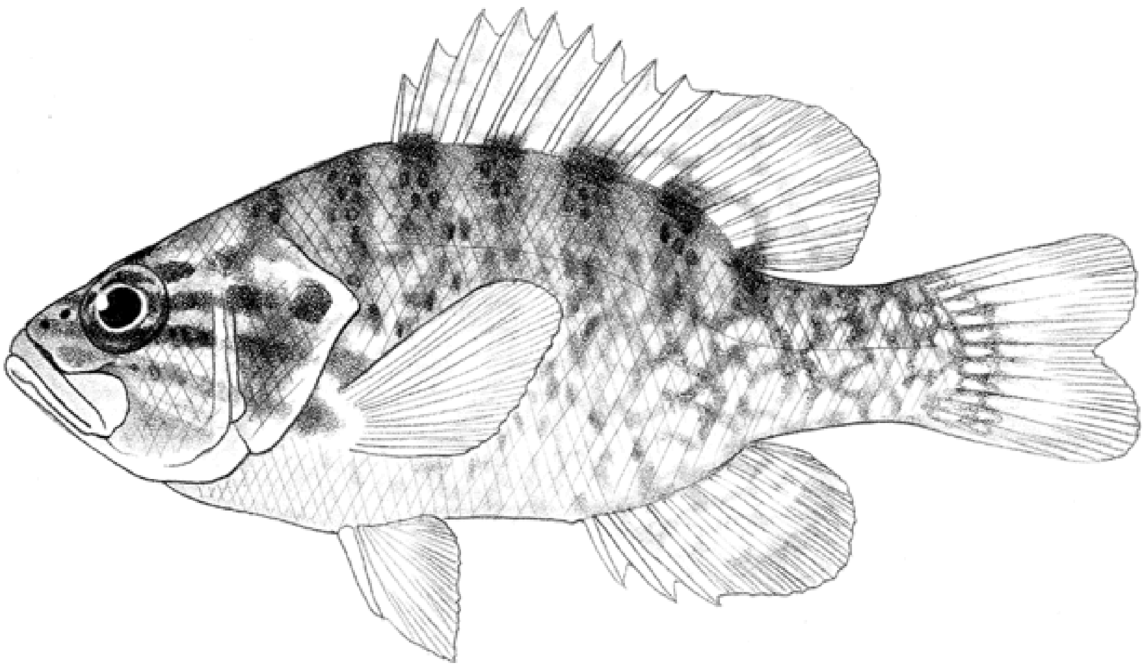
Maryland Biological Stream Survey Round Four Results
Investigating Potential Changes Over Time in Stream Conditions



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Executive Summary

The goal was to answer the question – "are Maryland's stream conditions improving or degrading over time?". To answer this question, Maryland Biological Stream Survey sampling protocols were used to collect ecological and water quality data from the same stream sites that were sampled twice within a 14- or 20-year interval. These sites were selected randomly to represent Maryland's stream conditions. Additionally, data from a set of high-quality (reference) streams were compared over the same 14-year interval.

Based on the results from examinations of representative and reference streams and in the context of other recent studies:

- Site-specific results varied substantially for all variables and types of streams.
- Some aspects of the representative stream condition and/or reference stream condition improved, some appeared to have degraded, and others showed no apparent change.
- Biological integrity did not change. However, slightly more signs of improvement than degradation were evident from the percentages of samples with higher or lower index of biotic integrity scores during the more recent periods.
- Certain specific indicators of biological diversity were lower during more recent years with sensitive, as well as rare, threatened, or endangered biota tending to be lower in abundance and distribution for most comparisons.
- Signs of declines in certain intolerant benthic macroinvertebrate taxa (specifically mayflies) tended to be consistent and pervasive, aligning with other recently documented global trends in insect abundance and diversity. In contrast, changes in the percentages of two groups of benthic macroinvertebrates (increases in intolerant caddisflies and decreases in tolerant collector taxa) indicated improvements in biological diversity.
- There were more non-native fish species and non-natives became more abundant and widespread over the 14- and 20-year periods.
- Although fish and benthic macroinvertebrate index of biotic integrity scores did not change significantly, increases in specific biodiversity-related indicators like tolerant species (such as non-natives) and decreases in certain intolerants may be early indicators of ecosystem degradation that are not yet reflected in these more generalized biotic indices and measures of general biological community composition.
- Maryland's representative and reference streams appear to have become warmer and saltier – but with less acidity and sulfate, and higher concentrations of orthophosphate.
- Except for orthophosphate, patterns in nutrient concentrations over time were weak and inconsistent. However, reference stream nutrient concentrations tended to be lower compared with representative streams.
- Since temperature data were only available for sites sampled in the 14-year interval, 20 years of potential temperature change in Maryland streams could not be evaluated.

- There were clear geography and site-type patterns in results showing stream temperature change over time. Central Maryland reference streams, as well as western reference and representative streams, did not demonstrate significantly warmer water during more recent sampling, while all other areas did.
- A multitude of environmental factors that were not included are likely to have potentially influenced stream aquatic life and/or have inherent relevance to water quality and physical habitat conditions. Although such other factors were likely also important, there is a strong weight of evidence from multiple scientific studies demonstrating the importance of the variables we examined to biological conditions in streams.

The results and discussions of change in stream conditions over time in this report are intended to support environmental policies, regulations, and resource management in Maryland relating to aquatic life, water quality, rare and invasive species, climate adaptation, and other uses as appropriate.

Background

Maryland's Streams

There are more than 16,000 miles of non-tidal streams in Maryland according to a 1:24,000 scale map (National Hydrography Dataset | U.S. Geological Survey). Depending on their locations, Maryland's streams are tributaries to the Chesapeake Bay, the Atlantic Coastal Bays, the Ohio River, or the Delaware River. The condition of these streams affects their ecosystem services and contributes to the health of downstream waters. Maryland's streams also possess significant inherent values.

Maryland's stream network is divided into 18 major river basins and more than 130 watersheds (often referred to as 8-digit watersheds because of the unique 8-digit number label designating each) consisting of non-tidal and tidal waters. This watershed diversity – along with physiographic, geologic, and stream size diversity – results in substantial stream biological diversity. There are three major stream ecoregions (Highlands, Eastern Piedmont, and Coastal Plain) in Maryland based on general distinctions in biological community structure (e.g., fish and benthic macroinvertebrates; Roth et al. 1998, Southerland et al. 2007; Figure 1). Eastern Piedmont is referred to simply as "Piedmont" throughout the remainder of this report. Nine Key Wildlife Habitats more specifically define and describe unique stream biological assemblages (Maryland Department of Natural Resources 2005).

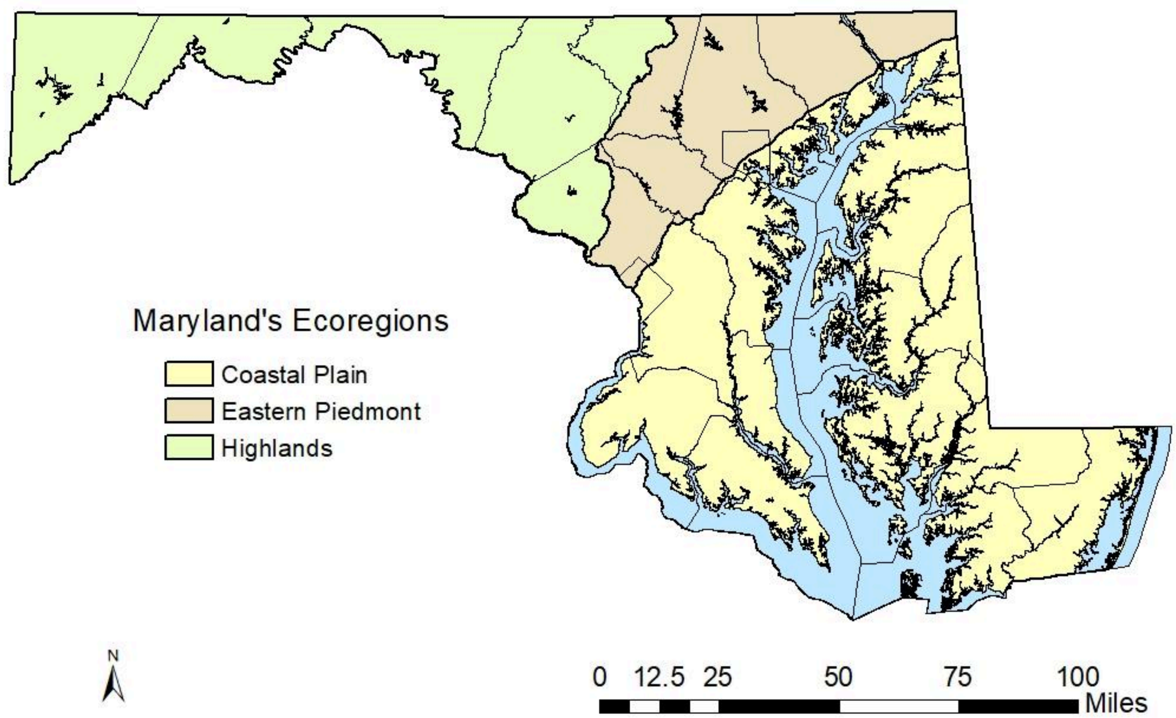

Figure 1. Map of Maryland's stream ecoregions with county boundaries and large water bodies.

Maryland's Stream Life

There are nearly 100 fish species, 16 native freshwater mussels, nine native crayfishes, eight stream salamanders, and hundreds of different aquatic insects and other benthic macroinvertebrates found in Maryland's streams. Despite the high diversity, stream species tend to be disproportionately prone to extinction and imperilment (Riccardi and Rassmusen 1999) compared with terrestrial species. Throughout the United States, freshwater animal groups such as freshwater mussels, crayfish, amphibians, and freshwater fish have the highest proportion of imperiled species. For example, 69% of freshwater mussels, 51% of crayfish, and 37% of freshwater fish species are considered at risk nationally (Master et al. 1998). Although all of these species need attention to help ensure their persistence, an especially significant Maryland example is the Maryland Darter (*Etheostoma sellare*). This species is known only from two

Maryland streams and, despite recent surveys in those and other nearby waters, none have been found for over 30 years.

Non-tidal streams and rivers also help support recreationally and commercially important species. For example, healthy coldwater streams are the principal habitats for trout in Maryland. Warmwater streams and rivers often support fisheries for smallmouth and largemouth bass, and other gamefish. Where no blockages inhibit access, several anadromous fish species migrating from the ocean or Chesapeake Bay (e.g., river herring and shad) rely on healthy Maryland streams and rivers for spawning habitat. The American Eel (*Anguilla rostrata*) is catadromous and relies on Maryland streams as places to live and grow, often for 20 years or more. While streams are important for sustaining the American Eel, this species also has important influences on stream ecosystems where it occurs (Stranko et al. 2014, Galbraith et al. 2018).

Stream biota are affected by various stressors from throughout a watershed upstream from where they occur. Even minor chemical, physical, hydrologic, biological, and/or landscape degradation can negatively alter stream biological communities and cause species displacement or extirpation, as well as reductions in abundance – especially of particularly sensitive species (Stranko et al. 2008, King et al. 2011). Due to the naturally insular nature of streams and stream species' habitats, alterations that cause further fragmentation increase imperilment risk and can exacerbate the influence of other stressors (Gido et al. 2012; Perkin et al. 2014).

State and federal requirements and policies stress the need for monitoring, as well as for protecting and restoring stream water quality and biota. Examples include Clean Water, Endangered Species, and Maryland Wild and Scenic Rivers Act standards. Additionally, land acquisition decisions by the Maryland Department of Natural Resources often consider important stream biota. Other laws exist that pertain to invasive species transportation, possession, and management in Maryland. Such species can negatively affect native species. Furthermore, coincident with the important focus of the Chesapeake Bay Program on reducing sediment and nutrient pollution from rivers and streams to the Bay, are equally important outcomes of the 2014 Chesapeake Bay Program Agreement about stream ecological protection and restoration through groups such as the Stream Health Workgroup, Healthy Watersheds Goal Implementation Team, and the Brook Trout Outcome.

The condition of stream aquatic life integrates and reflects environmental conditions in the stream (physical, chemical, and biotic properties) impacted by the landscape (through the influence of factors like land cover and geology), air (via atmospheric deposition), and subsurface (through the degree of connection and quality of groundwater), as well as due to changes in weather and climate. As such, monitoring stream ecological conditions is integral to environmental and natural resource management.

The Maryland Biological Stream Survey

The Maryland Department of Natural Resources, Maryland Biological Stream Survey (MBSS) is a statewide monitoring program designed to assess the status and trends in ecological conditions of wadeable, non-tidal streams in Maryland (Klauda et al. 1998, Roth et al. 2005). More specifically, the primary goal of the MBSS is to provide the best possible data and information to inform the protection and restoration of Maryland's stream ecological resources, thus helping meet the needs described above and contribute to effective, scientifically supported and rigorous stream ecological conservation and management. MBSS data are used to assess the condition of stream ecological resources, assist in identifying potential stressors to those resources, provide an inventory of Maryland stream biological diversity, guide stream-related management, and effectively communicate findings in scientific and nontechnical formats.

MBSS data were collected using standard protocols as described in field sampling manuals (e.g., Kazyak 2001, Stranko et al. 2019) and rigorous data quality assurance and control standards and training. Maryland, as with most states in the United States, developed biological indicators to assess stream health using a scientifically defensible and widely used approach (Southerland et al. 2007). Specifically in Maryland, benthic macroinvertebrate and fish indices of biotic integrity (IBI) scores are used to support state standard assessments (e.g., High Quality Waters - Antidegradation). Due to the rigorous taxonomic identifications and supportive ecological, distributional, and (often) abundance information provided by the MBSS, data also help contribute to decisions about the appropriate status of certain native stream-dwelling animals and contribute knowledge about certain aquatic non-native and invasive species.

Select chemistry, physical habitat, temperature, and landscape data were sampled at MBSS sites along with biological data. This information helps interpret biological sampling results and also contributes information about the condition of these factors in Maryland's streams. The chemistry variables that are analyzed as part of MBSS contribute to information about influences such as atmospheric deposition, nutrient concentrations, and certain ions. Physical habitat assessment rates the quality of habitat for stream fauna and documents riparian habitat and vegetation. Temperature monitoring is vitally important for determining species suitability and examining potential influences of climate change. Upstream landscape conditions (especially land cover) tend to be strongly related to stream biological, chemical, and physical habitat conditions.

Probability-based (randomly selected site) sampling via the MBSS allows the condition of non-sampled reaches to be inferred statistically with quantifiable precision (Southerland et al. 2009). Three statewide, probability-based stream assessments, herein known as Rounds, evaluated stream ecological conditions in Maryland during discrete time periods, at different watershed scales, and using different stream maps, sample stratification, and sample sizes. Although less than 2% of total stream miles were sampled during each Round, the probability-based sampling design allowed for the extrapolation to statewide and certain watershed-scale conditions (depending on the Round of sampling).

During Round One, 955 sites were selected from a 1:250,000 scale map for sampling over a three-year period (1995-1997) to assess the health of Maryland streams at a statewide and 18 major river basin scales. A total of 1,066 sites were sampled from a 1:100,000 scale map over five years (2000-2004) as part of Round Two, with a focus on statewide and 84 Primary Sampling Unit (watershed) scales. Substantial targeted site sampling was added coincident with Round Two, with locations selected to spatially and/or temporally coincide with a specific condition or action. This aspect of sampling continues to be part of the MBSS and is used to answer specific questions about the influence of a condition or action at those specific locations (e.g., a steam restoration project, a dam removal, weather and climate influences). Round Three consisted of sampling 252 sites with randomly selected locations between 2007 and 2009 on the same 1:100,000 scale stream map used for Round Two. The Round Three assessment focused on statewide and 12 major river basin scales.

MBSS Round Four Background and Design

Some of the most frequently asked and important questions to be answered by the MBSS pertain to changes in stream conditions over time (e.g., are stream conditions improving?; are stream conditions degrading?). Since 2000, the MBSS Sentinel Site Network (Prochaska 2005) has investigated natural annual variability in a subset of 28 high-quality Maryland streams. Each Round of MBSS sampling estimated statewide stream conditions during a discrete time interval. Although the estimates from these Rounds are statistically valid, trends over time are difficult to definitively discern from these results, especially when comparing results from Rounds that used different scale stream maps (Southerland et al. 2013) or different sampling designs. The EPA National Rivers and Streams Assessment incorporates repeat sampling of a subset of sites along with newly selected random sites (USEPA 2017) so that temporal trends are identified, and spatial representation is also accomplished. Sampling sites previously sampled during earlier Rounds reduces the variation among sites compared with new random sites and therefore provides the highest probability of detecting changes over time (Southerland et al. 2013).

Based on a power analysis applied to Round Two data and practical constraints on the number of sites that could be sampled, an approximate number of sites were resampled during Round Four (2014-2018) to detect a statewide change in benthic IBI (at 80% probability) of approximately 0.19 (Southerland et al. 2013). This consisted of revisiting 147 sites previously sampled during Round One (Figure 2) and 251 sites previously sampled during Round Two (Figure 3). Round One (1995-1997) random site resampling was conducted from 2015 to 2017 (sites were resampled one time 20 years after the original sampling). Round Two (2000-2004) random site resampling was conducted from 2014 to 2018 (sites were resampled one time 14 years after the original sampling). Results from Sentinel Site sampling during Round Two and Four were also compared over the same 14-year interval (Sentinel Sites were not sampled during Round One, so comparisons were not possible; Table 1). Sentinel Site analysis provided the opportunity to examine potential change over time in high-quality streams, as well as to help interpret results from random sample comparisons. The Sentinel Sites are referred to as reference sites throughout this report. Annual trends from these sites, as presented in Resource Assessment Service (2023), are also useful to help interpret results from this report.

Table 1. Maryland Biological Stream Survey sampling Rounds and associated sampling years. Notation is provided here for reference throughout the report. R4/1 refers to Round 1 resampling in Round 4; R4/2 refers to Round 2 resampling in Round 4.

Sampling Round	Years Sampled	Notation
Random Site Round 1	1995 - 1997	R1
Random Site Round 2	2000 - 2004	R2
Random Site Round 1 Resampling in Round 4	2015 - 2017	R4/1
Random Site Round 2 Resampling in Round 4	2014 - 2018	R4/2
Reference Site Round 2	2000 - 2004	S2
Reference Site Round 4	2014 - 2018	S4

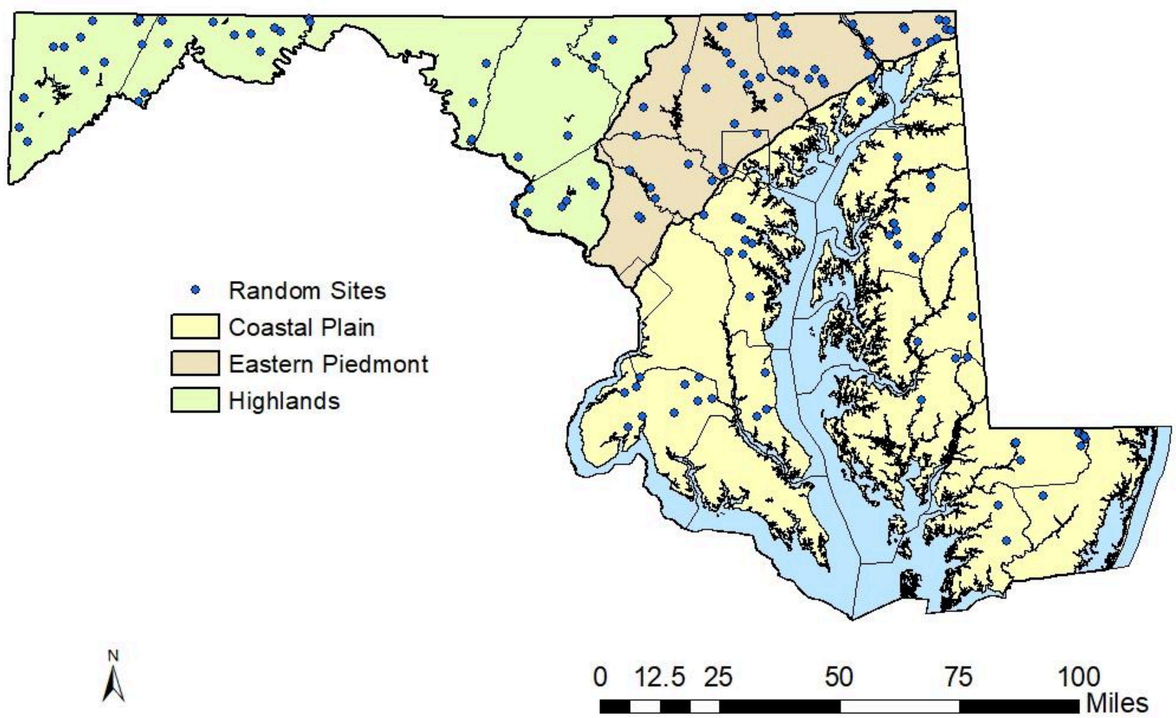

Figure 2. Map depicting the sampling locations of Round 4 vs Round 1 random sampling locations.

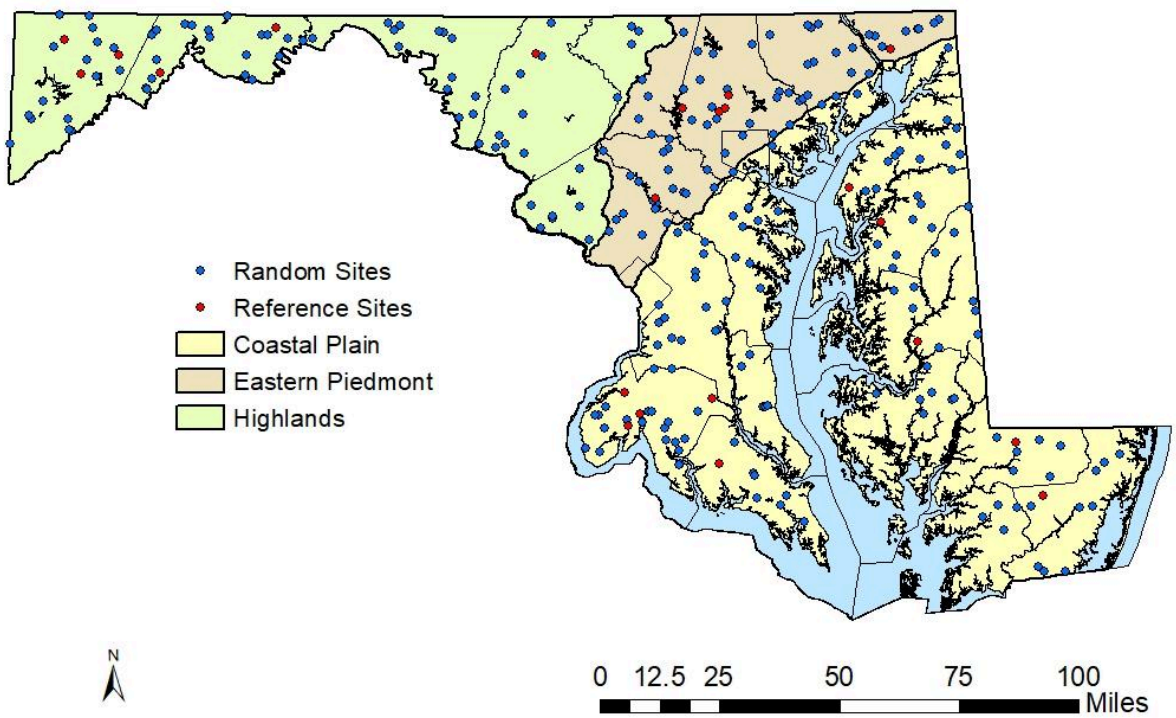

Figure 3. Map depicting the sampling locations of Round 4 vs Round 2 random sampling locations and reference site locations.

Change Over Time Methods Overview

MBSS Round One (R1) resampling consisted of stream sampling at 147 sites that were sampled once during 1995, 1996, or 1997 and again 20 years later during Round 4 (R4/1; 2015, 2016, or 2017). The locations of these sites were selected using stratified random sampling (Roth et al. 1999) from a 1:250,000 scale stream map. The subset of sites for resampling were randomly selected from 955 sites sampled during R1. A total of 251 stream sites originally sampled as part of Round Two (R2; 2000-2004) were resampled 14 years later as part of Round Four (R4/2; 2014-2018). The locations of these sites were selected using stratified random sampling (Roth et al. 2005) from a 1:100,000 scale stream map. The subset of sites for resampling were randomly selected from 1,109 sites sampled during R2. Data from 22 high-quality reference streams (Sentinel Sites; Prochaska 2005) were also compared between the Round Two (S2) and Round

Four (S4) time periods. All data were collected following standard MBSS sampling procedures (Kazyak 2001, Stranko et al. 2019, Resource Assessment Service 2022).

All variables could not necessarily be sampled during resampling visits from all sites, due to the stream being dry or other reasons. Thus, sample sizes for analyses and reporting varied by variable (Table 2). Additionally, minor changes in protocols inhibited comparisons of Round Four results for certain variables with the results from certain previous Rounds. This report details the changes in biological indicators based on benthic macroinvertebrate and fish assemblages, water chemistry, and water temperature.

Table 2. Sample size overview of the number of site pairs available for the four major sections investigated. Further descriptions of sample sizes are included within each section. N/A indicates no monitoring occurred.

Section	R1 vs. R4/1	R2 vs. R4/2	S2 vs S4
Benthic Macroinvertebrates	133	242	110
Fish	119	209	100
Water Chemistry	147	251	110
Temperature	N/A	92	58

We tested the hypothesis that benthic macroinvertebrate, fish, and temperature metrics, as well as water chemistry parameters, have not changed over time (i.e., $\mu_{present} - \mu_{past} = 0$). Prior to testing this hypothesis, we examined the differences in variance among all metrics and parameters with Quantile-Quantile (Q-Q) plots for deviations from paired t-test assumptions and the central limit theorem, which establishes that moderately large sample sizes approximate a normal distribution (Wilk and Gnanadesikan 1968). The equality of variance between sample populations for each time period was assessed with Bartlett's test. Since the majority of metrics and parameters failed normality testing (Bartlett's test p-values < 0.05), and there was ample evidence of non-normal data distributions based on visual examinations of Q-Q plots, all metrics and parameters were tested for statistical significance between Rounds using a nonparametric Wilcoxon signed rank test. A normal approximation with a continuity correction was used as necessary in some Wilcoxon tests (due to the presence of zeros and/or ties); these results are noted with an asterisk within each section. Given the use of multiple statistical tests, we deemed statistical significance at alpha = 0.01 to reduce the probability of making a Type I error. Statistical comparisons were made at the statewide scale for benthic macroinvertebrates, fish, and water chemistry, whereas the temperature analyses focused on comparisons at the statewide and ecoregion scales. Means, medians, 95% confidence intervals, and violin plots or boxplots (depending on the best method for data visualization) showing the distribution of all metrics and parameters, are included in respective appendices.

Round Four Results and Conclusions

Benthic Macroinvertebrates

Methods

This analysis incorporated benthic macroinvertebrate sample pairs where both samples contained at least 60 individuals. This resulted in a total of 133 sample pairs to compare between R1 and R4/1, and a total of 242 sample pairs for the R2 vs. R4/2 comparison. Data were available from all reference sites for the S2 vs. S4 comparison, resulting in a total of 110 reference sample pairs.

Since increases in taxonomic richness are indicative of improvements in stream conditions, differentiating richness increases due to taxonomy changes from actual changes in the number of taxa in a sample is critical for comparable examinations of stream health and biodiversity over time. We specifically addressed the potentially confounding influence of factors such as taxonomic splitting and name changes by modifying the taxonomy of more recent samples (i.e., Round Four) to match that of earlier samples (i.e., Round One or Round Two: Appendix A).

The following benthic community metrics were compared between Rounds: the Maryland family benthic macroinvertebrate IBI (Stribling et al. 1998), percent EPT, percent EPT without Baetidae or Hydropsychidae, percent Ephemeroptera, percent Ephemeroptera without Baetidae, percent Trichoptera, percent Trichoptera without Hydropsychidae, percent Plecoptera, percent Odonata, the percent of individuals intolerant to urbanization, and the percent of individuals that were collectors (Table 3; Appendix B). In addition to metric investigation, a non-metric multidimensional scaling technique was implemented to visualize potential changes in community composition over time.

Percentage-of-individual metrics were used here because percentages help normalize for differences in the total number of individuals among samples. Percent intolerant to urbanization is a component metric used consistently to calculate benthic macroinvertebrate IBI scores for all Maryland streams (Southerland et al. 2005). Insects in the mayfly (Ephemeroptera), stonefly (Plecoptera), and caddisfly (Trichoptera) orders are widely considered the most intolerant stream-dwelling benthic macroinvertebrates (Poff et al. 2006) and changes in their proportions may indicate more specific or subtle changes in stream condition over time that may not be reflected in benthic IBI scores. Focusing on the percentages of individuals within these insect orders independently directly examines these particularly sensitive aspects of stream biological diversity. The mayfly family Baetidae and the caddisfly family Hydropsychidae were excluded because these families are often considered more tolerant to pollution and habitat degradation compared with other members of their orders (Jackson et al. 2009, Lakew and Moog 2015, Boehme et al. 2016, Masese and Raburu 2017). Percent Odonata (dragonflies and damselflies) was examined in this study because other studies have observed recent declines in these taxa along with others in orders Ephemeroptera, Trichoptera, and Plecoptera (Sánchez-Bayo and Wyckhuys 2019, Eggleton 2020, Sánchez-Bayo and Wyckhuys 2021), and because large numbers of Odonates are listed as endangered or threatened in Maryland (Maryland Natural Heritage Program 2021). Percent collectors was examined because many taxa in this group are generalists and therefore less likely to respond negatively to alterations to their environment (Poff et al. 2006). As such, increases in the proportion of generalists (such as collectors) may indicate declining stream conditions.

The Maryland family benthic macroinvertebrate IBI (Stribling et al. 1998) was used, rather than the genus benthic IBI (Southerland et al. 2005), to avoid potentially confounding influences resulting from improvements in genus-level taxonomic identification skills over time. Unlike benthic community metric calculations that used samples with at least 60 individuals, family benthic macroinvertebrate IBIs were only calculated using samples with at least 80 individuals. The 80-individual limit ensured all samples would be within 20% of the target number of 100 individuals used for Maryland's benthic IBI.

Rarefaction methods described in Hurlbert (1971) were also employed to reduce the potential for differences in the numbers of individual benthic macroinvertebrates in samples to confound comparability of family IBI scores. We rarefied each sample to 100 individuals, or the lowest number of individuals per site pair, and the data were bootstrapped for 1000 iterations. The family IBI (and its component metrics) was calculated for each iteration. Further analyses were performed using the average over all iterations.

Results

No significant differences among any temporal comparisons were observed in the family benthic IBI (Table 3). Although family benthic IBI scores were not significantly lower or higher, a slightly higher percentage of samples with the narrative ranking of Good was observed in R4/1 compared to R1 (20 years previously). The percentage of samples with the narrative ranking of Good observed in R4/2 was the same in R2 (14 years previously; Figure 4). A slightly higher percentage of samples scoring in the Good range was observed in S4 compared to S2 (14 years previously). Site-specific family benthic IBI scores rarely changed from Good to Poor, or from Poor to Good during repeat sampling. Specifically, a total of four sites that initially scored Good during R1 sampling received Poor scores 20 years later during R4/1 repeat sampling. A total of six R2 samples and five S2 sites that initially scored Good received Poor scores 14 years later (during R4/2 or S4 repeat sampling). In contrast, a total of six sites initially sampled during R1 scored Poor then Good during R4/1 repeat sampling, while a total of three R2 sites and two S2 sites scored Poor then Good.

The percentages of sensitive benthic macroinvertebrates were lower in recent years compared with 20 or 14 years previously (Table 3). This was most apparent over the 20-year interval and in order-level community measures like percent EPT and percent mayflies, especially with the exclusion of Baetidae. Stoneflies and the percentage of individuals intolerant to urbanization were also lower in recent years (Table 3, Figure 5).

Percent Trichoptera was the only order-level measure of sensitive benthic taxa that was not lower during more recent sampling. Rather, it was significantly higher in all three temporal comparisons (Table 3, Figure 5). This was the case even with the exclusion of Hydropsychidae. The tolerant benthic macroinvertebrate measure percent collectors was significantly lower in the 14-year but not the 20-year comparison period (Table 3, Figure 5).

Table 3. Results of Wilcoxon signed rank tests applied to family benthic IBI scores and ten measures of benthic macroinvertebrate community composition at sites with randomly selected locations between 20-year (R1 vs. R4/1) and 14-year (R2 vs. R4/2) time intervals, as well as reference (S2 vs. S4) sites over a 14-year interval. All values for IBI and other measures below were derived by rarefaction to an abundance of 100 individuals or to the lowest abundance within each site pair. P-values < 0.01 highlighted in red indicate significantly lower values and in blue indicate significantly higher values during the more recent sampling. Further descriptive statistics and graphics displaying means of rarefied datasets for each metric are presented in Appendix A. EPT measures include individuals in orders Ephemeroptera, Plecoptera, and Trichoptera.

Benthic Macroinvertebrate Community Measure	**Random**	**Random**	**Reference**
	R1 vs. R4/1	**R2 vs. R4/2**	**S2 vs. S4**
Family Benthic IBI Score	p = 0.870	p = 0.662	p = 0.786
% EPT	p = 0.981	p = 0.133	p = 0.814
% EPT (Baetidae and Hydropsychidae excluded)	p < 0.001	p = 0.107	p = 0.232
% Ephemeroptera	p < 0.001	p = 0.003	p = 0.003
% Ephemeroptera (Baetidae excluded)	p < 0.001	p < 0.001	p = 0.001
% Trichoptera	p < 0.001	p < 0.001	p < 0.001
% Trichoptera (Hydropsychidae excluded)	p < 0.001	p < 0.001	p < 0.001
% Plecoptera	p = 0.004	p = 0.775	p = 0.563
% Odonata	p = 0.044	p = 0.146	p = 0.354
% Intolerant Urban	p < 0.001	p = 0.018	p = 0.166
% Collectors	p = 0.345	p < 0.001	p < 0.001

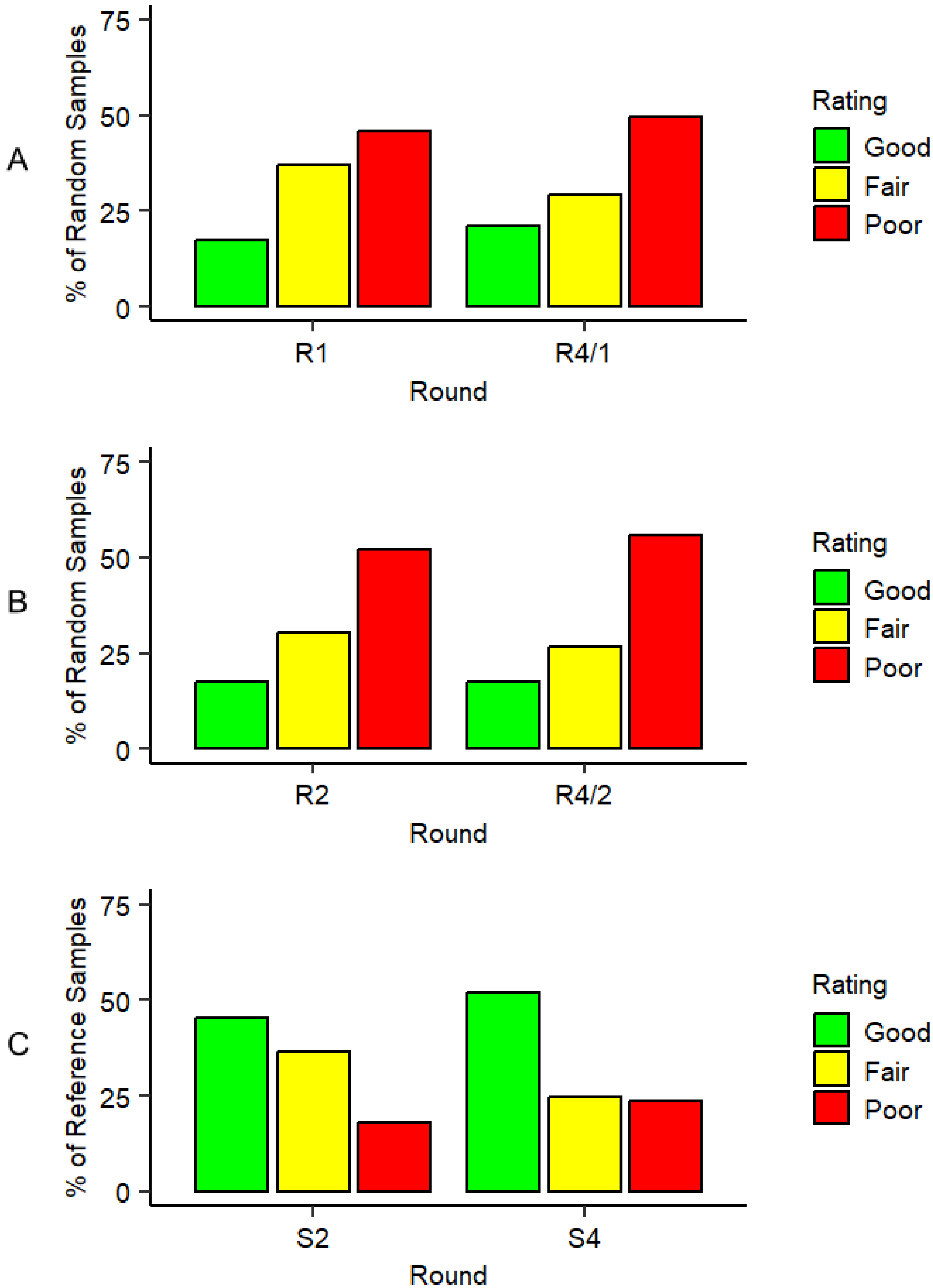

Figure 4. Percentages of samples with Good, Fair, and Poor family benthic Index of Biotic Integrity scores from randomly chosen locations between R1 and R4/1 (A), and R2 and R4/2 (B), as well as reference sites S2 and S4 (C).

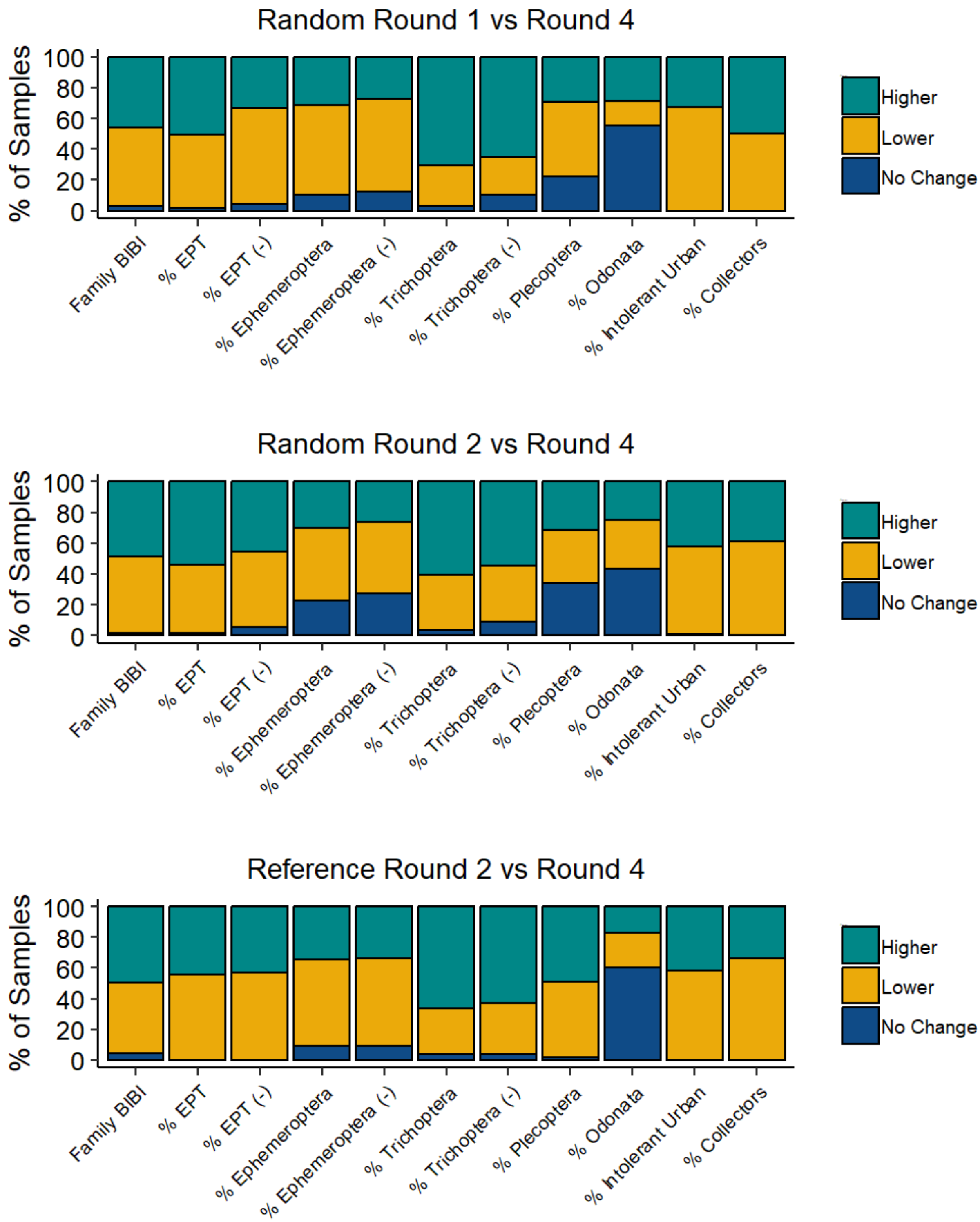

Figure 5. Percentages of samples with higher, lower, or no change in 11 benthic macroinvertebrate measures in the later period among the three temporal comparisons. The symbol (-) refers to the exclusion of insect families Hydropsychidae and/or Baetidae.

