Distribution of water chemistry, trace metals, and Chironomidae in coastal freshwater rock pools

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Abstract: Coastal rock pools of Lake Superior in North America are a mosaic of biologically rich ecosystems that vary in their physical morphology and chemistry. Occurring in bedrock depressions between the forest edge and the lake, the aquatic communities in these pools are susceptible to impacts from both international shipping lane traffic and changing climate patterns. Individual pools are small, but the number of pools across the coastal landscape provide an expansive network of variable habitats, with >70,000 pools found in the 48 km of shoreline in the study area. In this study, we aimed to understand the relationships between aquatic invertebrates and abiotic conditions in coastal pools. The Dipteran family Chironomidae were studied because of their ubiquity and diversity in freshwater systems. We used nonmetric multidimensional scaling to describe how Chironomidae diversity and abundance were related to seasonality, habitat, and environmental factors (water chemistry and trace metals). We observed 2 distinct chironomid assemblages associated with habitat type based on chemical characteristics and physical location on the shoreline, including numerous rheophilic species near the lake, where consistent wave wash mimics lotic conditions, and species preferring lentic-mesotrophic conditions near the forest edge. Conductivity and pH were higher in pools near the lake, whereas temperature and dissolved O_2 varied seasonally. Nutrients and metals differed between study sites and pools at each site. At 2 sites, naturally occurring Cu and Al were found at concentrations that exceeded estimated chronic toxicity for sensitive aquatic invertebrates. Pool separation of known tolerant or sensitive species indicated a potential response to elevated metal concentrations. Metals may be an important driver for sensitive species occupancy and survival in certain pools, raising more general concerns regarding community responses to natural conditions in pristine environments.

Key words: trace metals, nutrients, aluminum, copper, chronic toxicity, bedrock weathering, zonation, sensitive aquatic invertebrates, metapopulations, pupal exuviae, rheophilic, Isle Royale

Small waterbodies have long been studied as microcosms for broader ecological inquiry (Williams 2006). Marine intertidal coastal communities have contributed important knowledge regarding shoreline ecosystems, such as food webs and successional forces (Paine 1966, Berlow 1997). More recently, these habitats have been used to assess biotic response to stressors, such as contaminant disturbance and climate change (Holan et al. 2019, Menge et al. 2022). Colbo (1996) found that Chironomidae (Diptera) on marine shores were separated based on salinity and conductivity gradients, with marine species occupying low shore tidal habitats and brackish-tolerant freshwater species occupying pools above the high tide line where there was increased salinity due to storm waves and spray. Inland rock pools away from coastal environments have also been investigated as model systems (Vanschoenwinkel et al. 2007, Brendonck et al. 2010). Ecological studies on freshwater shorelines are scarce (Egan et al. 2014). Although similarities between marine and freshwater shorelines occur, such as strong community zonation, there are fundamental differences, particularly in water chemistry

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and hydrologic variation between predictable tidal inputs vs stochastic wave wash or precipitation events (Little et al. 2009, Egan et al. 2015b).

Characterizing species assemblages and their relations to environmental variables, such as trace metals, nutrients, and physical habitat, is key to understanding ecological relationships and providing baseline monitoring data (Downs et al. 2011, Jacks et al. 2021). Freshwater coastal rock pools are unique aquatic habitats, ideal for ecological observation because of their small size, lack of groundwater interactions, and often pristine ecology (De Meester et al. 2005, Brendonck et al. 2010). Such simplicity found in rock pools is helpful in reducing influences that can confound characterizing the natural ecology, particularly for aquatic invertebrates (Wilson and Gajewski 2004).

Physical habitat structure, water chemistry, and the potential for immigration have implications for rock pool ecology. For instance, rock pool water chemistry is known to be influenced by pool volume and duration (Anusa et al. 2012) as well as by diurnal fluctuations (Morris and Taylor 1983). In the case of coastal tidal rock pools, Wilson et al. (1992) found algal community structure was organized by colonization chance, and the size of rock pools did not appear to influence microbial diversity as much as within-pool habitat heterogeneity (Williams 2015, Tiettinen et al. 2021). Major factors driving macroinvertebrate community structure in rock pools often include hydroperiod (Washko and Bogan 2019, Mdidimba et al. 2021), seasonality (Gusha et al. 2021), and nonnative species invasions (Silva et al. 2021). Egan et al. (2014) found chironomid composition at the genus level was similar across deep and shallow coastal pools, whereas zooplankton responses varied, and diatoms showed mixed results with much similarity but some unique assemblages. Likewise, Tiettinen et al. (2021) found a negative relationship between diatom richness and freshwater pool volume, where smaller pools often had higher species richness. In contrast, they found no relationship between volume and species richness of any microbial group.

Water quality criteria, from basic chemical parameters to toxic chemicals and metals, help to evaluate the influence of many factors on the health of aquatic organisms (USEPA 2021). Benthic macroinvertebrates from the family Chironomidae are useful indicators of chemical variables and general aquatic health at various taxonomic levels, from communities to the genus and species levels (Rosenberg 1992, Lindegaard 1995, Wilson and Gajewski 2004, Justus et al. 2016). Chironomids can act as a biotic screening tool to detect impairments to the health of aquatic communities, from lethal effects that restructure populations to sublethal effects including physical deformities of larval structures, such as mouthparts or antennae (Vermeulen 1995). Larval chironomids, particularly the 1st instar, are often the least resistant life stage to toxic conditions (Gauss et al. 1985). However, chironomid responses to metal concentrations vary based on the subfamily and the type of deformity involved. The genus *Chironomus* is often cited as a robust indicator of toxic metal concentrations resulting in deformities; yet, it has a lower mortality rate, and synergistic effects appear to be more important than single factors (Vermeulen 1995, Di Veroli et al. 2014) when compared with chironomid genera such as *Paratanytarsus* and *Cladotanytarsus*, which have a sensitivity to metals and pH comparable with Ephemeroptera (Anderson et al. 1980, Bilyj and Davies 1989). For metals, environmental availability and subsequent uptake by aquatic organisms is influenced by many factors, including redox potential, water hardness, and pH (Hem 1978, Gauss et al. 1985, Krantzberg 1989), making knowledge of general water chemistry parameters as important as metal concentrations. Further, the differences in chironomid responses to metal concentration may be because of feeding and behavioral differences (Reynolds and Ferrington 2002). Organic matter and dissolved metals can form complexes, potentially increasing exposure for grazing organisms but, conversely, having the potential to reduce bioavailability (Smith et al. 2015). Chironomids that build retreats and consume organic matter may be more susceptible to Cu, which has a high affinity to organics (Teutsch et al. 1999). Free-living and predatory species may respond differently to metal concentrations based on their trophic level.

Investigation of chironomid assemblages and how they vary with habitat and water chemistry in rock pools, such as those found along shorelines of Lake Superior in North America, can inform biomonitoring efforts. Isle Royale, Michigan, USA, is an archipelago in Lake Superior consisting of 1 large island (544 km^2) and hundreds of smaller islands, with most of its forest- and wetland-dominated landscape protected as wilderness (Fig. 1). Along the eastern shoreline, exposed basaltic and andesitic lava flows form a general pattern of steeper slopes near the forest edge that grade to lower slopes along the lakeshore (Fig. 2A, B). Along this coast, up to 71,931 distinct pools have been measured, ranging from deep, permanent pools to shallow, ephemeral pools (Egan et al. 2015b). Local climate is influenced by Lake Superior (Peterson et al. 1998), and the lake also directly creates ecological zonation of the rock pools. Closer in proximity to the lake are splash zone pools with a dominant input of waves, whereas upslope, lichen zone pools typically have only rainfall and occasional upland seepage inputs (Egan et al. 2015b). This zonation, as well as factors such as wave disturbance, predation, and evaporation, likely accounts for assemblage variability in amphibians and macroinvertebrates (Smith 1983, Van Buskirk 1992).

