.

Werner, A. D., Bakker, M., Post, V. E., Vandenbohede, A., Lu, C., Ataie-Ashtiani, B., ... & Barry, D. A. (2013). Seawater intrusion processes, investigation and management: Recent advances and future challenges. Advances in water resources, 51, 3-26.

Wickham, Hadley. 2016. "Data Analysis." In Ggplot2: Elegant Graphics for Data Analysis, edited by Hadley Wickham, 189–201. Cham: Springer International Publishing.

Woodley, A., Hintz, L. L., Wilmoth, B., & Hintz, W. D. (2023). Impacts of water hardness and road deicing salt on zooplankton survival and reproduction. Scientific Reports, 13(1), 2975.

Yang, W., Wang, F., Liu, L. N., & Sui, N. (2020). Responses of membranes and the photosynthetic apparatus to salt stress in cyanobacteria. Frontiers in Plant Science, 11, 537220.

Zhou, M., Butterbach-Bahl, K., Vereecken, H., & Brüggemann, N. (2017). A meta-analysis of soil salinization effects on nitrogen pools, cycles and fluxes in coastal ecosystems. Global change biology, 23(3), 1338-1352.