Benthic Macroinvertebrate Assemblage

Nonmetric Multidimensional Scaling analysis (displayed as an ordination plot in Figure 6) showed considerable overlap in benthic macroinvertebrate genus-level community compositions between Rounds. These results are consistent with family benthic IBI score results indicating similar benthic communities between Rounds.

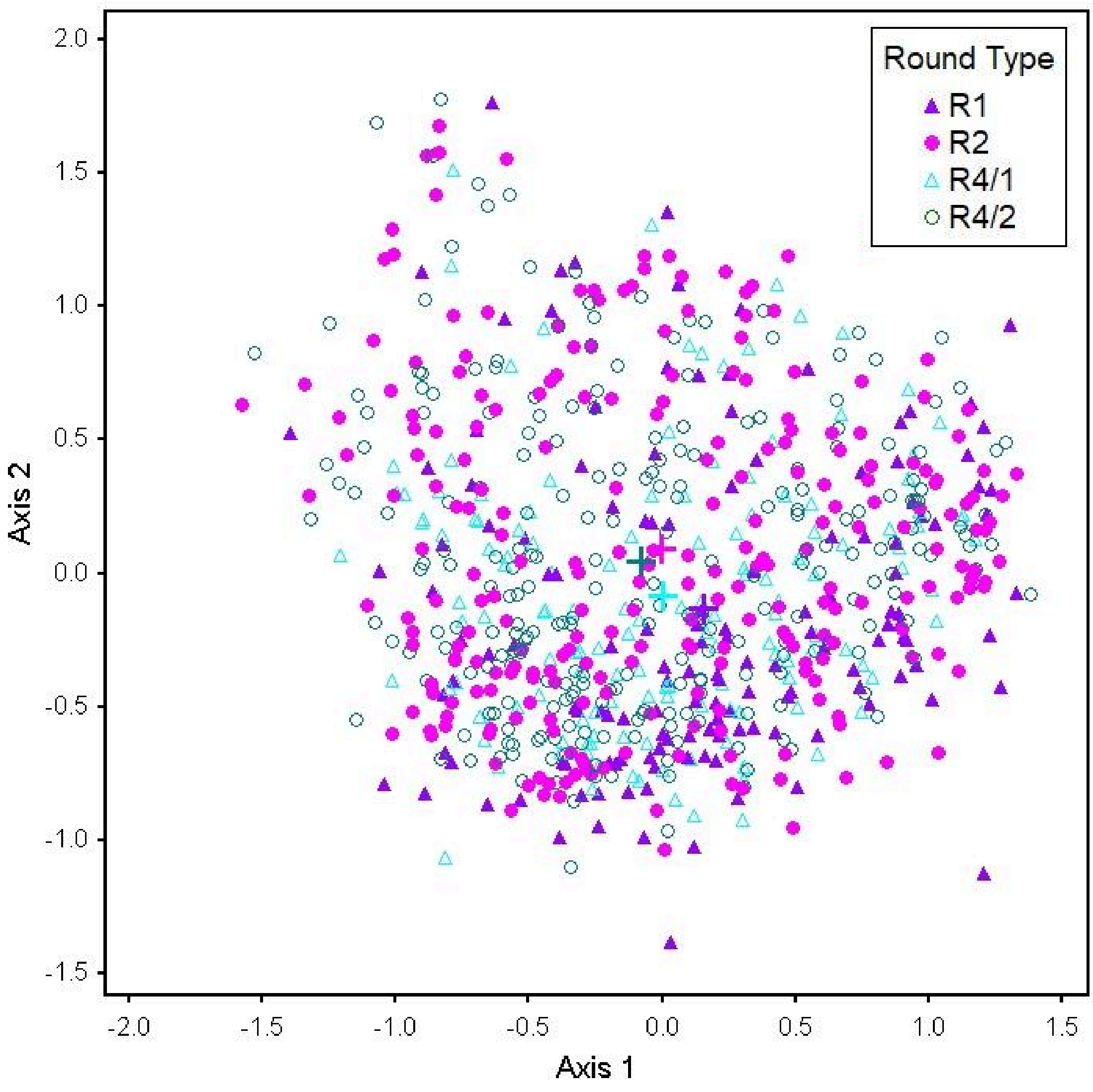

Figure 6. Non-metric Multidimensional Scaling (NMS) ordination results comparing the benthic macroinvertebrate assemblages between random sampling Rounds. Stress = 0.21 (less than adequate representation), plus signs indicate centroids by Round Type. Round 1 and Round 4/1 are represented by closed and open triangles respectively. Round 2 and Round 4/2 are represented by closed and open circles respectively.

Discussion

Certain benthic macroinvertebrate results indicate lower percentages of sensitive taxa over time, while others indicate no change or higher percentages of sensitive taxa. Although there are some contrasting results, there appears to be more evidence of declines than improvements in benthic macroinvertebrate measures examined in this study, particularly over the 20-year examination, and were observed more often at random sites compared with reference sites. Family benthic IBI and ordination results indicate no differences in general community composition between the periods examined here. However, the results from the relative abundances of specific insect orders, as well as select tolerance metrics, provide evidence of differences that may be too subtle to detect in the studied timeframes with community measures. Further monitoring in future years may show signs of change that can be detectable at the community level.

Observing lower numbers of sensitive benthic macroinvertebrates over time is consistent with other studies throughout the United States (Wang and Lyons 2003, Southerland et al. 2007, Kenney et al. 2009). These observations could be attributable to physical habitat alterations, changes in water chemistry such as increased conductivity, temperature, and increased urbanization (Nedeau et al. 2003, Echols et al. 2009, Cuffney et al. 2010, Rezende et al. 2014, Piggott et al. 2015). In contrast to declines in certain sensitive benthic macroinvertebrates, we observed higher percentages of caddisflies and lower percentages of collectors in recent years. These may indicate improvements in stream conditions such as less acidity and/or reductions in atmospheric deposition (Clean Air Act 2011). Positive correlations between pH and caddisfly richness and density have been observed in previous studies (Townsend and Hildrew 1984, Mackay and Kersey 1985, Rosemond et al. 1992). Although this report focused on examining statewide patterns in Maryland's stream conditions, results unique to individual regions, streams, stressor types, and other factors may help explain many of the patterns observed herein.

There is interest in dragonflies and damselflies as they appear to be in decline globally (Sánchez-Bayo and Wyckhuys 2019, Eggleton 2020, Sánchez-Bayo and Wyckhuys 2021). Contrary to other recent studies, however, significant differences in the percentages of these insects were not observed in recent years, indicating no change in their numbers over time in Maryland's streams. However, the relative rarity of dragonflies and damselflies in our dataset contributes to uncertainty in results and conclusions.

The importance and widespread use of stream-dwelling benthic macroinvertebrates as indicators of stream health is exemplified by the results of this study. In addition to evaluating a commonly used stream health indicator, our results contribute valuable information about certain Maryland benthic macroinvertebrates (especially sensitive taxa) over time. By adjusting for the potentially confounding influences of factors such as changes in taxonomy and inconsistencies in the numbers of individuals in samples, we were able to provide rigorous comparisons over two time periods (20 and 14 years) using randomly selected sites (as representative of Maryland's streams) and over 14 years using reference (high-quality) stream conditions. By examining specific groups of benthic macroinvertebrates, we were further able to elucidate patterns in biological community composition not evident at the community level and biological indicator scales. This combination of analyses should help inform stream management, as well as future monitoring and assessments. Ultimately, we learned that although overall steam health (as measured by a family-level benthic macroinvertebrate IBI) has not appeared to change substantially, the percentages of certain sensitive taxa seem to be lower while others were higher in the more recent period. Especially in the context of recent information about insect biodiversity trends, such patterns are important to understand and monitor. Moreover, they could also eventually manifest as trends in Maryland stream biological integrity.

Fish

Methods

Sites that could not be sampled (typically due to being dry) or where sampling occurred, but no fish were observed were excluded from all analyses, resulting in 119 R1 vs. R4/1 and 209 R2 vs. R4/2 sample pairs with fish data for analyses. Data were available from 20 of the 22 reference sites for the S2 vs. S4 comparison, resulting in a total of 100 reference sample pairs.

The following fish community metrics were compared between Rounds: the Maryland fish IBI (FIBI; Southerland et al. 2005), species richness and abundance, native species richness and abundance, non-native species richness and abundance, RTE (rare, threatened, or endangered) species richness and abundance, and gamefish species richness and abundance (Table 4). In addition to metric investigation, a non-metric multidimensional scaling technique was implemented to visualize potential changes in community composition over time. Indicator Species Analyses and associated Monte-Carlo statistical tests were also conducted to compare fish species and their relative abundances between random site Rounds and separately for reference sites.

Non-native fish consisted of species introduced to the Atlantic drainage in Maryland (all streams east of the Youghiogheny Watershed) and species introduced to the Ohio River drainage (which consists of the Youghiogheny in Maryland). Certain species are native to one of these drainages and have been introduced into the other. Other species are non-native to both drainage basins. Any species not native to the drainage where it was collected was considered non-native. RTE species consisted of any species included in the 2021 edition of the rare, threatened, and endangered animals of Maryland, regardless of status (Maryland Natural Heritage Program 2021). Gamefish were limited to any trout species as well as smallmouth and largemouth bass. The categories for all species by site type are shown in Appendices C-E.

Due to the potentially strong influence sampling effort can have on fish abundance and species detection, mean electrofishing seconds were compared between the original R1 and R2 sampling and the repeat samples 20 (R4/1) and 14 (R4/2) years later. The result was significantly different effort between R1 and R4/1, with R4/1 having higher average electrofishing time (mean R1 = 6,012 seconds and R4/1 = 9,571 seconds, $p < 0.001$). There was no significant difference between R2 and R4/2 ($p = 0.110$) or S2 and S4 ($p = 0.121$) electrofishing effort. Although significantly more electrofishing time was used during R4/1, there were no significant differences in mean abundance ($p = 0.109$) or abundance per square meter ($p = 0.220$) between samples from R1 and R4/1. Thus, no adjustments were made to abundance, richness, or any fish sampling results.

Results

Mean FIBI results were not significantly different among any temporal comparison; R1 to R4/1 ($p = 0.318$), R2 to R4/2 ($p = 0.029$), and S2 to S4 ($p = 0.089$). The percent of sites scoring within the Good narrative ranking (Southerland et al. 2005) remained about the same across all temporal comparisons. However, the percentage of sites with Poor scores was slightly lower, with concomitantly more Fair scores, in the R1 vs. R4/1 and the S2 vs. S4/2 comparisons (Figure 7).

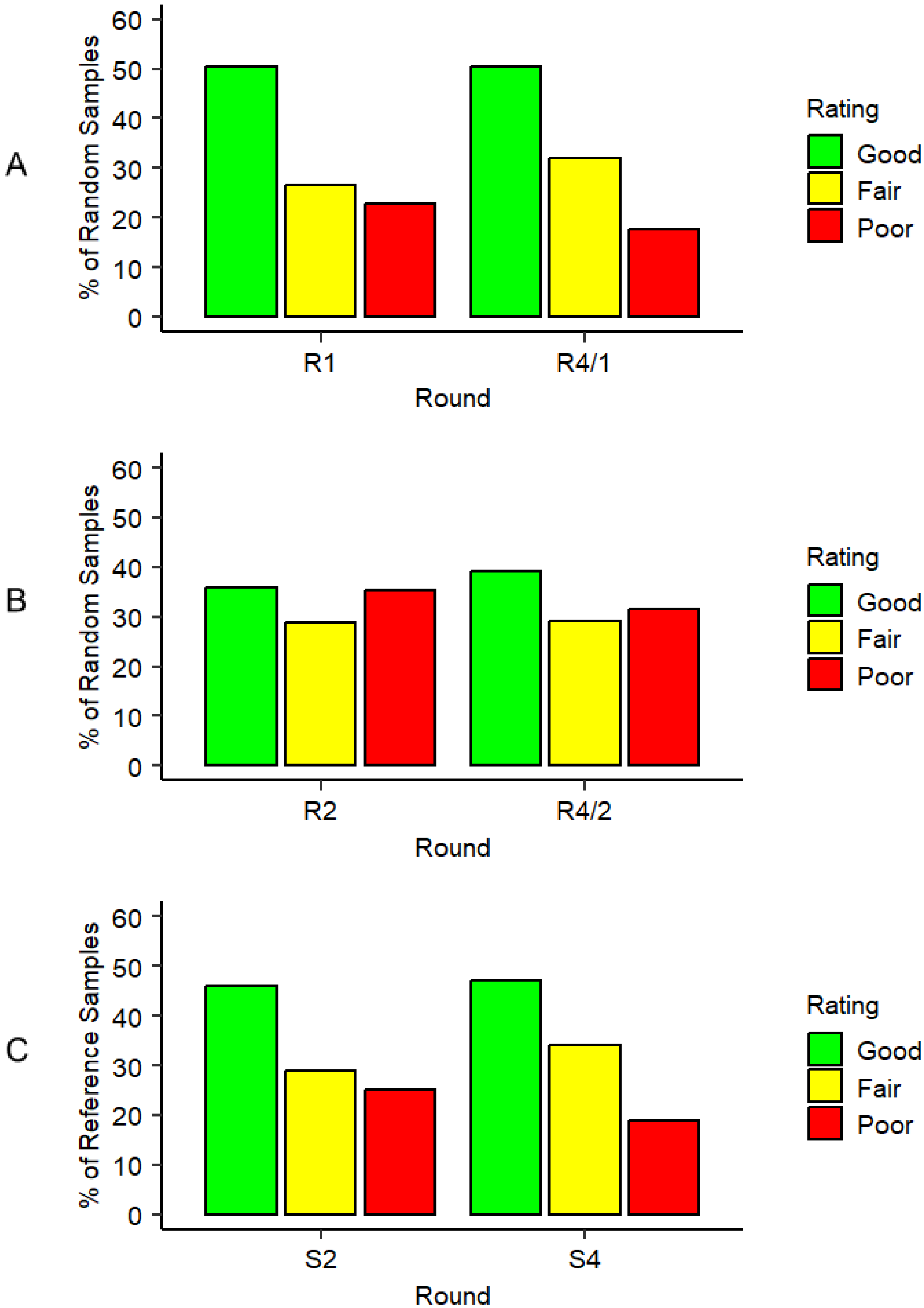

Figure 7. Percentages of samples with Good, Fair, and Poor fish Index of Biotic Integrity scores from randomly chosen locations between R1 and R4/1 (A), and R2 and R4/2 (B), as well as reference sites S2 and S4 (C).

Consistent with narrative ranking patterns, a greater percentage of samples had higher FIBI scores during the most recent sampling period compared with sites that had lower or the same scores (Figure 8). Changes from Good to Poor or Poor to Good were rare and are likely the most informative and ecologically meaningful changes. One site that scored Good during R1 and two sites that scored Good during R2 scored Poor during the more recent Round Four sampling (R4/1 and R4/2, respectively). Alternatively, four sites from R1 and three from R4/1 scored Poor then Good. One reference site scored Poor during the S2 sampling period then Good during S4. None of the reference sites scored Good in earlier Rounds then later scored as Poor.

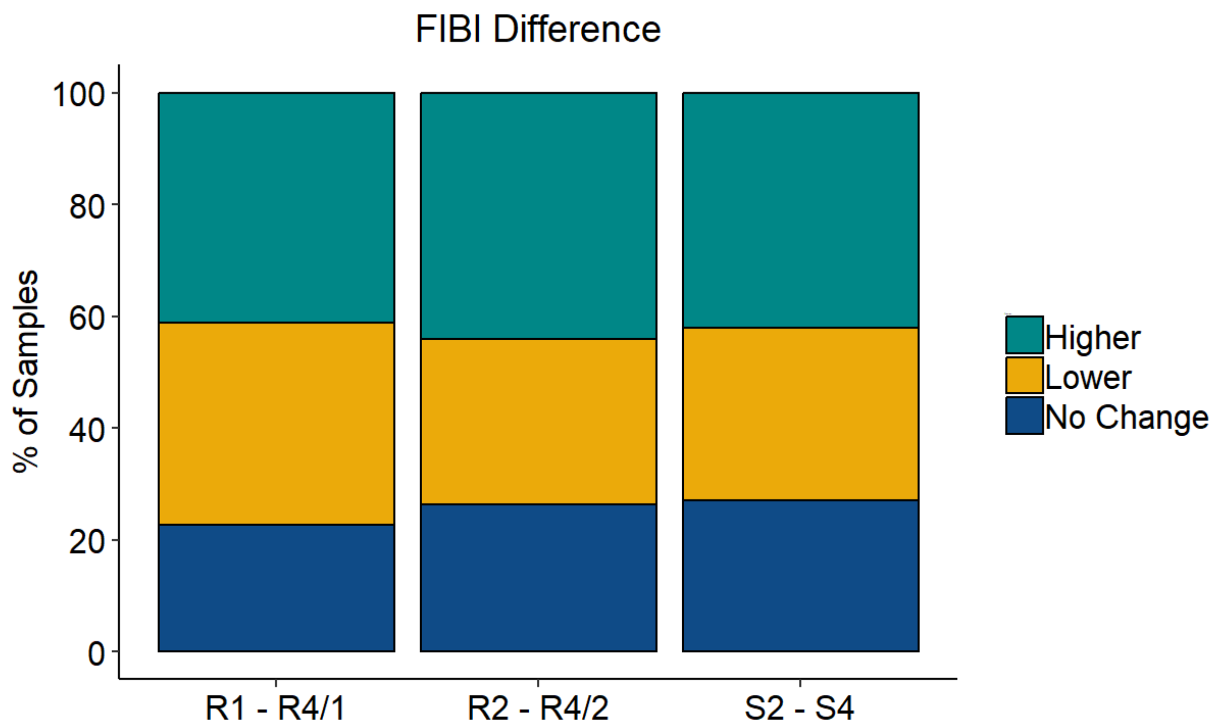

Figure 8. Percentages of samples with higher, lower, or no change in fish IBI scores in the later sampling period among the three temporal comparisons.

Fish Assemblage

A Non-Metric Multidimensional Scaling (NMS) ordination analysis applied to the random site fish data by Round (Figure 9) showed results consistent with the FIBI score results, indicating there are no distinct patterns of difference between general fish assemblages between Rounds. The centroids of all Rounds are close to one another, and overlap among sites is high in ordination space.

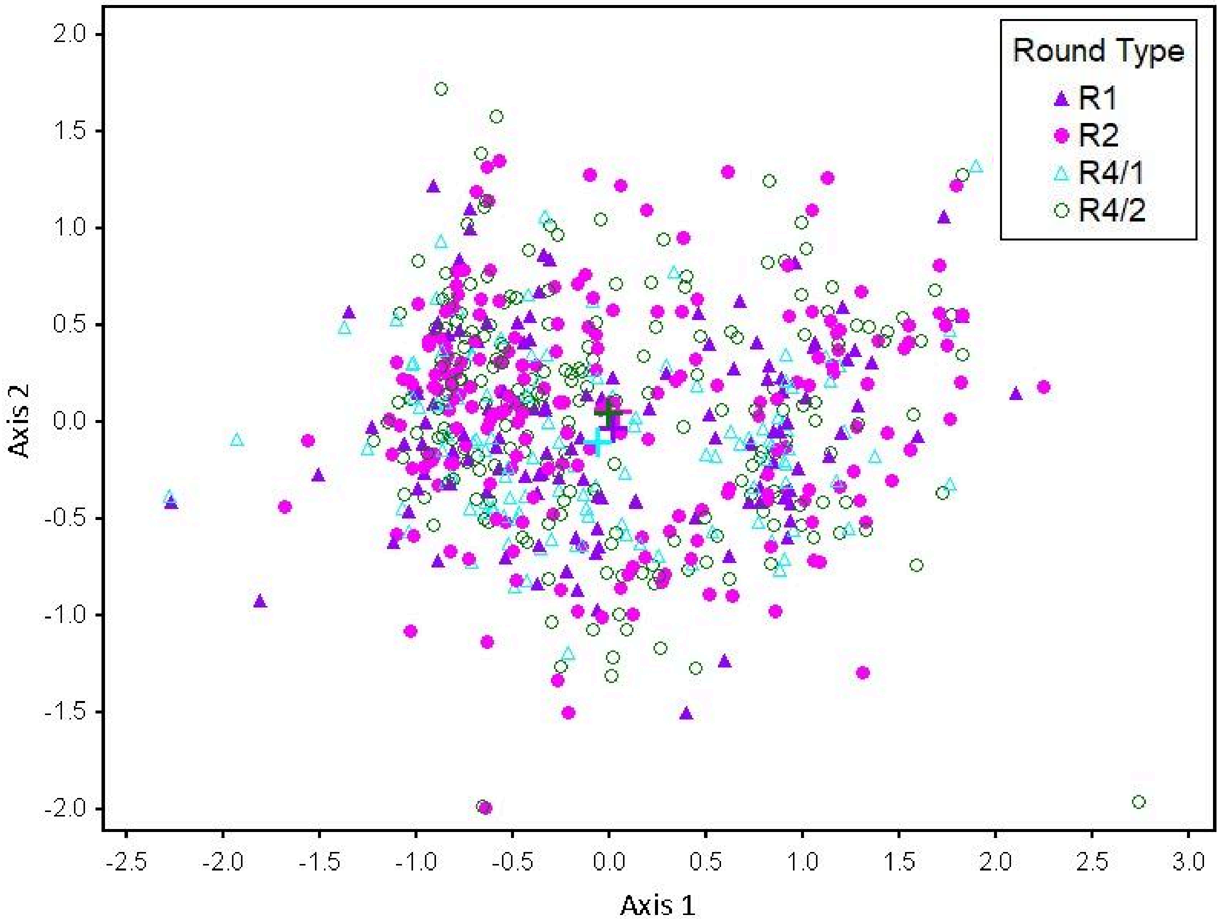

Figure 9. Non-Metric Multidimensional Scaling (NMS) ordination results comparing the fish assemblages between random sampling Rounds. Stress = 0.19 (fair representation), plus signs indicate centroids by Round Type. Round 1 and Round 4/1 are represented by closed and open triangles respectively. Round 2 and Round 4/2 are represented by closed and open circles respectively.

Based on Indicator Species Analyses values, no species were strong indicators of any particular random sampling Round. This supports the other (FIBI and NMS) assemblage results. According to reference site Indicator Species Analysis results, one species (Green Sunfish; *Lepomis cyanellus*) was indicative of differences between Rounds at reference sites (Indicator Value = 21.9, p = 0.008). As Green Sunfish is non-native to all Maryland streams except the western Maryland Youghiogheny River watershed, this may be representative of higher relative abundances of non-native species during more recent years.

Table 4. Results of Wilcoxon signed rank tests for all fish combined, native fish, non-native fish, RTE (rare, threatened, or endangered) fish, and gamefish species at sites with randomly selected locations between 20-year (R1 vs. R4/1) and 14-year (R2 vs. R4/2) time intervals, as well as reference (S2 vs. S4) site sampling over the 14-year time interval. P-values < 0.01 are highlighted in red to indicate significantly lower values and in blue to indicate significantly higher values during the more recent sampling. Asterisks indicate metrics where a continuity correction was applied to the Wilcoxon test. Further descriptive statistics and graphics for each metric are presented in Appendix F.

Fish Metrics		**Random**	**Random**	**Reference**
		R1 vs. R4/1	**R2 vs. R4/2**	**S2 vs. S4**
All	Richness	p = 0.040	p < 0.001	p = 0.007
	Abundance	p = 0.109	p = 0.554	p = 0.007
Native	Richness	p = 0.634	p = 0.001	p = 0.266
	Abundance	p = 0.233	p = 0.731	p < 0.001
Non-Native	Richness	p < 0.001	p < 0.001	p < 0.001*
	Abundance	p < 0.001	p = 0.011	p = 0.005
RTE	Richness	p = 0.008*	p = 0.888*	p = 0.208*
	Abundance	p = 0.041	p = 0.489	p = 0.007*
Gamefish	Richness	p = 0.242	p = 0.479	p = 0.309*
	Abundance	p = 0.004	p = 0.983	p = 0.034

Results from random site comparisons indicate higher numbers of non-native species richness, and higher total species richness during more recent sampling. RTE species richness over the 20-year period was lower, and non-native species richness and abundance over the 14-year and 20-year periods were higher.

Similar to patterns observed at random sites, the number of non-native species and abundances were higher during recent years at the reference sites (Figure 10, Table 4). The number of samples with RTE species was significantly lower during R4/1 compared to R1, but no significant change was detected for the R2 vs. R4/2 or S2 vs. S4 comparisons (Figure 11, Table 4).

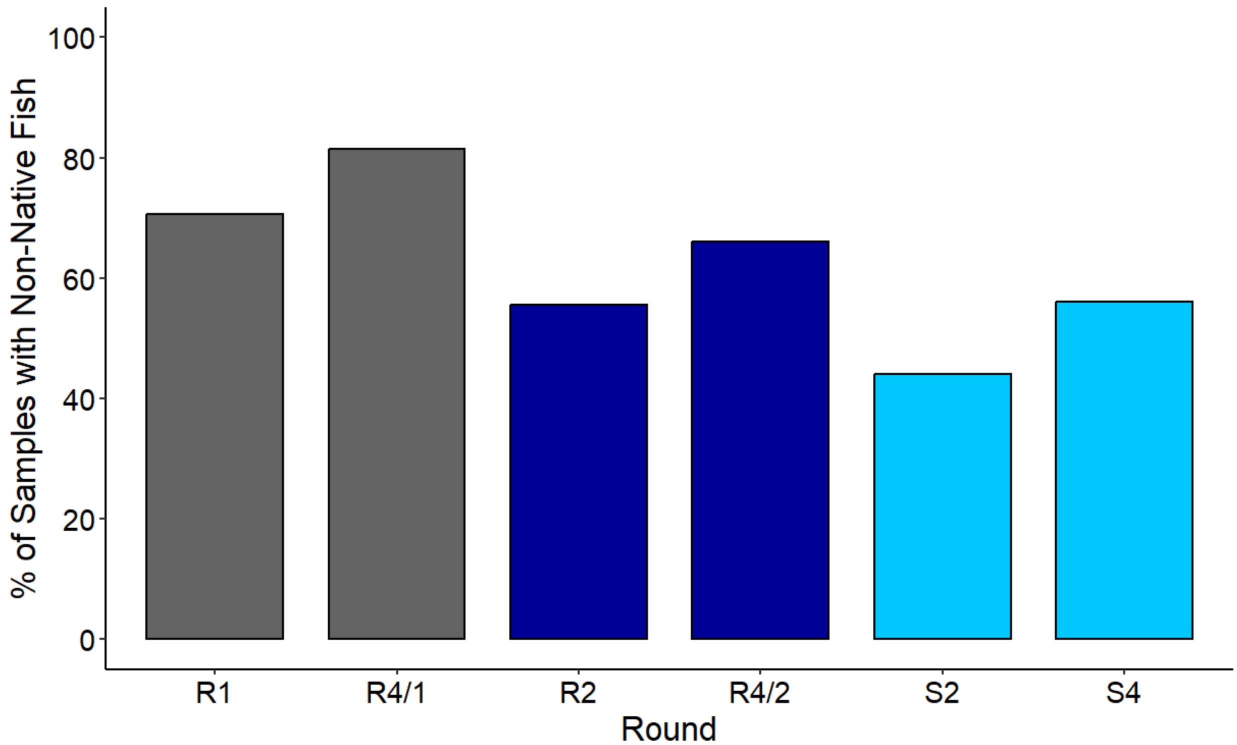

Figure 10. Percentages of samples during each sampling round with non-native fish species present.

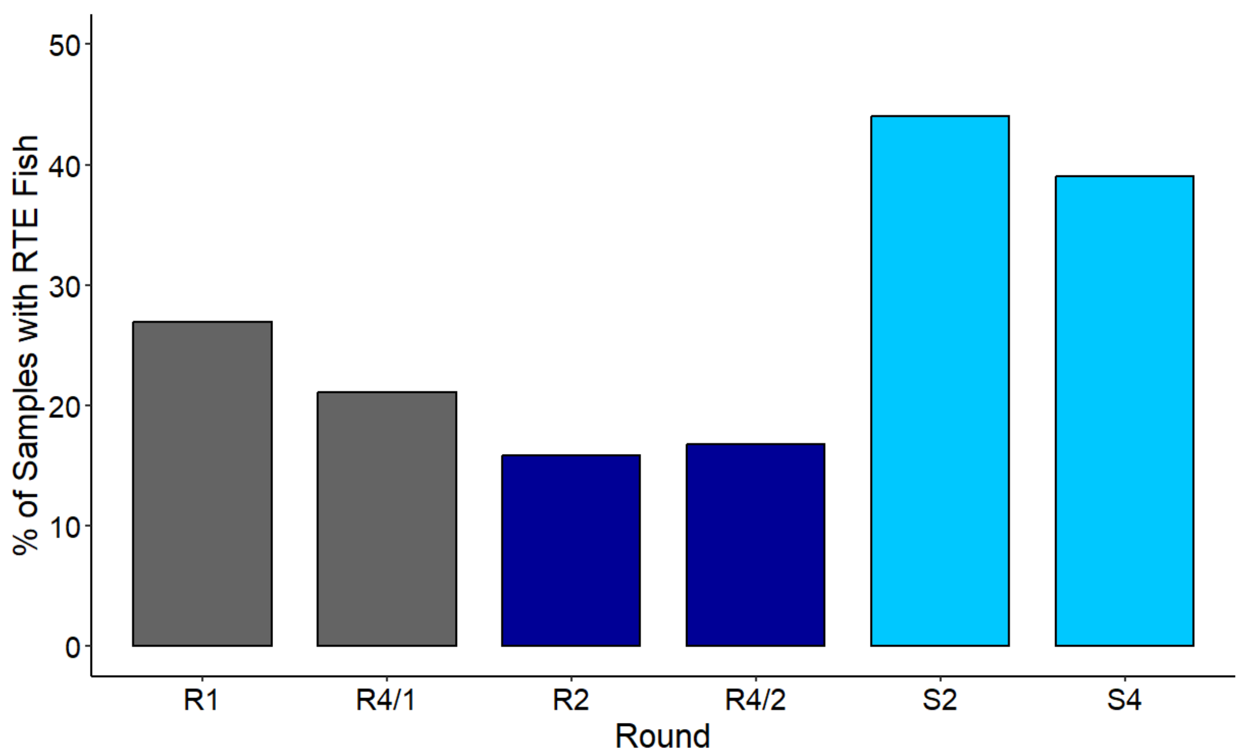

Figure 11. Percentage of samples during each sampling round with RTE (rare, threatened, or endangered) fish species present.

RTE and Non-Native Species Abundances

The percentage of total fish abundance (from all sites by Round combined) consisting of non-native species was consistently higher in recent years (Figure 12). The percentage of total combined fish abundance consisting of RTE species was lower in recent years at R4/1 compared with R1 random sites, and S4 compared with S2 reference sites, but was slightly higher at R4/2 compared with R2 random sites.

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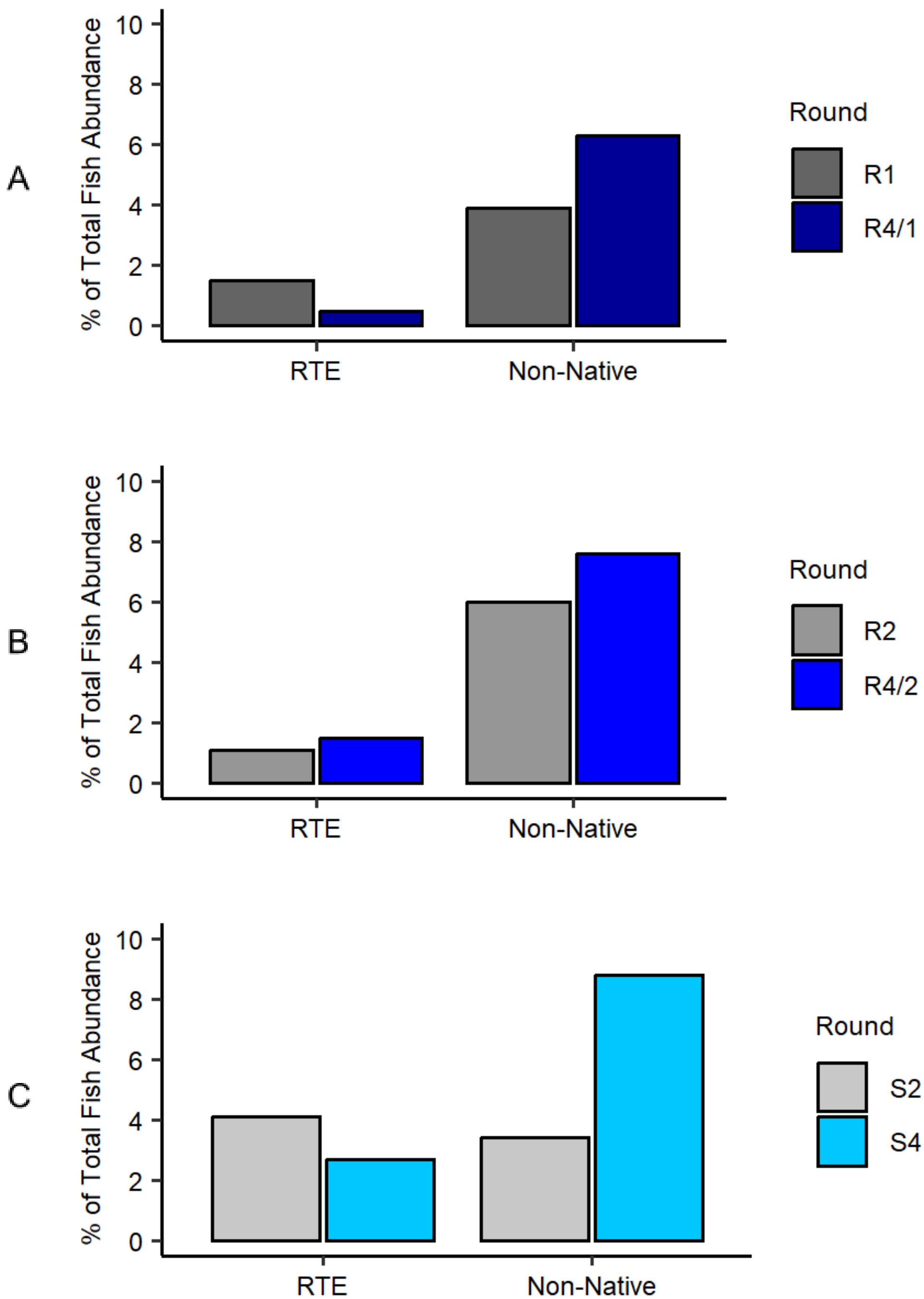

Figure 12. Percentages of total fish abundance consisting of RTE (rare, threatened, or endangered) and non-native species during R1 and R4/1 (A), R2 and R4/2 (B), and S2 and S4 (C).

The total number of non-native species collected was higher during R4/1 and R4/2 compared to the original sampling 20 and 14 years earlier during R1 and R2 (Figure 13). The lowest number of non-native species collected from random sites was during the 1995-1997 R1 sampling. Ten non-native species were collected during R4/1 and R4/2 combined that were not observed during the earlier R1 or R2. Those included: Bluehead Chub (*Nocomis leptocephalus*), Flathead Catfish (*Pylodictis olivaris*), Channel Catfish (*Ictalurus punctatus*), Mimic Shiner (*Notropis volucellus*), Redear Sunfish (*Lepomis microlophus*), Tiger Trout (*Salmo trutta* × *Salvelinus fontinalis*), Tiger Muskellunge (*Esox lucius* × *masquinongy*), Northern Snakehead (*Channa argus)* which were introduced to Atlantic Drainage streams, as well as Yellow Perch (*Perca flavescens*) and Pumpkinseed *(Lepomis gibbosus*) which were introduced to the Youghiogheny watershed. One non-native species (Cutthroat Trout: *Oncorhynchus clarkii*) was found during R1 and not during more recent R4/1 or R4/2 sampling. See Appendices C-E for species-specific details.

More RTE species were collected during R1 (1995-1997) than any other period (Figure 14). Fourteen RTE species were collected during R4/1. One less RTE species was collected during R2 compared with R4/2, 14 years later. One RTE species (Ironcolor Shiner: *Notropis chalybaeus*) was collected twice during the first sampling period (R1) and not during any subsequent periods. No RTE fish species were collected only during the 2014-2018 Round Four sampling period and not during previous sampling.

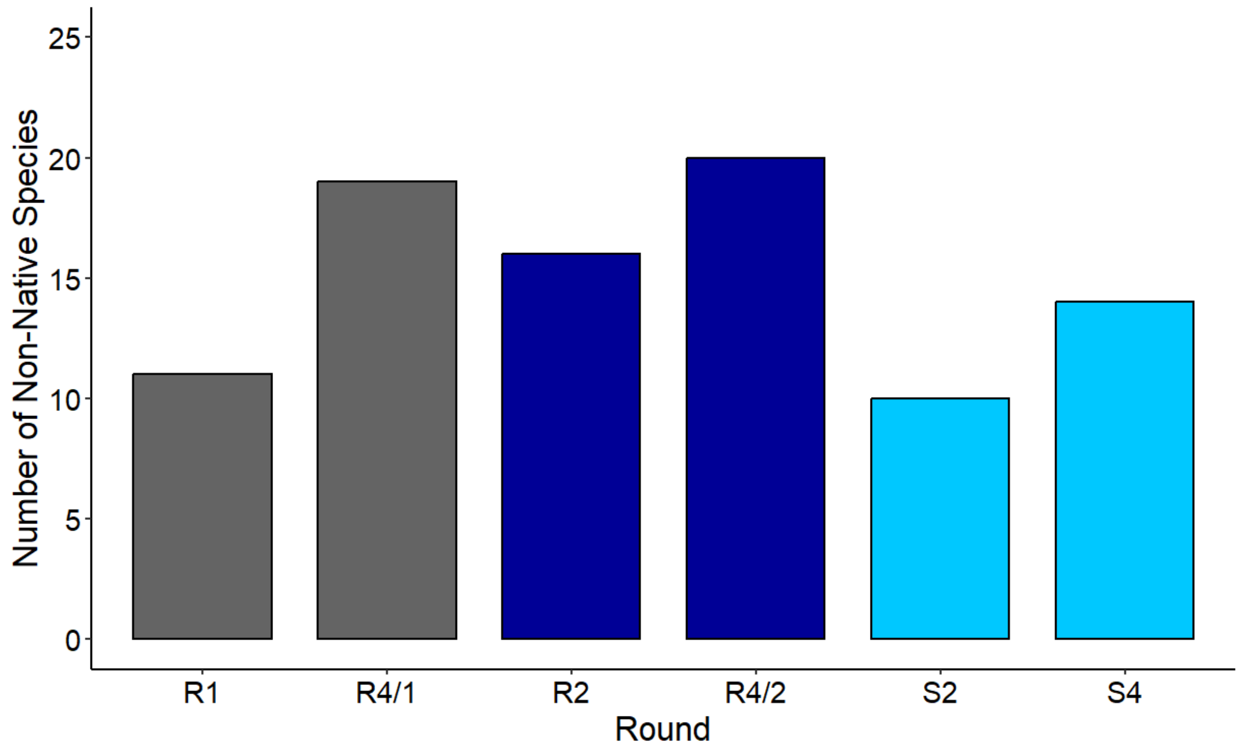

Figure 13. Total number of non-native species collected from all sites by sampling Round.