We examined chironomid relations to seasonality, water chemistry, and trace metal variables. Our objectives were to describe 1) the distribution of rock pool geomorphometry and water chemistry, including nutrients and trace metals, across sites and 2) patterns of chironomid assemblage composition between habitat zones and along gradients of chemical

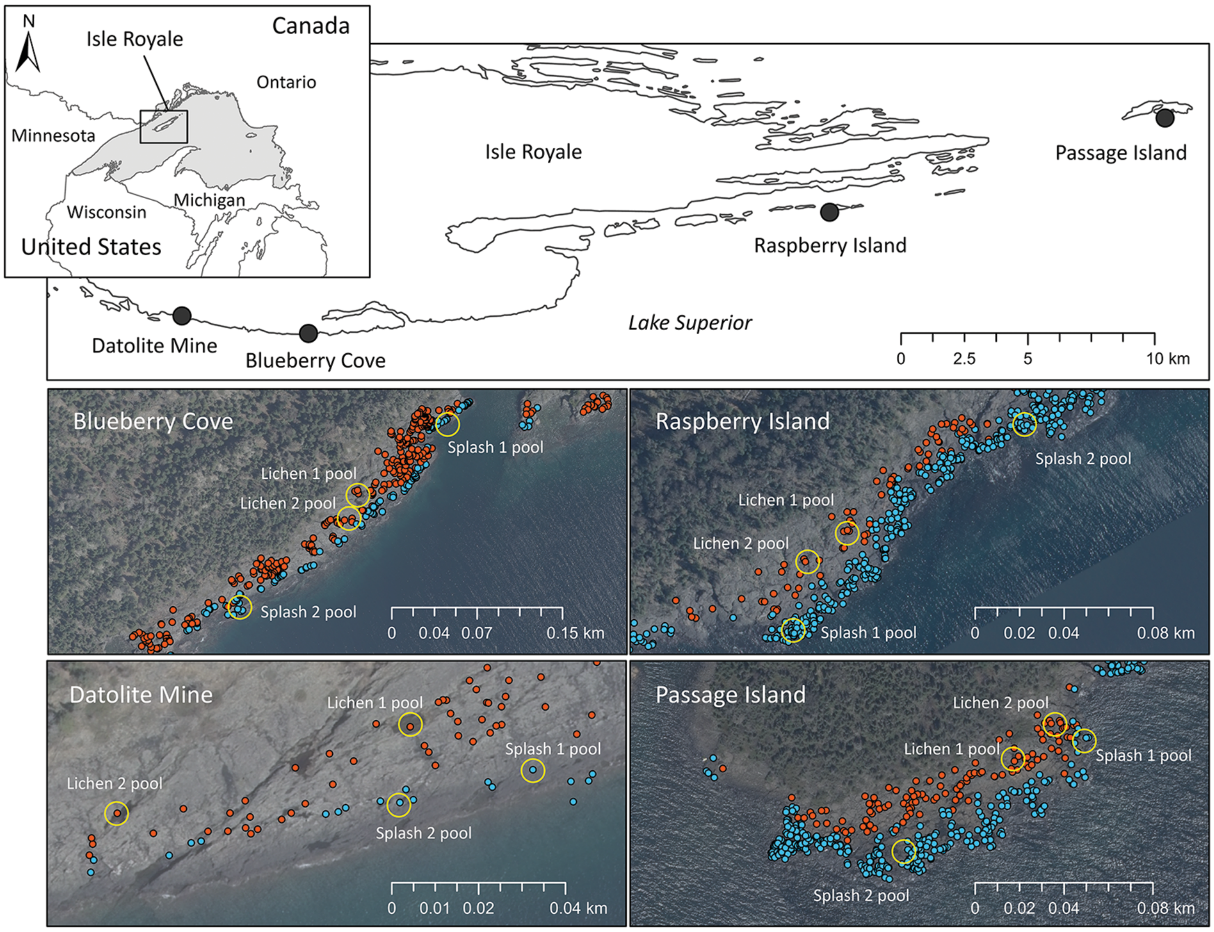

Figure 1. Isle Royale, Michigan, USA, location in the Lake Superior region and study sites in the northeast ½ of the park. Site maps show all mapped pools (blue = splash zone, orange = lichen zone), including locations of the 16 study pools (within yellow circles).

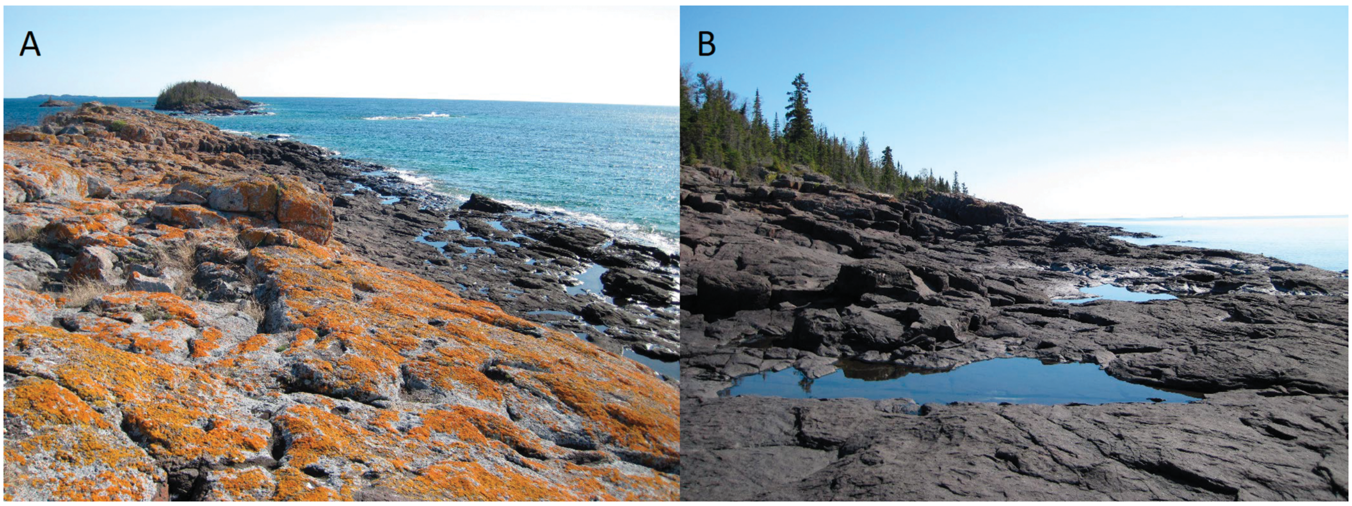

Figure 2. Shorelines of Isle Royale, Michigan, USA, looking northeast, showing the long, sloping bedrock and distinct zonation defined by presence or absence of lichen (A) and transition from lake edge to forest edge (B).

concentrations. Our primary hypothesis was that chironomids would respond to shoreline gradients such as disturbance and mesotrophic-to-oligotrophic conditions. We had no a priori hypothesis for trace metals because we did not anticipate elevated metal concentrations.

METHODS

To address our research objectives, we conducted a field survey of rock pools at 4 Lake Superior study sites, capturing both splash zone and lichen zone habitat types. We collected chironomid pupal exuviae as well as rock pool geomorphometry and water chemistry data from spring (April) to autumn (October) in 2010. We then used multivariate analyses to visualize the relationships among Chironomidae assemblages, geomorphometry, and water chemistry variables between sites and habitat zones.

Study sites

Using high-definition aerial photography, we randomly selected 4 study sites from 3 landscape strata: a distant offshore island (Passage Island, hereafter Passage), nearshore barrier islands (Raspberry Island, hereafter Raspberry), and the main island (Blueberry Cove and Datolite Mine, hereafter Blueberry and Datolite, respectively) (Fig. 1). At each location, we randomly selected 4 pools of ≥15-cm maximum depth (z_{max}) for 16 total pools. Two pools at each site were located within a splash zone, as defined by a lack of surrounding lichens and presumably with a strong influence from wave inputs and winter ice scour from Lake Superior. The other 2 pools were located within a lichen zone, surrounded by crustose or foliose lichens and occasionally some moss or flora but not located at the forest edge (Fig. 2A, B). Zones were parallel to the shoreline with variation in pool densities by zone and site (e.g., Datolite with few pools and Passage with abundant pools; Fig. 1; Egan et al. 2015b).