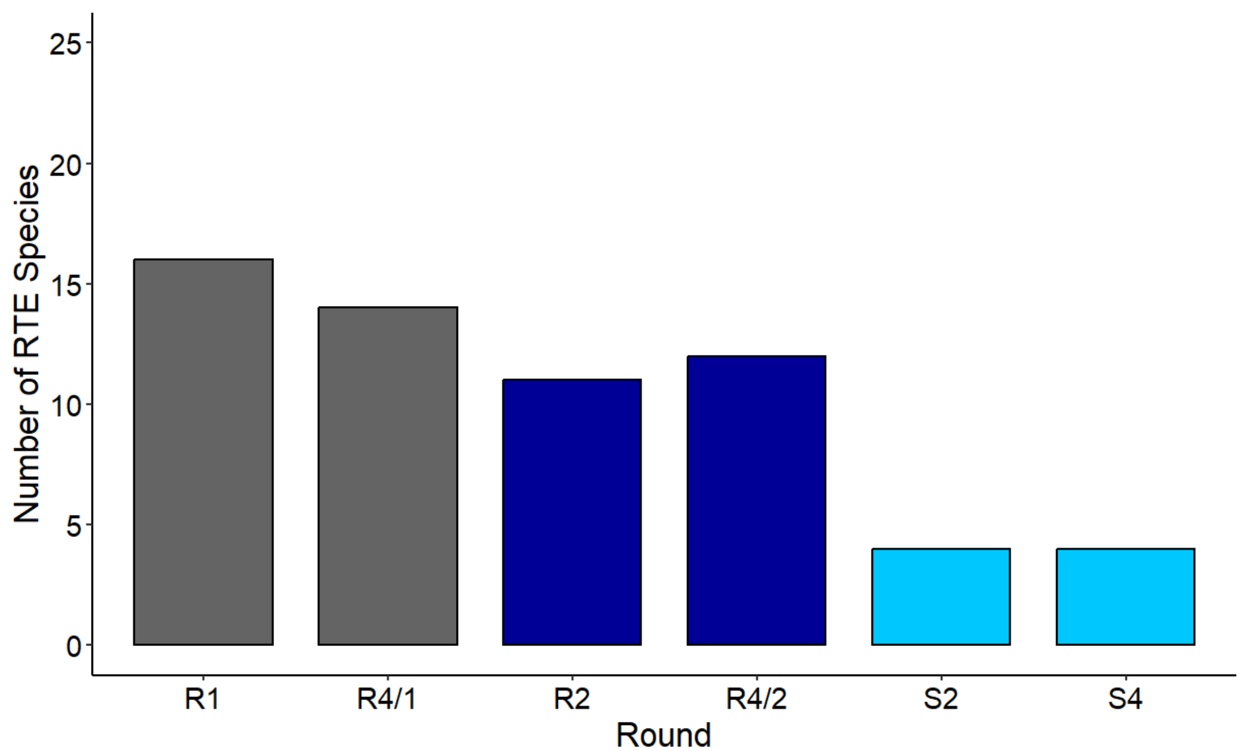

Figure 14. Total number of RTE (rare, threatened, or endangered) species by sampling round.

Discussion

Results from resampling Maryland streams that were sampled 20 and 14 years previously indicate more non-native fish species and fewer RTE species in more recent years. Higher percentages and abundances of non-native species in more recent sampling were more evident and consistent compared with declines in RTE species. These patterns were observable from resampling stream sites with randomly selected locations representative of Maryland's stream conditions, and reference streams representing high-quality streams. The magnitude of these patterns also seems to be larger over the 20-year interval than the 14-year interval resampling. Such increases in non-natives and declines in rare species are consistent with patterns in stream fish community changes observed in many other studies (McKinney and Lockwood 1999, Ricciardi and Rasmussen 1999, Rahel 2002, Burkhead 2012, Kuczynski et al. 2018, Gavioli et al. 2019, Sleezer et al. 2021) and, along with studies from other parts of North America, provide evidence of widespread biotic homogenization in stream ecosystems (Lockwood and McKinney 2001, Petsch 2016).

Declines in the abundances of all types of fish species, except for non-native species, were observed at the reference sites. As these sites were sampled annually for at least 14 years, repeated disturbance from successive sampling might have negatively influenced fishes (Putman 1995, Snyder 2003, Ellender et al. 2016). It is also possible that even minor stress in these high-quality streams may have negatively affected abundance. Ultimately, observed declines at reference sites that are not reflected in other streams are enigmatic. Further investigation may help explain these results and elucidate potential concomitant natural or anthropogenic sources of stress or variability.

There were no significant differences in mean FIBI scores among any of the temporal comparisons, although the R2 to R4/2 comparison could have been significantly higher ($p = 0.029$) in the later sampling period if we decided to be less conservative on the alpha level used. Additionally, slightly higher proportions of sites showed higher FIBI scores during the more recent period compared with initial sampling. There were no observed differences in community compositions (based on NMS and Indicator Species analyses), except for increases in Green Sunfish (a non-native fish species) at reference sites in the later sampling period. Although FIBI scores did not decrease in Maryland streams, increases in tolerant species (such as non-natives) and decreases in intolerant species (such as RTE) are sometimes early indicators of ecosystem alterations not yet reflected in other metrics (Morgan and Cushman 2005, Sleezer et al. 2021).

Despite the differences observed over time, mean non-native and RTE species percentages by site were each less than 10, with standard errors of the mean less than 2.0, for all Rounds – except for S2, when the mean percentage of RTE species was 11.2. Based on the results described herein, changes in these species groups that make up a small percentage of the total assemblage were not sufficient to indicate community or assemblage differences. However, although non-native and RTE species make up small portions of ecosystems, they are sometimes considered to have disproportionately high ecological importance (Leitão et al. 2016). In Maryland (as in many other areas), minimizing the losses of RTE species as well as inhibiting increases in non-native species are important natural resource management goals. Thus, by combining an examination of assemblage and biological index data with separate examinations of patterns in non-native and RTE species, we were able to provide information to support various biological integrity and biological diversity-related natural resource management goals.

The complete absence of the Ironcolor Shiner from recent sampling also supports the concept that particularly rare and sensitive species are likely declining. However, based on additional recent Maryland Department of Natural Resources sampling, this species is known to occur in Maryland's streams not sampled as part of this study. Moreover, abundant conservation efforts throughout the Maryland Department of Natural Resources and other entities in Maryland are focused on protecting critical habitats, streams, and watersheds specifically for the benefit of this and other important stream species and habitats.

Sites sampled during R1 (selected randomly from first- through third-order streams on a 1:250,000 scale map) were larger (mean catchment area = 8,160 acres) compared with sites sampled during R2 (selected randomly from first- through fourth-order streams on a 1:100,000 scale map with a mean catchment area of 5,847 acres). Stream size could influence the total number of fish species, as well as non-native and RTE species – with more species tending to occur in larger systems (Fausch et al. 1990, Roth et al. 1998). However, mean catchment areas were not statistically different according to an unequal two-sample t-test ($p = 0.080$).

Although there were differences in maps and sampling designs, results from sampling over time were largely consistent. Notable exceptions include a larger magnitude of increase in non-native species in the R1 vs. R4/1 comparison, and a slight increase, rather than decrease, in RTE species in the R2 vs. R4/2 comparison. We cannot determine definitively if these differences are due to time, different stream maps, different stream sizes, sampling design differences, differences in anthropogenic influence, or perhaps other factors associated with the particular streams that were sampled as part of each unique sampling Round. The consistent patterns in non-native, RTE, and FIBI score temporal comparison results at reference sites, however, support the concept that these patterns are likely representative of changes over time in Maryland's streams. However, the potential influence that successive annual sampling may have had on the data from these reference sites could be potentially confounding.

Although significantly and substantially different effort (mean electrofishing seconds) was employed during R1 compared with the repeat sampling 20 years later (R4/1), we chose to not adjust abundance results by effort (e.g., using catch per unit of effort) because abundances were not significantly different. Such adjustments, if implemented, may change the results observed herein. Along with a lack of statistical difference in mean effort employed within the other datasets, the consistency in results with these other datasets lends support to the results from the Round One comparison without adjustment.

Over 20- and 14-year periods, non-native fish species appear to have been added and RTE species lost from certain Maryland streams. Concomitantly, however, results did not change sufficiently to manifest a negative change in stream health (biological integrity) as measured by the Maryland FIBI.

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Water Chemistry

Methods

All sample pairs between Rounds had available water chemistry data for comparison, resulting in 147 sample pairs in the R1 vs R4/1 comparison, and 251 sample pairs in the R2 vs R4/2 comparison. Data were available from all reference sites for the S2 vs. S4 comparison, resulting in a total of 110 reference site pairs.

Water chemistry sampling at all sites followed standard MBSS sampling protocols (Kazyak 2001, Stranko et al. 2019). Each sample consisted of a one-time water chemistry grab collected during the Spring Index period between March 1 and April 30. Samples were collected in deep flowing water, when possible, and upstream of any disturbance from other sampling. Bottles used were leached in deionized water prior to sample collection. Any syringes used were new with packaging unopened. Water samples were kept on ice and shipped within 48 hours of collection to the UMCES Appalachian Lab in Frostburg, Maryland. All samples were tested for specific conductivity, acid neutralizing capacity (ANC), pH, dissolved organic carbon (DOC), sulfate, and nitrate-N. R2, S2, R4/2, and S4 samples were additionally tested for chloride, total nitrogen (TN), ammonium-N, total phosphorus (TP), and orthophosphate-P (Table 6). Since nitrite-N averages were close to laboratory detection limits, nitrite-N was not analyzed in this study.

The percentages of sites from each round that exceeded select water chemistry thresholds were also compared. The threshold used for Acid Neutralizing Capacity (ANC) was adopted from Southerland et al. (2007), in which values less than 50 µeq/L are considered to demonstrate chronic (highly sensitive to acidification) exposures to aquatic organisms. pH thresholds were derived from COMAR (2014) indicating biological degradation at levels below 6.5 and above 8.5. The remaining thresholds were adopted from Morgan et al. (2006; Table 5) in which critical values were derived from significant quantile (50th) regression equations based on the 3.0 Benthic IBI score delineating sites deemed as Poor (1.0 - 2.99) and Fair (3.0 - 3.99). Water chemistry measurements greater than these values indicate potential detrimental effects on biological communities (Morgan et al. 2006).

Table 5. Water chemistry parameters investigated, associated thresholds used, and threshold sources. A (—) indicates no threshold was used. ANC = Acid Neutralizing Capacity, DOC = Dissolved Organic Carbon, TN = Total Nitrogen, TP = Total Phosphorus, PO4 = Orthophosphate-P.

	Water Chemistry Thresholds	
Parameter	**Threshold**	**Threshold Source**
Conductivity	> 247 µS/cm	Morgan et al. (2006)
ANC	< 50 µeq/L	Southerland et al. (2007)
pH	< 6.5	COMAR (2014)
pH	>8.5	COMAR (2014)
Sulfate	—	—
DOC	—	—
Nitrate-N	> 0.86 mg/L	Morgan et al. (2006)
Chloride	> 50 mg/L	Morgan et al. (2006)
TN	> 1.3 mg/L	Morgan et al. (2006)
Ammonium-N	> 0.18 mg/L	Morgan et al. (2006)
TP	> 0.043 mg/L	Morgan et al. (2006)
PO4	> 0.052 mg/L	Morgan et al. (2006)

Results

Conductivity, chloride, ANC, pH, and orthophosphate were all significantly higher ($p < 0.001$ for all comparisons), and sulfate was significantly lower in more recent sampling from random sites in both Rounds and at reference sites ($p < 0.001$ for all comparisons; Table 6; Appendix G). In addition, ammonium-N and TP were significantly lower during R4/2 random sampling compared with R2, but were not significantly different at the reference sites. TN was significantly lower at reference sites during the more recent sampling, but no change was evident at the random sites (S4; Table 6). There was no significant difference in nitrate-N or DOC among any of the three temporal periods.

Conductivity, chloride, ANC, pH, and orthophosphate-P were higher in large percentages of the more recent sampling during all Rounds (Figure 15). In contrast, sulfate was lower at large percentages of samples. The percentages of samples with higher or lower values during recent sampling for other nutrients and DOC varied across Rounds but tended to be around 50% (Figure 15).

Although orthophosphate-P levels tended to be low (R2 mean = 0.01 mg/L; S2 mean < 0.01 mg/L), there appeared to be consistently higher concentrations at both random and reference sites over the 14-year period in R4/2 and S4. Moreover, the percentages of sites with higher orthophosphate-P concentrations during recent years were greatest among all parameters investigated in the S2 vs. S4 comparison (Figure 15).

Table 6. Results of Wilcoxon signed rank tests comparing median chemistry values at sites with randomly selected locations between 20-year (R1 vs. R4/1) and 14-year (R2 vs. R4/2) time intervals, as well as reference (S2 vs. S4) sites over the 14-year interval. P-values < 0.05 are highlighted in red to mark significantly lower medians and in blue to mark significantly higher medians during the more recent sampling. A (—) indicates a metric was not sufficiently sampled to enable a comparison in the given time interval. Further descriptive statistics and graphics for each parameter are presented in Appendix G.

Water Chemistry Parameter	**Random**	**Random**	**Reference**
	R1 vs. R4/1	**R2 vs. R4/2**	**S2 vs. S4**
Conductivity	$p < 0.001$	$p < 0.001$	$p < 0.001$
ANC	$p < 0.001$	$p < 0.001$	$p < 0.001$
pH	$p < 0.001$	$p < 0.001$	$p < 0.001$
DOC	$p = 0.026$	$p = 0.180$	$p = 0.030$
Sulfate	$p < 0.001$	$p < 0.001$	$p < 0.001$
Nitrate-N	$p = 0.749$	$p = 0.732$	$p = 0.412$
Chloride	—	$p < 0.001$	$p < 0.001$
TN	—	$p = 0.483$	$p = 0.007$
Ammonium-N	—	$p < 0.001$	$p = 0.380$
TP	—	$p < 0.001$	$p = 0.970$
Orthophosphate-P	—	$p < 0.001$	$p < 0.001$

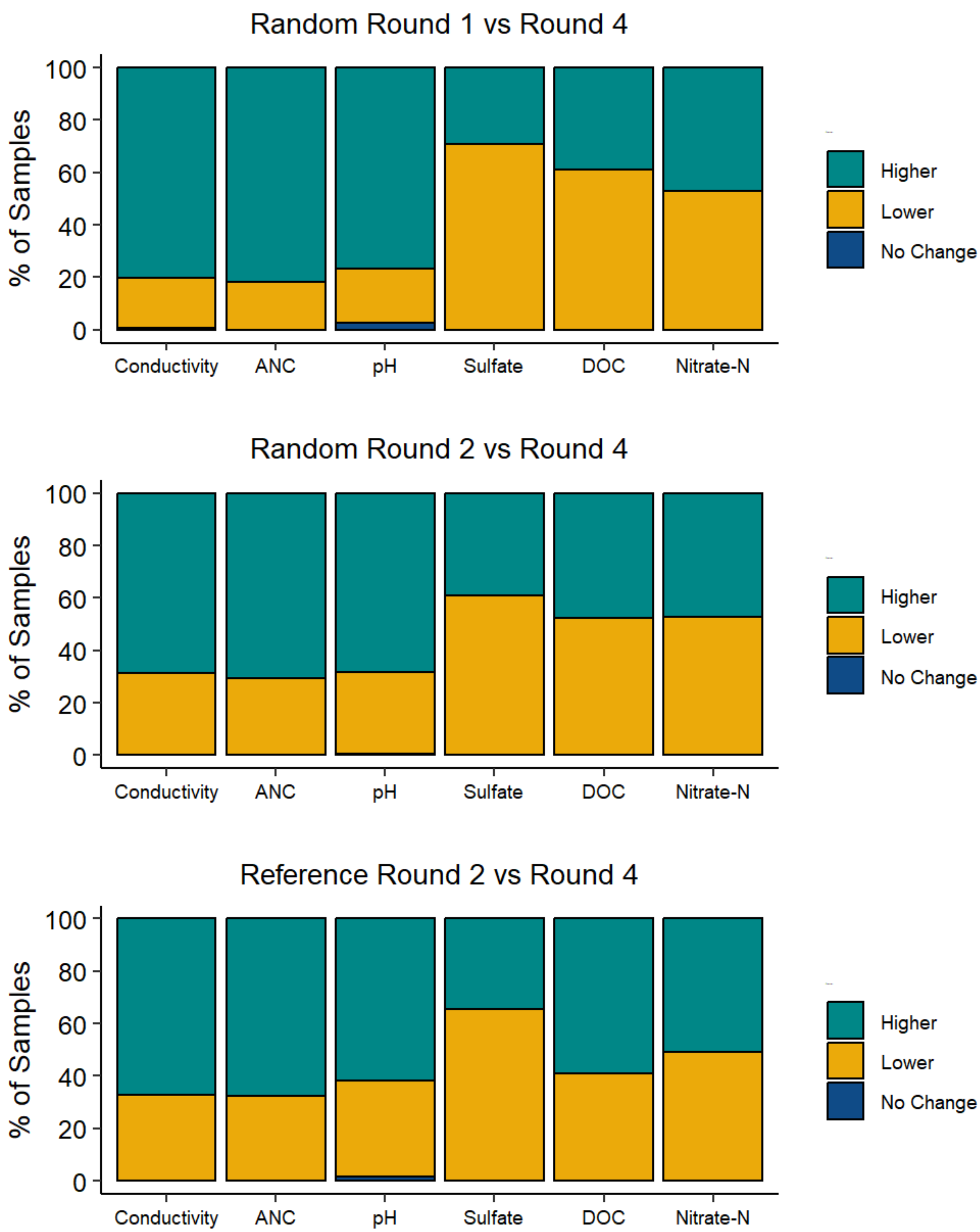
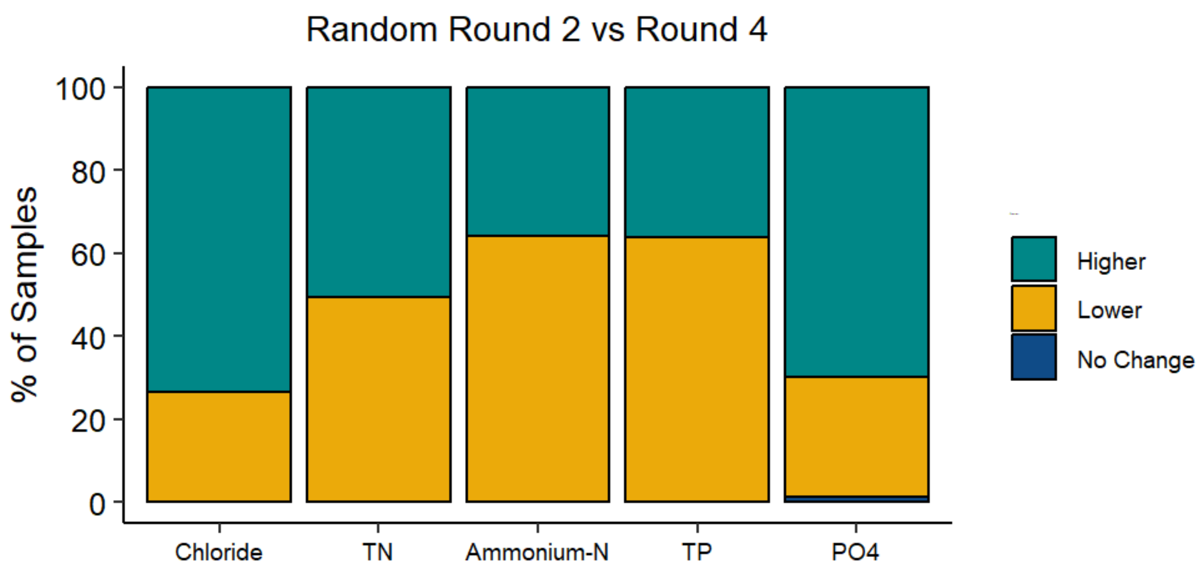


Figure 15. Percentages of samples with lower, higher, or no change in chemistry parameters in the later sampling period among the three temporal comparisons. ANC = Acid Neutralizing Capacity, DOC = Dissolved Organic Carbon.



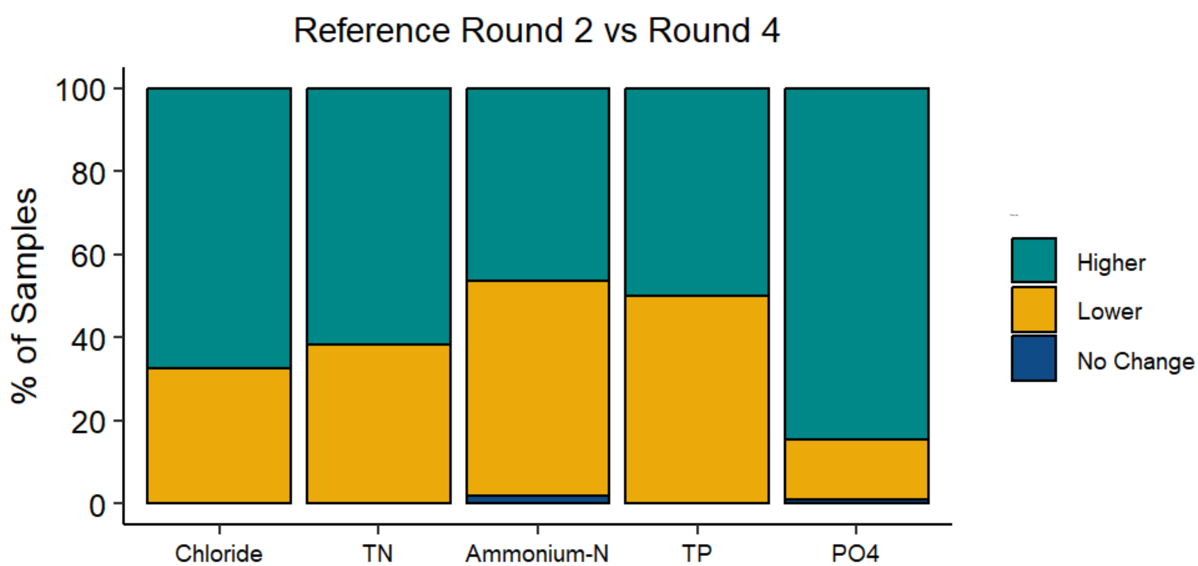


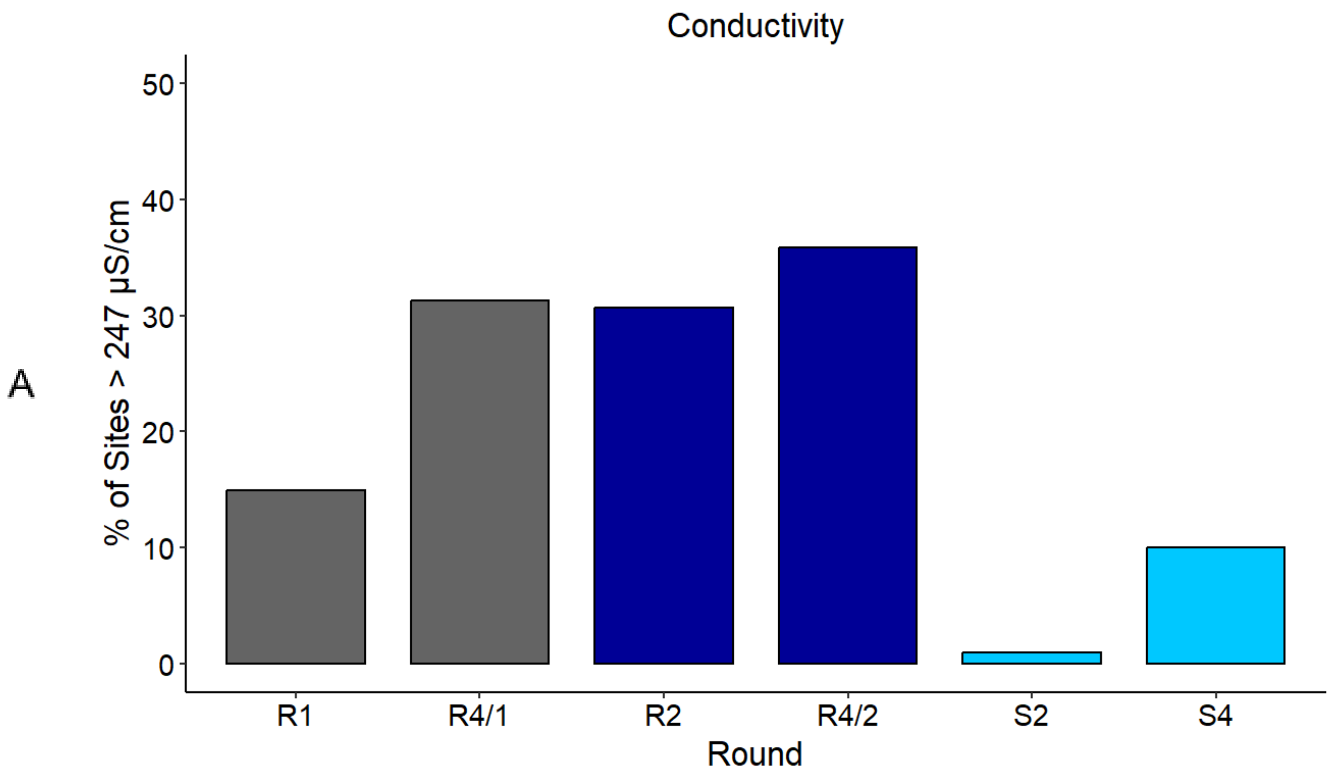
Figure 15 (continued). Percentages of samples with lower, higher, or no change in chemistry parameters in the later sampling period among Round Two vs. Round Four temporal comparisons. TN = Total Nitrogen, TP = Total Phosphorus, PO4 = Orthophosphate-P.

Water Chemistry Thresholds

Consistent with other results, more sites exceeded conductivity and chloride thresholds during more recent sampling compared with original sampling (Figure 16A, Figure 17A). Although only one reference site (0.91%) exceeded thresholds for conductivity and none exceeded chloride thresholds during S2, 10% exceeded conductivity thresholds and 7% exceeded chloride thresholds during S4 (Figure 16A, Figure 17A). In contrast, fewer sites exceeded acidic pH thresholds (< 6.5) during more recent sampling for all three temporal comparisons (Figure 16D). Less than 3% of random sites and no reference site exceeded alkaline pH thresholds (> 8.5) in any sampling period. Consistently fewer sites showed signs of being acid-sensitive based on ANC results from more recent sampling for all three temporal periods (Figure 16B).

Although orthophosphate-P levels were significantly higher in recent years, the percentage of sites that exceeded orthophosphate-P thresholds was less than 4% at random sites (and was slightly lower during R4/2 compared with R2) and less than 1% at reference sites (Figure 17E). In addition, there were fewer sites exceeding TP thresholds in the more recent sampling period compared with original sampling in both the R2 vs. R4/2 and S2 vs. S4 comparison (Figure 17D). While there was no significant difference in nitrate-N or TN between random sampling Rounds, the percentage of sites exceeding nitrate-N and TN thresholds was greater than 50% in both random sampling Rounds. In contrast, fewer sites exceeded ammonium-N thresholds in the later random sampling Round compared with original sampling (Figure 17C).

Percentages of sites exceeding water chemistry thresholds were lower in some comparisons in recent years, while higher in others. Reference sites had lower percentages of samples exceeding nutrient thresholds compared with random sites, though there were greater percentages of samples exceeding TN and ammonium-N thresholds during recent sampling compared with previous sampling.



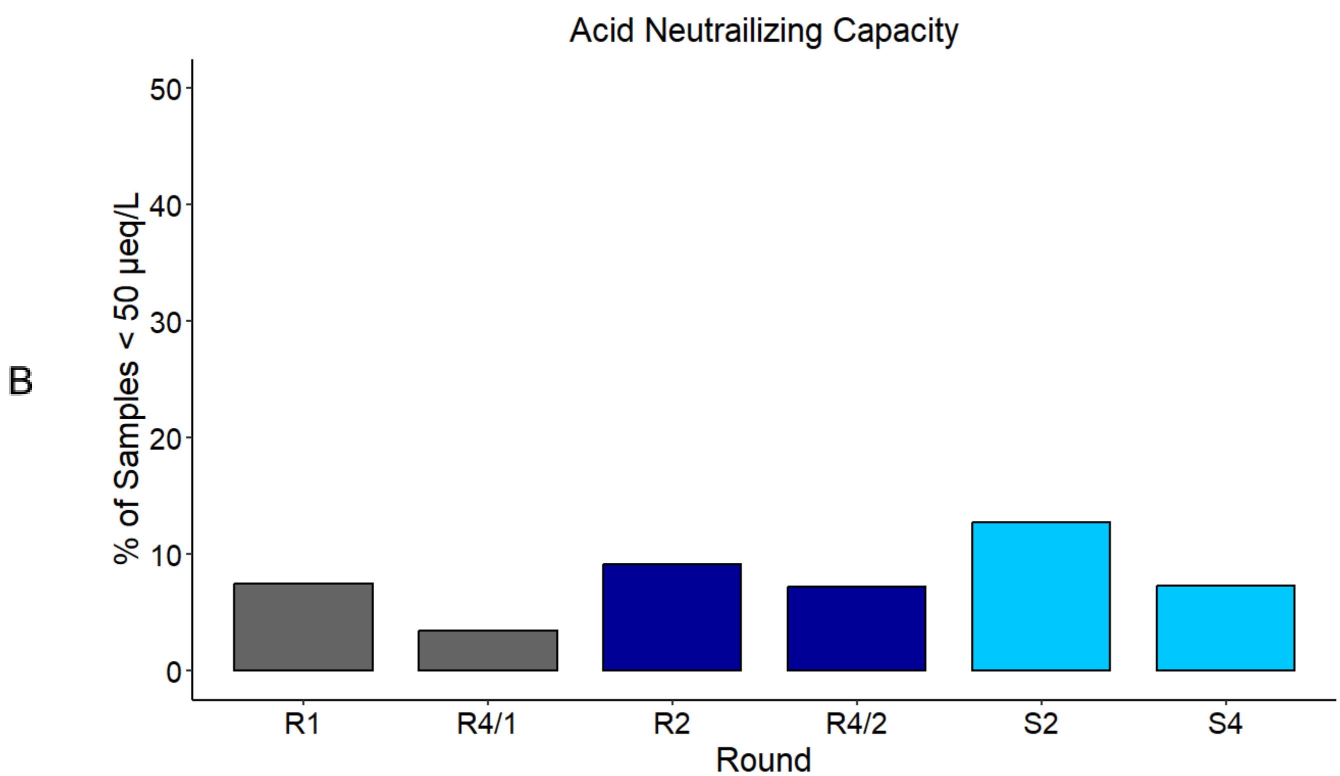


Figure 16. Percentages of samples collected during each MBSS sampling round exceeding thresholds for select water chemistry parameters (Panels A = Conductivity, B = Acid Neutralizing Capacity, C = pH > 8.5, D = pH < 6.5, and E = Nitrate-N) among all temporal comparisons.

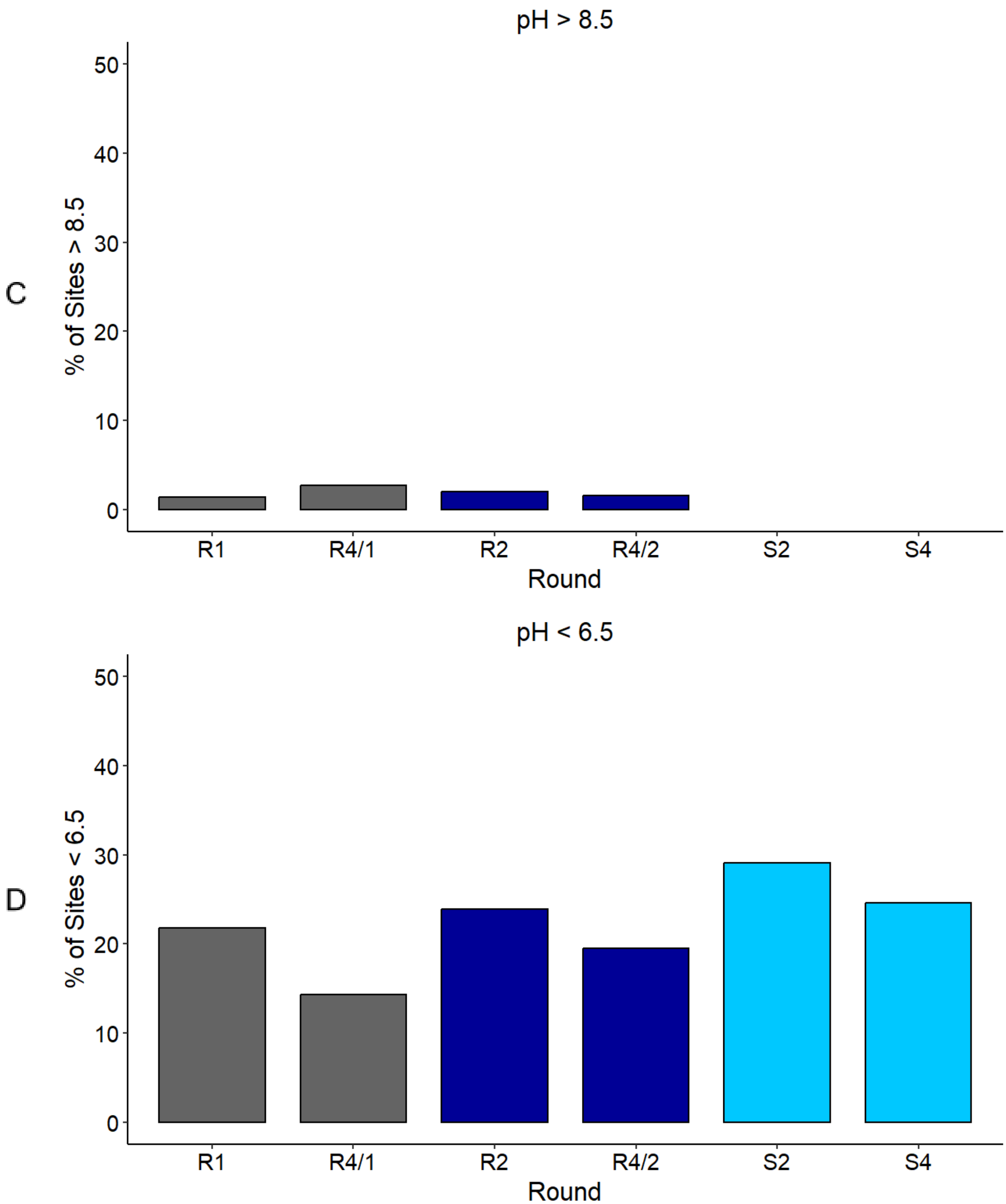


Figure 16 (Continued). Percentages of samples collected during each MBSS sampling Round exceeding thresholds for select water chemistry parameters (Panels A = Conductivity, B = Acid Neutralizing Capacity, C = pH > 8.5, D = pH < 6.5, and E = Nitrate-N) among all temporal comparisons.

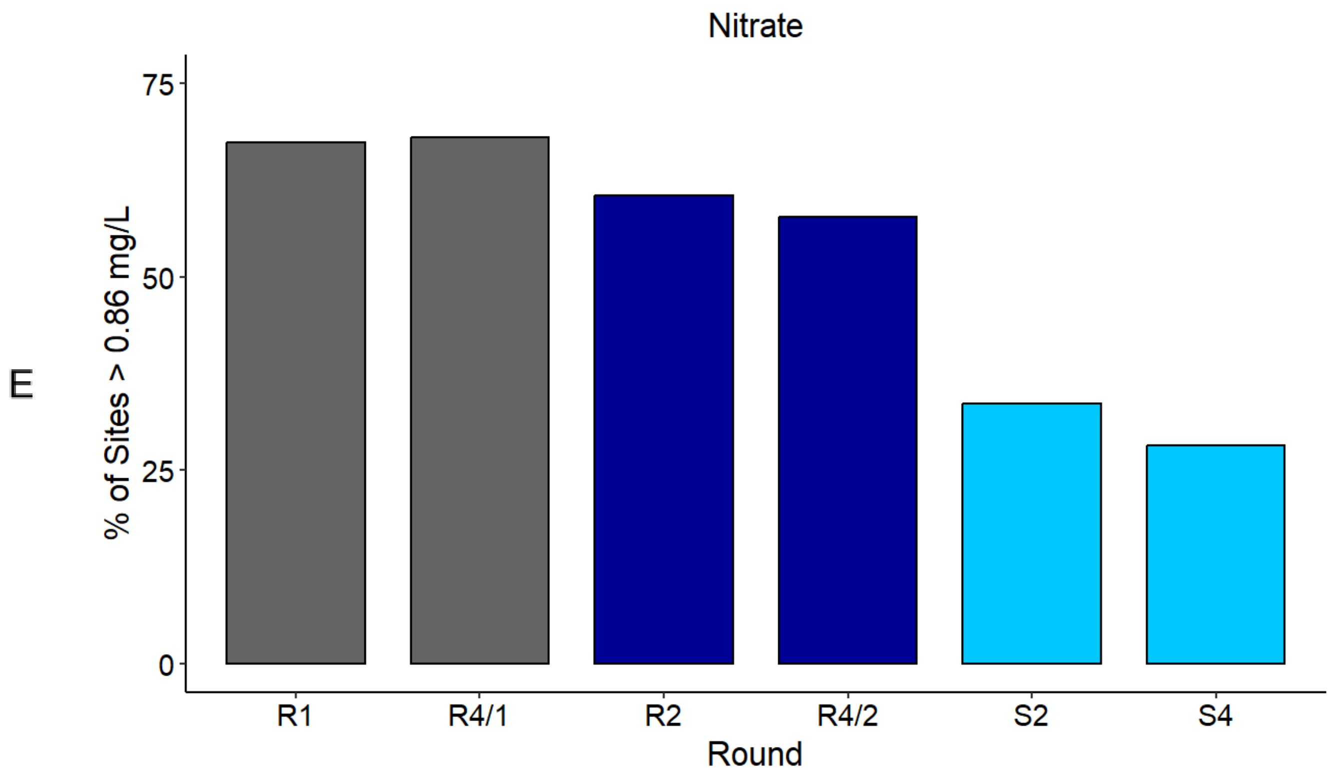


Figure 16 (Continued). Percentages of samples collected during each MBSS sampling round exceeding thresholds for select water chemistry parameters (Panels A = Conductivity, B = Acid Neutralizing Capacity, C = pH > 8.5, D = pH < 6.5, and E = Nitrate-N) among all temporal comparisons.