Chironomidae

Chironomid adult emergence is a key lifecycle endpoint, and because of environmental and chemical pressures on the larval state, chironomid pupal exuviae are well suited for community studies. Exuviae have additional benefits, including collection efficiency because they accumulate along shorelines following eclosion; ability to detect species, including cryptic species, that emerge from microhabitats; ability to estimate both richness and relative abundance; and identifiability, usually to species or morphotype (Coffman 1973, Verneaux and Aleya 1999, Bouchard and Ferrington 2011). Limitations with exuviae sampling include reduced taxonomic resolution for some genera (although morphotype allows diversity analyses to remain robust) and lower resolution of realized larval niche because pupal exuviae are aggregated at the water surface, which is particularly important when exuviae are moved from a different habitat (e.g., upslope, upstream, or via wave wash).

Six sampling rounds occurred from April to October 2010, generally spaced at monthly intervals. In each sample round, we collected surface-floating pupal exuviae of Chironomidae from each of the 16 pools. We used a pan and sieve to collect exuviae for 5 min at each pool based on methods in Kranzfelder et al. (2015). Monthly sampling for Chironomidae can be adequate for detecting up to ~70% of species present in lotic habitats, even within a single year (e.g., Coffman 1973, Bouchard and Ferrington 2011). Subsampling was often unnecessary because most or all of the pool surface was accessible and specimen numbers were limited. When samples had many exuviae (generally >100), we subsampled by randomly removing ½ of the individuals from a gridded tray, followed by removing representatives of morphologically unique specimens, which were not included in the random subsample (Egan et al. 2014). This approach allowed us to obtain relative abundances from each sample while maximizing the estimation of true assemblage richness. We permanently slide-mounted specimens and used keys in Wiederholm (1986) and Ferrington et al. (2019) to identify them to genus, followed by the most appropriate keys and descriptions available for each genus (Egan and Ferrington 2015). Voucher specimens are curated at the University of Minnesota insect collection, with additional material at Keweenaw National Historical Park, Michigan.

Pool geomorphometry

We took measurements of physical characteristics of the pools to capture pool geomorphometry variables. We used a measuring tape or laser range finder to measure the pool length and width and a measuring tape to measure pool depth. We estimated surface area as (L × W) / 2 because of the irregular shape of many pools.

Water chemistry

We collected basic water chemistry variables from each pool during the May, July, August, and October sample rounds. Anions, cations, metals, and nearshore Lake Superior samples were collected in May, July, and October from each site. We used a Eureka Manta 2 multiprobe, calibrated daily, to collect in situ variables including temperature, pH, dissolved O_2 (mg/L and % saturation), and specific conductance (EC25, electrical conductivity converted to the reference temperature of 25°C). We analyzed grab samples of pool water for chlorophyll *a* (Chl *a*), nutrients (total P [TP], total N [TN], soluble reactive P, NH_4^+, $NO_3^- + NO_2^-$), dissolved inorganic and organic C (DIC, DOC), anions, cations, and trace metals (Table S1). We collected grab samples elbow-deep into three 1-L acid-washed amber polypropylene bottles near the center of each pool, with caps opened under water. We transported the samples on ice in an opaque soft-sided cooler to a field lab and processed them the same day. We collected unfiltered TP and TN samples in clear high-density polyethylene bottles and froze them. We collected Chl *a* samples with a hand vacuum pump with a glass microfiber grade GF/C filter, treated them with saturated $MgCO_3$ solution, stored them in foil, and froze them. Other samples were refrigerated with no head space following filtration through a hand vacuum pump with a 0.4-μm polycarbonate track etch-membrane filter and handled in the following manner: cations and metals in a clear low-density polyethylene bottle and HNO_3 preservative; anions in an amber polypropylene bottle with no preservative; NO_3^- + NO_2^-, soluble reactive P, and NH_4^+ in an amber high-density polyethylene bottle with H_2SO_4 preservative; and DIC and DOC in an amber glass bottle with $CuSO_4$ preservative and an electrical tape cap seal. Analyses were done at the Science Museum of Minnesota's St Croix Watershed Research Station and University of Minnesota's Department of Earth Sciences Aqueous Geochemistry Laboratory. Both are research laboratories that run robust quality assurance and quality control procedures; however, they are not certified because they are not agency supported nor do they have regulatory authority. All laboratory analyses included field duplicates, lab duplicates, lab spikes, and blanks as appropriate.

Trace metals can be toxic in aquatic systems at relatively low concentrations depending on physical and chemical characteristics of the water and metal speciation (Adams et al. 2020). Metals bind with a biotic ligand (a biochemical receptor site, such as a fish gill), upon which an accumulation threshold can be reached where a lethal or sublethal toxicological response occurs (Smith et al. 2015). We compared trace metal results with normalized chronic and acute criterion values for Cu and Al based on United States Environmental Protection Agency (USEPA) water quality criteria recommendations (USEPA 2021). To estimate Cu toxicity, we used the mechanistic biotic ligand model, which predicts acute and chronic Cu effects on many organisms across a wide range of water quality conditions (USEPA 2007, Smith et al. 2015). Ten parameters are required for model inputs: temperature, pH, DOC, major cations (Ca^{2+}, Mg^{2+}, Na^+, K^+), major anions (SO_4^{2-} and Cl^-), and alkalinity/DIC. We estimated the humic acid fraction of DOC at 10% for this model. Al is a more complex metal that acts through multiple toxic mechanisms and is less suited to a biotic ligand model (Adams et al. 2020). We estimated Al toxicity using the USEPA's Aluminum Criteria Calculator V.2.0.Macro (https://www.epa.gov/sites/default/files/2018-12/aluminum-criteria-calculator-v20.xlsm) with calculations based on pH, DOC, and hardness (in mg/L of $CaCO_3$) (USEPA 2018). Toxicity endpoints include potential adverse effects on growth, reproduction, and survival on a range of aquatic organisms, including surrogates for a diverse community.

Differences in geomorphometry and water chemistry between sites and habitat zones

To determine patterns in environmental conditions among the 4 sites and 2 habitat types, we used hypothesis testing and multivariate ordination analyses. To test for differences between lichen and splash zone water chemistry parameters, we used Wilcoxon matched-pairs signed-rank tests and the estimated effect sizes. To describe the variability in habitat, basic water quality, nutrients, and metals among rock pools and nearshore water of Lake Superior, we used nonmetric multidimensional scaling (NMDS; 20 random starts, Bray–Curtis dissimilarity). We analyzed each of these data categories separately with the *vegan* package (version 2.6.4; Oksanen et al. 2013) in R (version 4.2.3; R Project for Statistical Computing, Vienna, Austria) using R Studio (version 2023.12.0). The NMDS solutions were evaluated using stress levels outlined by Clarke (1993), where stress > 0.2 was not considered a good representation of the data, stress values between 0.2 and 0.05 were acceptable, and stress < 0.05 was considered an excellent representation of the data. We visualized ordinations with the R package *ggplot2* (version 3.4.2; Wickham 2016).

Relationships between chironomid assemblages and environmental variables

To assess patterns of chironomid assemblage distribution, we used NMDS as described above. Chironomid count data ($n = 46$ species) were converted to relative abundance. Abundant taxa ($n = 11$ species) were selected for analysis based on having ≥1 occurrence with >1% relative abundance and being present in ≥3 samples, resulting in 32 samples for analyses. We transformed chironomid relative abundances with the square root transformation (Hellinger method) to reduce the outlier effects of very dominant and rare taxa and then applied a Bray–Curtis similarity calculation for NMDS analysis. Next, to examine the relationships, and possible gradients, of environmental variables with the distribution of the chironomid assemblage ordination, we used generalized additive models with the *vegan* package's ordisurf function.

RESULTS

Environmental variables

Geomorphological characteristics varied among study pools. Pools ranged from 1.0 to 12.5 m in length (mean = 5.1 m), 0.5 to 11.0 m in width (mean = 2.5 m), 0.2 to 0.6 m in z_{max} (mean = 0.4 m), 0.39 to 68.75 m^2 in surface area (mean = 9.7 m^2), and 0.1 to 30.3 m^3 in volume (mean = 4.6 m^3) (see Table S1 for details). Based on geomorphological data, the rock pools were distributed along the 1st NMDS axis ($k = 2$, stress = 0.03) by decreasing z_{max} and increasing surface area and volume. The 2nd NMDS axis was defined by decreasing pool width and increasing length in the positive direction (Figs S1, S2A, B). The NMDS did not identify any geomorphological clustering across the sites or zones.