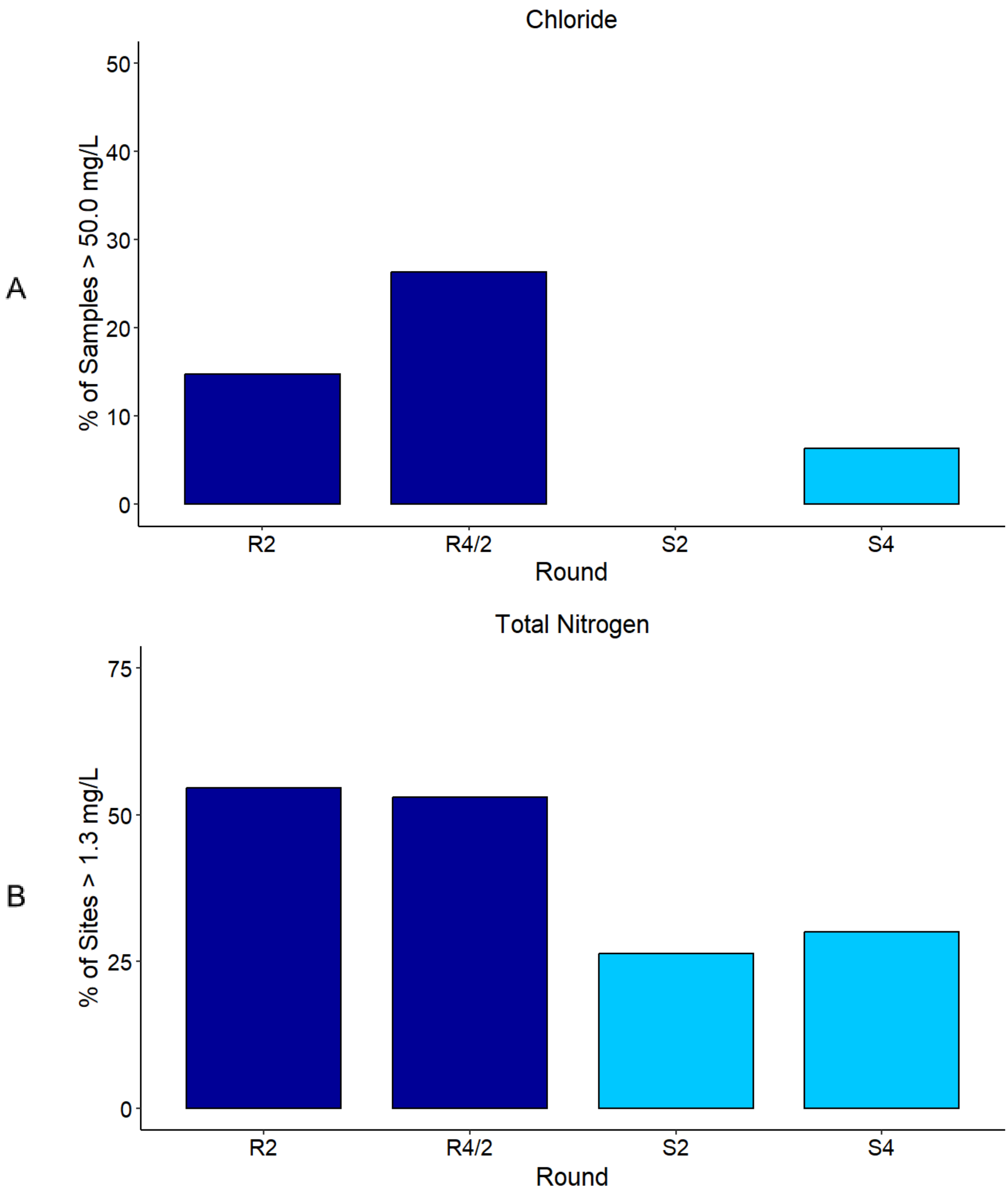
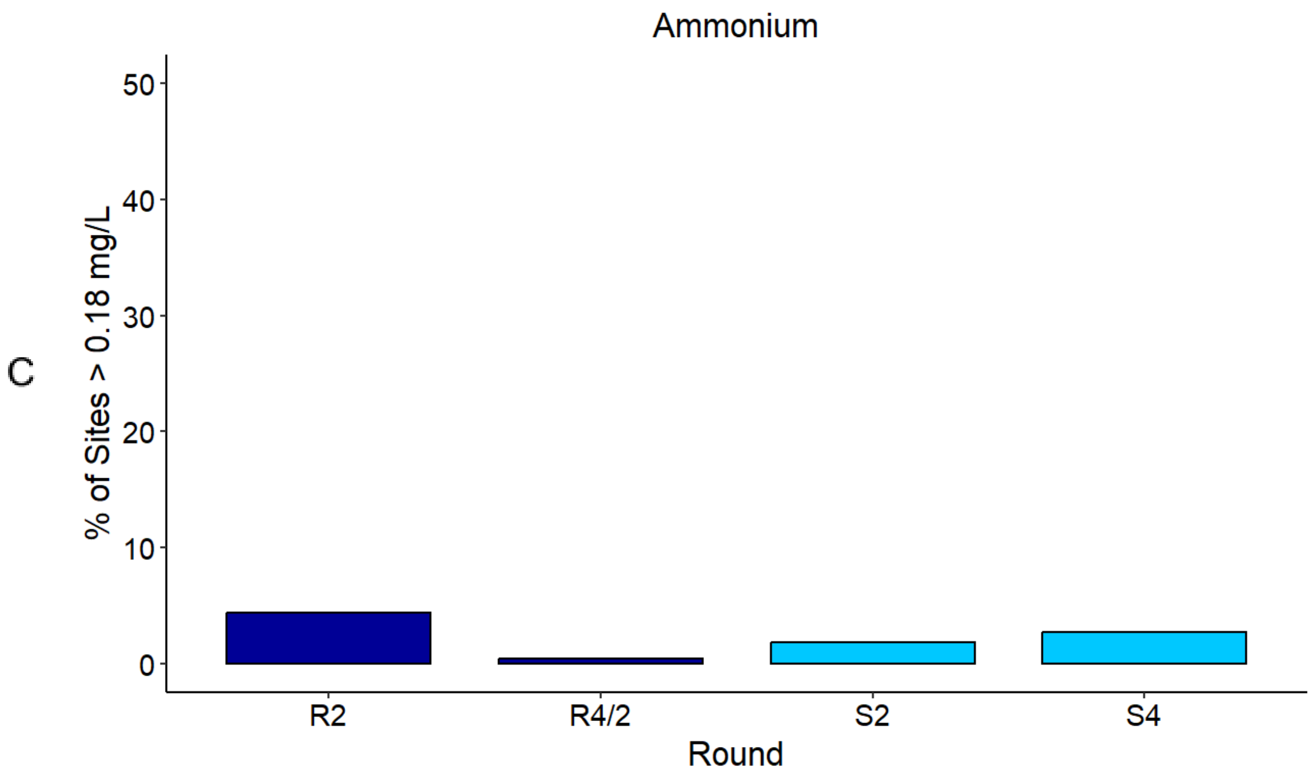


Figure 17. Percentages of samples collected during R2, R4/2, S2, and S4 exceeding thresholds for select water chemistry parameters. Panels A = Chloride, B = Total Nitrogen, C = Ammonium-N, D = Total Phosphorus, and E = Orthophosphate-P.



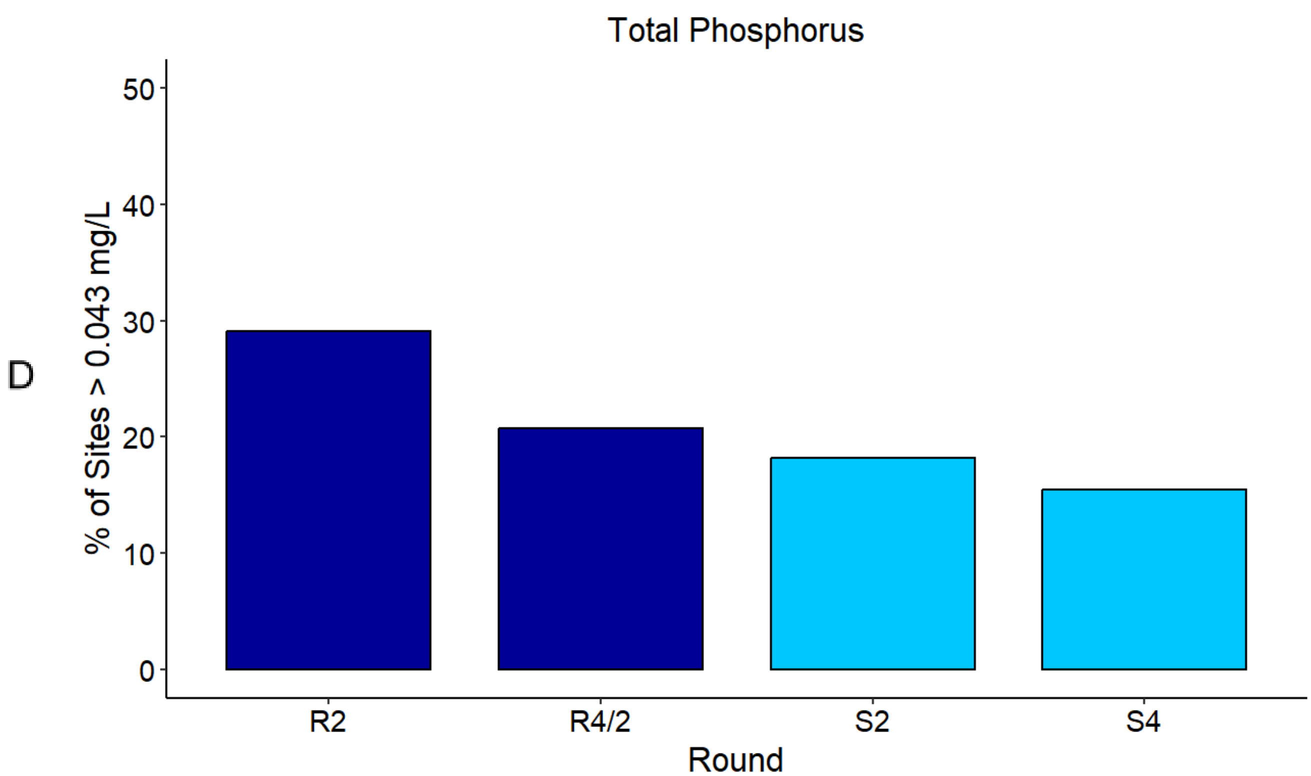


Figure 17 (Continued). Percentages of samples collected during Rounds 2, 4/2, S2, and S4 exceeding thresholds for select chemistry variables. Panels A = Chloride, B = Total Nitrogen, C = Ammonium-N, D = Total Phosphorus, and E = Orthophosphate-P.

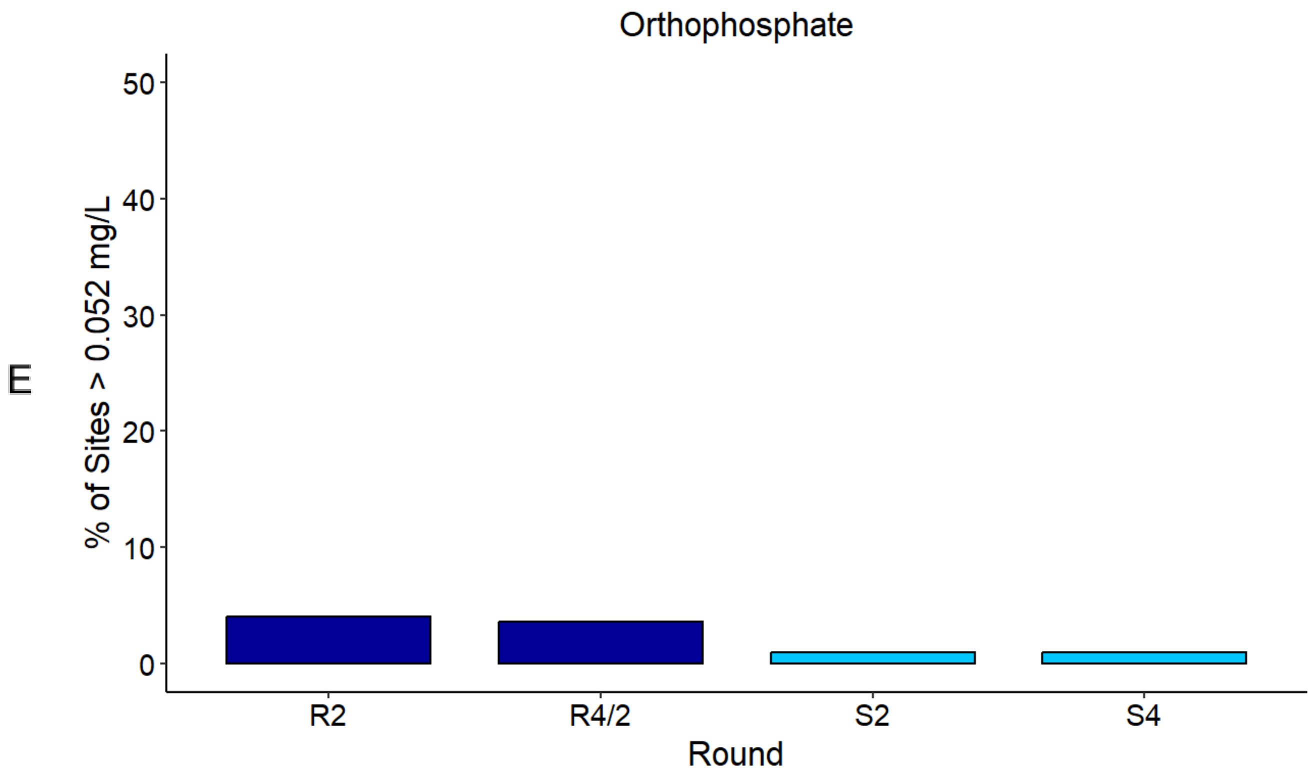


Figure 17 (Continued). Percentages of samples collected during Rounds 2, 4/2, S2, and S4 exceeding thresholds for select water chemistry parameters. Panels A = Chloride, B = Total Nitrogen, C = Ammonium-N, D = Total Phosphorus, and E = Orthophosphate-P.

Variables Associated with Acid Sensitivity

Streams with ANC less than 200 µeq/L can be considered acid-sensitive (Schindler 1988). Other water chemistry parameters may elucidate potential reasons for acid sensitivity (Roth et al. 1998). DOC can indicate natural (i.e, organic) sources of acidity, while sulfate can be indicative of atmospheric deposition or acid mine drainage. Depending on the watershed and land uses, nitrate-N can be indicative of atmospheric deposition, fertilizer, or septic/sewerage sources. We determined the percentages of sites from each Round with ANC less than 200 µeq/L and either DOC greater than 8.0 mg/L, sulfate greater than 50 mg/L, or nitrate greater than 5.0 mg/L (Figure 18). Low percentages (less than 30%) of samples showed any indication of low ANC and an indication of influence from these other parameters. DOC greater than 8.0 mg/L was the most common indication concomitant with ANC less than 200 µeq/L. The largest percentages of samples with DOC greater than 8.0 mg/L were from reference sites. No reference sites had sulfate greater than 50 mg/L or nitrate greater than 5.0 mg/L. Small percentages of samples (less than 10%) from random sites had sulfates greater than 50 mg/L or nitrates greater than 5.0 mg/L along with ANC < 200 µeq/L. Only western Maryland samples had sulfate greater than 50 mg/L.

No consistent pattern was evident that higher or lower percentages of samples with ANC less than 200 μeq/L had substantial sulfate, nitrate, or DOC concentrations above thresholds during original or recent sampling rounds.

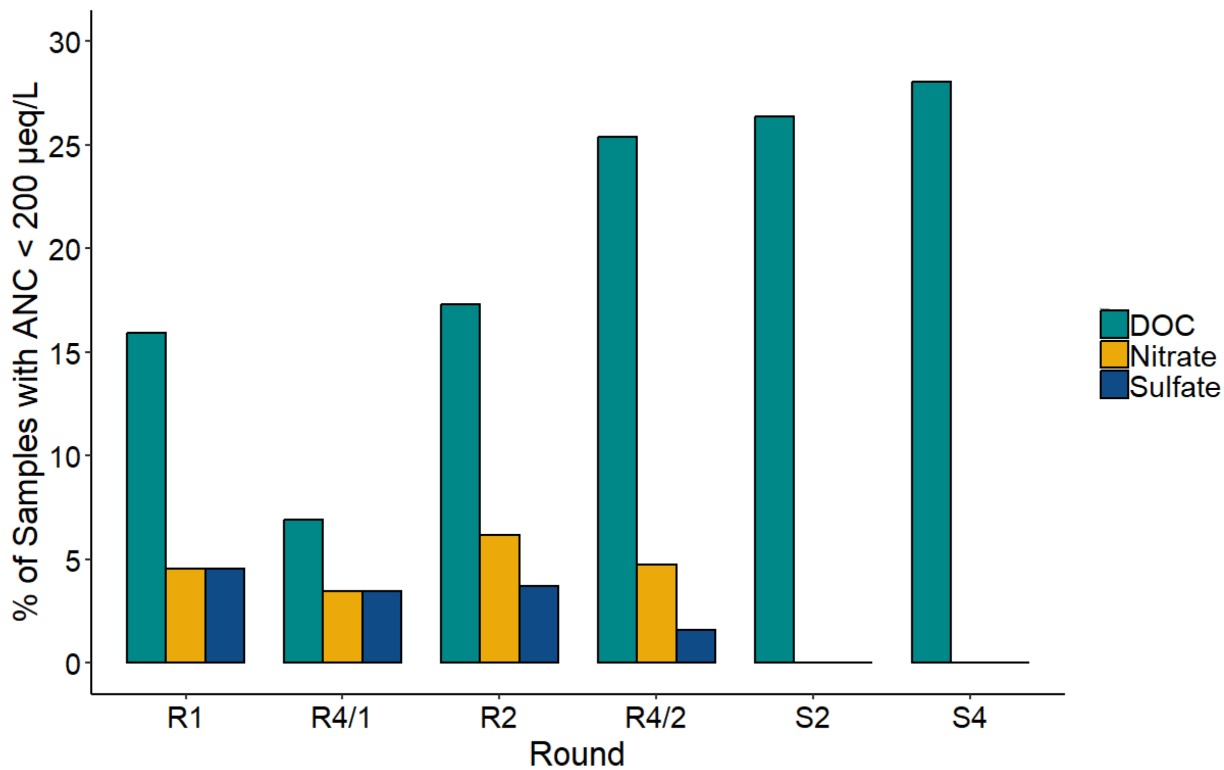


Figure 18. Percent of samples with Acid Neutralizing Capacity (ANC) less than 200 μeq/L, indicating potentially acid sensitive streams, that also exceeded 8.0 mg/L Dissolved Organic Carbon (DOC), indicating potential sources of natural acidity; 50 mg/L Sulfate indicating potential mine drainage; and/or 5.0 mg/L Nitrate, indicating potential atmospheric deposition and/or fertilizer inputs to the streams sampled.

Discussion

Maryland streams appear to have become saltier and less acidic with higher conductivity, chloride, ANC, pH, and orthophosphate in recent years compared to past conditions at randomly selected stream sites and reference sites. Levels of all these parameters have been linked to a variety of anthropogenic activities including road salt, mining, agriculture, and concrete weathering (Kaushal et al. 2013, Kaushal et al. 2017, Kaushal et al. 2018). Higher pH and ANC, indicating reduced acidity, may be associated with some of these same factors, but could also separately or simultaneously be linked to recent amendments to the Clean Air Act in 1990 that required reductions in annual sulfur dioxide and nitrogen oxide emissions (Clean Air Act 2011).

Atmospheric deposition is a major contributor to sulfate in streams (Southerland et al. 2005). Based on indications from sulfate patterns (but without definitive evidence), influence from atmospheric deposition may have potentially declined compared to 14 or 20 years ago due to Clean Air Act amendments, assuming sulfur dioxide emissions have been reduced (Kline et al. 2007; Eshelman et al. 2008). Some of the highest sulfate concentrations, as well as some of the largest decreases in sulfate concentrations, were from western Maryland, which hosts the state's coal mining operations. Therefore, it is possible that amelioration of acid mine drainage also may have contributed to the reductions in sulfate observed. However, certain streams in the Coastal Plain region – where no coal mining occurs – also had lower concentrations, supporting the notion that sulfate reductions may be at least partially attributable to factors other than mine drainage.

While there is an extensive effort to reduce nutrients in Maryland's streams and rivers, there were not clear and consistent results indicating lower nitrogen-related nutrient concentrations in recent samples compared with original samples collected 20 or 14 years prior. These nutrients were consistently lower in reference site samples compared to random site samples, regardless of sampling year, supporting the notion that higher-quality streams with more forested catchments typically have lower nutrients. Others working in Maryland have reported reductions in nitrate-N concentrations in both predominantly-forested and mixed land use watersheds attributable to the 1990 Clean Air Amendments (Eshelman et al. 2013; Eshelman et al. 2016), so it is somewhat surprising that those trends were not observed here.

Orthophosphate-P levels tended to be low and sites infrequently exceeded the 0.052 mg/L threshold (less than 10% of samples among all comparisons). However, higher orthophosphate-P levels and a greater proportion of sites exceeding orthophosphate-P thresholds may indicate increasing inputs from the landscape (Perillo et al. 2021) or from stream bank erosion (Fox et al. 2016), especially during high flow events (Frazar et al. 2019). Additional and site-specific investigation into orthophosphate-P changes may help better explain the patterns observed in this analysis.

ANC and pH were higher in recent years and the percentages of sites with ANC and pH levels beyond thresholds important to stream biota (MDE 2014) were lower in recent years. Such improvements may have positively affected biota in some streams. Increases to pH, however, could have negative influences in naturally acidic streams where endemic (and often rare) biota occur. Lower pH, ANC, and higher DOC levels in certain Coastal Plain reference streams may be indicative of such distinctive stream conditions.

While increases in conductivity and chloride appeared to be widespread, this pattern was most obvious in the more populated areas of the state. Furthermore, although reference streams are less impacted by most anthropogenic influences, some of these sites exceeded important conductivity and chloride thresholds in recent years when none had in previous years. This finding may suggest an impending future threat to the biodiversity and biological integrity of such high-quality streams.

These chemistry results indicate certain conditions may have become more beneficial to aquatic life, such as reduced acidity, as well as certain conditions that could be detrimental, such as increased conductivity and chloride. A more detailed understanding of these and other chemical conditions along with associated biological responses could likely help inform successful stream water quality and biological protection and restoration strategies.

Temperature

Methods

No temperature loggers were deployed during Round One sampling, therefore no temperature comparisons over the 20-year interval were feasible. After accounting for lost loggers and those that failed QC procedures outlined in Maryland Department of Natural Resources (2016), 92 comparisons of temperature logger data at random sites and 58 comparisons at reference sites were available over the same 14-year period from Round Two to Round Four. The term "sample" in this section refers to the full temperature dataset collected at a site in a given year.

Temperature loggers recorded water temperature every 20 minutes from June 1 through August 31 following methods described in field sampling manuals (Kazyak 2001, Stranko et al. 2019; Quality Assurance Document for Temperature Monitoring). The 20-minute water temperature readings were summarized by calculating the average daily mean temperature, maximum temperature, minimum temperature, the percent of readings greater than 20°C, and the percent of readings greater than 24°C. Analysis of the percent of temperature readings metrics was based on thresholds used by MDE for Use III nontidal cold water streams that could potentially support brook trout populations; exceedance of these thresholds might indicate a stream does not provide suitable conditions for this species (Heft 2006, MDE 2023). Given the strong influence of geography on water temperature (Caissie 2006), comparisons were conducted by ecoregions (i.e., Coastal Plain, Eastern Piedmont, Highlands; Table 7) in addition to statewide comparisons.

Table 7. Number of random and reference site sample comparisons with temperature logger data over 14-year time periods by region.

	Random	Reference
Total	92	58
Highlands	24	15
Eastern Piedmont	27	13
Coastal Plain	41	30

Daily mean temperature data from a subset of 145 of the more recent (R4/2 and S4) samples were investigated to represent the relationships between air and water temperatures using linear regression models. Air temperature data was not collected for five of the more recent samples. R2 and S2 data were not included as air temperature was not recorded during this time period. The regression line slope and the degree of correlation described in these models can indicate the relative influence of air temperature on stream water temperatures (Caissie 2006, O'Driscoll and DeWalle 2006, Hilderbrand et al. 2014) and thus help interpret this potential influence on stream temperature differences over time. The slope indicates the rate of change in water temperature for a given change in air temperature, and is therefore a measure of a stream's thermal sensitivity (Kelleher et al. 2012). The coefficient of determination (R^2) indicates the amount of variance in water temperature explained by air temperature. While ecoregional patterns differed for these two outcomes, samples with higher R^2 values tended to have steeper slopes. It is important to note that MBSS air temperature data could contain some inconsistencies in air logger locations among sites, which could be a potentially confounding factor in interpreting these data.

Results

Warmer stream temperatures were observed among random and reference samples in Round Four compared to Round Two (Table 8). Based on examinations of statewide data, both random and reference samples experienced significantly higher average daily temperatures, minimum temperatures, and percentages of temperature readings above 20°C. From a statewide perspective, neither maximum temperatures nor percent of readings above 24°C appeared to significantly change in random or reference samples.

Higher temperatures during recent years at random Piedmont streams, and at random and reference Coastal Plain streams appeared to drive the statewide pattern of higher water temperatures. Temperature metrics in the Piedmont ecoregion indicated divergent results, with warming across four of five metrics in random samples and no statistically significant changes observed for reference samples. Notably, random Piedmont samples were the only ecoregion-level group that appeared to experience higher maximum temperatures in more recent sampling. Evidence of warming was observed in the same three temperature metrics in both random and reference Coastal Plain samples: average daily mean, minimum, and percent of readings above 20°C. Among the Highlands samples, temperature metrics were unchanged for both groups with the notable exception of significantly lower maximum temperatures in Highlands random samples in R4/2 ($p = 0.004$). Temperature readings did not exceed 24°C in Highlands reference samples in either Round.

Table 8. Results of statewide and ecoregion-level Wilcoxon signed rank tests on five temperature metrics at sites with randomly selected locations (R2 and R4/2) and reference (S2 and S4) sites over a 14-year interval. P-values < 0.01 are highlighted in blue to indicate significantly higher values in the more recent sampling, and highlighted in red to indicate significantly lower values. NA denotes no readings exceeding 24°C. * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction. Further descriptive statistics and graphics for each metric are presented in Appendix H.

Temperature Metric	Statewide		Highlands		Piedmont		Coastal Plain	
	R2 vs. R4/2	S2 vs. S4	R2 vs. R4/2	S2 vs. S4	R2 vs. R4/2	S2 vs. S4	R2 vs. R4/2	S2 vs. S4
Average Daily	p < 0.001*	p < 0.001*	p = 0.114	p = 0.229	p < 0.001	p = 0.027	p = 0.002	p < 0.001
Maximum	p = 0.529*	p = 0.626*	p = 0.004	p = 0.015	p = 0.004	p = 0.588	p = 0.404	p = 0.058
Minimum	p < 0.001*	p < 0.001*	p = 0.214*	p = 0.035	p = 0.002	p = 0.094	p < 0.001*	p = 0.001
Percent >20°C	p < 0.001*	p < 0.001*	p = 0.751*	p = 0.541*	p < 0.001	p = 0.376	p = 0.001	p < 0.001
Percent >24°C	p = 0.023*	p = 0.019*	p = 0.490*	NA	p = 0.032*	p = 0.584*	p = 0.346*	p = 0.013*

Among random samples, the Highlands ecoregion had the lowest percentages of samples that exhibited warmer temperatures across all five metrics in R4/2 compared to R2. These percentages were lower by only slim margins, however, for average daily mean and minimum temperatures (Figure 19). A temperature metric in a given sample was considered higher or lower if it had changed by at least ±0.01°C (average daily temperature, maximum temperature, and minimum temperature), or ±0.01 percentage point (percentage of temperature readings above 20°C and percentage of temperature readings above 24°C) in R4/2. Random Highlands sites also had notably larger percentages of samples with lower metrics in R4/2 compared to both other ecoregions. About 75% of random Highlands samples experienced lower maximum temperatures, and 45.8% of samples showed lower percentages of readings above 20°C in R4/2. In addition, nearly 71% of random Highlands samples had either lower or unchanged percentages of readings above 24°C in more recent sampling. This combined percentage of lower and unchanged samples was greater than the combined percentages for Coastal Plain (51.2%) and Piedmont samples (59.3%). The Piedmont ecoregion showed the greatest proportions of warmer random samples in two temperature metrics. The vast majority of Piedmont random samples experienced higher average daily mean temperatures (96.3% of samples) and percentages of readings above 20°C (88.9% of samples) in R4/2.

Among reference samples, differences among the ecoregions were most pronounced for the maximum temperature and the percentages of readings metrics (Figure 20). In the Highlands ecoregion, 80% of reference samples had lower maximum temperatures. In contrast, slight majorities of reference Coastal Plain (56.7%) and Piedmont (61.5%) samples experienced higher maximum temperatures in S4 compared to S2. Reference Highlands sites also experienced lower percentages of readings above 20°C in 46.7% of samples, and another 33.3% of samples experienced no change. Clear majorities of reference Coastal Plain and Piedmont samples, however, experienced higher percentages of readings above 20°C in S4 (83.3% and 69.2%, respectively). Reference Highlands samples notably did not have temperature readings above 24°C in either Round and therefore experienced no change in this metric. A majority of reference Piedmont samples (69.2%) also did not exceed 24°C in either Round and therefore did not experience significant change. The remaining four reference Piedmont samples were evenly split with two samples that were higher and two that were lower in S4. In contrast, 70% of Coastal Plain reference samples exhibited higher percentages of readings above 24°C, while 26.7% of samples were lower and 3.3% of samples did not change.

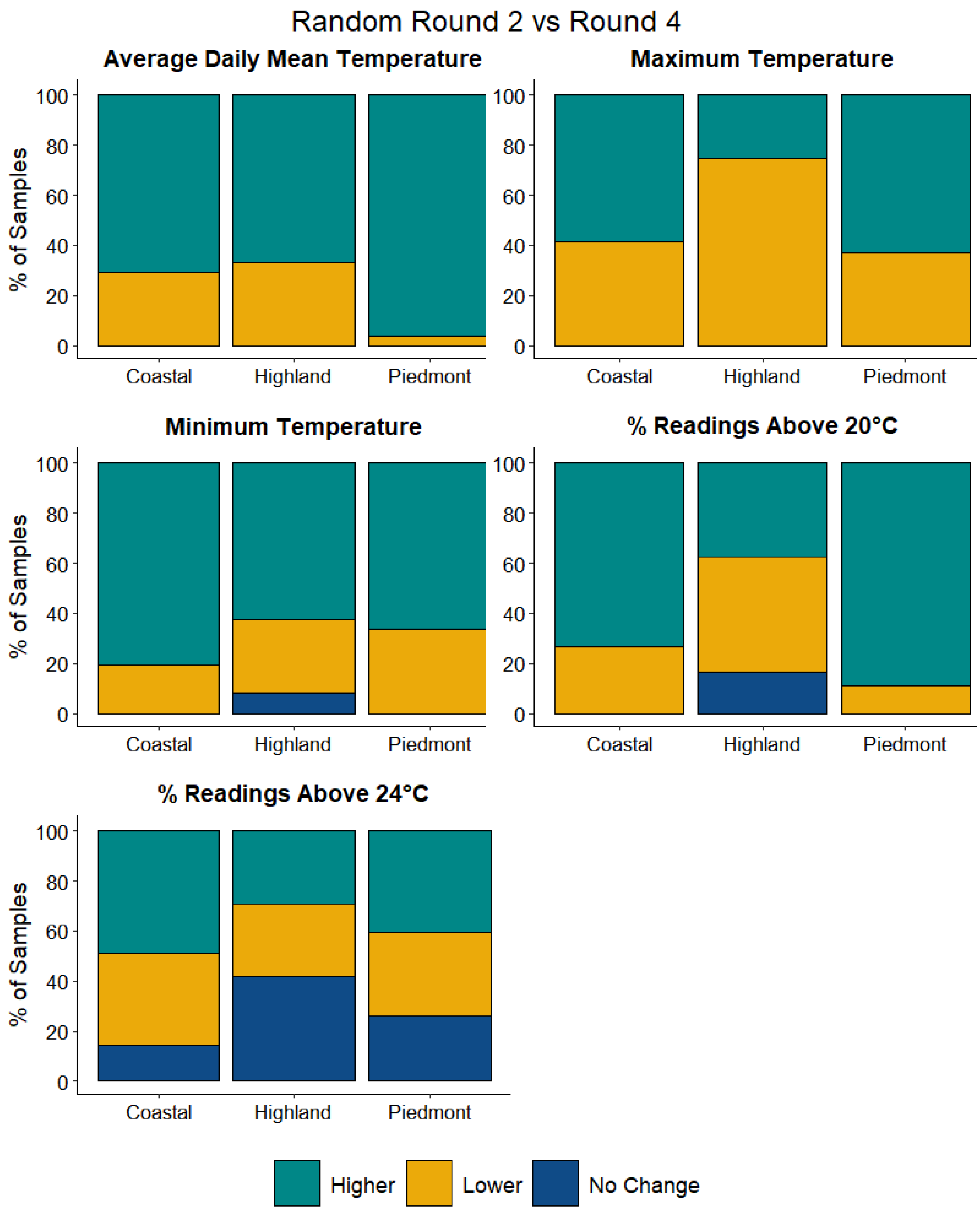


Figure 19. Percent of random samples, grouped by ecoregion, with higher, lower, or no change in temperature metrics in R4/2 compared to R2. A sample was considered higher or lower if the average daily mean, maximum, or minimum temperature changed by at least ± 0.01°C, and if the percent of readings above 20°C or 24°C changed by at least ± 0.01 percentage point.

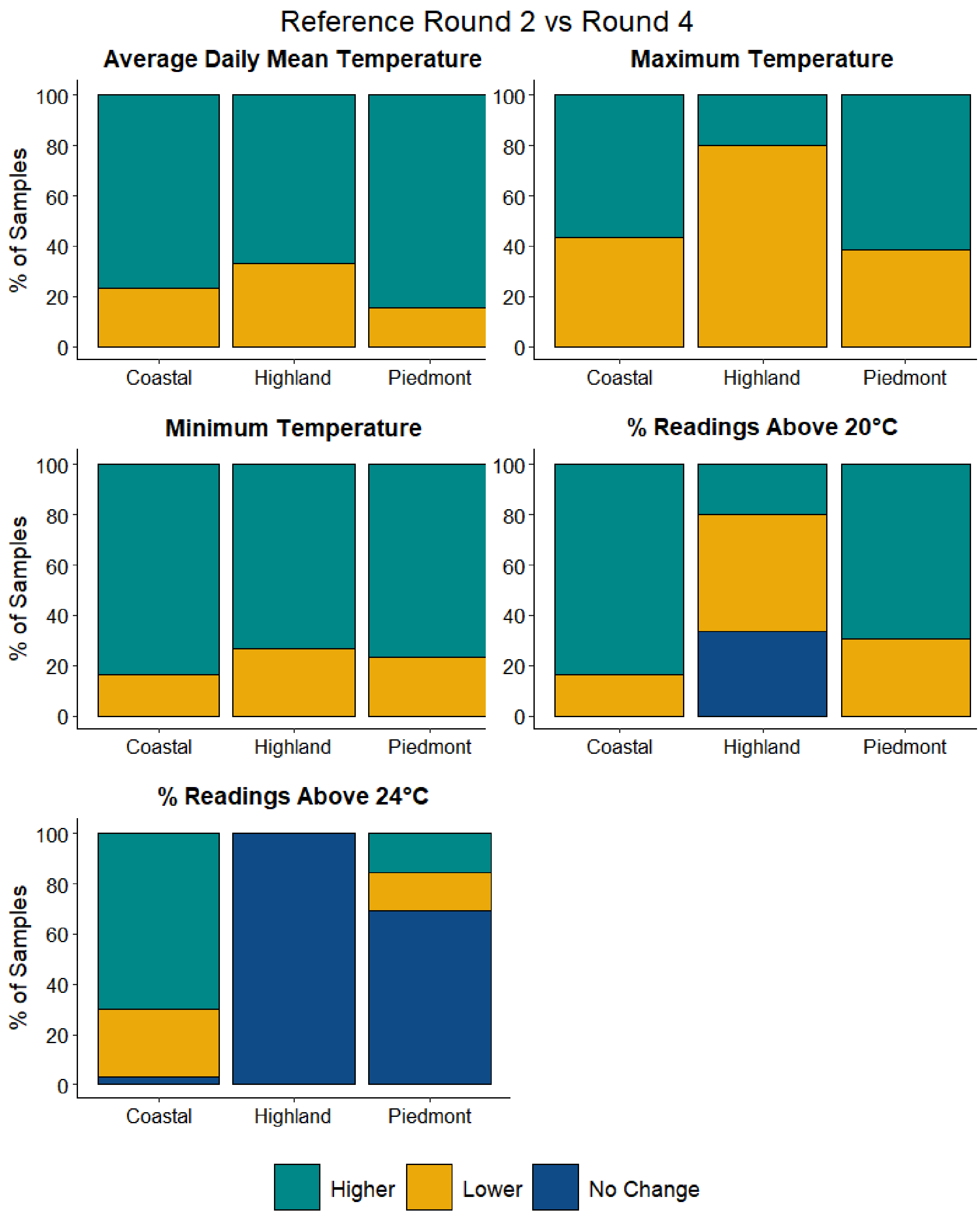


Figure 20. Percent of reference samples, grouped by ecoregion, with higher, lower, or no change in temperature metrics in R4/2 compared to R2. A sample was considered higher or lower if the average daily mean, maximum, or minimum temperature changed by at least ± 0.01°C, and if the percent of readings above 20°C or 24°C changed by at least ± 0.01 percentage point.

Air vs. Water Temperature Regression Models

All regression model results were statistically significant ($p < 0.01$). Two regression model outcomes help describe the linear relationship between air and water temperatures: the coefficient of determination (R^2) indicates the amount of variance in water temperature explained by air temperature, and the slope indicates the rate of change in water temperature for a given change in air temperature. Higher slopes indicate greater responses by water temperature to a given change in air temperature. These factors varied across samples, ecoregions, and site types. Samples with higher R^2 values tended to, but did not necessarily, have steeper slopes.

We observed relatively low average slopes for the two reference sample groups that did not exhibit any significant warming in S4 (Figure 21). These results indicate that air temperature might be less influential on water temperature for these samples. Among all the random and reference ecoregion groups, reference Highlands samples had the lowest average slope of 0.46 (Table 9). Reference Piedmont samples were tied (with random Coastal samples) for the second lowest average slope of 0.53 among all ecoregion groups (Table 9). Two ecoregion groups that experienced significant warming in multiple metrics had the highest average slopes, indicating water temperature might be more sensitive to air temperature changes; the average slope was 0.56 for reference Coastal samples, and 0.57 for random Piedmont samples (Table 9). Though they also experienced significant warming, random Coastal samples had a relatively low average slope of 0.53. This slope was slightly lower than the average slope for random Highlands samples (mean slope = 0.54; Table 9), and equal to the mean slope for reference Piedmont samples – two groups that did not exhibit significant warming in more recent years.

Based on R^2 values of the regression models, our results showed that the relationship between daily mean water and air temperatures appeared to be weaker in reference samples compared to random samples in each ecoregion (Table 9). Air temperature showed the lowest level of explanatory power for stream temperature changes in reference Highlands samples (mean R^2 = 0.52; Figure 23). The Piedmont ecoregion showed the strongest relationship among reference samples (mean R^2 = 0.66; Figure 25), followed by reference Coastal Plain samples (mean R^2 = 0.60; Figure 27). Among random samples, the relationship was also weakest in the Highlands ecoregion (mean R^2 = 0.61; Figure 22), moderate in the Coastal ecoregion (mean R^2 = 0.64; Figure 26) and strong in the Piedmont ecoregion (mean R^2 = 0.70; Figure 24).

Table 9. Mean R^2 and slope values from regression models describing the linear relationship between stream water temperature and air temperature.