Wilcoxon matched-pairs signed-rank tests determined that TP, soluble reactive P, TN, DOC, and Chl *a* were higher in lichen pools, whereas pH, specific conductivity, and DIC

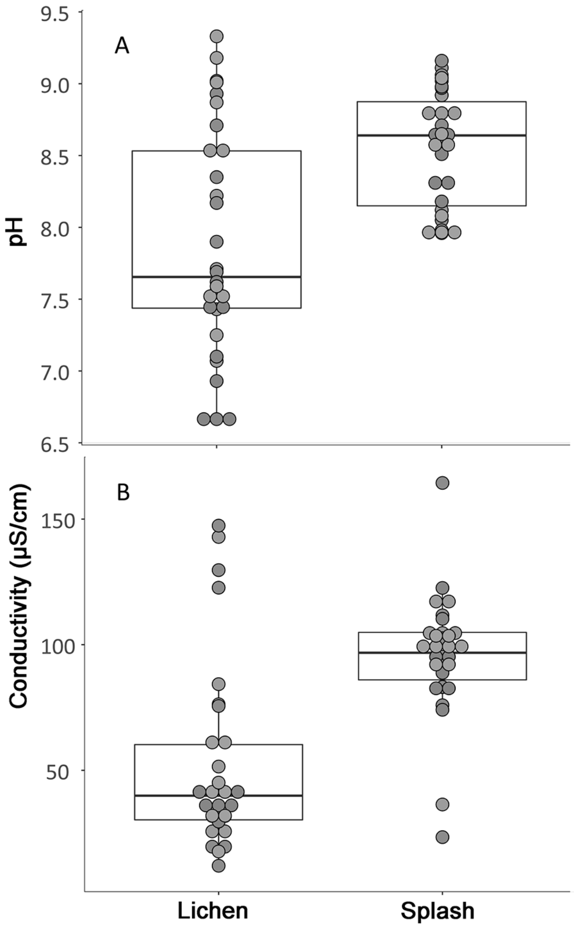

Figure 3. Boxplots for pH (A) and specific conductivity data (µS/cm) (B) for Isle Royale, Michigan, USA, coastal rock pool lichen and splash zones. Wilcoxon matched-pairs signed-rank tests indicated that habitat zones differed in both pH ($V = 11$, $p < 0.001$, effect size = 0.805) and specific conductivity ($V = 14$, $p < 0.001$, effect size = 0.786). Boxes encompass the interquartile range, the horizontal line indicates the median, whiskers represent the 1^{st} and 4^{th} quartiles, and overlain dots represent individual samples.

were higher in splash pools. For example, on average, pH was 0.5 higher ($V = 11$, $p < 0.001$, effect size = 0.805; median ± SD: 8.64 ± 0.39 vs 7.66 ± 0.78; Fig. 3A) and specific conductivity was 43 µS/cm higher in the splash zone than in the lichen zone ($V = 14$, $p < 0.001$, effect size = 0.786; mean ± SD: 95 ± 24.29 vs 52 ± 36.38 µS/cm; Fig. 3B). Basic water chemistry values for each sample can be found in Table S2. NMDS of Isle Royale rock pools with basic water chemistry measurements ($k = 2$, stress = 0.057; Figs 4A, B, S3A, B) separated the lichen and splash zones along the 1^{st} axis and splash zone sites corresponding with higher conductivity. Monthly sample groups progressed from October to May, July, and finally August along the 2^{nd} axis, following a decreasing gradient of dissolved O_2 concentrations and an increasing gradient of temperature.

Nutrients and trace metals in the pools of the lichen zone separated strongly compared with those in the splash zone pools and Lake Superior samples (NMDS stress = 0.06, $k = 3$, Figs 5, S4A, B), with the solutes in the splash zone more like those in Lake Superior water samples. Within the lichen zone pools, each site clustered together and generally separated from the other sites. Lake Superior sites nested within the splash zone sites, and these sites were the nearest clusters to the centroids of N analytes NO_3-N, NO_x, and DIN:TP. Lichen zone sites of Datolite and Raspberry clustered near the centroids of Cu, P, and Chl *a*, and the lichen zone sites of Blueberry and Passage were clustered in the direction of many metal centroids, including Al.

Only 2 metals, Al and Cu, had concentrations within the range of estimated chronic toxicity known to lead to lethal or sublethal effects for some aquatic invertebrates (Fig. 6A, B). The highest Al values were in Passage and Blueberry lichen zone pools (Table S3, Figs 5, 6A), but no Al concentrations were near potential acute values (i.e., criterion maximum concentration) for toxicity. At Passage, all results were near or exceeded estimated chronic toxicity criteria that may result in harm to sensitive species (i.e., criterion continuous concentration [CCC]), ranging from 530 to 810 CCC and 533.3 to 900.25 µg/L (Table S3). At Blueberry, 1 sample was near estimated chronic toxicity (640 CCC/605 µg/L), and 2 others showed elevated concentrations with values of 790 and 910 CCC (573 and 580 µg/L, respectively).

Cu did not show a strong gradient, although higher concentrations were evident in the lichen zone (Figs 5, 6B). Lichen pools at Raspberry and Passage had similar Cu concentrations (6–15 and 8–9 µg/L, respectively), whereas lichen pools at Datolite and Blueberry had distinctly different concentrations (2–17 and 6–31 µg/L, respectively), highlighting the variability of conditions in individual pools (Table S4). Using the biotic ligand model for Cu, none of the metal concentrations would predict acute effects (final acute value; Table S4) based on estimated values for zooplankton (*Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*) or most aquatic insects (USEPA 2007). However, for the sensitive chironomid *Paratanytarsus dissimilis*, a widespread Nearctic species that was not detected in our pools, Cu concentrations in 2 pools have the potential to be either problematic for larval growth or mortality (Fig. 6B; Anderson et al. 1980, as *Tanytarsus dissimilis*).

Chironomid distribution and relationships with environmental variables

Of the 29 species included in analyses, 8 were exclusively found in the lichen zone, 12 were exclusively found in the splash zone, and 9 were found in both zones. Of the 9 in both zones, 4 (14%) appeared to strongly prefer 1 zone, and 5 species (17%) did not exhibit a clear preference (Fig. 7).

As with base water chemistry variables, chironomid species clustered in NMDS (stress = 0.07, $k = 2$; Fig. S5) by zone and locality (Table 1, Fig. 8A). Furthermore, as for nutrients

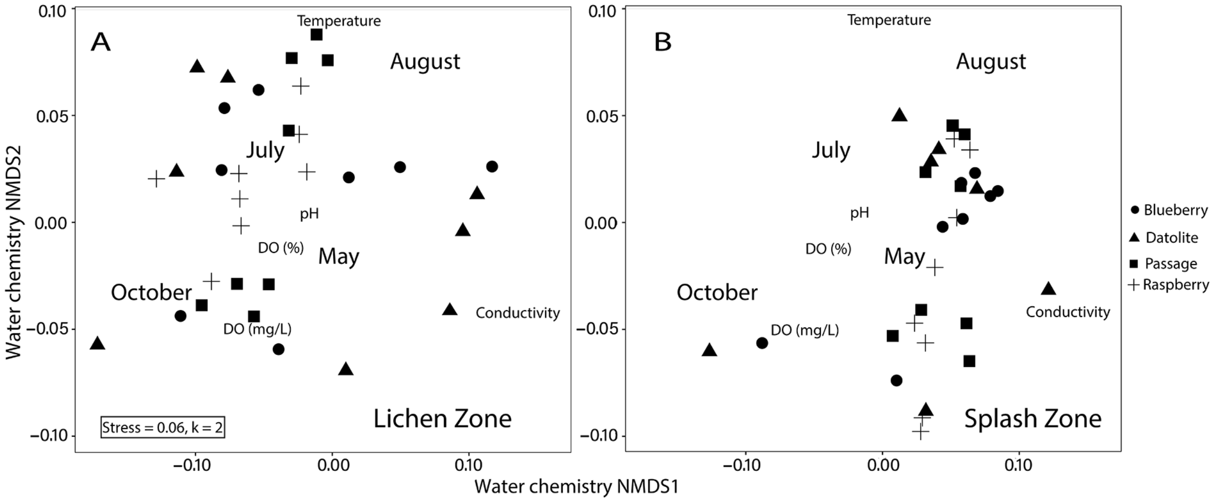

Figure 4. Coastal rock pools of Isle Royale, Michigan, USA, ordinated by basic water chemistry parameters with nonmetric multidimensional scaling (NMDS; stress = 0.06, k = 2), visualized separately by habitat: lichen zone (A) and splash zone (B). Sites are indicated by symbols: Blueberry Cove (circle), Datolite Mine (triangle), Passage Island (square), and Raspberry Island (cross). DO (%) = dissolved O_2 (% saturation).

and trace metals, the Blueberry and Passage sites clustered together, as did the Datolite and Raspberry sites. The latter assemblages were distinguished by *Psectrocladius subsensilis*, *Paratanytarsus laccophilus*, *Ablabesmyia pulchripennis*, and *Psectrocladius limbatellus*. The Blueberry and Passage assemblages were distinguished by *Psectrocladius sensilipes*, *Psectrocladius pilosus*, *Cricotopus intersectus*, and *Chironomus* sp. IR 1. Splash zone assemblages were characterized by *Corynoneura doriceni*, *Corynoneura arctica*, *Eukiefferiella coerulescens*, and *Orthocladius dubitatus*.