	Reference		**Random**	
	Mean R^2	**Slope**	**Mean R^2**	**Slope**
Coastal Plain	0.60	0.56	0.64	0.53
Piedmont	0.66	0.53	0.70	0.57
Highlands	0.52	0.46	0.61	0.54

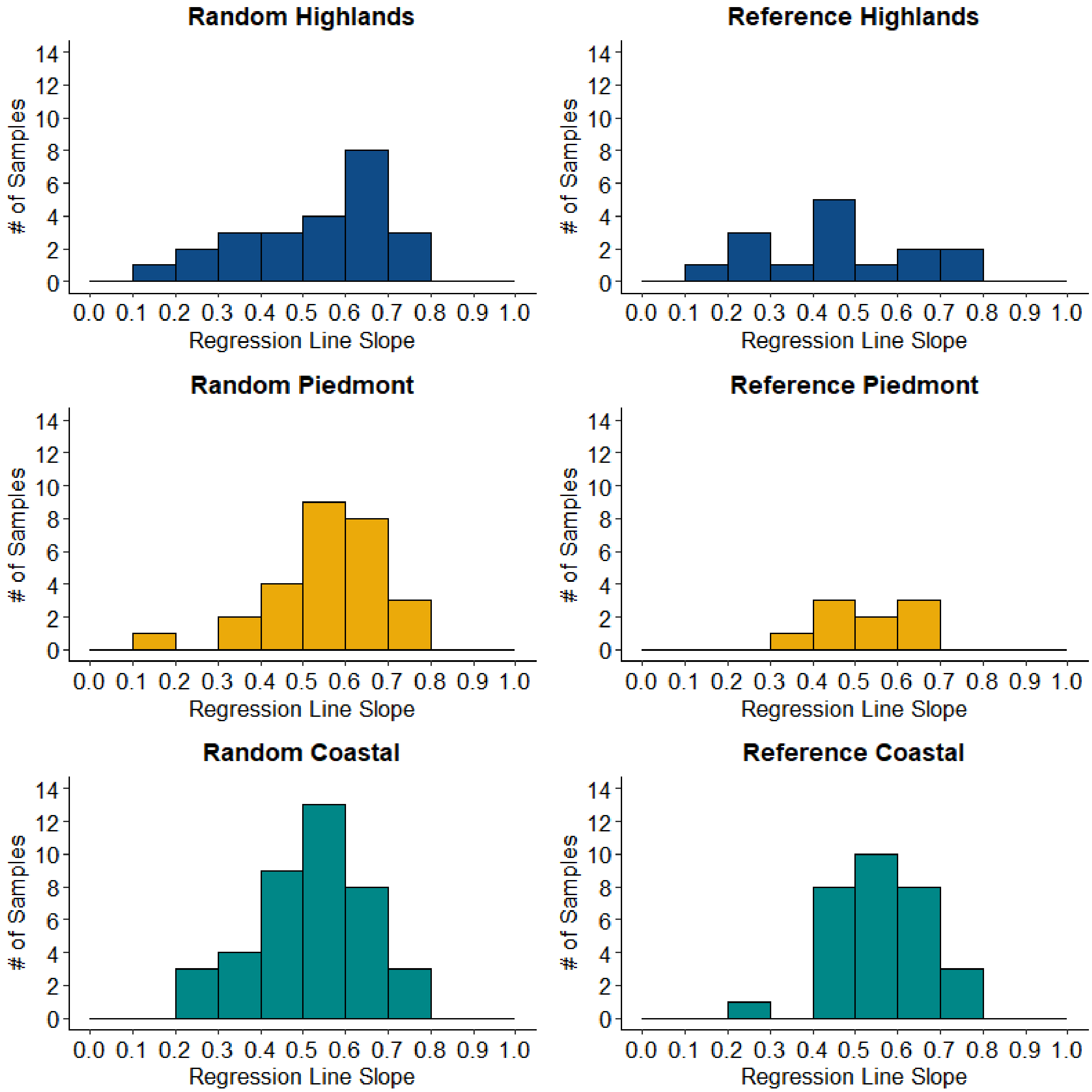


Figure 21. Numbers of samples within select ranges of water versus air temperature regression line slopes for random and reference samples by ecoregion. Higher regression line slopes generally indicate that water temperatures are more responsive to air temperature.

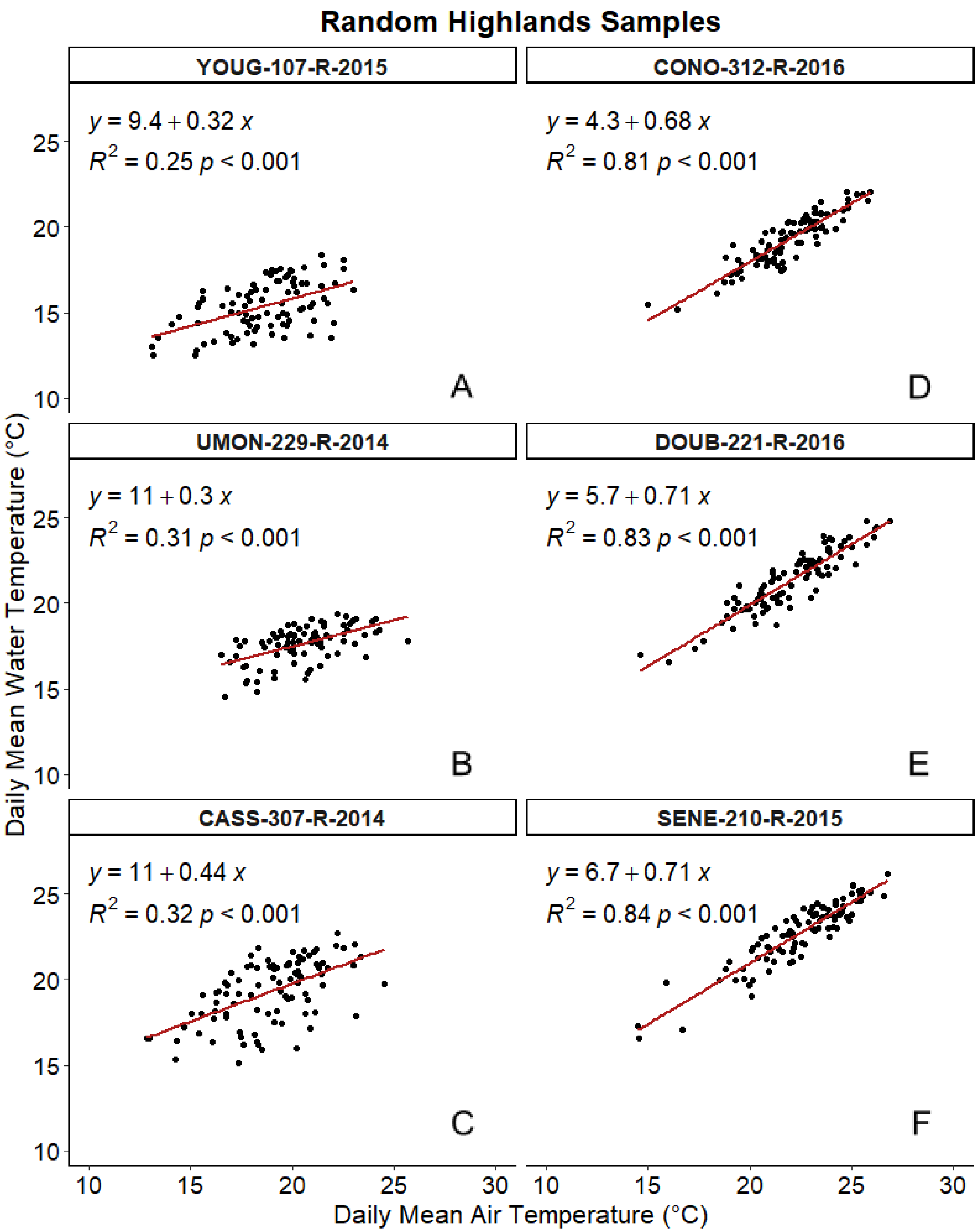


Figure 22. Examples from random Highlands samples representing relatively weak (A, B, C) and strong (D, E, F) relationships between daily mean water and air temperatures.

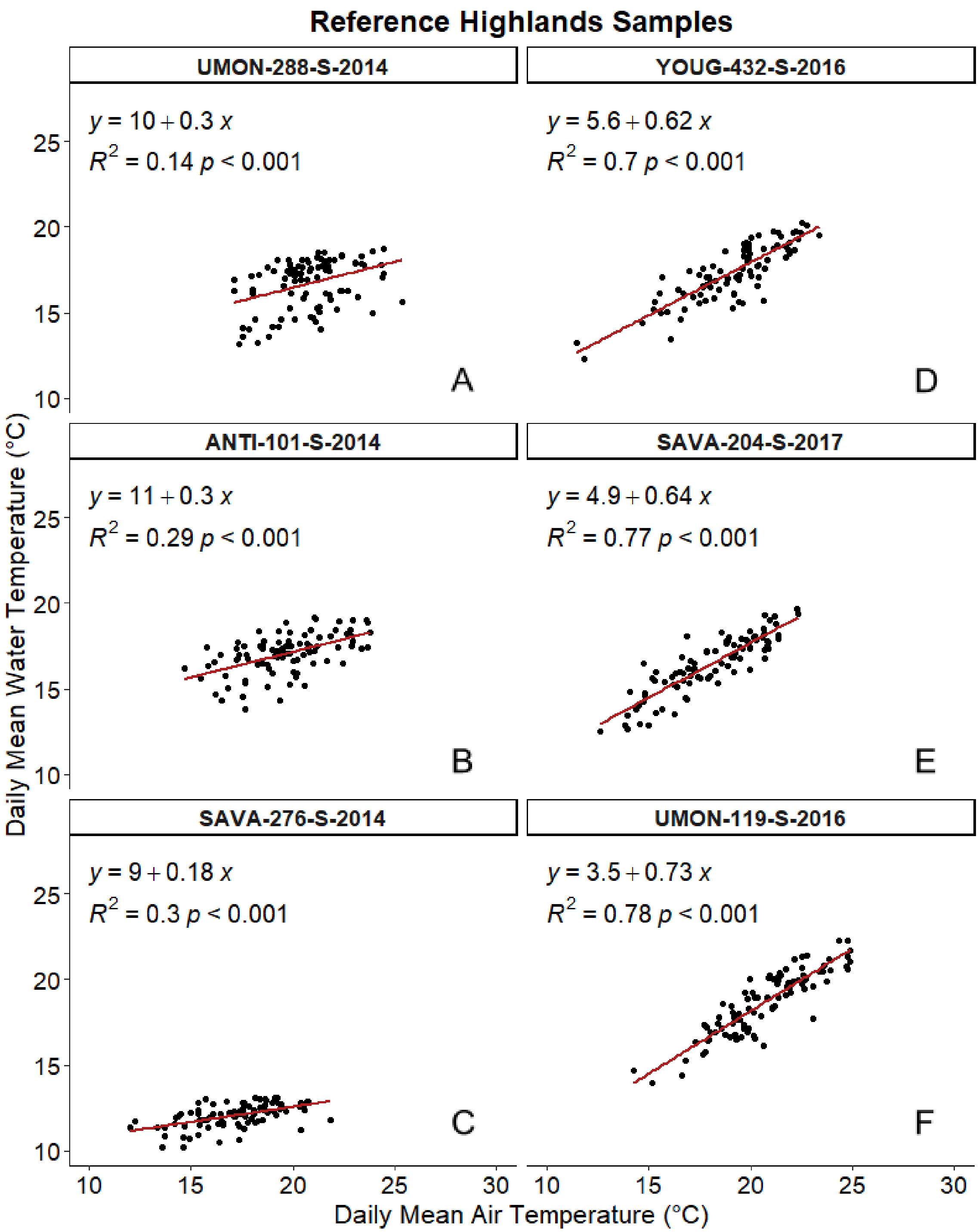


Figure 23. Examples from reference Highlands samples representing relatively weak (A, B, C) and strong (D, E, F) relationships between daily mean water and air temperatures.

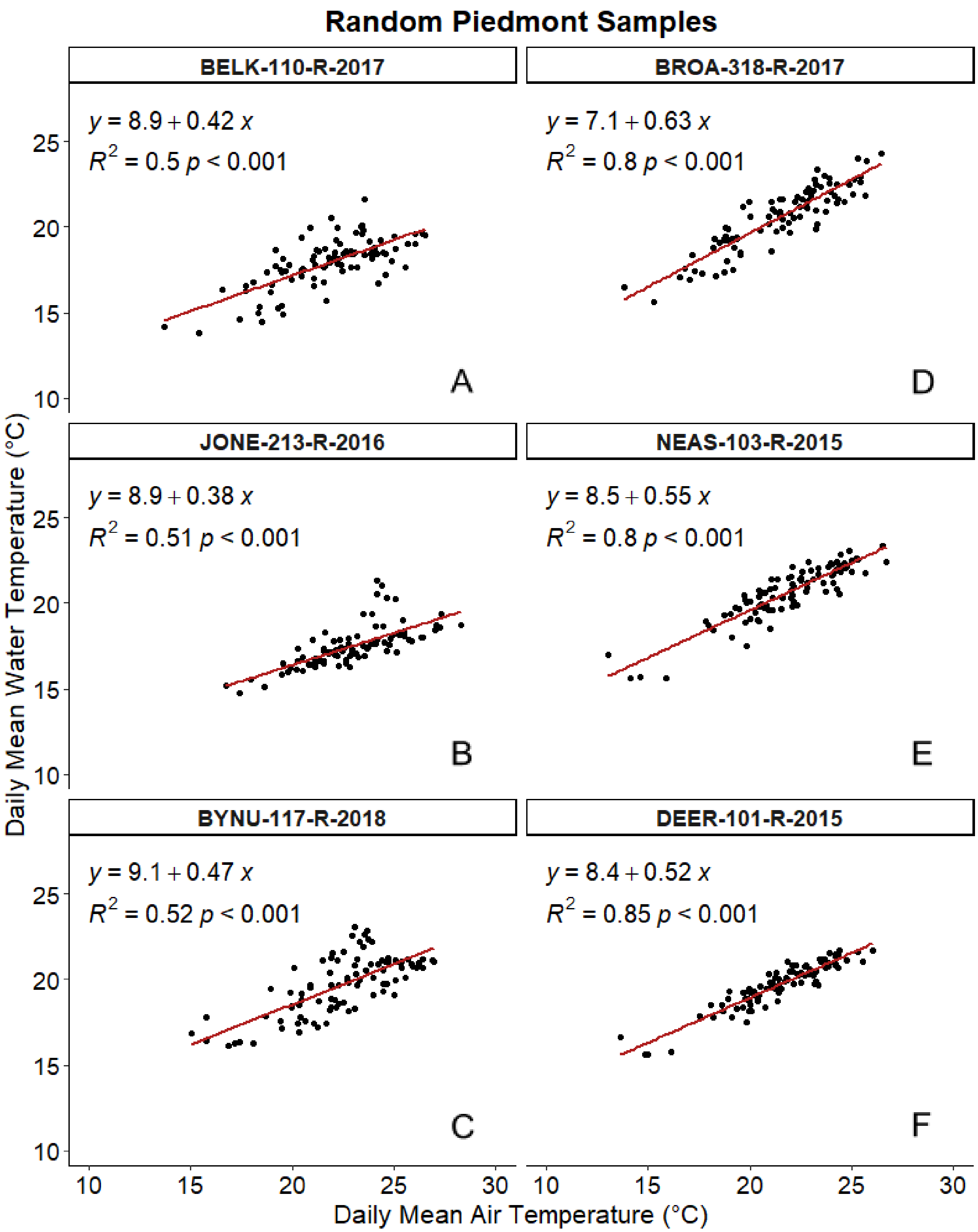


Figure 24. Examples from random Piedmont samples representing relatively weak (A, B, C) and strong (D, E, F) relationships between daily mean water and air temperatures.

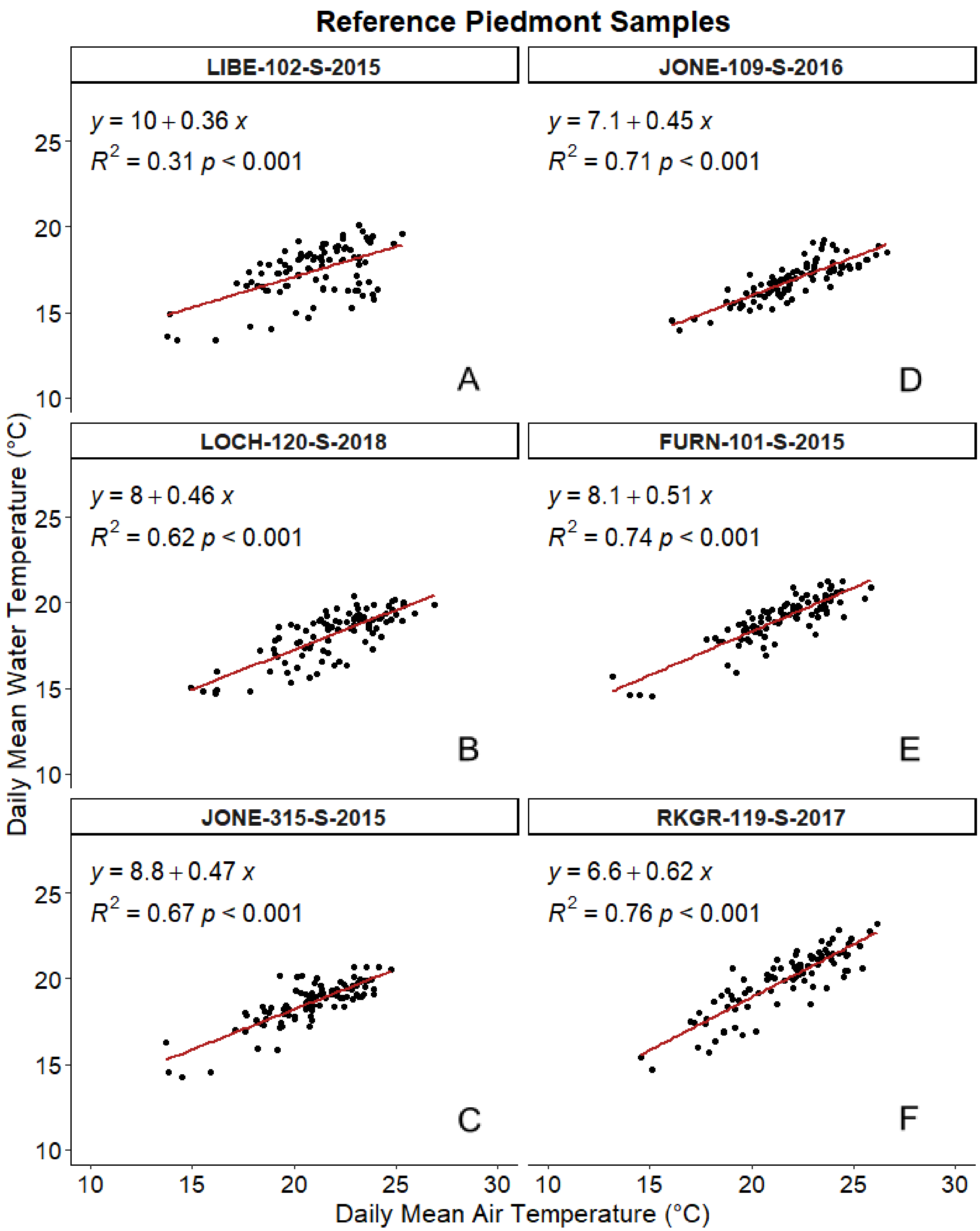


Figure 25. Examples from reference Piedmont samples representing relatively weak (A, B, C) or strong (D, E, F) relationships between daily mean water and air temperatures.

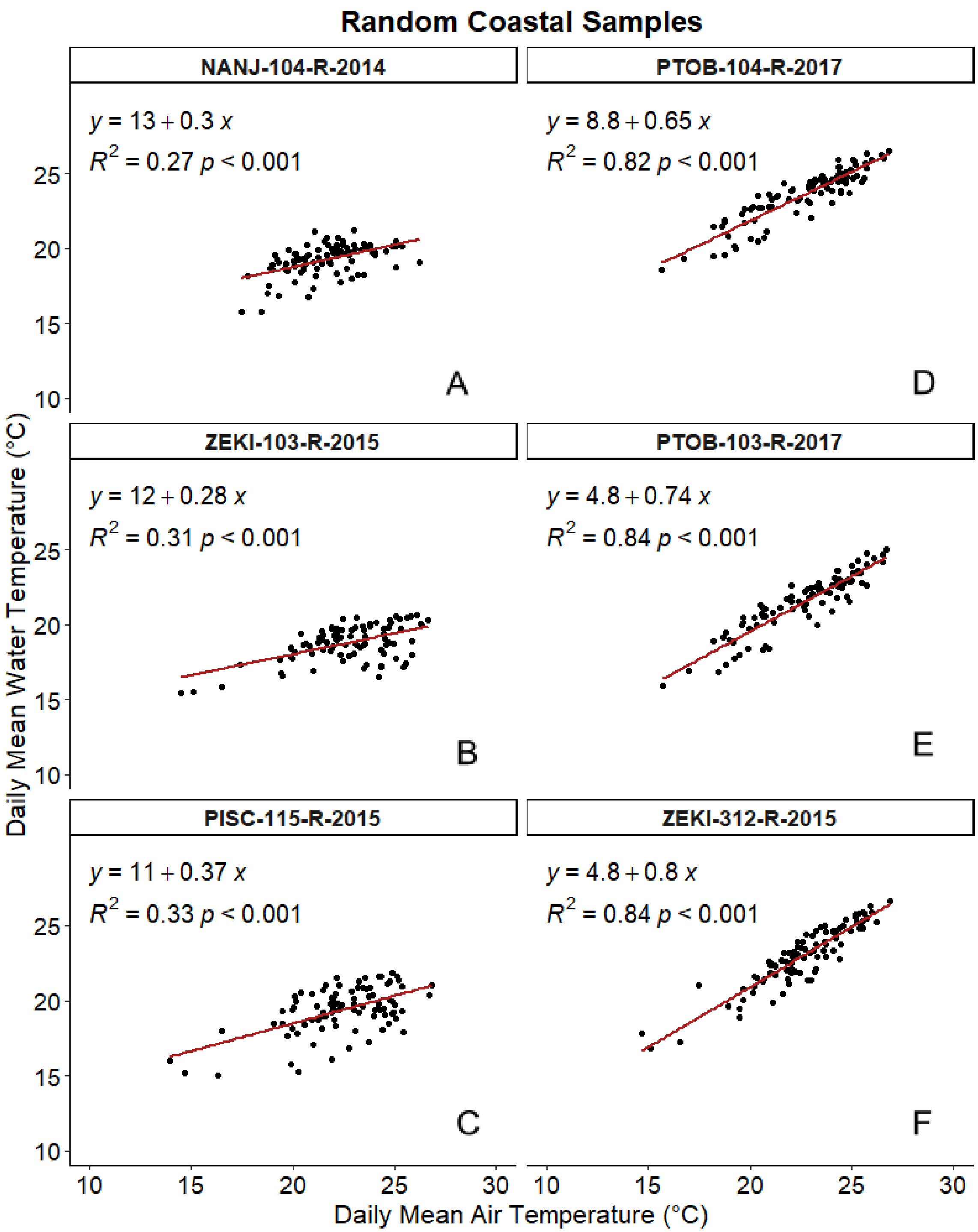


Figure 26. Examples from random Coastal Plain samples representing relatively weak (A, B, C) and strong (D, E, F) relationships between daily mean water and air temperatures.

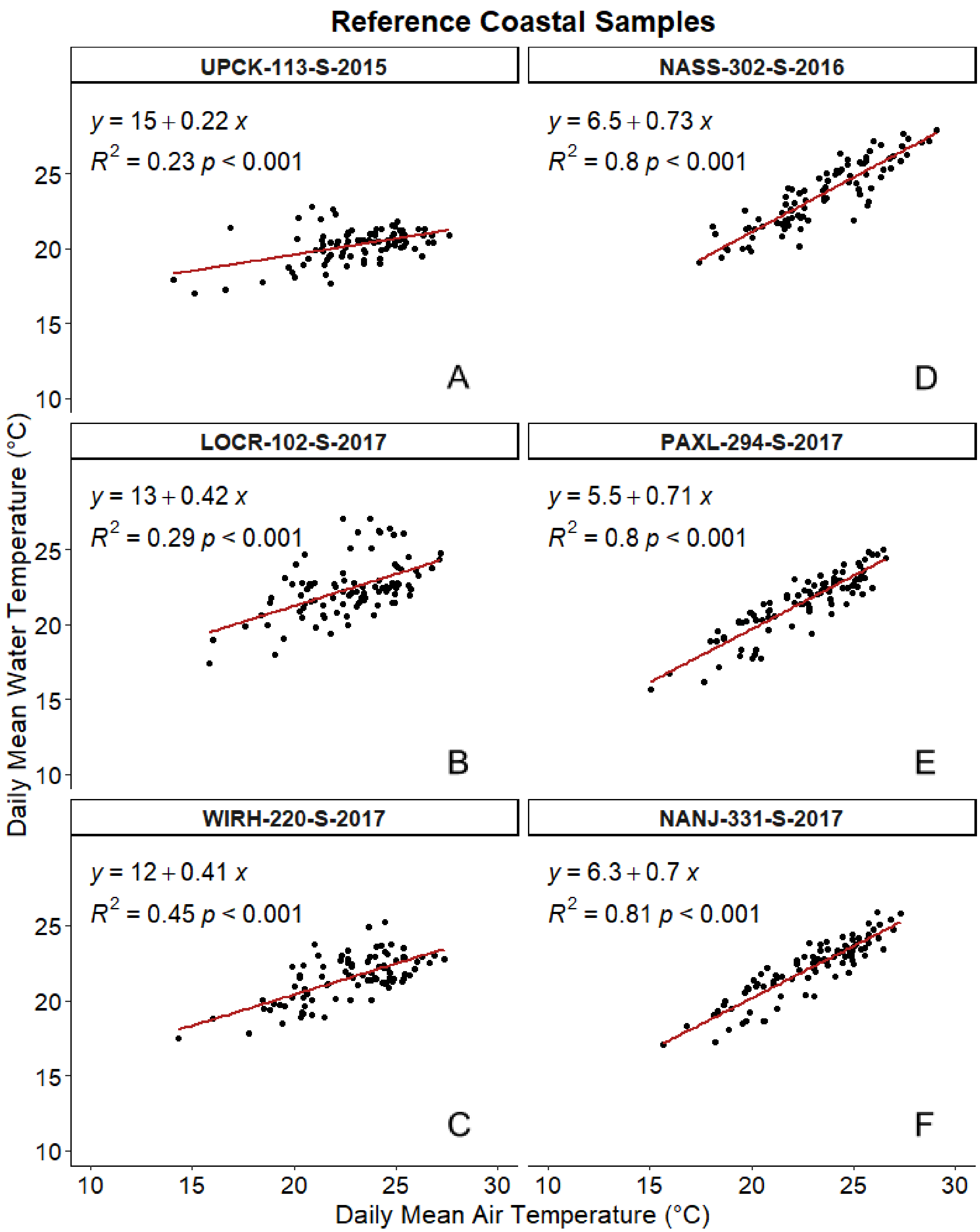


Figure 27. Examples of reference Coastal Plain samples representing relatively weak (A, B, C) and strong (D, E, F) relationships between daily mean water and air temperatures.

Discussion

The overall higher stream temperatures among random and reference MBSS samples from Round Two to Round Four are generally consistent with findings from other studies analyzing stream temperature trends in Maryland, the Chesapeake Bay watershed, and across the U.S. Reflective of climate change's widespread impacts, air temperatures in Maryland have increased and are likely contributing to warmer stream temperatures. The state has experienced an increase in air temperatures of approximately 2.5°F, or 1.4°C, since the early 1900s, with further significant warming projected in some scenarios by 2100 (Runkle et al. 2022). Rice and Jastram (2015) found that air temperature – which underwent a median rate change of 0.023°C each year from 1960 to 2010 across 64 mid-Atlantic sites – was significantly related to water temperature in the Chesapeake Bay watershed region. In the same time period, stream water temperature changed at a median rate of 0.028°C each year at 57 sites in and adjacent to the region, including multiple sites in Maryland (Rice and Jastram 2015). A similar pattern of warming streams accompanied by warming annual mean air temperatures emerged across many sites analyzed around the U.S. (Kaushal et al. 2010). Kaushal et al. (2010) observed a trend of significantly warming water temperatures spanning decades in 20 of 40 studied major streams and rivers across the U.S., including the Potomac, Patuxent, and Gunpowder rivers in or near Maryland. The Potomac River warmed at a markedly greater pace than the half-century median rate reported by Rice and Jastram (2015), increasing at a rate of 0.046°C per year from 1922 to 2006 (Kaushal et al. 2010).

Given such recent influences of global climate change, particularly in urban areas, it would be expected that MBSS' ecoregional temperature results reflected more consistent warming across site types and in streams potentially impacted by development. Instead, temperatures in reference Piedmont samples appeared to remain constant despite high levels of urban development in the central Maryland ecoregion, even as random Piedmont samples experienced significant warming across four of five metrics. While higher temperatures were observed at both random and reference samples in the Coastal Plain, no significant changes were evident in maximum temperature or percentage of readings above 24°C at either the ecoregion's random or reference samples. It is possible that our analysis was limited by small sample sizes and not able to detect all significant temperature changes. The results from the Highlands ecoregion showing constant temperatures – and even potentially cooling maximum temperatures in random samples – may be supported by findings in Kaushal et al. (2010) that the Mid-Atlantic region's urban areas experienced the greatest rates of stream and river temperature increases in the country, as the Highlands is the least developed ecoregion of Maryland.

The influence of air temperature on water temperature among R4/2 and S4 samples might help explain the changes – or lack thereof in the Highlands ecoregion – observed in this study. Regression models of air temperature and water temperature with weaker correlations and lower slopes can be indicative of stream water that is less responsive to air temperature changes (Caissie 2006, O'Driscoll and DeWalle 2006, Hilderbrand et al. 2014, Hitt et al. 2023). Certain regression model results were consistent, and others contrasted with findings in Hilderbrand et al. (2014), which limited their analysis to mostly forested watersheds. Hilderbrand et al. (2014) found that Highlands sites had the lowest mean slope and weakest relationship between air and water temperature based on R^2 values, and Coastal Plain sites had the highest mean slope and strongest relationship. Our results showed the same pattern for slope values among reference samples, but differed for random samples; random Coastal Plain samples had the lowest mean slope and random Piedmont samples had the highest mean slope. The low slope among random Coastal Plain samples was particularly surprising given the warming observed in that ecoregion. Our analysis of R^2 values indicated that both reference and random Highlands samples had the weakest relationship in their respective groups, similar to Hilderbrand et al. (2014). In contrast to the other study, however, we found that reference and random Piedmont samples had the strongest relationship in their respective groups.

Based on the slope and/or R^2 values we observed, there were possible signs that some individual samples with potentially greater degrees of air temperature influence generally experienced more warming between Rounds. Overall, random samples with R^2 values around 0.8 or higher, and (to a lesser degree) reference samples with an R^2 value of 0.76 or higher appeared potentially more likely to have experienced substantial warming in three or more metrics. At the ecoregion-level, temperature changes in random samples from the more developed Piedmont ecoregion showed the clearest potential signal of air temperature influence; the majority of the most substantial warming in R4/2 occurred in samples with a regression line slope of 0.55 and higher, or an R^2 value above 0.60. Also, among reference Coastal samples, relatively moderate and larger changes in four or five warmer temperature metrics appeared to more commonly occur when the sample slope was around 0.6 or higher. Any pattern among sample-specific changes, however, was not clear in other ecoregion groups based on slope or R^2 values. For example, some individual random Highlands samples with higher slopes and R^2 values experienced greater degrees of warming in multiple metrics, while others experienced greater degrees of cooling.

Greater levels of groundwater influence may be one factor among many contributing to the relatively weaker air-water temperature relationships and regression line slopes observed in the Highlands, helping explain the general lack of warming in samples from the ecoregion (O'Driscoll and DeWalle 2006, Snyder et al. 2015, Hitt et al. 2023). Cooler stream temperatures can prevail when and where groundwater discharge is significant, even potentially buffering streams from warming air temperatures (Chu et al. 2008, Kanno et al. 2014) and seasonal extremes (O'Driscoll and DeWalle 2006), or in areas lacking other potential mitigating factors such as forested riparian zones (Simmons et al. 2015). Yet the depth of the aquifer source might limit the longevity of this buffering effect, as groundwater stemming from shallow deposits may be more sensitive to air temperature increases tied to climate change (Snyder et al. 2015).

Piedmont and Coastal Plain streams in more urbanized catchments face potential warming effects, perhaps tied to greater extents of impervious surfaces. Stream sites in more urbanized areas can experience higher daily average stream temperatures and more frequent temperature surges from storm runoff (Nelson and Palmer 2007), and increases in impervious surfaces can block precipitation from underground aquifers, reducing recharge of groundwater that can buffer stream temperatures (Erickson and Stefan 2007). Notably, however, while potentially coupled warming stream water and air temperature trends have been observed near urban areas in the mid-Atlantic region (Kaushal et al. 2010), Rice and Jastram (2015) did not find evidence that urban land use played a significant role in how air temperature might have affected water temperature at sites in the Chesapeake Bay region.

The presence of forests in riparian areas and within the watershed could also help maintain cooler stream temperatures at the Highlands sites. By roughly 2018, the western Maryland region had approximately 70% tree canopy cover in riparian buffer areas, compared to the state's overall coverage of 58% (Minnemeyer et al. 2022). Riparian buffers with canopy cover have been shown to have cooling effects on mean and maximum stream temperatures compared to streams without similar buffers (Bowler et al. 2012, Simmons et al. 2015). In the Chesapeake Bay region, shaded deciduous forests appeared to slow the rise of stream water temperature compared to air temperature, while streams surrounded by agricultural land use with reduced shading experienced warming that outpaced air temperature (Rice and Jastram 2015). In addition, Ouyang et al. (2019) showed that – contrary to other comparisons of forest and agricultural land impacts – forest vegetation and ground litter that capture precipitation and reduce surface water runoff could potentially contribute to more groundwater recharge compared to agricultural land in a humid subtropical region.

It is important to strengthen our understanding of the various factors influencing Maryland's stream temperatures, particularly amid warming air temperatures associated with climate change. These results indicate that stream temperatures in the state have generally risen in less than two decades, creating warmer conditions that could result in impacts such as reduced dissolved oxygen levels, increased nutrient pollution inputs sourced from sediment (Duan and Kaushal 2013), and changes or declines in the distribution, development, and survival of aquatic species including some aquatic insects (Pyne and Poff 2017) and coldwater fish (Stranko et al. 2008, Lyons et al. 2010, Hester and Doyle 2011, Isaak et al. 2012). Our findings also echo studies showing that some streams appear less sensitive to changes in air temperature, potentially due to the cooling effects of groundwater, differences in land cover, or other factors.

Annual Sentinel Site Temporal Trends Report Results Comparison

A report evaluating annual temporal trends by site for many of the same individual reference sites (MBSS Sentinel) also used in this report was recently published (Resource Assessment Service 2023; henceforth referred to as the Sentinel Site report). Results from the Sentinel Site report largely align with reference site results from this report. The primary distinction between these two reports relates to the type of temporal analysis conducted. This report statistically compared data from pairs of samples from the same sites collected 14 years apart during two different time periods (2000-2004 and 2014-2018); the Sentinel Site report correlated year (for 20 years of annual sampling 2000-2020) with metric values for each site individually. The Sentinel Site report examined temporal trends only at Sentinel Sites (referred to as reference sites in this report). Thus, the comparison between reported results in the Sentinel Site report is only for reference sites.

Biology

The Sentinel Site report did not use non-native, RTE, game, or any richness information for fish. Other fish results are primarily consistent with the results in this report. For example, more sites in the Sentinel Site report showed significant temporal decreases in fish abundance, and this finding was more evident compared with other fish data trends. The Fish IBI and most other fish metrics examined were higher over time at some sites while lower at others. Consistent with this report, the Sentinel Site report also found that the percent intolerant to urbanization benthic macroinvertebrate metric had the largest proportion of sites exhibiting significant declines over time. According to the Sentinel Site report, percent Ephemeroptera was one of three metrics that was not significantly higher at any site in recent years and only exhibited significant declines from two sites. In this report however, percent Ephemeroptera was significantly lower in the most recent time period examined. Interestingly, the number of Ephemeroptera taxa metric declined at three of four sites with significant trends in the Sentinel Site report. This is different from the results for other richness metrics (number of total taxa, scraper taxa, and EPT taxa) in that report which significantly increased at large proportions of sites. Richness metrics were not investigated in this report due to the potential influence of different numbers of individuals, as well as certainty in genus-level identification (especially during the 1990s), may have on such metrics.

Water Chemistry

As with biology results, most water chemistry results were similar between the Sentinel Site report and this report. The Sentinel Site report results revealed more region-specific results. Similarly to this report, sulfate declined at many sites, but those sites were largely in western

Maryland. Conductivity, chloride, orthophosphate, and pH increased at many of the sites with a significant temporal trend in these variables with time in the Sentinel Site report. In addition, ANC showed a consistent increase over time at many sites in the Sentinel Site report, primarily in the Piedmont ecoregion of central Maryland. Total nitrogen was the only water chemistry parameter with generally inconsistent results between these two reports. While this report indicates significantly lower values at reference sites over the time period examined, the Sentinel Site report results show five sites with significantly increasing trends and none with lower. While we do not know the reason for these differences, it may be due to the different time periods examined, analysis techniques, or the use of all years versus only a subset of years.

Temperature

The Sentinel Site report examined temperature data from all seasons of the year. Since summer was the only time when data were available from random sites, only summer data (June 1 - August 31) were used for the reference sites in this report. According to the Sentinel Site report, many sites showed significantly higher temperature metrics over time, with no significant decreases in any summer water temperature metric. Consistent with the results in this report, very few sites from western Maryland showed any increase based on summer data. Somewhat inconsistent with this report – which showed significantly higher temperatures at random Piedmont sites but not reference Piedmont sites – several reference Piedmont sites demonstrated significant increases in summer temperatures. Combined, however, the results from both reports indicate increasing summer stream temperatures on the eastern shore, southern Maryland, and some sites in central Maryland, but little to no significant increases in western Maryland.

Summary of Comparison

Although there are some minor distinctions, the results from these two reports complement each other well. Such agreement in results provides a weight of evidence to show how Maryland's high-quality (reference) streams have likely changed over time. Although they used different analyses and examined distinct time periods, both reports indicate that reference streams have likely become warmer (with the clear exception of western Maryland), with higher conductivity, pH, and orthophosphate, but with lower concentrations of sulfate. In addition, certain intolerant benthic taxa and fish abundance seem to have declined.

Conclusions

Change over Time?

A common question pertaining to ecosystem health and long-term monitoring is "are conditions improving or degrading?" Continued random sampling by the MBSS has and will continue to produce estimates of stream conditions in Maryland that can be compared over time. However, since a unique set of sites from a subset of streams is selected each year, variability in condition estimates is high, and detecting trends is sometimes difficult unless large changes occur. Thus, answering important questions about change over time is challenging. By resampling large subsets of representative sites, we were able to provide data and results best suited for answering this question. We did this for two time periods and two map scales with different sampling designs. Although these paired datasets and analyses results are not entirely conclusive, there is evidence of improvement, degradation, and no change depending on the aspect of stream condition investigated over the 20- and 14-year time periods.

Biology

Stream aquatic life integrates and reflects environmental conditions from the landscape (through the influence of factors like land cover and geology), air (via atmospheric deposition), and subsurface (through the degree of connection and quality of groundwater), as well as due to changes in weather and climate. In this report, we assessed aquatic life using measures of biological integrity and more detailed evaluations of biological diversity compositions including taxa that are considered sensitive or tolerant to anthropogenic stressors. Biological integrity was primarily unchanged at the statewide scale. Certain sensitive/intolerant and tolerant biota from more specific and detailed metrics tended to show signs of degrading conditions and biotic homogenization while other such taxa provided indications of improving stream conditions. Rare fish species tended to be lower in abundance and distribution, except for a slight increase in the proportion of samples with RTE fish species over the 14-year random site comparison. Concomitantly, non-native fish species were consistently higher in every way measured and every time period examined. The absence of change in biological integrity assessments could, at least in part, be due to inconsistencies in biodiversity patterns. However, increases in tolerant species and decreases in sensitive species may be early indicators of ecosystem alterations not yet reflected in more generalized biological indices.

Water Chemistry

Maryland's streams appear to have become saltier – but with less acidity and sulfate, and higher orthophosphate. Reductions in acidity and sulfate are encouraging, tend to be beneficial to stream ecology, and could perhaps be attributed to reductions in emissions and consequential improvements in air quality. Sulfate reductions could also have been achieved through coal mine drainage remediation that would also likely reduce acidity. While some of these improvements likely occurred over the time periods examined, increasing conductivity and chloride (likely due to road salt) and a potential increase in the weathering of buildings and other alkaline materials likely also contributed. Depending on particular streams and the factors influencing them, beneficial decreases in acidity may be accompanied by increases in less desirable factors such as conductivity, for example. However, reductions in acidity may have resulted in some ecological improvements. An exception may be where high biological diversity (e.g., rare acid endemic biota) is associated with naturally acidic conditions.

Although water quality conditions tended to be better at reference than at random sites, increases in conductivity and chloride were evident at reference sites, even to the point where important thresholds were exceeded during more recent sampling. Understanding the propensity for such patterns to exist is important for interpreting results from these sites intended to provide reference conditions consistent with minimally degraded stream conditions.

Temperature

Since temperature data were only available for the 14-year interval, 20 years of potential temperature change could not be evaluated. Given the recent widespread influences of global climate change, temperature results would be expected to reflect consistent warming throughout Maryland streams. Instead, there appear to be clear patterns associated with geography and site type. Central (Piedmont ecoregion) reference samples, as well as western (Highland ecoregion) reference and random samples, did not demonstrate significantly warmer water during more recent sampling, while samples from other areas (Coastal Plain reference and random, as well as Piedmont random) did demonstrate warming. As air temperatures have increased, relative groundwater, landscape conditions, gradient, or other factors may have contributed to different region and site type results for stream water temperature changes over time. A better understanding of the unique factors associated with streams where temperature changed and did not change is critical for planning effective climate change-related water quality and ecological protection and adaptation efforts.

Importance of Consistent Data

Even though adjustments were required for certain variables to ensure comparability, the use of consistent monitoring protocols made this rigorous examination of stream conditions over time possible. There are other variables monitored by the MBSS that we were not able to use due to imprecision or inconsistencies in data collection over time. While many site-specific variables could also contribute substantially to examinations of changes in stream conditions over time, physical habitat assessment scores are likely the most blatant omission. MBSS physical habitat assessment scores are strongly correlated with stream biological conditions and thus provide useful information about potential stressors to aquatic life. However, physical habitat variables were not used here because, while an increased emphasis on training and certification by the MBSS has reduced variability among assessors in recent years, it has also revealed substantial historical inconsistency among assessors.

Land cover is an important factor in determining stream condition. Land cover within catchments (land area upstream) of each site was derived for MBSS sites. However, we did not use these land cover data in our evaluation of potential change over time due to inconsistencies in the data between the time periods of interest, lack of precision in available data for the individual years needed, and lack of data from throughout entire catchments with sufficient resolution for all sites.

Differences in weather (especially rainfall) and resulting variations in stream flow can subsequently cause variations in water quality, physical habitat, and ultimately biological condition. While rainfall and flow data exist for many locations throughout Maryland, sufficient spatial precision was not available to determine the exact responses of specific (especially small) streams examined here, without performing involved (and imprecise) modeling and extrapolation. However, according to NOAA rainfall data for Maryland, the mean annual rainfall totals were similar between the multiple year sampling periods used in this report (R1 = 45.76; R2 = 45.75; R4/1 = 43.24; R4/2 = 47.82). While this is not a detailed, rigorous comparison, it indicates that results were likely not influenced by differences in rainfall. Additionally, although we cannot say for certain, we assume comparing pairs of sites sampled over several years (three for the Round One comparison and five for the Round Two comparison) and conducting two separate temporal comparisons, reduced the potential for confounding influences of variations in weather and rainfall on results.

Other Important Variables

In addition to physical habitat, land use, and flow, there are many additional biological, physical, chemical, landscape, and other factors important to streams that were not sampled, assessed, or considered in this evaluation of change over time in Maryland's stream conditions. Certain additional biological factors would help more comprehensively represent the biological diversity of Maryland's streams. A multitude of other environmental factors are likely to influence (be potential stressors to) stream aquatic life and/or have inherent relevance to water quality and physical habitat conditions. While it is not feasible to sample every parameter, certain factors may be worth considering in current and future MBSS assessments. However, most additional factors could obviously not be compared with the data from 20 and 14 years ago as examined here.

Potential Reasons for Biological Conditions

Although additional information can always be useful, the benthic macroinvertebrate and fish biological indices and biological indicators of tolerance to anthropogenic stressors used here are consistent with methods used throughout the United States and beyond to assess stream conditions. While these indices and tolerance metrics provide a representation of overall environmental conditions, definitively defined stressors and other explanatory conditions are challenging to derive. It is possible the actual causal factor(s) may not have been measured (or even measurable) due to a lack of sufficient spatial or temporal precision and/or timing in measurements, and/or effects that may result from synergistic influences from multiple factors.

Despite the challenge in definitively defining factors responsible for observed biological conditions, there is a strong weight of evidence from multiple scientific studies associating the variables we examined with biological conditions, as well as describing mechanisms for associations. Specifically, pH, conductivity, chloride, and temperature are strongly correlated with stream conditions. While we still cannot conclude that these are definitive factors, such studies lend support to their potential role in contributing to Maryland's stream biological conditions. Of particular relevance may be the observed changes in what are considered biologically meaningful thresholds exceeded for these factors.

Along with abundant additional factors and analyses that can lend information to stream condition interpretations, the results presented here represent an assessment of only general stream conditions. There are many region, stream, and site-specific considerations yet to be investigated and revealed through continued examination of this rich and rigorous dataset. Such investigations will help develop effective conservation strategies at appropriate spatial scales.

Intended Purpose

The results and discussions of change in stream conditions over time in this report are intended to add information to the extensive applications of MBSS data and results used to support environmental policies, regulations, and resource management in Maryland relating to aquatic life, water quality, rare and invasive species, climate adaptation, and other uses as appropriate. Results so far, as presented here, describe improvements and/or degradation depending on the specific streams and specific indicators examined. While future stream monitoring in Maryland will focus on expanded representation of Maryland's streams through the use of a finer scale (1:24,000 scale) map than was used in either survey analyzed here, overlap of the more detailed map with the Round Two (1:100,000 scale) map ensures comparability with this historical sample frame. Additionally, stream monitoring collaboration with other state agencies, county agencies, and researchers (using the same stream map) has expanded substantially in recent years. Such collaboration will enhance the sharing and use of the information herein, as well as provide important evaluation and scrutiny of the results we present.

Ultimately, this report is a substantial contribution toward an important goal of the MBSS – to provide the best possible data and information to inform the protection and restoration of Maryland's stream ecological resources. More specifically, to provide the most rigorous possible assessment of change over time in overall stream conditions and answer the question - "are streams improving or degrading in Maryland?".

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Appendices

APPENDIX A
Benthic Macroinvertebrate Taxonomic Name Translations

Appendix A. List of taxonomic name conversions used in this study to increase comparability in benthic samples over time. Cladocera, Copepoda, and Ostreacoda were removed from all samples in accordance with Maryland Biological Stream Survey protocols.

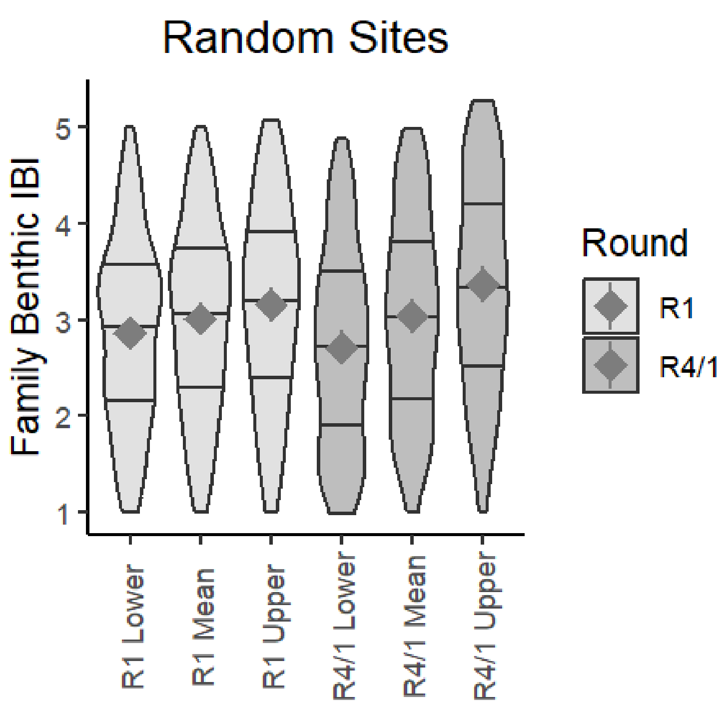
Current Name	Simplified Name Used in Study
Labiobaetis	Baetis
Pseudocloeon	Baetis
Plauditis	Baetis
Anafroptilum	Centroptilum
Teloganopsis	Serratella
Ceratopsyche	Hydropsyche
Maccaffertium	Stenonema
Macromiidae	Corduliidae
Polycentropus Group	Polycentropus
Bezzia/Palpomyia	Bezzia
Faxonius	Orconectes
Girardia	Dugesiidae
Goniobasis	Pleurocera
Leptohyphidae	Tricorythidae
Neoporus	Hydroporus
Physella	Physa
Pisidiidae	Sphaeriidae
Thienemannimyia	Thienemannimyia Group
Cricotopus	Cricotopus/Orthocladius
Orthocladius	Cricotopus/Orthocladius
Orthocladiinae A	Orthocladiinae
Orthocladiinae B	Orthocladiinae
Cladocera	Removed from samples
Copepoda	Removed from samples
Ostracoda	Removed from samples

APPENDIX B
Benthic Macroinvertebrate Metric Statistics and Violin Plots

R1 vs. R4/1 Comparisons

Family Benthic IBI

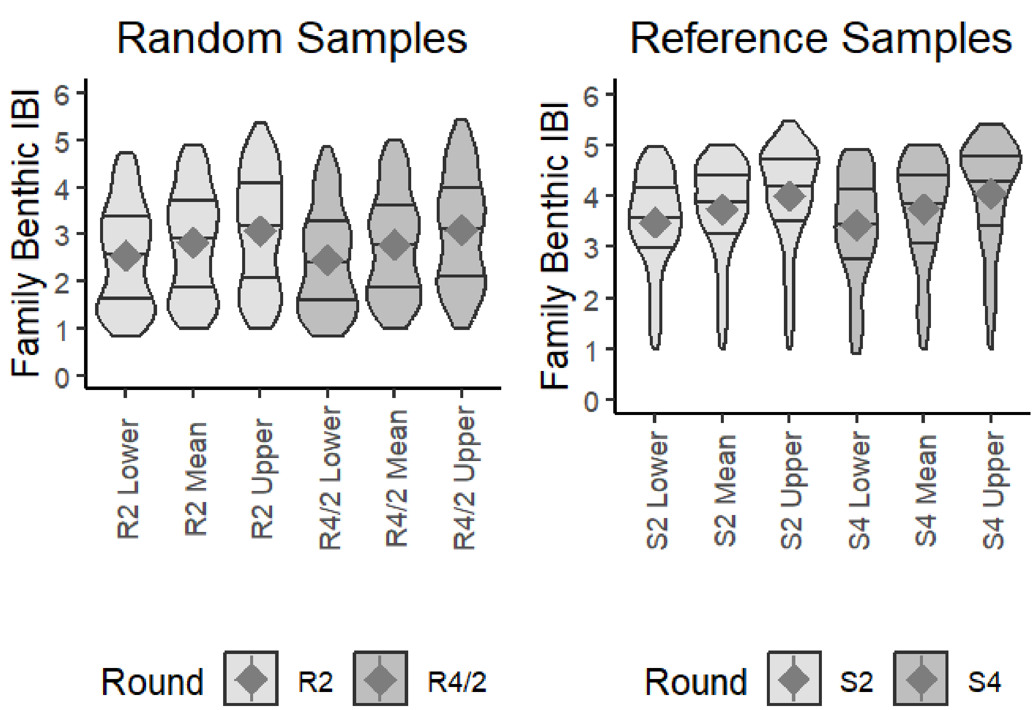
	RANDOM	
	R1	**R4/1**
N (Samples)	133	133
Unique Sites	133	133
Mean	3.01	3.04
Median	3.00	3.00
St. Dev.	0.98	1.04
Range	1.0 - 5.0	1.0 - 4.99
Wilcoxon test 95% CI Direction	0.974 - 0.16, 0.20 **No Change**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Family Benthic IBI

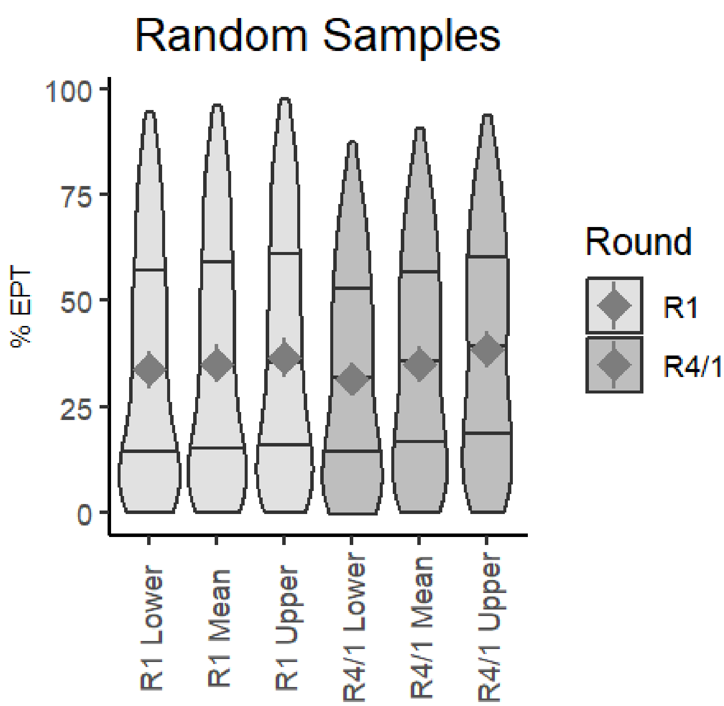
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	242	242	110	110
Unique Sites	242	242	22	22
Mean	2.83	2.79	3.75	3.74
Median	2.93	2.78	3.87	4.01
St. Dev.	1.13	1.10	0.94	1.03
Range	1.0 - 4.89	1.0 - 4.99	1.0 - 5.0	1.0 - 4.99
Wilcoxon test	0.662		0.786	
95% CI	- 0.12, 0.08		- 0.13, 0.16	
Direction	**No Change**		**No Change**	



R1 vs. R4/1 Comparisons

% EPT

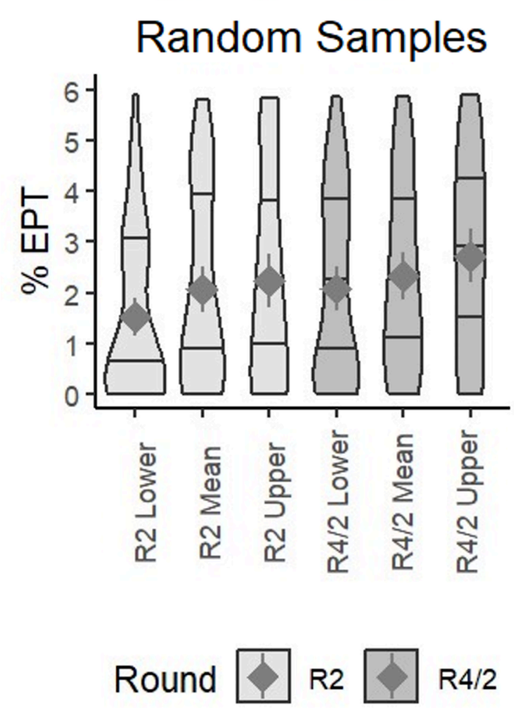
	RANDOM	
	R1	R4/1
N (Samples)	144	144
Unique Sites	144	144
Mean	35.03	34.92
Median	30.08	31.50
St. Dev.	27.66	24.96
Range	0 - 95.89	0 - 90.49
Wilcoxon test	0.981	
95% CI	-3.53, 3.61	
Direction	**No Change**	

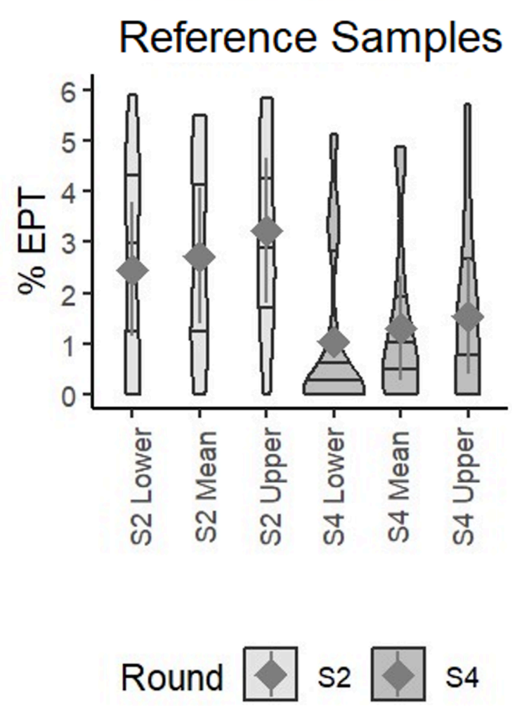


R2 vs. R4/2 & S2 vs. S4 Comparisons

% EPT

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	28.69	30.56	50.14	50.15
Median	22.62	24.49	55.01	58.18
St. Dev.	25.92	27.26	26.67	25.71
Range	0 - 92.98	0 - 93.22	0 - 92.45	0 - 87.42
Wilcoxon Test 95% CI Direction	0.133 -4.63, 0.87 **No Change**		0.814 -4.17, 3.88 **No Change**	

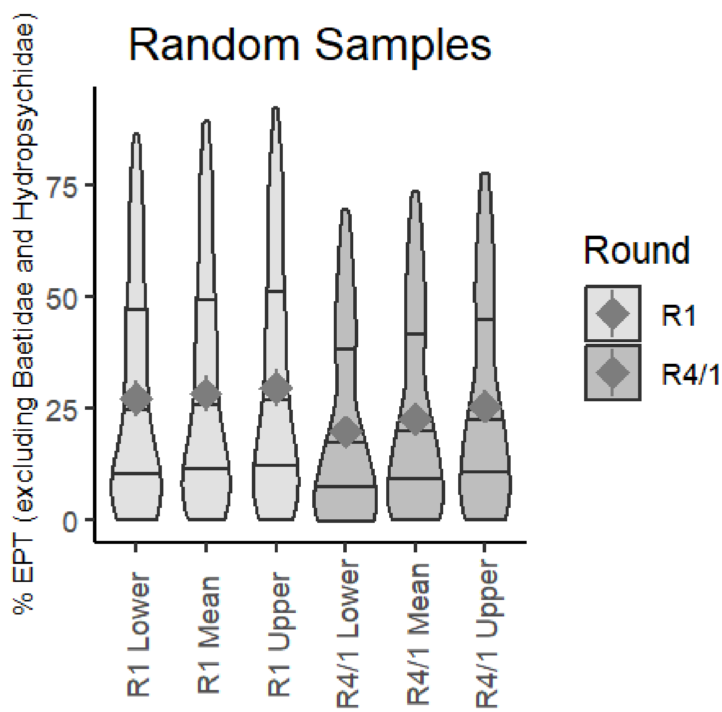




R1 vs. R4/1 Comparisons

% EPT (excluding Baetidae and Hydropsychidae)

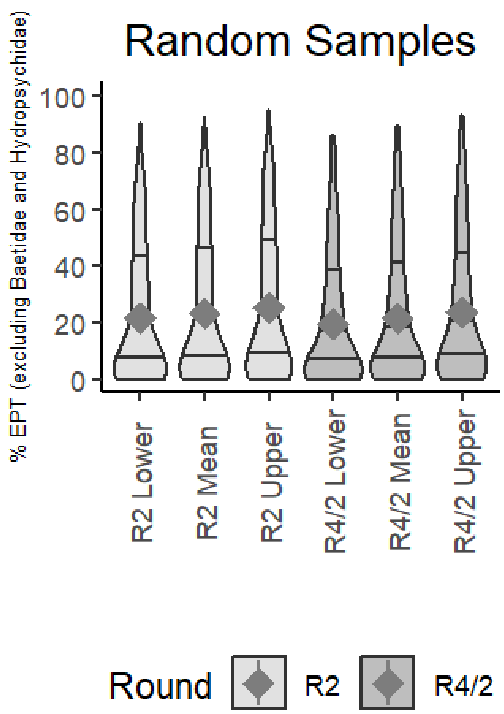
	RANDOM	
	R1	R4/1
N (Samples)	144	144
Unique Sites	144	144
Mean	28.22	22.51
Median	20.42	16.02
St. Dev.	25.19	21.29
Range	0 - 89.2	0 - 73.5
Wilcoxon test 95% CI Direction	< 0.001 - 9.57, -3.20 **Lower**	

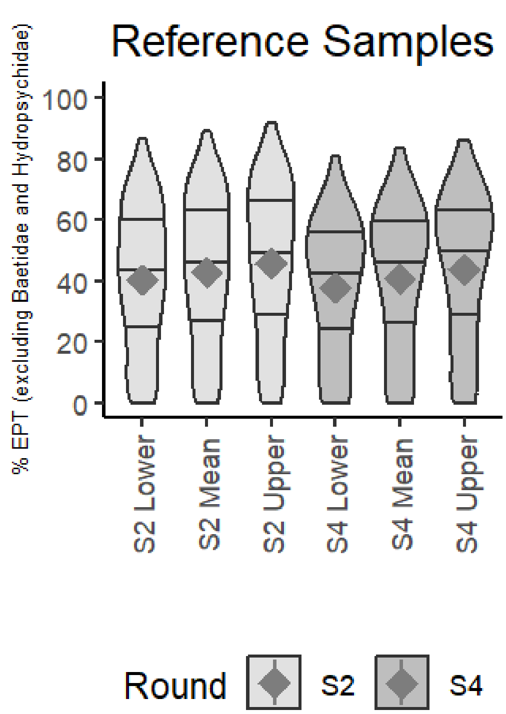


R2 vs. R4/2 & S2 vs. S4 Comparisons

% EPT (excluding Baetidae and Hydropsychidae)

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	23.1	21.4	42.9	40.6
Median	13.0	11.1	45.3	46.4
St. Dev.	24.3	23.7	23.9	22.9
Range	0 - 93.0	0 - 89.7	0 - 89.0	0 - 83.4
Wilcoxon Test	0.107		0.232	
95% CI	-3.24, 0.31		-6.57, 1.55	
Direction	**No Change**		**No Change**	

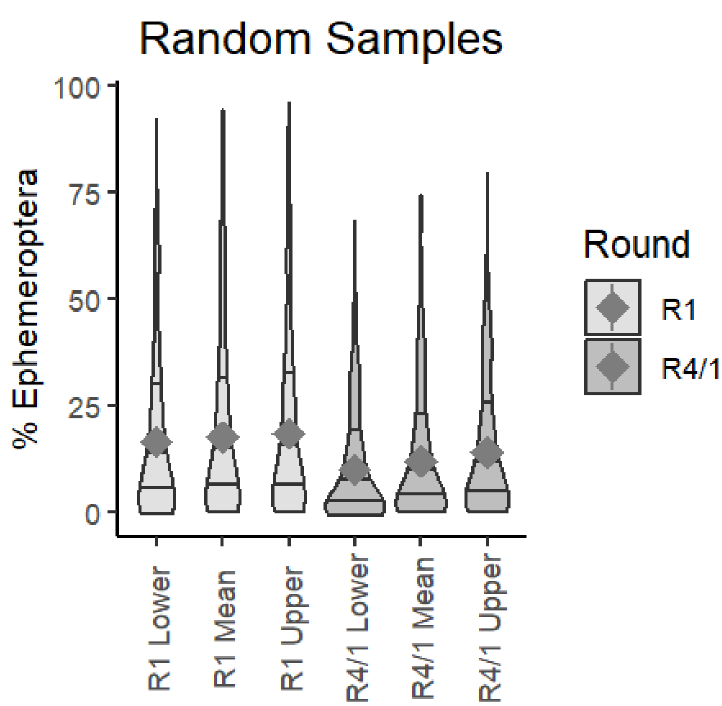




R1 vs. R4/1 Comparisons

% Ephemeroptera

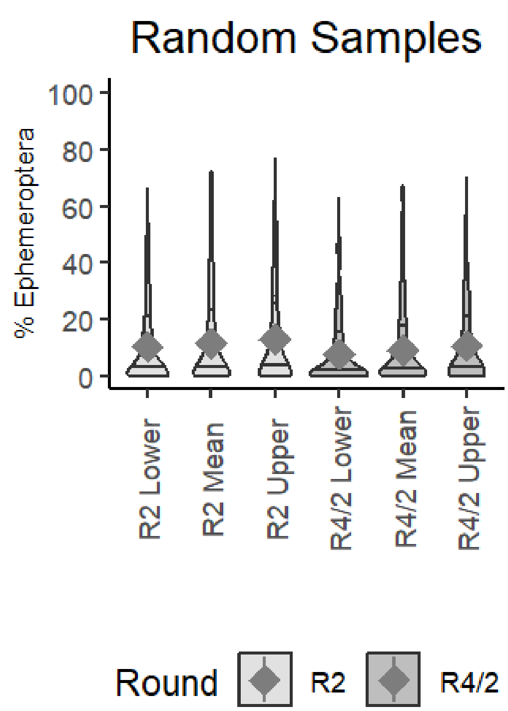
	RANDOM	
	R1	**R4/1**
N (Samples)	144	144
Unique Sites	144	144
Mean	17.6	12.2
Median	8.75	6.31
St. Dev.	20.8	14.6
Range	0 - 94.3	0 - 74.2
Wilcoxon Test	< 0.001	
95% CI	-8.28, -2.29	
Direction	**Lower**	

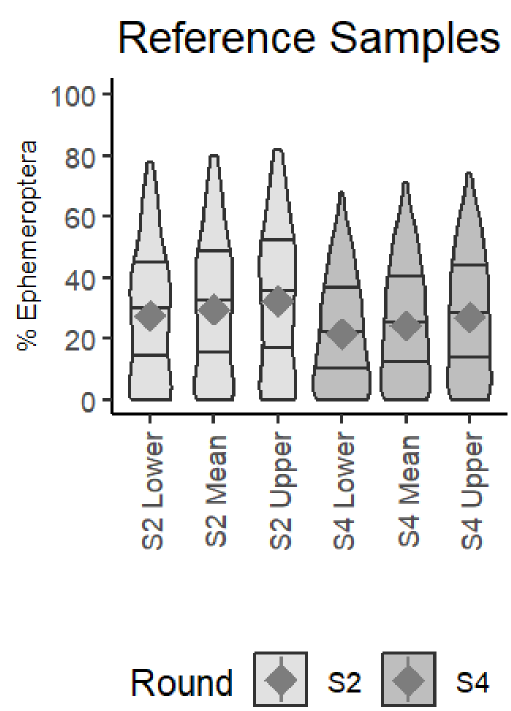


R2 vs. R4/2 & S2 vs. S4 Comparisons

% Ephemeroptera

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	11.4	8.93	29.7	24.3
Median	3.75	2.68	29.0	22.0
St. Dev.	15.6	12.8	22.60	18.7
Range	0 - 71.9	0 - 66.8	0 - 79.6	0 - 71.1
Wilcoxon Test	0.003		0.003	
95% CI	-3.93, -0.78		-9.54, -1.88	
Direction	**Lower**		**Lower**	

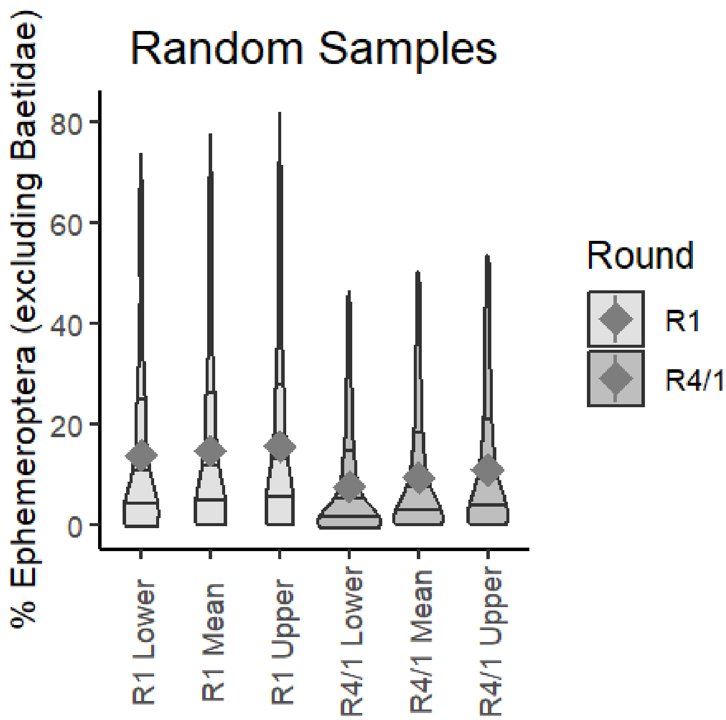




R1 vs. R4/1 Comparisons

% Ephemeroptera (excluding Baetidae)

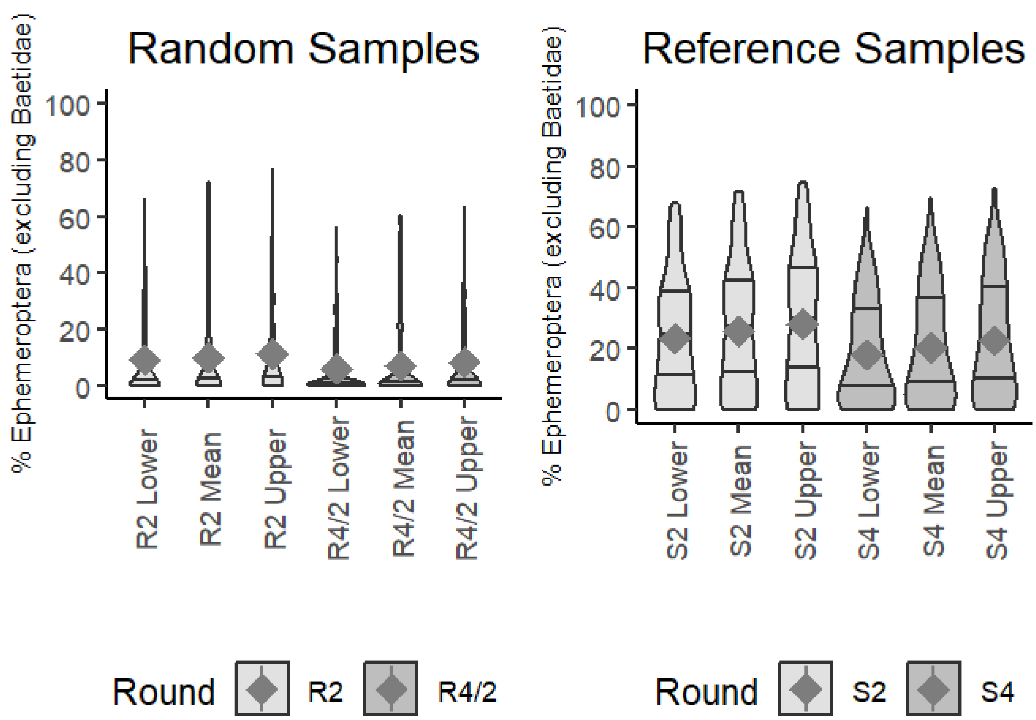
	RANDOM	
	R1	**R4/1**
N (Samples)	144	144
Unique Sites	144	144
Mean	14.82	9.45
Median	6.53	4.06
St. Dev.	18.47	12.52
Range	0 - 77.8	0 - 50.0
Wilcoxon Test	< 0.001	
95% CI	-7.63, -2.59	
Direction	**Lower**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

% Ephemeroptera (excluding Baetidae)

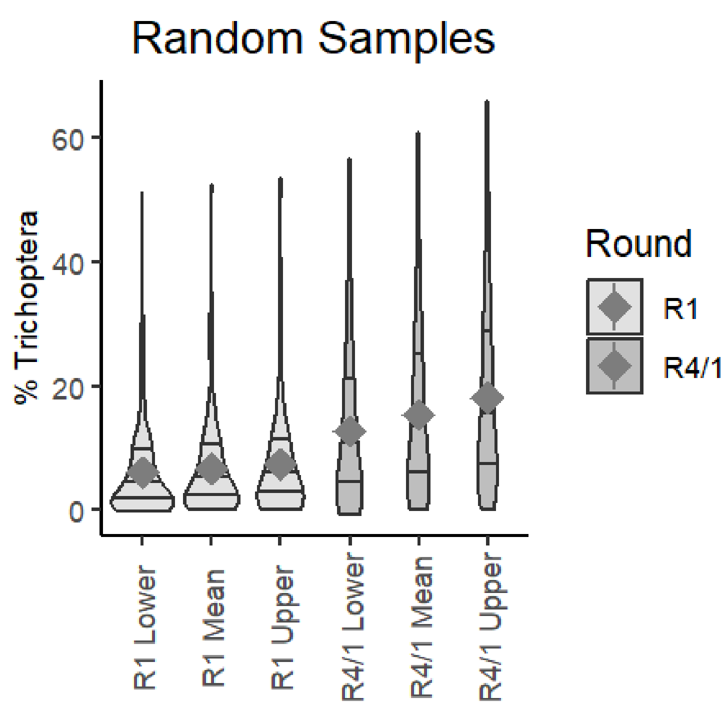
	RANDOM		**REFERENCE**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	10.1	7.07	25.6	20.2
Median	2.91	1.57	22.9	16.2
St. Dev.	14.9	11.7	20.6	17.8
Range	0 - 71.9	0 - 60.1	0 - 71.3	0 - 69.3
Wilcoxon Test	< 0.001		0.001	
95% CI	-4.26, -1.33		-8.00, -1.92	
Direction	**Lower**		**Lower**	



R1 vs. R4/1 Comparisons

% Trichoptera

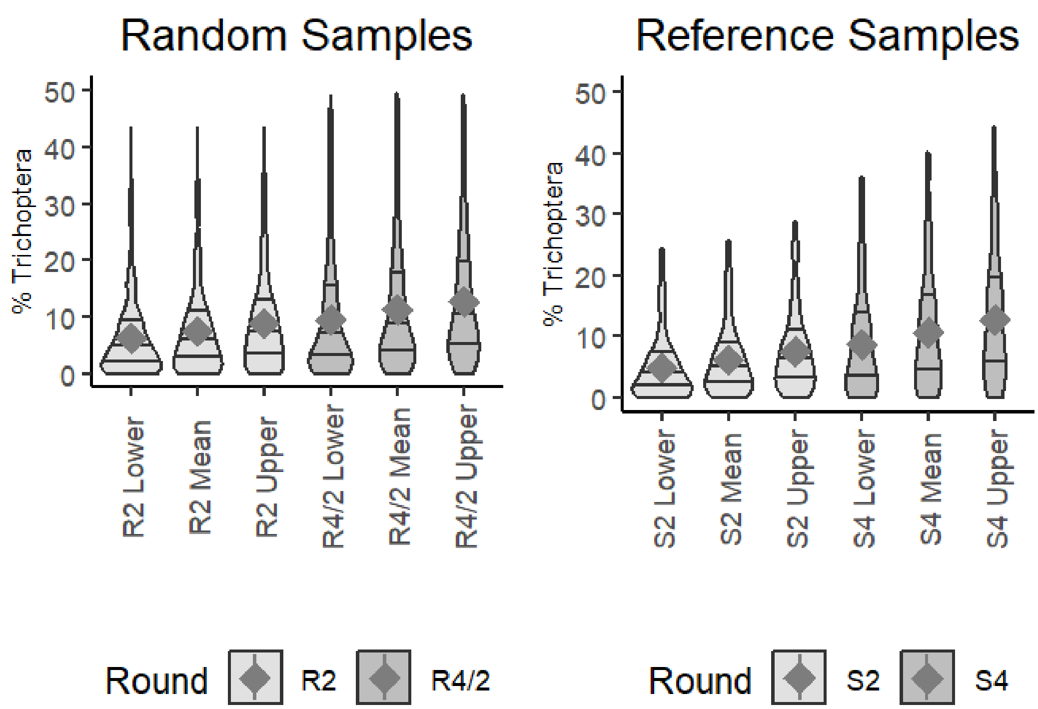
	RANDOM	
	R1	**R4/1**
N (Samples)	144	144
Unique Sites	144	144
Mean	6.74	15.5
Median	4.18	9.84
St. Dev.	8.15	14.8
Range	0 - 52.5	0 - 60.9
Wilcoxon Test	< 0.001	
95% CI	4.87, 10.5	
Direction	**Higher**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

% Trichoptera

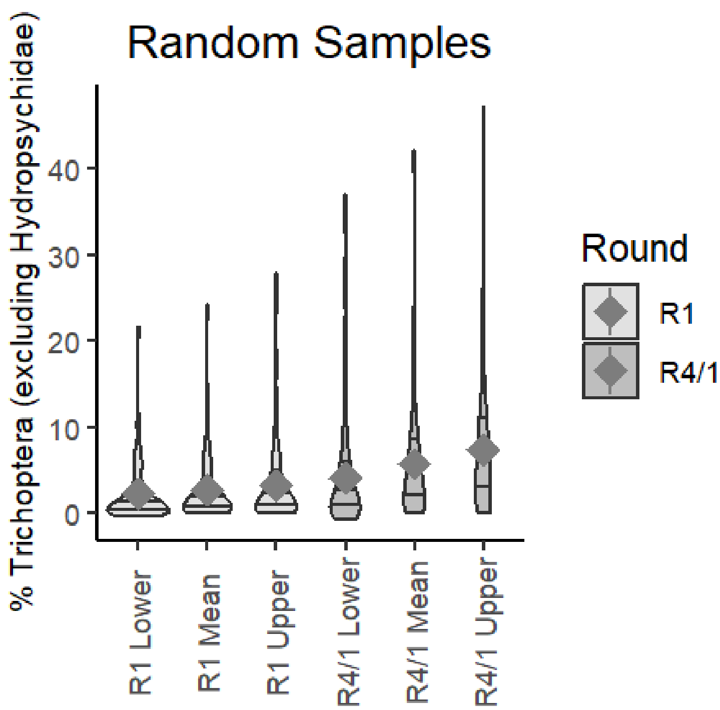
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	7.71	12.0	6.18	10.7
Median	4.88	6.70	4.44	7.95
St. Dev.	9.04	13.0	6.06	9.52
Range	0 - 68.8	0 - 73.0	0 - 25.5	0 - 40.2
Wilcoxon Test	< 0.001		< 0.001	
95% CI	1.86, 4.36		2.40, 5.63	
Direction	**Higher**		**Higher**	



R1 vs. R4/1 Comparisons

% Trichoptera (excluding Hydropsychidae)

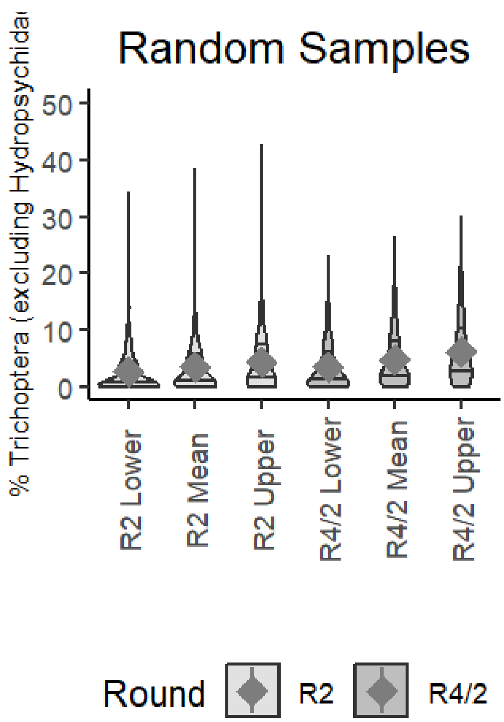
	RANDOM	
	R1	**R4/1**
N (Samples)	144	144
Unique Sites	144	144
Mean	2.74	5.75
Median	1.16	3.60
St. Dev.	4.12	6.92
Range	0 - 24.2	0 - 42.2
Wilcoxon Test	< 0.001	
95% CI	1.79, 3.64	
Direction	**Higher**	

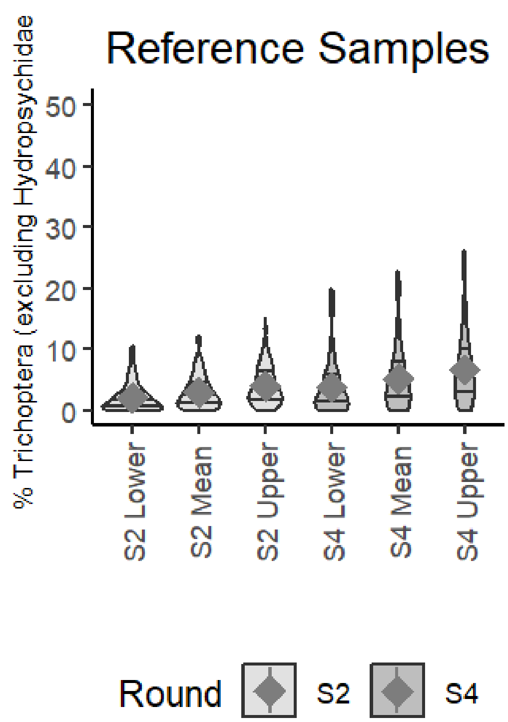


R2 vs. R4/2 & S2 vs. S4 Comparisons

% Trichoptera (excluding Hydropsychidae)

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	3.41	4.73	2.97	5.22
Median	1.71	3.04	2.22	3.40
St. Dev.	4.89	4.99	2.70	5.14
Range	0 - 38.6	0 - 26.6	0 - 12.0	0 - 22.8
Wilcoxon Test	< 0.001		< 0.001	
95% CI	0.69, 1.99		0.77, 2.77	
Direction	**Higher**		**Higher**	