Generalized additive models fitted to the chironomid assemblage ordination illustrate influential geochemical gradients of specific conductivity and Cu concentration across assemblage clustering (Fig. 8B, C). Specific conductivity decreased and Cu concentrations increased toward the lichen pool chironomid assemblages of the Datolite and Raspberry sites (p = 0.01 and 0.005, respectively).

DISCUSSION

We explored the distribution of geomorphological and water chemistry variables in freshwater coastal rock pools at Isle Royale, Lake Superior, as well as their associations with chironomid assemblages. We found that the pools varied chemically both temporally and between splash and lichen zones, reflecting interactions with lake and precipitation inputs. Furthermore, within the lichen zone, these rock pools are differentiated by trace metal influences from weathering processes. Distinct chironomid assemblages in pools are clustered based on zone and water chemistry. In addition to physical and chemical parameters structuring communities, sensitive chironomid species may be affected by chronic Al or Cu concentrations in a subset of lichen zone pools. Results from data presented here, combined with previous studies, reveal a high degree of physical, chemical, and biological complexity in these coastal rock pools. The lack of

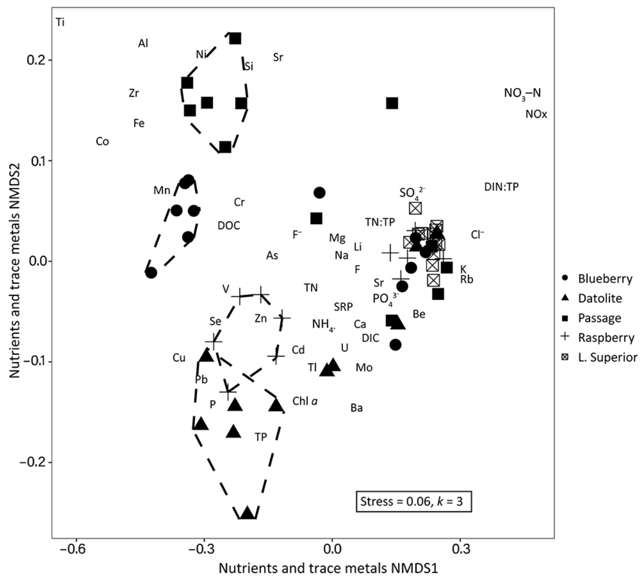

Figure 5. Coastal rock pools of Isle Royale, Michigan, USA, ordinated by nutrients and trace metals with nonmetric multidimensional scaling (NMDS; stress = 0.06, k = 3). The first 2 axes of a 3-dimensional solution are shown. Lichen zone pools are encircled by dashed lines, and splash zone pools are not encircled. Sites are indicated by symbols: Blueberry Cove (circle), Datolite Mine (triangle), Passage Island (square), Raspberry Island (cross), and Lake (L.) Superior (square with x). Centroids for nutrients and metals are represented by elemental alphanumeric abbreviations.

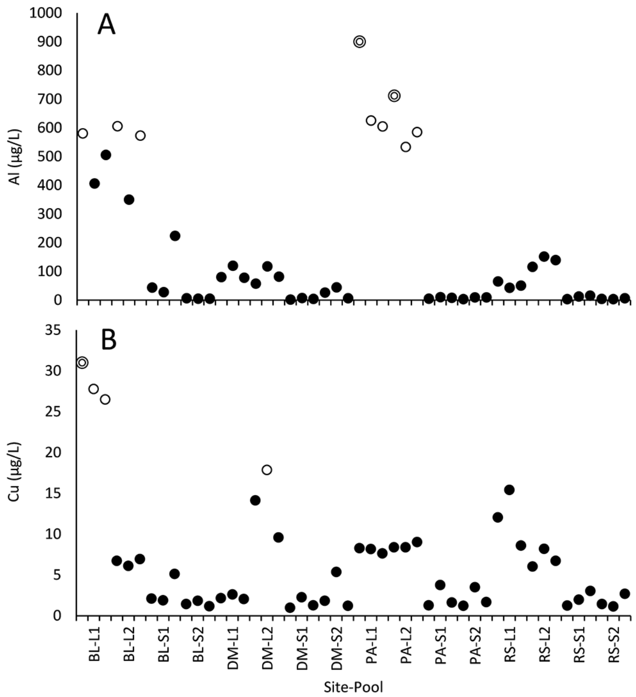

Figure 6. Al (µg/L) (A) and Cu (µg/L) (B) in coastal rock pools at Isle Royale, Michigan, USA, sampled in May, July, and October 2010. Individual pool results are clustered as 3 adjacent points above the site-pool label. For Al, open circles indicate elevated concentrations near chronic toxicity, and double circles indicate model results that exceeded chronic toxicity for some sensitive aquatic species. For Cu, open circles indicate conditions leading to reduced larval growth (16 µg/L), and the double circle indicates 100% mortality (28 µg/L) for the chironomid *Paratanytarsus dissimilis* (Anderson et al. 1980).

geomorphological distribution patterns indicates a limited across-site effect, reinforcing the importance of chemical differences within sites.

Seasonality and proximity to Lake Superior were key factors in determining rock pool water chemistry. Warmer water temperatures were observed in July and August, and cooler temperatures in May and October, with an expected inverse relationship between temperature and dissolved O_2. Lake Superior drove zonation differences in water chemistry and temperature. Splash zone pools were generally colder and higher in conductivity compared with lichen zone pools, and they displayed less variability within each month, indicating the influence of wave recharge on the chemistry of pools in the splash zone. Along Isle Royale coastlines, Lake Superior EC25 ranges from 98 to 103 µS/cm from May to August (AE, unpublished data), which is consistent with historical nearshore observations in Lake Superior (Yurista et al. 2009). These values are higher than most lichen zone observations and consistent with the splash pool mean specific conductivity. Furthermore, low specific conductivity values in the lichen zone reflect the influence of rainfall recharge and the generally inert nature of the basaltic and andesitic bedrock.

Nutrients and trace metals

Nutrients and metals further illustrate conspicuous zonal and site differences in rock pools. Nutrients, such as DOC and TP, appear to be primary components distinguishing the mesotrophic lichen zone and oligotrophic splash zone pools. Splash zone pools, although strongly influenced by the lake, show variability that indicates weathering and upslope influences when water replacement from waves is not occurring. In our ordination, base cations (Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}) clustered with splash zone pools. These cations are often mobile, and their central placement in the NMDS biplot indicates short gradients across study sites. Several more-resistant elements that commonly weather from basalts and andesites (Si, Al, Fe; Colman 1982) are important in creating the strong gradient on NMDS axis 2. These results may be due to elemental concentrations at each site, relative mobilities of each element from bedrock or soils, increased rates of weathering in the presence of lichens, or the influence of local climate on weathering rates (Colman 1982, Eggleton et al. 1987, Chen et al. 2000). In the future, it seems unlikely that bedrock weathering of metals due to acid deposition will increase, or that pool pH will decrease, because there is a widespread regional decline in sulfate deposition (Ramstack Hobbs et al. 2022) and because of the buffering capacity of the pools (total hardness mean 32,336 µg/L as $CaCO_3$; Table S3). However, slight declines have been occurring in the pH of Isle Royale inland lakes, which may be the result of reduced sulfate leading to mobilization of DOC and a consequent increase in organic acids from wetlands in the watershed (Gorham 1998, Ramstack Hobbs et al. 2022). Therefore, the small proportion of pools that have upland influences (7% of 71,931 pools mapped; Egan et al. 2015b) may respond in a similar manner.