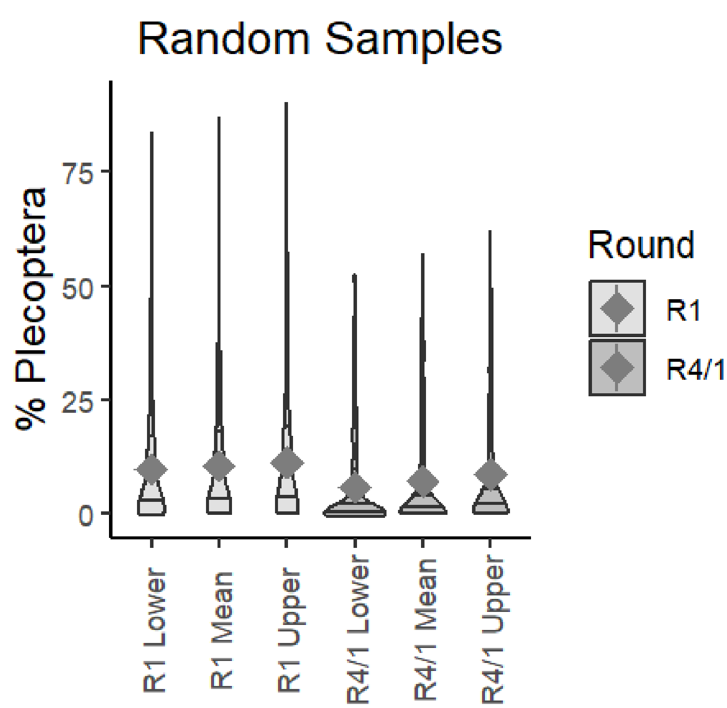




R1 vs. R4/1 Comparisons

% Plecoptera

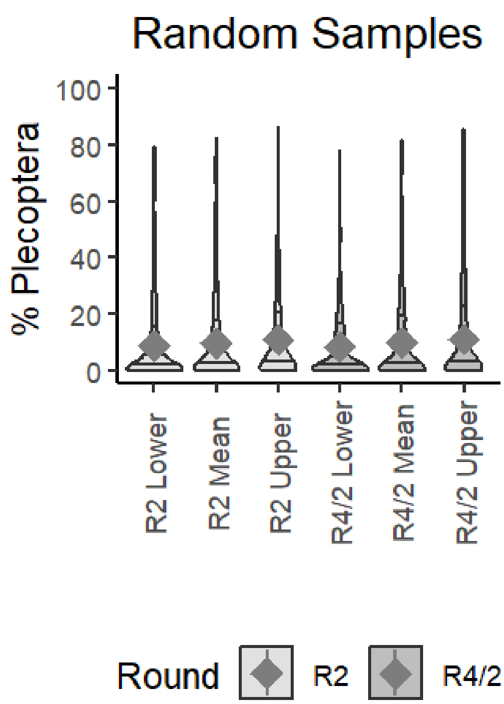
	RANDOM	
	R1	**R4/1**
N (Samples)	144	144
Unique Sites	144	144
Mean	10.67	7.31
Median	4.43	1.59
St. Dev.	15.30	11.81
Range	0 - 86.98	0 - 57.22
Wilcoxon Test	0.004	
95% CI	-5.90, -0.98	
Direction	**Lower**	

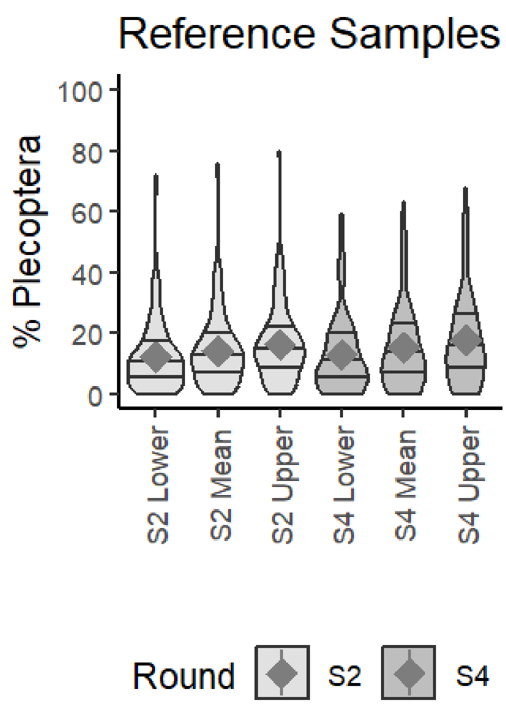


R2 vs. R4/2 & S2 vs. S4 Comparisons

% Plecoptera

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	9.62	9.62	14.2	15.2
Median	1.95	0.88	12.3	11.0
St. Dev.	15.7	16.1	11.8	14.0
Range	0 - 82.9	0 - 81.8	0 - 75.8	0 - 63.3
Wilcoxon Test	0.775		0.563	
95% CI	-1.58, 1.24		-1.66, 3.28	
Direction	**No Change**		**No Change**	

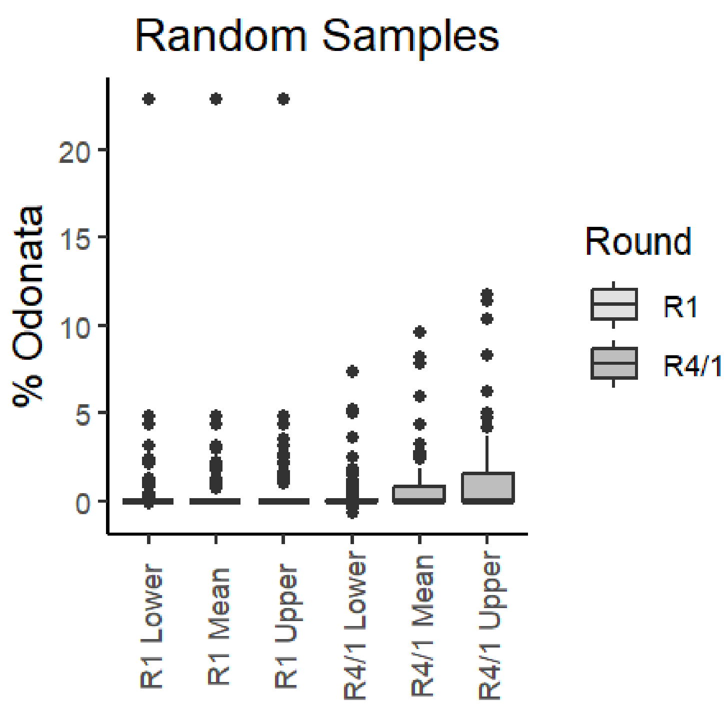




R1 vs. R4/1 Comparisons

% Odonata

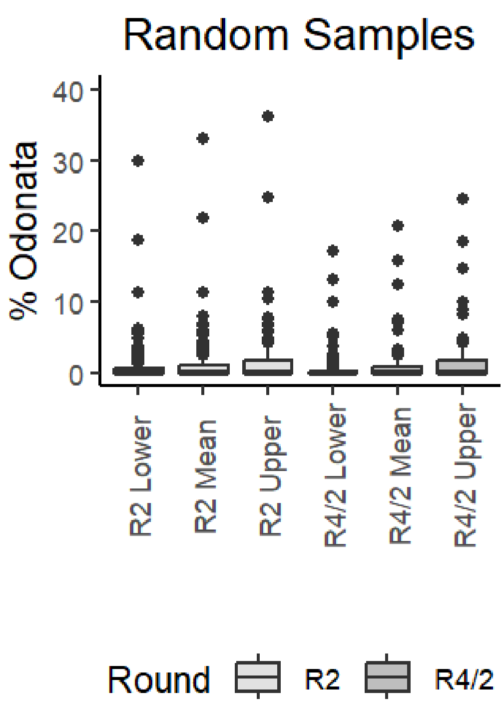
	RANDOM	
	R1	**R4/1**
N (Samples)	144	144
Unique Sites	144	144
Mean	0.46	0.66
Median	0	0
St. Dev.	2.04	1.49
Range	0 - 22.9	0 - 9.54
Wilcoxon Test	0.044	
95% CI	0.005, 1.129	
Direction	**No Change**	

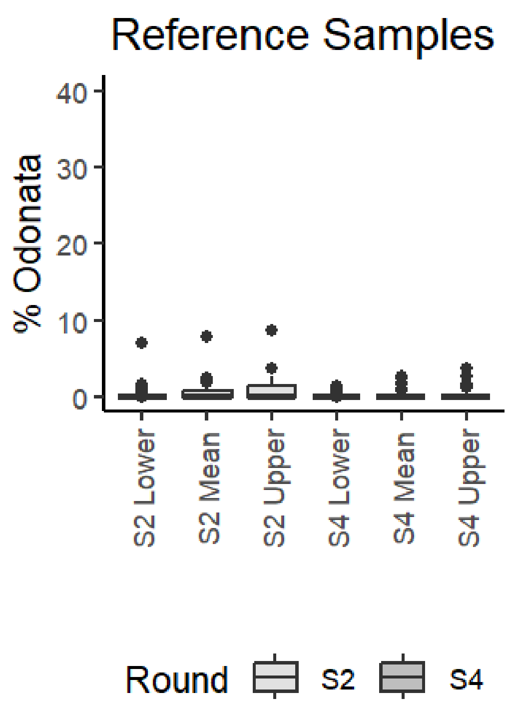


R2 vs. R4/2 & S2 vs. S4 Comparisons

% Odonata

	RANDOM		REFERENCE	
	R2	R4/2	S2	S4
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	1.00	0.80	0.37	0.27
Median	0	0	0	0
St. Dev.	2.90	2.13	0.92	0.60
Range	0 - 33.1	0 - 20.8	0 - 7.85	0 - 2.61
Wilcoxon Test	0.146		0.354	
95% CI	-0.55, 0.05		-0.77, 0.33	
Direction	**No Change**		**No Change**	

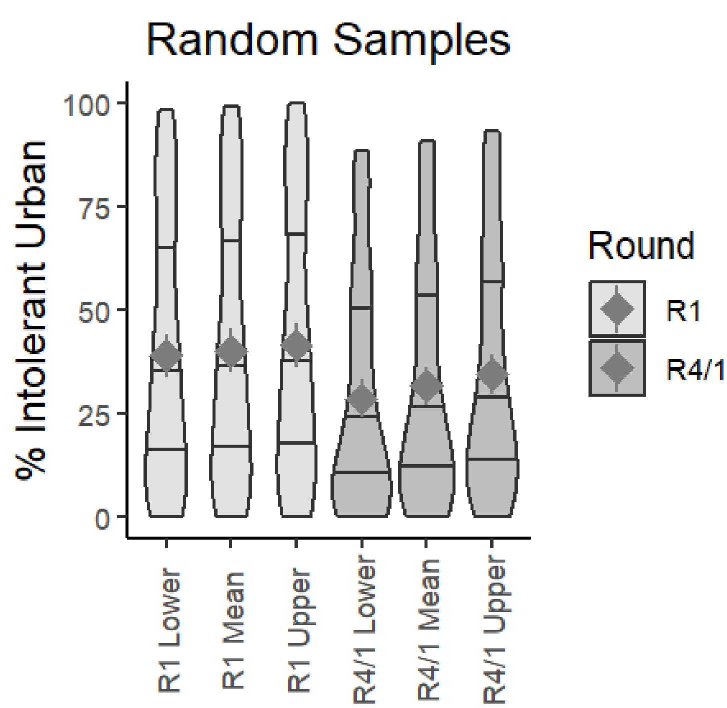




R1 vs. R4/1 Comparisons

% Intolerant Urban

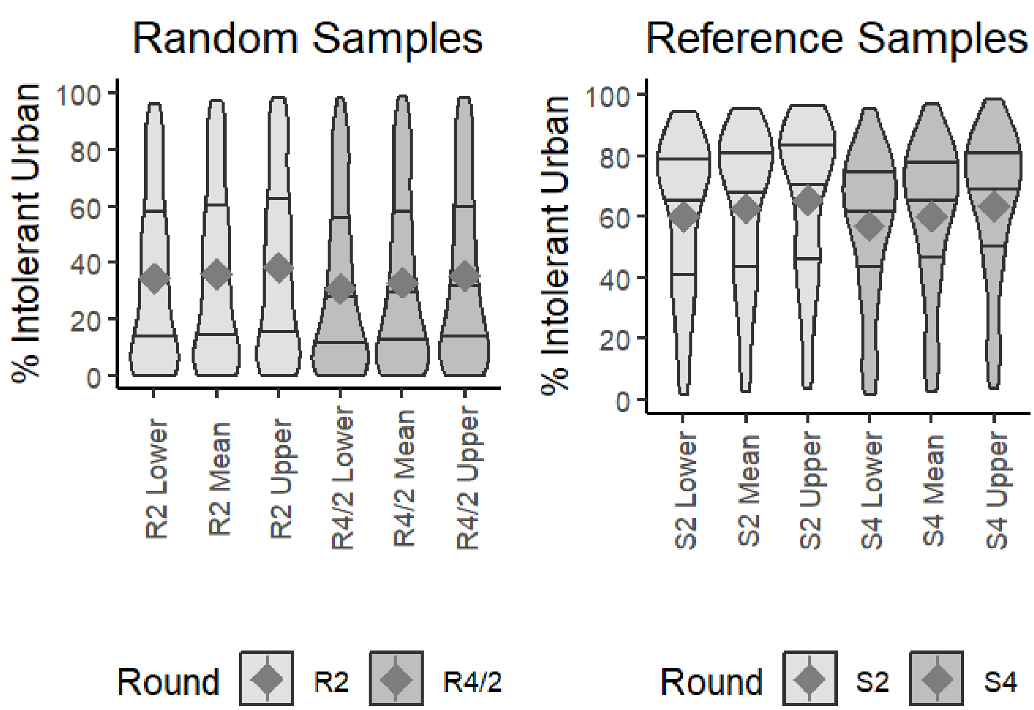
	RANDOM	
	R1	**R4/1**
N (Samples)	144	144
Unique Sites	144	144
Mean	40.3	31.6
Median	31.7	22.6
St. Dev.	31.5	28.3
Range	0 - 99.2	0 - 90.9
Wilcoxon Test	< 0.001	
95% CI	-11.5, -4.38	
Direction	**Lower**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

% Intolerant Urban

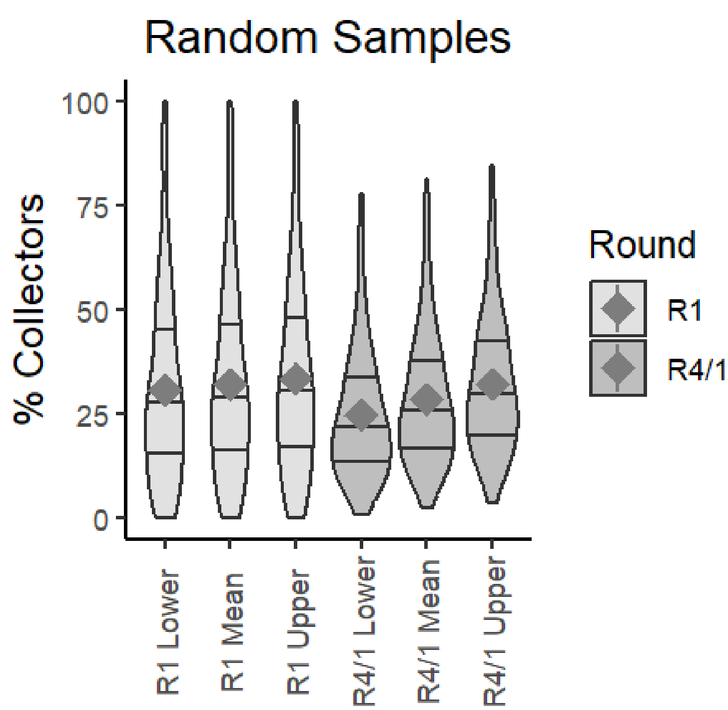
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	35.9	32.9	62.7	60.2
Median	30.3	24.3	71.6	66.0
St. Dev.	30.1	29.6	25.4	24.0
Range	0 - 97.5	0 - 99.3	2.56 - 95.4	2.62 - 97.0
Wilcoxon Test	0.018		0.166	
95% CI	-4.97, -0.42		-6.81, 1.23	
Direction	**No Change**		**No Change**	



R1 vs. R4/1 Comparisons

% Collectors

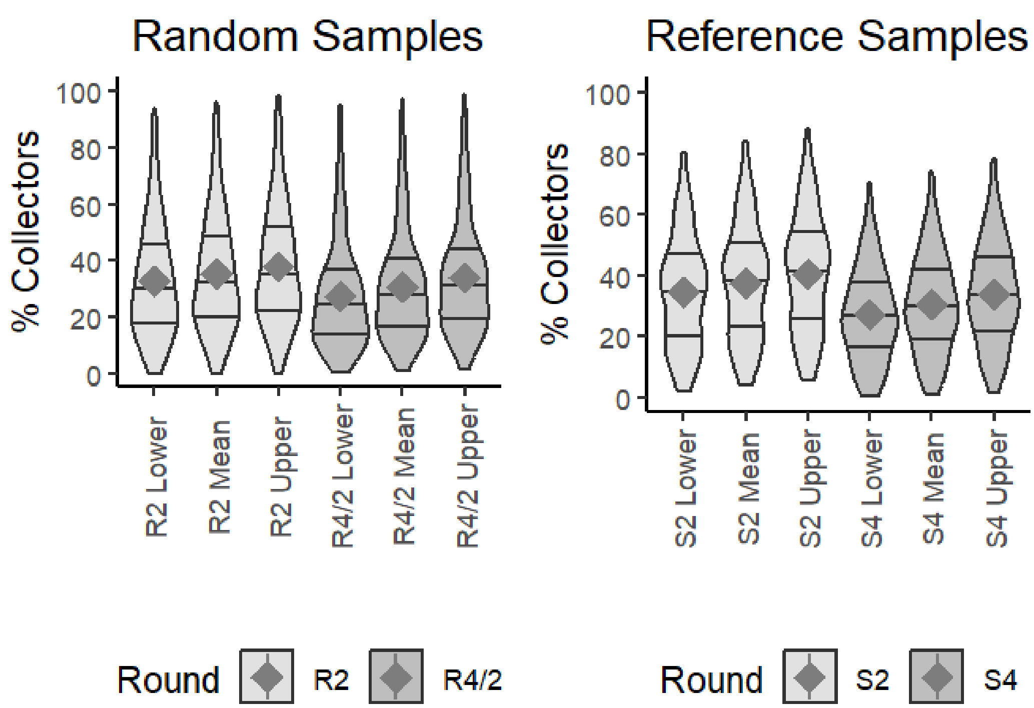
	RANDOM	
	R1	**R4/1**
N (Samples)	144	144
Unique Sites	144	144
Mean	32.2	28.6
Median	27.4	25.2
St. Dev.	23.0	16.1
Range	0 - 100	2.39 - 81.2
Wilcoxon Test	0.345	
95% CI	-6.25, 2.06	
Direction	**No Change**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

% Collectors

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	243	243	110	110
Unique Sites	243	243	22	22
Mean	35.2	30.7	37.5	30.6
Median	31.7	27.7	38.9	29.6
St. Dev.	20.7	19.5	18.7	16.0
Range	0 - 96.1	0.85 - 97.1	3.93 - 84.0	0.91 - 74.2
Wilcoxon Test 95% CI Direction	< 0.001 -8.53, -2.54 **Lower**		< 0.001 -10.7, -2.69 **Lower**	



APPENDIX C - E
Raw Fish Counts by Round Type

Appendix C. Frequency of occurrence (number of sites observed) for fish species collected during Round One (1995 - 1997) random site sampling and 20 years later during Round Four repeat sampling of select Round One random sites (2015 - 2017).

Common Name	Scientific Name	Species Type	R1	R4/1
American Brook Lamprey	*Lethenteron appendix*	RTE	1	0
American Eel	*Anguilla rostrata*		69	69
Banded Killifish	*Fundulus diaphanus*		4	3
Banded Sunfish	*Enneacanthus obesus*	RTE	6	1
Black Crappie	*Pomoxis nigromaculatus*	Non-native Chesapeake	3	2
Blacknose Dace	*Rhinichthys atratulus*		79	78
Blue Ridge Sculpin	*Cottus caeruleomentum*		28	30
Bluegill	*Lepomis macrochirus*	Non-native Chesapeake	57	67
Bluehead Chub	*Nocomis leptocephalus*	Non-native	0	3
Bluespotted Sunfish	*Enneacanthus gloriosus*		16	10
Bluntnose Minnow	*Pimephales notatus*		20	18
Brook Trout	*Salvelinus fontinalis*	RTE, Game	7	5
Brown Bullhead	*Ameiurus nebulosus*		20	14
Brown Trout	*Salmo trutta*	Non-native, Game	16	19
Central Stoneroller	*Campostoma anomalum*		16	19
Chain Pickerel	*Esox niger*		19	17
Channel Catfish	*Ictalurus punctatus*	Non-native Chesapeake	0	1
Checkered Sculpin	*Cottus sp.*		2	2
Chesapeake Logperch	*Percina bimaculata*	RTE	1	1
Comely Shiner	*Notropis amoenus*	RTE	1	1
Common Carp	*Cyprinus carpio*	Non-native	2	2
Common Shiner	*Luxilus cornutus*		31	30
Creek Chub	*Semotilus atromaculatus*		66	72
Creek Chubsucker	*Erimyzon oblongus*		32	32
Cutlip Minnow	*Exoglossum maxillingua*		30	32
Cutthroat Trout	*Oncorhynchus clarkii*	Non-native, Game	1	0
Cyprinid Hybrid	—		2	1
Eastern Mosquitofish	*Gambusia holbrooki*		3	19
Eastern Mudminnow	*Umbra pygmaea*		42	41
Eastern Silvery Minnow	*Hybognathus regius*		3	0
Fallfish	*Semotilus corporalis*		32	32
Fantail Darter	*Etheostoma flabellare*		12	16
Fathead Minnow	*Pimephales promelas*	Non-native	2	3
Flathead Catfish	*Pylodictis olivaris*	Non-native	0	1
Flier	*Centrarchus macropterus*	RTE	2	3
Glassy Darter	*Etheostoma vitreum*	RTE	1	1
Golden Redhorse	*Moxostoma erythrurum*		0	2
Golden Shiner	*Notemigonus crysoleucas*		23	15
Goldfish	*Carassius auratus*	Non-native	0	1
Green Sunfish	*Lepomis cyanellus*	Non-native Chesapeake	23	47
Greenside Darter	*Etheostoma blennioides*		9	10
Inland Silverside	*Menidia beryllina*		0	1
Ironcolor Shiner	*Notropis chalybaeus*	RTE	2	0
Johnny Darter	*Etheostoma nigrum*	RTE	1	1
Largemouth Bass	*Micropterus salmoides*	Non-native Chesapeake, Game	29	43
Least Brook Lamprey	*Lampetra aepyptera*		20	21
Lepomis Hybrid	—		1	6
Longear Sunfish	*Lepomis megalotis*	Non-native Chesapeake	0	2
Longnose Dace	*Rhinichthys cataractae*		44	48
Longnose Gar	*Lepisosteus osseus*		1	0

Margined Madtom	*Noturus insignis*		35	34
Mottled Sculpin	*Cottus bairdii*		5	5
Mud Sunfish	*Acantharchus pomotis*	RTE	3	1
Mummichog	*Fundulus heteroclitus*		2	2
Northern Hog Sucker	*Hypentelium nigricans*		31	31
Northern Snakehead	*Channa argus*	Non-native	0	2
Notropis Hybrid	—		0	1
Pearl Dace	*Margariscus margarita*	RTE	1	1
Pirate Perch	*Aphredoderus sayanus*		27	26
Potomac Sculpin	*Cottus girardi*		9	11
Pumpkinseed	*Lepomis gibbosus*	Non-native Youghiogheny	48	45
Rainbow Darter	*Etheostoma caeruleum*	Non-native	0	3
Rainbow Trout	*Oncorhynchus mykiss*	Non-native, Game	6	3
Redbreast Sunfish	*Lepomis auritus*		38	46
Redear Sunfish	*Lepomis microlophus*	Non-native Chesapeake	0	2
Redfin Pickerel	*Esox americanus*		29	21
River Chub	*Nocomis micropogon*		15	23
Rock Bass	*Ambloplites rupestris*	Non-native Chesapeake	9	10
Rosyface Shiner	*Notropis rubellus*		6	12
Rosyside Dace	*Clinostomus funduloides*		49	49
Satinfin Shiner	*Cyprinella analostana*		17	21
Sea Lamprey	*Petromyzon marinus*		10	15
Shield Darter	*Percina peltata*	RTE	8	3
Silverjaw Minnow	*Notropis buccatus*		5	8
Smallmouth Bass	*Micropterus dolomieu*	Non-native Chesapeake, Game	17	18
Spotfin Shiner	*Cyprinella spiloptera*		3	12
Spottail Shiner	*Notropis hudsonius*		14	14
Striped Shiner	*Luxilus chrysocephalus*	RTE	1	1
Swallowtail Shiner	*Notropis procne*		25	23
Swamp Darter	*Etheostoma fusiforme*	RTE	5	3
Tadpole Madtom	*Noturus gyrinus*		19	19
Tessellated Darter	*Etheostoma olmstedi*		67	71
Tiger Trout	*Salmo trutta* × *Salvelinus fontinalis*	Non-native, Game	0	1
Warmouth	*Lepomis gulosus*	RTE	5	5
White Catfish	*Ameiurus catus*		2	0
White Perch	*Morone americana*		0	1
White Sucker	*Catostomus commersonii*		69	66
Yellow Bullhead	*Ameiurus natalis*		15	24
Yellow Perch	*Perca flavescens*	Non-native Youghiogheny	13	7

Appendix D. Frequency of occurrence (number of sites observed) for fish species collected during Round Two (2000-2004) random site sampling and 14 years later during Round Four repeat sampling of select Round Two random sites (2014 - 2018).

Common Name	Scientific Name	Species Type	R2	R4/2
Alewife	*Alosa pseudoharengus*		1	0
American Brook Lamprey	*Lethenteron appendix*	RTE	3	4
American Eel	*Anguilla rostrata*		104	114
Banded Killifish	*Fundulus diaphanus*		7	5
Banded Sunfish	*Enneacanthus obesus*	RTE	3	2
Black Crappie	*Pomoxis nigromaculatus*	Non-native Chesapeake	6	5
Blacknose Dace	*Rhinichthys atratulus*		131	148
Blue Ridge Sculpin	*Cottus bairdii*		45	43
Blueback Herring	*Alosa aestivalis*		1	84
Bluegill	*Lepomis macrochirus*	Non-native Chesapeake	73	5
Bluespotted Sunfish	*Enneacanthus gloriosus*		18	15
Bluntnose Minnow	*Pimephales notatus*		32	32
Brook Trout	*Salvelinus fontinalis*	RTE, Game	9	10
Brown Bullhead	*Ameiurus nebulosus*		25	25
Brown Trout	*Salmo trutta*	Non-native, Game	13	14
Central Stoneroller	*Campostoma anomalum*		28	33
Chain Pickerel	*Esox niger*		19	18
Channel Catfish	*Ictalurus punctatus*	Non-native Chesapeake	0	2
Checkered Sculpin	*Cottus sp. 7*	RTE	2	2
Common Carp	*Cyprinus carpio*	Non-native	2	1
Common Shiner	*Luxilus cornutus*		30	30
Creek Chub	*Semotilus atromaculatus*		106	115
Creek Chubsucker	*Erimyzon oblongus*		42	41
Cutlip Minnow	*Exoglossum maxillingua*		28	32
Cyprinid (Unknown)	—		1	2
Cyprinid Hybrid	—		1	2
Eastern Mosquitofish	*Gambusia holbrooki*		10	30
Eastern Mudminnow	*Umbra pygmaea*		79	70
Eastern Silvery Minnow	*Hybognathus regius*		4	2
Fallfish	*Semotilus corporalis*		33	32
Fantail Darter	*Etheostoma flabellare*		26	32
Fathead Minnow	*Pimephales promelas*	Non-native	8	7
Flier	*Centrarchus macropterus*	RTE	0	1
Gizzard Shad	*Dorosoma cepedianum*		1	1
Glassy Darter	*Etheostoma blennioides*	RTE	1	0
Golden Redhorse	*Moxostoma erythrurum*		1	2
Golden Shiner	*Notemigonus crysoleucas*	Non-native Youghiogheny	30	24
Goldfish	*Carassius auratus*	Non-native	3	5
Green Sunfish	*Lepomis cyanellus*	Non-native Chesapeake	43	86
Greenside Darter	*Etheostoma nigrum*		18	13
Johnny Darter	*Etheostoma vitreum*	RTE	1	1
Largemouth Bass	*Micropterus salmoides*	Non-native Chesapeake, Game	48	51
Least Brook Lamprey	*Lampetra aepyptera*		34	33
Lepomis Hybrid	—		3	11
Longear Sunfish	*Lepomis megalotis*	Non-native Chesapeake	2	4
Longnose Dace	*Rhinichthys cataractae*		61	62
Margined Madtom	*Noturus insignis*		30	36
Mimic Shiner	*Notropis volucellus*	Non-native	0	1
Mottled Sculpin	*Cottus girardi*		4	4
Mud Sunfish	*Acantharchus pomotis*	RTE	3	2

Mummichog	*Fundulus heteroclitus*		3	8
Northern Hogsucker	*Hypentelium nigricans*		31	32
Northern Snakehead	*Channa argus*	Non-native	0	3
Notropis Hybrid	—		0	1
Pearl Dace	*Margariscus margarita*	RTE	3	3
Pirate Perch	*Aphredoderus sayanus*		27	25
Potomac Sculpin	*Cottus caeruleomentum*		22	20
Pumpkinseed	*Lepomis gibbosus*	Non-native Youghiogheny	58	52
Rainbow Darter	*Percina peltata*	Non-native	7	9
Rainbow Trout	*Oncorhynchus mykiss*	Non-native, Game	6	5
Redbreast Sunfish	*Lepomis auritus*		49	53
Redfin Pickerel	*Esox americanus*		35	21
River Chub	*Nocomis micropogon*		23	23
Rock Bass	*Ambloplites rupestris*	Non-native Chesapeake	19	17
Rosyface Shiner	*Notropis rubellus*		8	12
Rosyside Dace	*Clinostomus funduloides*		62	67
Satinfin Shiner	*Cyprinella analostana*		15	12
Sea Lamprey	*Petromyzon marinus*		15	18
Shield Darter	*Etheostoma fusiforme*	RTE	4	6
Silverjaw Minnow	*Notropis buccatus*		5	8
Smallmouth Bass	*Micropterus dolomieu*	Non-native Chesapeake, Game	28	31
Spotfin Shiner	*Cyprinella spiloptera*		13	11
Spottail Shiner	*Notropis hudsonius*		11	21
Striped Bass	*Morone saxatilis*		1	2
Striped Shiner	*Luxilus chrysocephalus*	RTE	0	1
Sunfish (Hybrid)	—		0	3
Swallowtail Shiner	*Notropis procne*		27	31
Swamp Darter	*Etheostoma caeruleum*	RTE	8	5
Tadpole Madtom	*Noturus gyrinus*		16	18
Tessellated Darter	*Etheostoma olmstedi*		87	93
Tiger Muskellunge	*Esox lucius × masquinongy*	Non-native	0	1
Warmouth	*Lepomis gulosus*	RTE	4	4
White Catfish	*Ameiurus catus*		0	2
White Perch	*Morone americana*		2	1
White Sucker	*Catostomus commersonii*		95	89
Yellow Bullhead	*Ameiurus natalis*		29	45
Yellow Perch	*Perca flavescens*	Non-native Youghiogheny	3	2

Appendix E. Frequency of occurrence (number of samples observed) for fish species collected during five annual Round Two (S2; 2000-2004) reference site samples and 14 years later during Round Four annual repeat sampling of Round Four reference sites (S4; 2014 - 2018).

Common Name	Scientific Name	Species Type	S2	S4
Alewife	*Alosa pseudoharengus*		1	0
American Eel	*Anguilla rostrata*		33	37
American Shad	*Alosa sapidissima*		0	1
Banded Sunfish	*Enneacanthus obesus*	RTE	3	4
Black Crappie	*Pomoxis nigromaculatus*	Non-native Chesapeake	3	6
Blacknose Dace	*Rhinichthys atratulus*		61	64
Blue Ridge Sculpin	*Cottus caeruleomentum*		31	30
Bluegill	*Lepomis macrochirus*	Non-native Chesapeake	29	30
Bluespotted Sunfish	*Enneacanthus gloriosus*		4	4
Bluntnose Minnow	*Pimephales notatus*		5	5
Brook Trout	*Salvelinus fontinalis*	RTE, Game	34	29
Brown Bullhead	*Ameiurus nebulosus*		19	13
Brown Trout	*Salmo trutta*	Non-native, Game	12	17
Central Stoneroller	*Campostoma anomalum*		6	6
Chain Pickerel	*Esox niger*		14	10
Channel Catfish	*Ictalurus punctatus*	Non-native Chesapeake	0	1
Common Carp	*Cyprinus carpio*	Non-native	0	1
Common Shiner	*Luxilus cornutus*		3	3
Creek Chub	*Semotilus atromaculatus*		40	42
Creek Chubsucker	*Erimyzon oblongus*		23	20
Cutlip Minnow	*Exoglossum maxillingua*		13	15
Eastern Mosquitofish	*Gambusia holbrooki*		8	9
Eastern Mudminnow	*Umbra pygmaea*		40	40
Fallfish	*Semotilus corporalis*		11	12
Fantail Darter	*Etheostoma flabellare*		10	10
Golden Shiner	*Notemigonus crysoleucas*	Non-native Youghiogheny	13	13
Green Sunfish	*Lepomis cyanellus*	Non-native Chesapeake	11	30
Greenside Darter	*Etheostoma blennioides*		5	5
Largemouth Bass	*Micropterus salmoides*	Non-native Chesapeake, Game	15	21
Least Brook Lamprey	*Lampetra aepyptera*		15	22
Lepomis Hybrid	--		0	7
Longnose Dace	*Rhinichthys cataractae*		25	22
Longnose Gar	*Lepisosteus osseus*		1	0
Margined Madtom	*Noturus insignis*		11	11
Mottled Sculpin	*Cottus bairdii*		5	5
Mud Sunfish	*Acantharchus pomotis*	RTE	2	2
Northern Hogsucker	*Hypentelium nigricans*		2	0
Pirate Perch	*Aphredoderus sayanus*		17	15
Potomac Sculpin	*Cottus girardi*		10	10
Pumpkinseed	*Lepomis gibbosus*	Non-native Youghiogheny	24	24
Rainbow Darter	*Etheostoma caeruleum*	Non-native	0	1
Rainbow Trout	*Oncorhynchus mykiss*	Non-native	2	1
Redbreast Sunfish	*Lepomis auritus*		4	19
Redear Sunfish	*Lepomis microlophus*	Non-native Chesapeake	0	1
Redfin Pickerel	*Esox americanus*		15	12
River Chub	*Nocomis micropogon*		0	2
Rock Bass	*Ambloplites rupestris*	Non-native Chesapeake	5	5
Rosyside Dace	*Clinostomus funduloides*		38	34
Satinfin Shiner	*Cyprinella analostana*		1	0

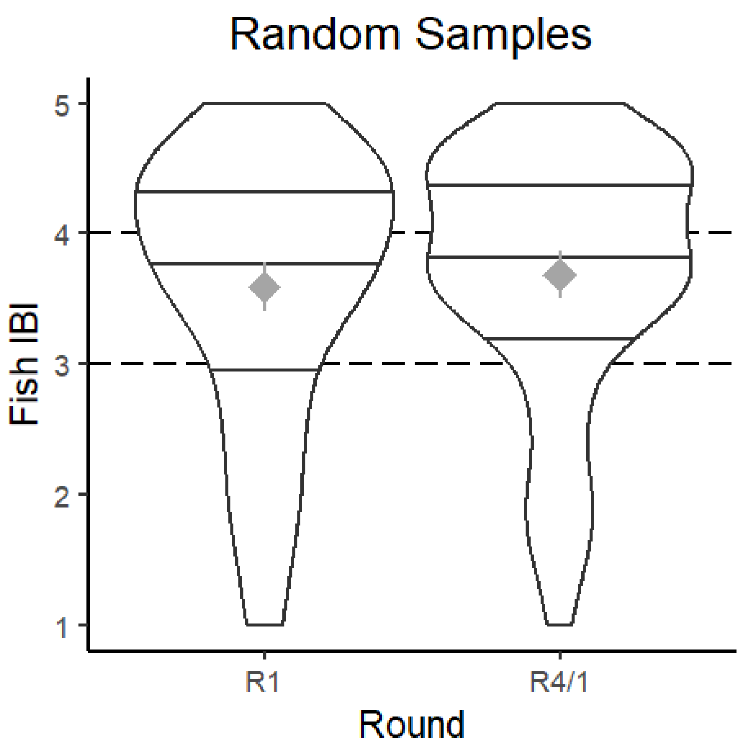
Sea Lamprey	*Petromyzon marinus*		5	13
Smallmouth Bass	*Micropterus dolomieu*	Non-native Chesapeake, Game	3	4
Spottail Shiner	*Cyprinella spiloptera*		6	3
Striped Bass	*Morone saxatilis*		1	0
Swallowtail Shiner	*Notropis procne*		1	0
Tadpole Madtom	*Noturus gyrinus*		10	12
Tessellated Darter	*Etheostoma olmstedi*		30	37
Warmouth	*Lepomis gulosus*	RTE	7	6
White Catfish	*Ameiurus catus*		1	1
White Perch	*Morone americana*		3	3
White Sucker	*Catostomus commersonii*		37	35
Yellow Bullhead	*Ameiurus natalis*		13	18
Yellow Perch	*Perca flavescens*		6	6

APPENDIX F
Fish Metric Statistics and Violin Plots

R1 vs. R4/1 Comparisons

Fish IBI

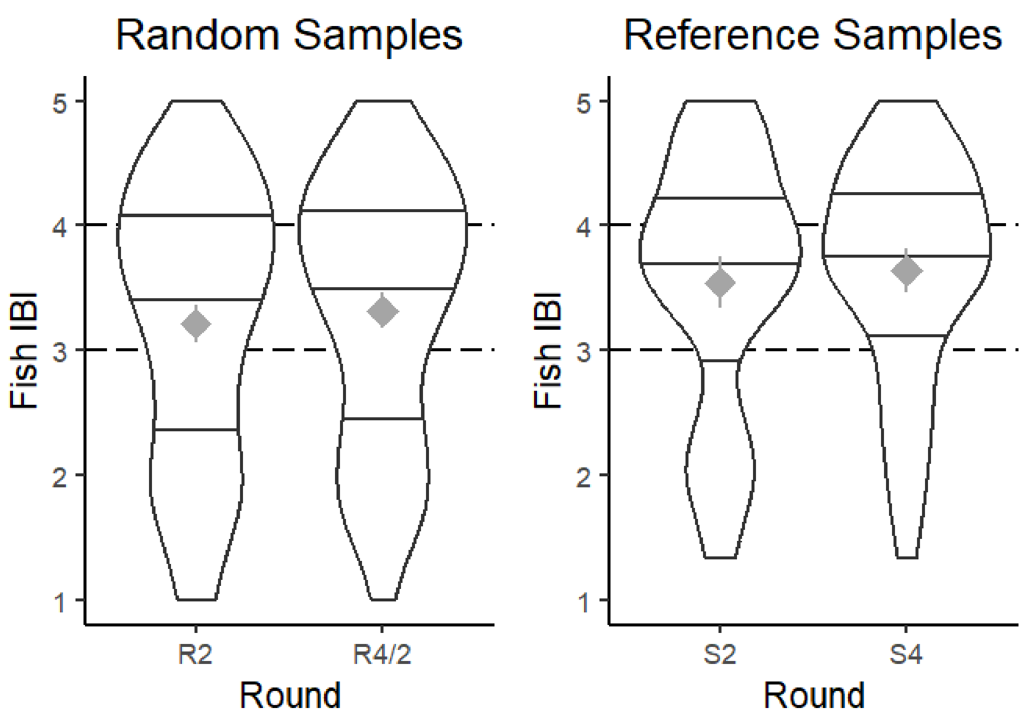
	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	3.59	3.68
Median	4.0	4.0
St. Dev.	1.05	0.98
Range	1.0 - 5.0	1.0 - 5.0
Wilcoxon test 95% CI Direction	0.318 -0.23, 0.048 **No Change**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Fish IBI