Across the lichen zone, rock pools separated in the NMDS ordination based on trace metals, with only the single exception of a Raspberry pool overlapping with the Datolite pool cluster. Metals associated with specific sites included Ni and Si at Passage; Mn and Co at Blueberry; and Cu, P, Pb, and Se at Datolite and Raspberry. Combustion and industrial emissions can be key anthropogenic sources of trace metals to the environment, yet natural sources can also be important (Nriagu and Pacyna 1988). Even though Cu mining and small settlements occurred at Isle Royale, both prehistorically and historically (Winchell 1881), there are no obvious local sources for anthropogenic metals in the study pools. Abrupt ridge-and-valley topography common at Isle Royale, along with limited overland flow or seepage through bedrock cracks to shoreline pools, creates shoreline structure where movement of water from historical mining sites to shoreline pools appears unlikely. Mining sites would have been placed at localities of higher Cu concentrations, creating

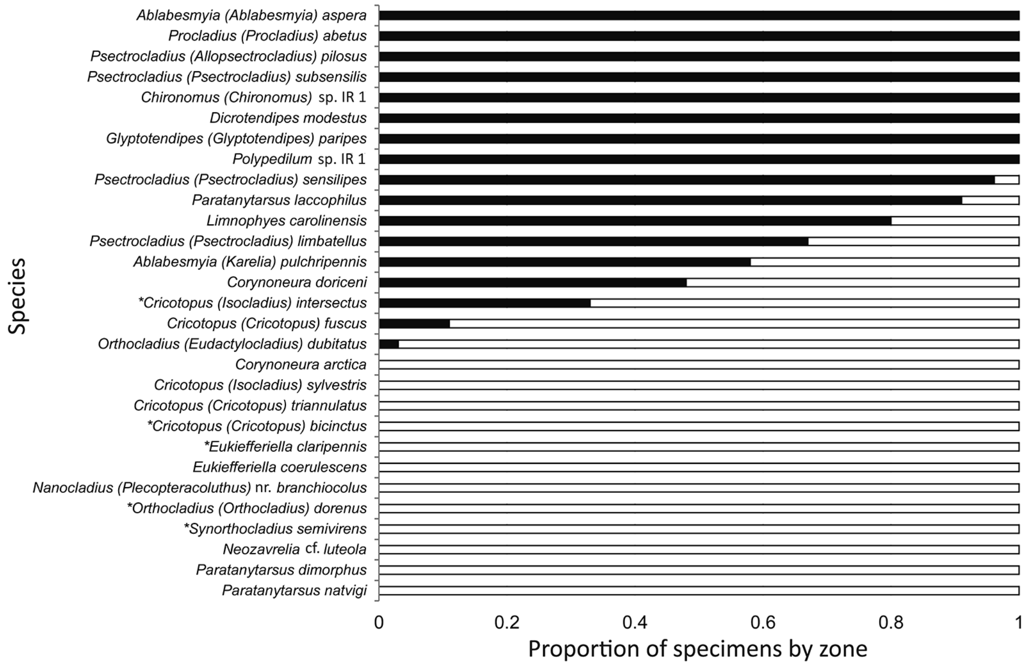

Figure 7. Proportion of Chironomidae by zone (when $n > 2$) from pupal exuviae collected in rock pools at Isle Royale, Michigan, USA, in 2010. Black bar = lichen zone, white bar = splash zone. Species with * are typical of lotic habitats.

a context for colocation of mining activities and a greater weathering potential of Cu. Considering the low metal concentrations we found and the strong site separation, we believe that upland soil chemistry or local chemical weathering of shoreline bedrock are the primary sources of trace metals in pools. Above certain concentrations, some of these metals have toxic effects on aquatic life, but in this study, Cu and Al were the only metals where concentrations in rock pools were near or exceeded levels at which adverse effects are known to occur in sensitive aquatic invertebrates (USEPA 2021). Rainfall can help dilute high concentrations of heavy metals to biologically manageable levels (Hammarstrom et al. 2003), which may be the case where rainfall strongly influences pools higher on the shore, and wave wash is an additional contributor to lower pools.

Cu in some lichen zone pools reached concentrations where deformities in tolerant species have been measured in experimental studies, leading us to speculate that sensitive species may be affected, even in this pristine locality with no obvious pollution sources. Cu is common in surface freshwater, often in a naturally occurring range of 0.2 to 30 μg/L (Bowen 1985), which corresponds with Isle Royale values. Cu is found in irregular deposits at Isle Royale (Thornberry-Ehrlich 2008), and mining activities occurred at numerous times parkwide, including near the Datolite study pools and a prospect pit near the Raspberry pools.

Cu toxicity estimates for the zooplankton *Daphnia* and *Ceriodaphnia*, the caddisfly *Clistoronia magnifica*, and amphibian tadpoles of *Anaxyrus boreas* would not predict that rock pool concentrations exceeded problematic acute values (USEPA 2007). Estimated acute and chronic toxicity ranges for *Chironomus tentans* (Nebeker et al. 1984a) and *Chironomus decorus* (Kosalwat and Knight 1987) were well above conditions in rock pools. However, 4^{th}-instar larvae are often used in *Chironomus* toxicity studies. In *C. tentans*, the 4^{th} instar was observed to be more tolerant to Cu than the 1^{st} instar, which had a sensitivity comparable with highly Cu-sensitive *D. pulex* (Gauss et al. 1985). Relatively elevated dissolved Cu occurs in lichen zone pools at all 4 sites, and some values exceed concentrations that can lead to the inability to complete the lifecycle for *C. magnifica* (13–17 μg/L at pH 7.2–7.4 and 14.5–16°C; Nebeker et al. 1984b). In laboratory conditions of pH 7.3 to 7.7 at 22°C, the chironomid *P. dissimilis* had reduced growth in larval stages at Cu >16.3 μg/L and 100% mortality >28 μg/L (Anderson et al. 1980), illustrating that not all chironomids are resistant to metals exposure. Cu concentrations may be a chronic problem for sensitive macroinvertebrates in individual lichen zone pools, as indicated by concentrations in both lichen zone pools at Blueberry (6–31 μg/L), Raspberry (6–15 μg/L), and Passage (7–9 μg/L) and 1 of the pools at Datolite (9–18 μg/L). One lichen pool and all splash pools had concentrations ≤5 μg/L. For comparison with lab studies, Isle Royale pools ranged from 10 to 30°C and 6.65 to 9.33 pH (Table S2).

Al had the strongest gradient out of the metals, with elevated concentrations at Blueberry (573–605 μg/L) and Passage (533–900 μg/L). As with other metals in rock pools, the most likely source of Al is chemical weathering or soil leaching

Table 1. Chironomidae pupal exuviae collected from coastal rock pools ≥15 cm deep (maximum depth) at Isle Royale, Michigan, USA, April to October 2010. Data reported are the number of individuals of each species and the number of samples in which each species was present by habitat zone. – indicates no individuals found. IR indicates the number given to distinct pupal exuviae morphotypes identified within a genus at Isle Royale.

Subfamily	Genus (Subgenus) species	Lichen individuals (no.)	Lichen samples (no.)	Splash individuals (no.)	Splash samples (no.)	Total individuals (no.)	Total samples (no.)
Tanypodinae	*Ablabesmyia* (*Ablabesmyia*) *aspera* Roback, 1959	10	4	–	–	10	4
	Ablabesmyia (*Karelia*) *pulchripennis* (Lundbeck, 1898)	19	14	10	7	29	21
	Conchapelopia (*Conchapelopia*) *fasciata* Beck & Beck, 1966	–	–	2	1	2	1
	Helopelopia cornuticaudata (Walley, 1925)	–	–	1	1	1	1
	Procladius (*Procladius*) *abetus* Roback, 1971	3	2	–	–	3	2
	Thienemannimyia (*Thienemannimyia*) *norena* (Roback, 1957)	–	–	2	1	2	1
Diamesinae	*Potthastia gaedii* (Meigen, 1838)	–	–	2	2	2	2
Orthocladiinae	*Corynoneura arctica* Kieffer, 1923	–	–	17	10	17	10
	Corynoneura doriceni Makarchenko & Makarchenko, 2006	11	3	12	4	23	7
	Cricotopus (*Cricotopus*) *bicinctus* (Meigen, 1818)	–	–	8	1	8	1
	Cricotopus (*Cricotopus*) *curtus* Hirvenoja, 1973	–	–	1	1	1	1
	Cricotopus (*Cricotopus*) *fuscus* (Kieffer, 1909)	2	2	17	6	19	8
	Cricotopus (*Cricotopus*) *triannulatus* (Macquart, 1826)	–	–	8	3	8	3
	Cricotopus (*Isocladius*) *intersectus* (Staeger, 1839)	36	13	73	9	109	22
	Cricotopus (*Isocladius*) *sylvestris* (Fabricius, 1794)	–	–	54	4	54	4
	Eukiefferiella claripennis Lundbeck, 1898	–	–	6	2	6	2
	Eukiefferiella coerulescens Kieffer, 1926	–	–	16	4	16	4
	Heterotrissocladius oliveri Saether, 1975	–	–	1	1	1	1
	Limnophyes carolinensis Saether, 1975	4	2	1	1	5	3
	Metriocnemus ursinus (Holmgren, 1869)	1	1	–	–	1	1
	Nanocladius (*Plecopteracoluthus*) nr. *branchicolus* Saether, 1977[a]	–	–	3	2	3	2
	Orthocladius (*Eudactylocladius*) *dubitatus* Johannsen, 1942	4	3	170	17	174	20
	Orthocladius (*Orthocladius*) *dorenus* (Roback, 1957)	–	–	4	2	4	2
	Orthocladius (*Orthocladius*) *obumbratus* Johannsen, 1905	1	1	–	–	1	1
	Parakiefferiella sp. IR 1	–	–	1	1	1	1
	Psectrocladius (*Allopsectrocladius*) *pilosus* Roback, 1957	27	6	–	–	27	6
	Psectrocladius (*Psectrocladius*) *limbatellus* (Holmgren, 1869)	4	4	2	2	6	6
	Psectrocladius (*Psectrocladius*) *sensilipes* Saether & Langton, 2011	69	16	3	3	72	19

Table 1. (*Continued*)

Subfamily	Genus (Subgenus) species	Lichen individuals (no.)	Lichen samples (no.)	Splash individuals (no.)	Splash samples (no.)	Total individuals (no.)	Total samples (no.)
	Psectrocladius (*Psectrocladius*) *subsensilis* Saether & Langton, 2011	79	17	–	–	79	17
	Pseudorthocladius rectilobus Saether & Sublette, 1983	1	1	–	–	1	1
	Smittia sp. IR 1	–	–	1	1	1	1
	Synorthocladius semivirens (Kieffer, 1909)	–	–	10	2	10	2
	Thienemanniella lobapodema Hestenes & Saether, 2000	–	–	1	1	1	1
Chironominae	*Chironomus* (*Chironomus*) sp. IR 1	26	10	–	–	26	10
	Chironomus (*Chironomus*) sp. IR 3	2	2	–	–	2	2
	Chironomus (*Chironomus*) sp. IR 4	1	1	–	–	1	1
	Chironomus (*Chironomus*) cf. *aberratus* Keyl, 1961	1	1	–	–	1	1
	Chironomus (*Lobochironomus*) sp. IR 14	2	2	–	–	2	2
	Dicrotendipes modestus (Say, 1823)	232	10	–	–	232	10
	Endochironomus nigricans (Johannsen, 1905)	1	1	–	–	1	1
	Glyptotendipes (*Glyptotendipes*) *paripes* (Edwards, 1929)	23	10	–	–	23	10
	Neozavrelia cf. *luteola* (Goetghebuer, 1941)	–	–	51	4	51	4
	Paratanytarsus cf. *dimorphus* Reiss, 1965	–	–	3	2	3	2
	Paratanytarsus laccophilus (Edwards, 1929)	152	14	15	2	167	16
	Paratanytarsus cf. *natvigi* (Goetghebuer, 1933)	–	–	3	2	3	2
	Polypedilum sp. IR 1	38	3	–	–	38	3

[a] *Nanocladius* nr. *branchicolus* likely originated from Lake Superior. See text for details.

from natural sources, which has been found in studies from other national parks with historical mining (Hammarstrom et al. 2003). Natural weathering is the most common Al source in the aquatic environment, particularly as runoff from acidic precipitation, heavy rains, and snow melt. Local concentrations can vary widely, even within a locality, because of geology and vegetation characteristics (as summarized in USEPA 2018). Although atmospheric transportation and deposition occur widely, there are no local anthropogenic sources, and variable site data suggest distant sources are not an important input to coastal pools.

Passage pools were well below estimated acute toxicity ranges for invertebrates such as chironomids (*Chironomus plumosus*, *Chironomus riparius*, *Chironomus anthracinus*, *P. dissimilis*) or zooplankton (*Hyalella azteca*, *D. magna*, *C. dubia*; see sources in USEPA 2018). Concentrations reaching or nearing potential chronic toxicity ranges for some species were related to pH ranging from 6.65 to 7.45, the low end of pool pH in this study. Most pools were neutral to basic, ranging from pH 7.5 to 9.33. As mentioned above, many toxicity studies on chironomids include species that are tolerant of pollutants or degraded conditions (Rosenberg 1992). Acute Al sensitivity was observed in *Daphnia catawba* (1000 μg/L at pH 6.5), with chronic responses and reproductive problems possible at lower concentrations for this and other *Daphnia* species (Havas and Likens 1985). Although Havas and Likens (1985) suggested that acidification and the consequent leaching of Al from soils was unlikely to cause adverse sublethal effects in *Chironomus* and *Chaoborus* species (Diptera:Chaoboridae), they did not include more sensitive dipteran genera in their study. In addition, USEPA (2018) criteria indicates chronic effects to aquatic organisms can occur in the range of 300 to 1000 μg/L Al. Sensitivity to pH within the chironomid genus *Cladotanytarsus* ranged from *Cladotanytarsus muricatus* disappearing from an experimental lake at pH 6.49 and the acidophilic *Cladotanytarsus aeiparthenus* becoming dominant in the range of pH 5.02 to 5.16 (Bilyj and Davies 1989), though these effects were

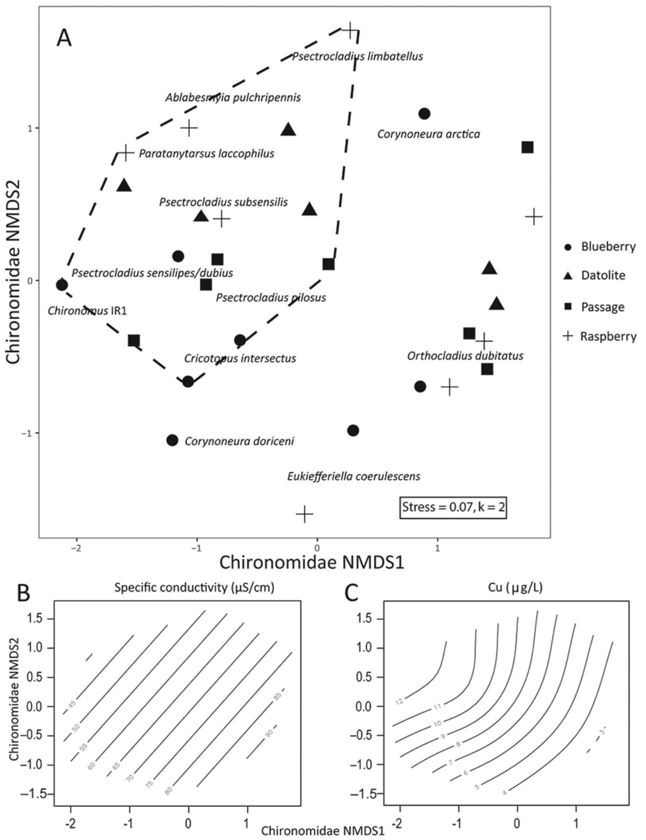

Figure 8. Coastal rock pools of Isle Royale, Michigan, USA, ordinated by Chironomidae relative abundances with nonmetric multidimensional scaling (NMDS; stress = 0.07, $k = 2$) (A). The lichen zone pools are encircled by a dashed line. Sites are indicated by symbols: Blueberry Cove (circle), Datolite Mine (triangle), Passage Island (square), and Raspberry Island (cross). Not all pools are represented because of infrequent or low taxon abundances. Fitted generalized additive models for specific conductivity ($p = 0.01$) (B) and Cu ($p = 0.005$) (C) are overlaid onto duplicate chironomid NMDS coordinate planes to highlight important environmental gradients across assemblage variation.

not directly related to Al concentrations. Based on ranges of pH, Al in some pools, particularly at Passage Island, may affect occupancy or survival for sensitive invertebrates.

Some of our Al results should be viewed with caution because pH was often higher than the range for the model. Although higher pH can cause speciation, leading to increased bioavailability, USEPA criteria for Al are not well understood for pH values >7 (Gensemer and Playle 1999), complicating interpretation of current and future responses of pool communities under different climatic conditions. In addition, Cu–DOC complexes change in the presence of Al because of competition for ligand sites, which is not accounted for in the biotic ligand model (Smith et al. 2015).

All pools that were near or above chronic effects criteria for Al also had DOC concentrations that exceeded model inputs. Higher DOC can bind Al into complexes and make it less bioavailable (USEPA 2018), potentially mitigating the negative effects of Al toxicity. Widespread increases in DOC have been linked to declines in atmospheric sulfur and catchment buffering (Monteith et al. 2007), which can be exacerbated by hydrological patterns of drought and subsequent DOC pulses from heavy rain (Tiwari et al. 2022). Isle Royale lakes have had variable concentrations and responses to DOC in recent decades (Ramstack Hobbs et al. 2022). We have observed drought having a desiccating influence on the hydroperiod of coastal pools, particularly in the lichen zone, but with generally restricted catchments, it is unclear how pool chemistry and metal bioavailability will respond to changing climate. Increased DOC and pH are key in Cu chronic toxicity estimates for *D. magna* (de Schamphelaere and Janssen 2004), indicating that changes in DOC and pH will be important for bioavailability of both Al and Cu to aquatic invertebrates (Smith et al. 2015).

Ecological endpoints for USEPA criteria are designed to protect "approximately 95 percent of a group of diverse taxa" from negative effects to growth, reproduction, or survival (USEPA 2018, p. 18). Laboratory tests, as summarized in USEPA recommendations and other studies cited here, are expected to be appropriate measures of species responses in similar field situations. With these endpoints in mind, we can predict that in the Isle Royale coastal pools with elevated metal concentrations, a proportion of sensitive species are likely to experience adverse effects from Al and Cu.

Chironomid assemblages

Most chironomids in coastal pools at Isle Royale differed between zones, and we expect that many variables played a role in that separation, including base water chemistry, nutrients, metals, physical disturbance, predation (Egan et al. 2015a), and immigration (Ranta 1982). Chironomid assemblages are known to respond to nutrient and chemical gradients (e.g., Vermeulen 1995, Wilson and Gajewski 2004). For example, it has been suggested that dissolved O_2 is a more important driver of benthic macroinvertebrate richness than temperature is (Croijmans et al. 2021) and is perhaps a previously unexplained driver of chironomid emergence from Isle Royale rock pools (Egan et al. 2015a).

We were surprised to find that elevated metal concentrations reached levels where chronic toxicity may be important in assemblage composition. Survival in contaminated sites may be due to either genetic adaptations or physiological mechanisms, such as binding of metals to metallothionein proteins (Postma and Groenendijk 1999). Populations exposed to metal contamination often develop a tolerance to the pollution, though it can be difficult to attribute tolerance to adaptive genetic resistance (Klerks and Weis 1987). Postma and Groenendijk (1999) found that natural exposure of the midge *C. riparius* to Cd in a lotic system did lead to genetic adaptations at a contaminated site within a few generations. However, larval drift from nonadapted upstream populations was also influential on selective pressure and gene flow in that study, and this effect may also apply to Isle Royale rock pools, where aerial dispersal of adults can genetically connect populations from nearby pools.

The abundance of certain species may dramatically increase with metal concentration, but diversity may simultaneously plummet (see review by Lindegaard 1995). Within Chironomidae, distinct differences in sensitivity to environmental contaminants does occur, such as those observed between the apparently more tolerant genus *Chironomus* (depending on the life stage; Gauss et al. 1985) and the more sensitive genus *Paratanytarsus* (Darville and Wilhm 1984, as *Tanytarsus*). Of 5 *Chironomus* morphotypes in our study, all were found in the lichen zone where Al and Cu metal concentrations were elevated, yet only 2 of 3 *Paratanytarsus* species were exclusive to the splash zone, where metal concentrations were very low. The presence of *P. laccophilus* in both Passage and Blueberry lichen zone pools suggests this species may be less sensitive to Al and Cu than others in the genus. It would be useful to have data on acute and chronic metals toxicity across a broader array of chironomid genera. Lacking such data complicates interpretation in studies like ours, which is a longstanding issue that has not been comprehensively addressed (Lindegaard 1995).

Many chironomid species in Isle Royale pools are eurytopic in habitat requirements; however, there is a general shift from lichen zone species (e.g., *Procladius abetus*, *P. subsensilis*, *Dicrotendipes modestus*, *Glyptotendipes paripes*), which typically occupy lentic or mesotrophic waters, to splash zone species (e.g., *Cricotopus bicinctus*, *Eukiefferiella claripennis*, *Orthocladius dorenus*, *Synorthocladius semivirens*), which commonly inhabit lotic systems. The influence of consistent wave wash in the splash zone creates habitat that mimics flowing water. Many species that occupied shallow, ephemeral pools in the splash zone are strictly rheophilic (e.g., in the genera *Diamesa*, *Potthastia*, and *Cricotopus*; see Egan and Ferrington 2015). Although they were not included in analyses because of lack of water chemistry data from shallow pools, their presence reinforces the model of a unique assemblage occurring in the splash zone.

The influence of Lake Superior on splash pools is apparent chemically, so we expect that chironomids preferring oligotrophic conditions will occupy both the littoral zone of the lake and splash pools, with aerial and hydrologic dispersal between the 2 zones. The relative abundance and biomass of chironomids in the nearshore Lake Superior environment is relatively low (Mehler et al. 2018). However, a factor we were not able to control—movement of pupal exuviae from the lake into splash zone pools via waves—was clearly observed on occasion (i.e., thousands of pupal exuviae in a small pool that could not realistically support such abundance). This effect can produce false positives for species occupancy. To reduce the possibility of including exuviae that washed in from Lake Superior, we collected and identified larvae from their algal retreats in the study pools. Nearly all genera that were identified from pupal exuviae were also confirmed as present in the pools based on larvae (which are generally not identifiable to species). One species that was probably a false positive is *Nanocladius* nr. *branchicolus*, which is in a subgenus known to be phoretic on Plecoptera. Stoneflies are common in the lake and only incidentally occupy coastal pools while mating on the shoreline.

The density of coastal rock pools at Isle Royale creates a complex mosaic of naturally discrete habitats with variable and dynamic water chemistry, trace metals, and chironomid assemblages that all fluctuate at landscape and local scales. As we expected, chironomid populations varied with seasonality, water chemistry, and shoreline position. The most unexpected finding was that concentrations of Al and Cu in some study pools reached levels known to be chronically toxic to sensitive aquatic invertebrates, which was likely a naturally occurring result of bedrock weathering. Metal toxicity at low concentrations can be both a concern and a natural component of community structuring in aquatic ecosystems. Across Isle Royale shorelines, it's likely that metapopulations will continue to interact and maintain a healthy, robust metacommunity (Hanski and Gilpin 1991), but in localities where habitat is limited, there could be subtle responses to low concentrations of metals that would typically not be measured. These findings suggest that remote and pristine sites globally can offer a useful context for comparing species and community responses with stressors, such as chronic toxicity, even at the scale of individual pools. Therefore, many valuable ecological findings are likely to arise from study of these and similar habitats and populations.

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