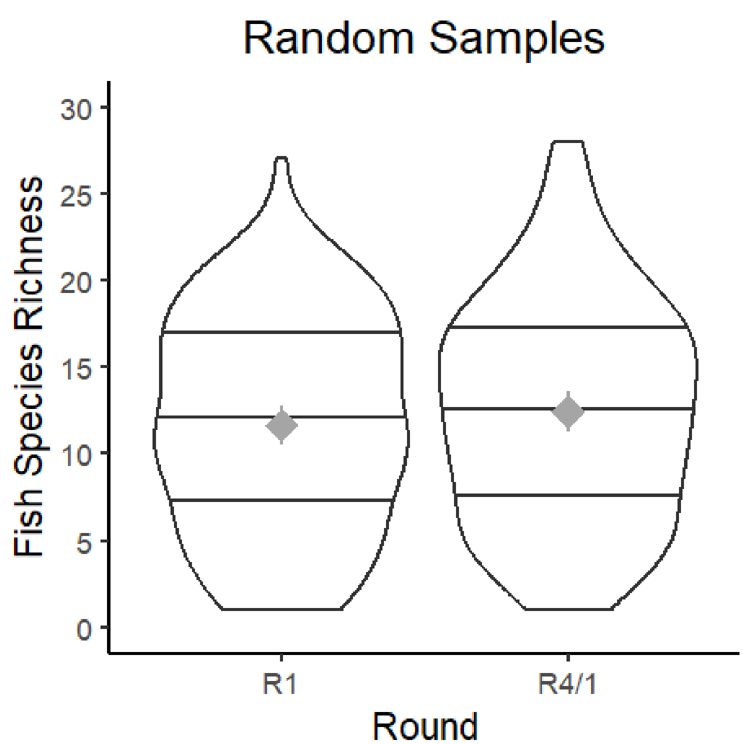
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	3.21	3.32	3.55	3.64
Median	3.33	3.67	3.67	3.67
St. Dev.	1.10	1.06	1.03	0.90
Range	1.0 - 5.0	1.0 - 5.0	1.33 - 5.0	1.33 - 5.0
Wilcoxon test 95% CI Direction	0.029 -0.20, -0.0084 **No Change**		0.089 -0.22, 0.02 **No Change**	



R1 vs. R4/1 Comparisons

Fish Species Richness

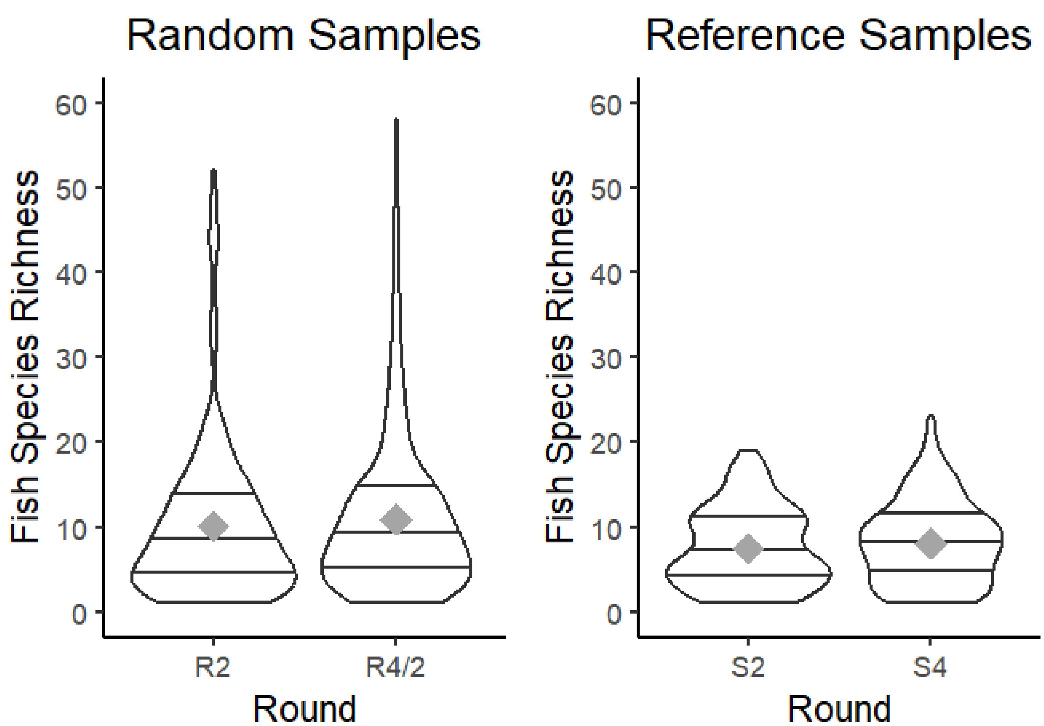
	RANDOM	
	R1	R4/1
N (Samples)	119	119
Unique Sites	119	119
Mean	11.66	12.45
Median	11.0	13.0
St. Dev.	6.21	6.41
Range	1 - 27	1 - 28
Wilcoxon test	0.040	
95% CI	-1.46, -0.14	
Direction	**No Change**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Fish Species Richness

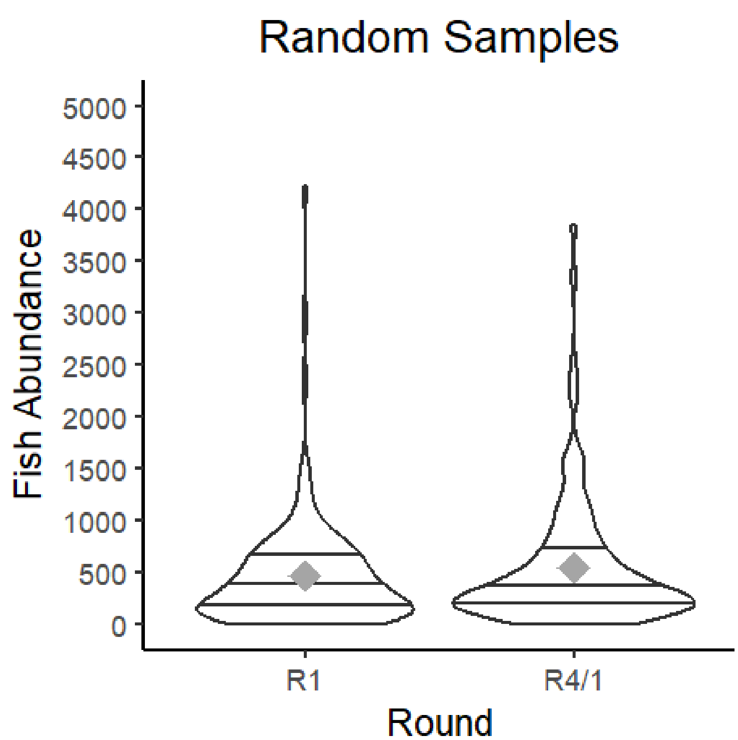
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	10.10	10.90	7.47	8.09
Median	8.0	9.0	6.0	7.5
St. Dev.	9.37	9.26	4.65	4.92
Range	1 - 52	1 - 58	1 - 19	1 - 23
Wilcoxon test 95% CI Direction	<0.001 -1.25, -0.35 **Higher**		0.007 -1.03, -0.21 **Higher**	



R1 vs. R4/1 Comparisons

Fish Abundance

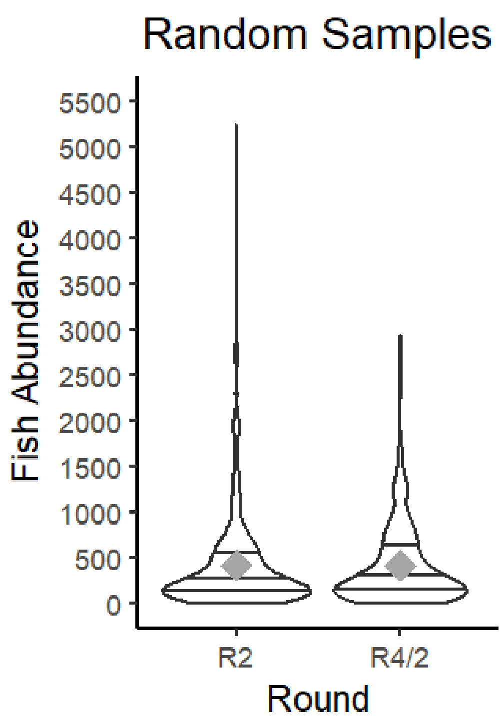
	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	463.97	545.67
Median	333	320
St. Dev.	561.28	632.86
Range	1 - 4,226	1 - 3,848
Wilcoxon test 95% CI Direction	0.109 -102.00, 9.50 **No Change**	

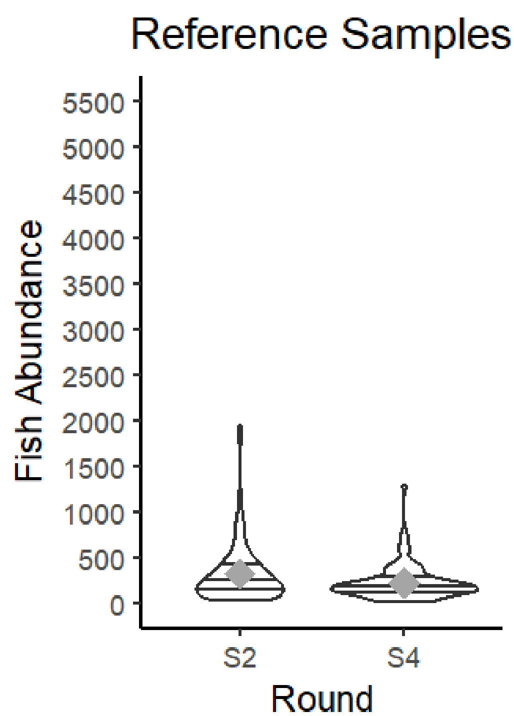


R2 vs. R4/2 & S2 vs. S4 Comparisons

Fish Abundance

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	414.94	412.22	320.25	222.94
Median	224	235	216.5	177
St. Dev.	580.95	469.23	326.64	191.74
Range	2 - 5,240	1 - 2,924	23 - 1,947	9 - 1,282
Wilcoxon test	0.554		0.007	
95% CI	-38,50, 19.50		30.00, 101.00	
Direction	**No Change**		**Lower**	

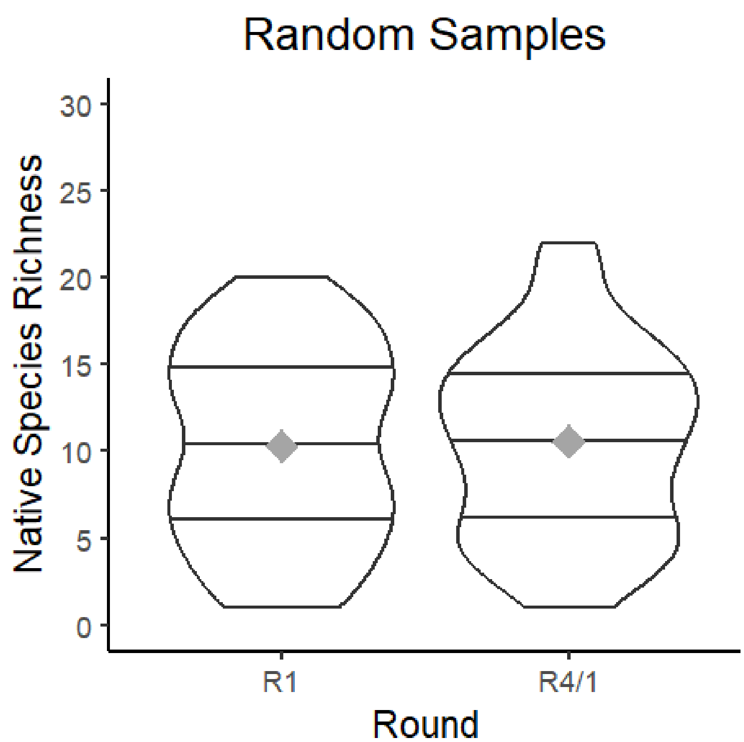




R1 vs. R4/1 Comparisons

Native Species Richness

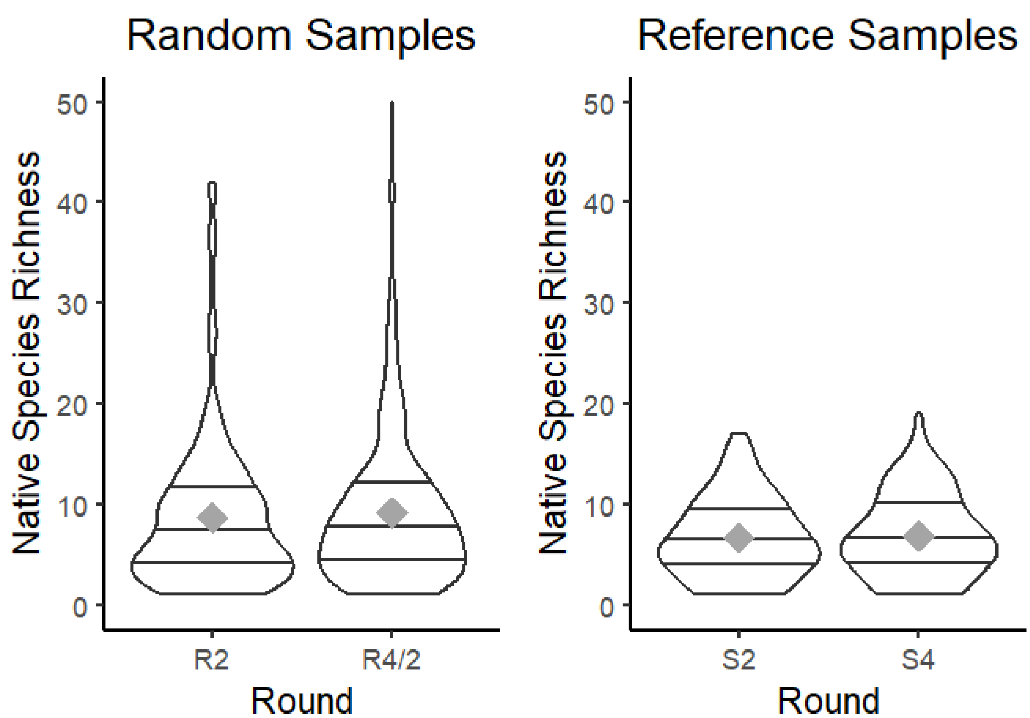
	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	10.27	10.51
Median	10.0	11.0
St. Dev.	5.52	5.42
Range	1 - 20	1 - 22
Wilcoxon test 95% CI Direction	0.634 -0.80, 0.32 **No Change**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Native Species Richness

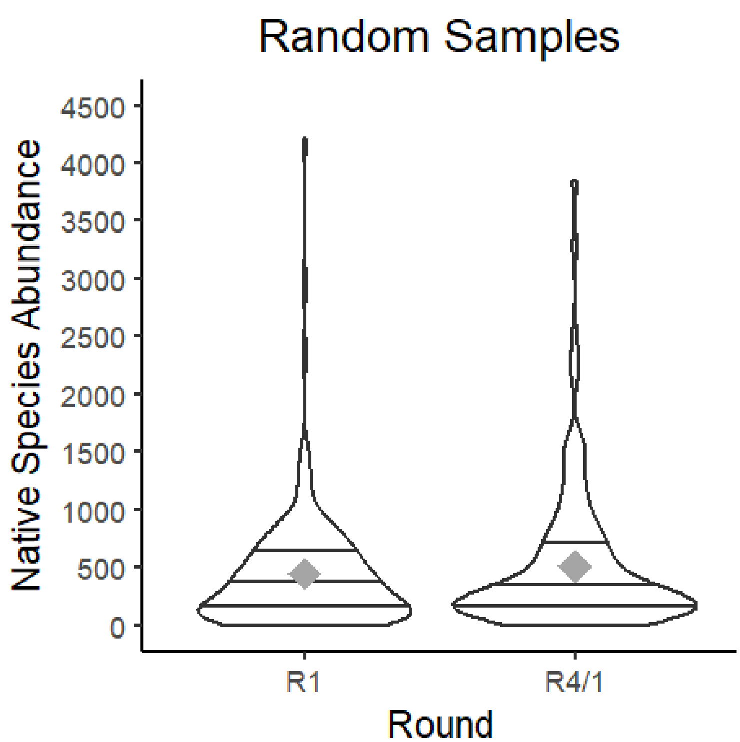
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	8.69	9.12	6.67	6.9
Median	7.0	7.0	6.0	6.0
St. Dev.	7.71	7.61	3.94	4.12
Range	1 - 42	1 - 50	1 - 17	1 - 19
Wilcoxon test 95% CI Direction	0.001 -0.84, -0.02 **Higher**		0.266 -0.57, 0.11 **No Change**	



R1 vs. R4/1 Comparisons

Native Species Abundance

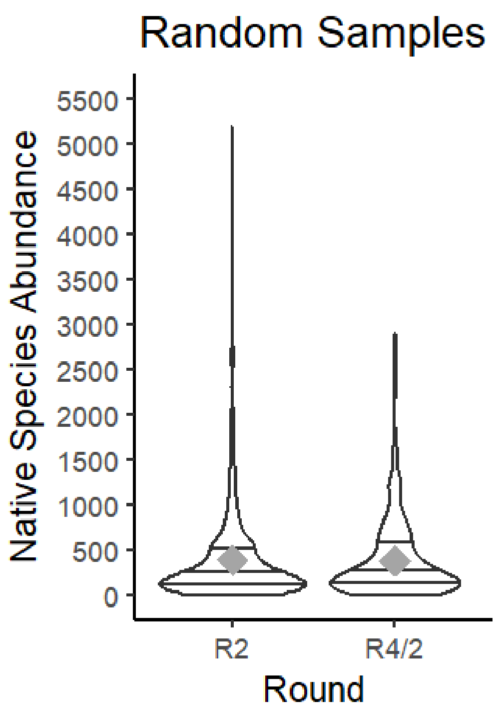
	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	445.99	511.94
Median	331	267
St. Dev.	560.75	629.69
Range	1 - 4218	1 - 3840
Wilcoxon test 95% CI Direction	0.233 -82.50, 21.00 **No Change**	

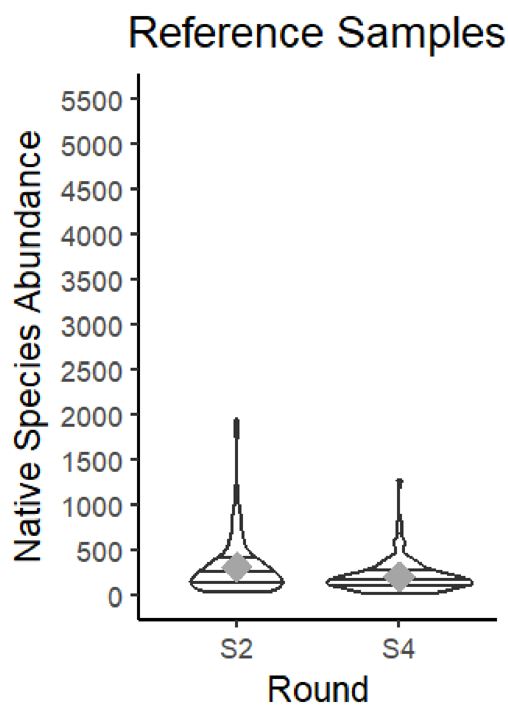


R2 vs. R4/2 & S2 vs. S4 Comparisons

Native Species Abundance

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	388.81	381.12	309.46	203.26
Median	211	215	210.5	164.5
St. Dev.	562.13	451.25	325.13	184.40
Range	2 - 5,190	1 - 2,890	23 - 1,947	9 - 1,277
Wilcoxon test 95% CI Direction	0.731 -33.50, 22.50 **No Change**		<0.001 38.00, 107.00 **Lower**	

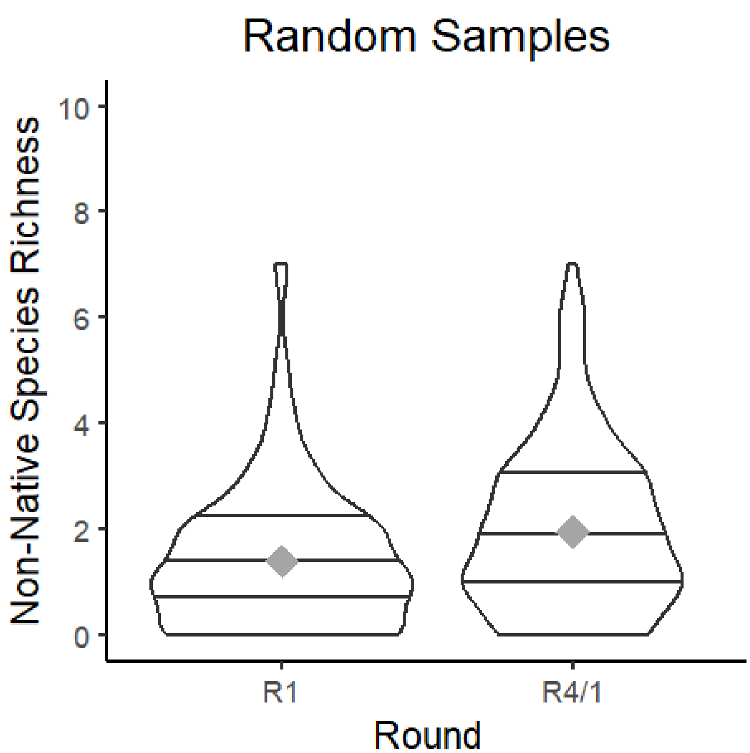




R1 vs. R4/1 Comparisons

Non-Native Species Richness

	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	1.39	1.94
Median	1.0	2.0
St. Dev.	1.38	1.58
Range	0 - 7	0 - 7
Wilcoxon test 95% CI Direction	< 0.001 -0.79, -0.32 **Higher**	

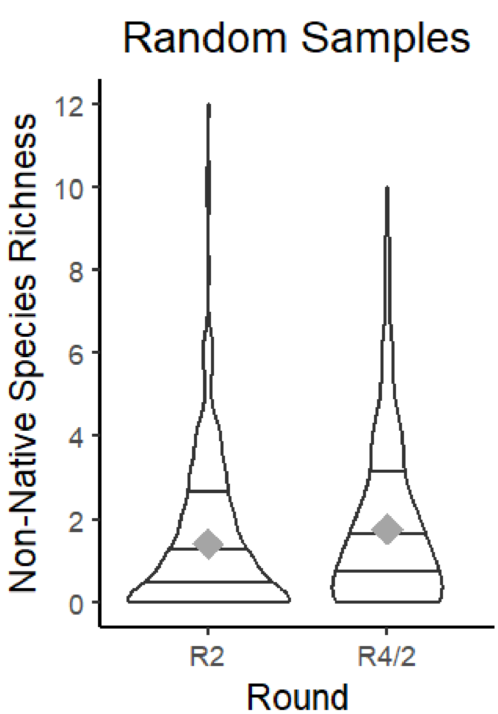


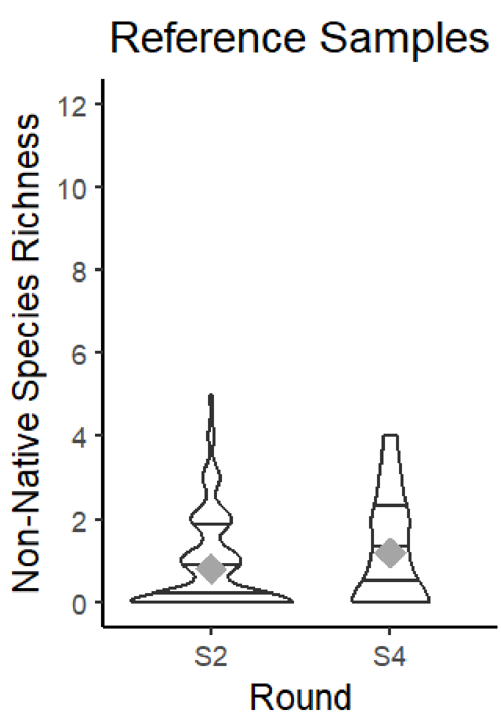
R2 vs. R4/2 & S2 vs. S4 Comparisons

Non-Native Species Richness

Note: * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	1.41	1.77	0.8	1.19
Median	1.0	1.0	0.0	1.0
St. Dev.	1.95	2.06	1.12	1.30
Range	0 - 12	0 - 10	0 - 5	0 - 4
Wilcoxon test 95% CI Direction	<0.001 -0.53, -0.19 **Higher**		<0.001* -0.58, -0.20 **Higher**	

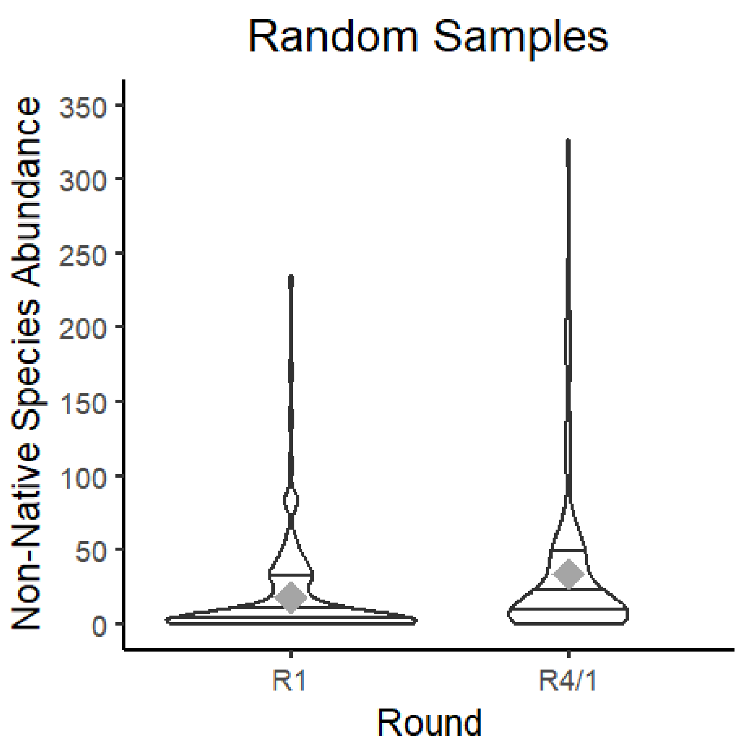




R1 vs. R4/1 Comparisons

Non-Native Species Abundance

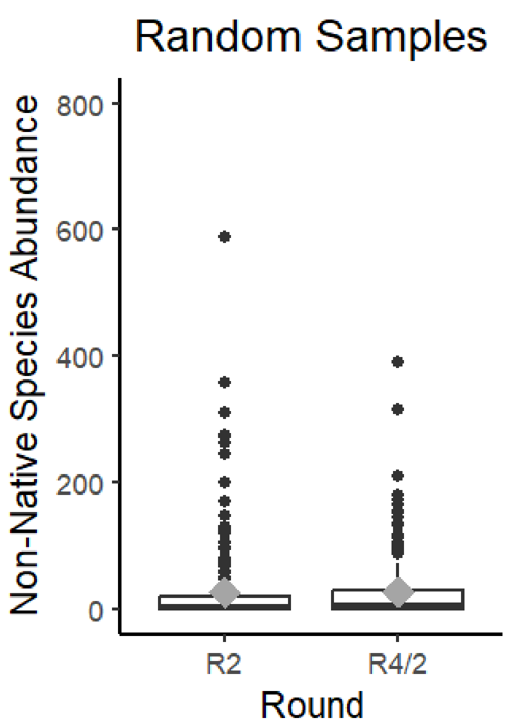
	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	17.97	33.74
Median	4	13
St. Dev.	34.56	54.06
Range	0 - 234	0 - 326
Wilcoxon test 95% CI Direction	< 0.001 -19.00, -7.00 **Higher**	

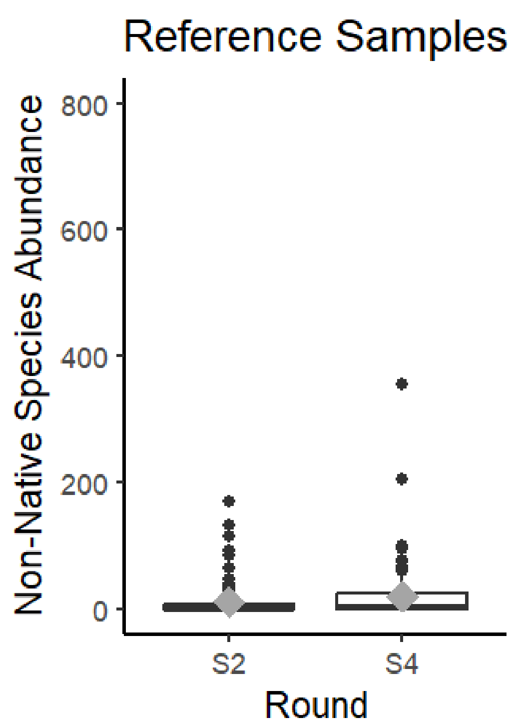


R2 vs. R4/2 & S2 vs. S4 Comparisons

Non-Native Species Abundance

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	26.13	31.08	10.79	19.68
Median	1	4	0	1
St. Dev.	68.42	77.96	28.10	45.56
Range	0 - 588	0 - 857	0 - 170	0 - 356
Wilcoxon test	0.011		0.005	
95% CI	-10.00, -1.00		-15.00, -2.50	
Direction	**No Change**		**Higher**	



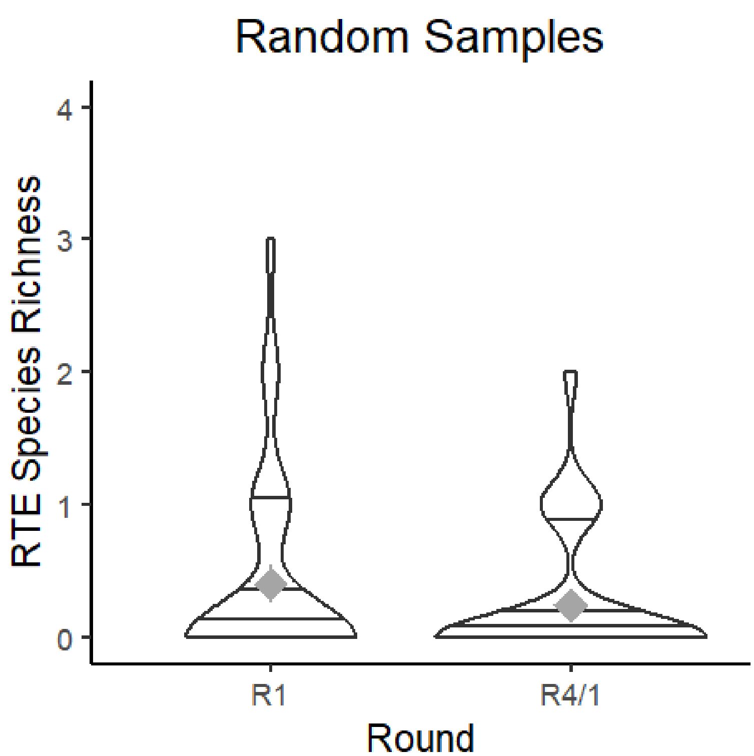


R1 vs. R4/1 Comparisons

RTE Species Richness

Note: * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	0.40	0.24
Median	0.0	0.0
St. Dev.	0.76	0.50
Range	0 - 3	0 - 2
Wilcoxon test 95% CI Direction	0.008* 0.04, 0.28 **Lower**	

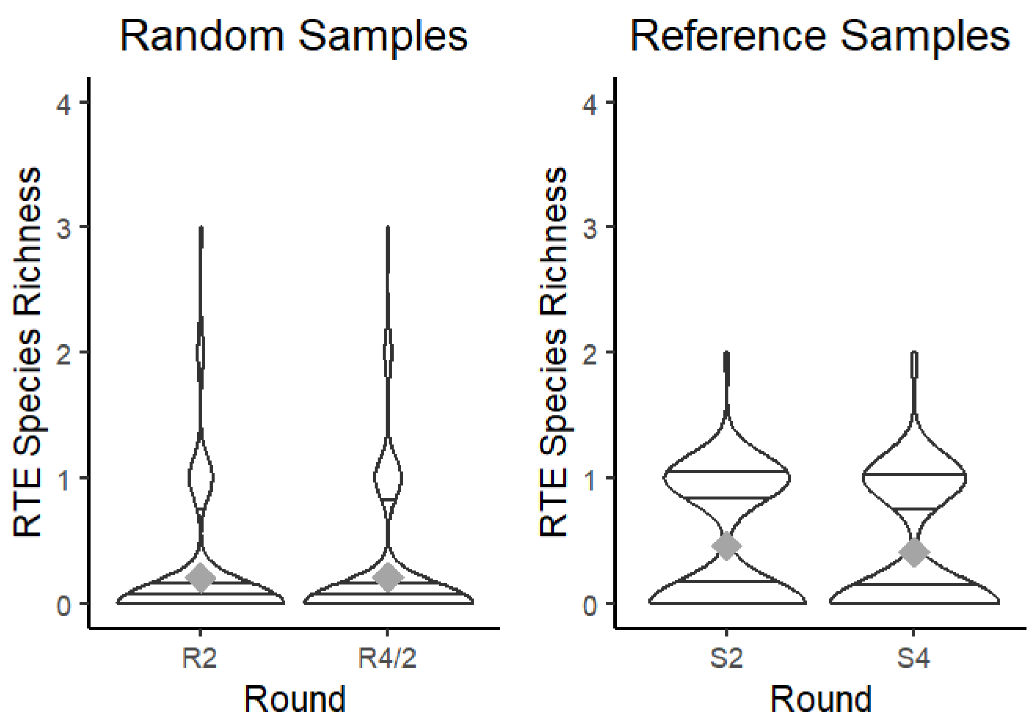


R2 vs. R4/2 & S2 vs. S4 Comparisons

RTE Species Richness

Note: * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

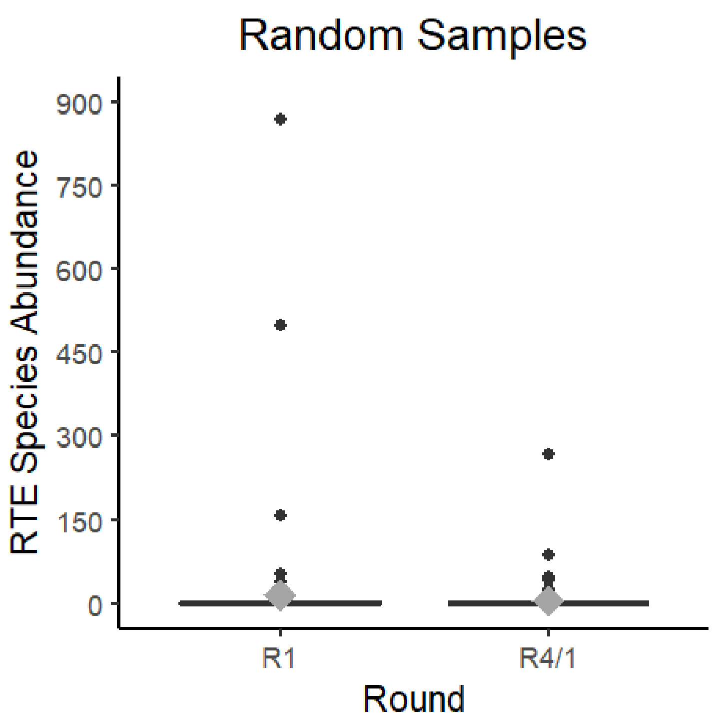
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	0.21	0.21	0.46	0.41
Median	0.0	0.0	0.0	0.0
St. Dev.	0.53	0.51	0.52	0.53
Range	0 - 3	0 - 3	0 - 2	0 - 2
Wilcoxon test 95% CI Direction	0.888* -0.05, 0.04 **No Change**		0.208* -0.03, 0.12 **No Change**	



R1 vs. R4/1 Comparisons

RTE Species Abundance

	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	15.37	5.12
Median	0	0
St. Dev.	92.32	26.29
Range	0 - 867	0 - 266
Wilcoxon test	0.041	
95% CI	<0.0001, <0.001	
Direction	**No Change**	

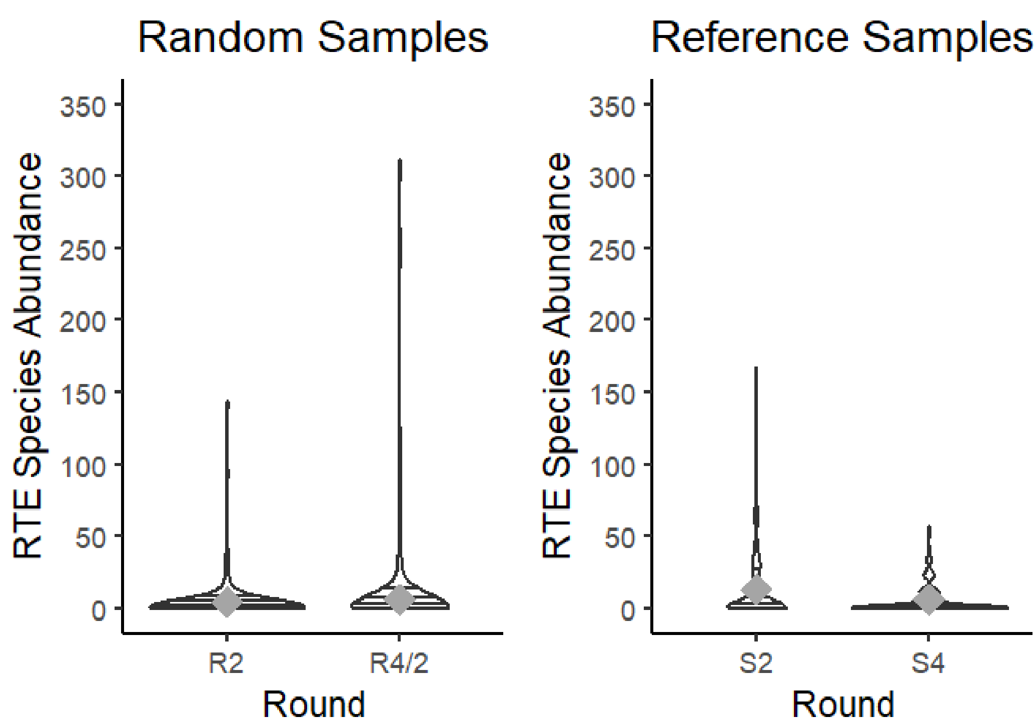


R2 vs. R4/2 & S2 vs. S4 Comparisons

RTE Species Abundance

Note: * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

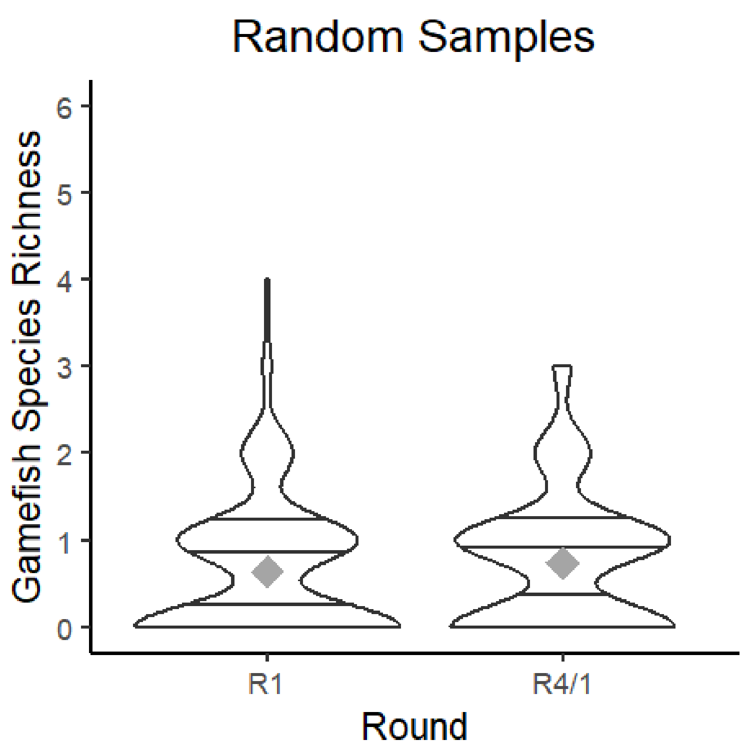
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	4.45	5.92	13.24	5.94
Median	0	0	0	0
St. Dev.	18.66	30.26	27.87	11.37
Range	0 - 143	0 - 311	0 - 167	0 - 57
Wilcoxon test 95% CI Direction	0.489 -11.00, 3.00 **No Change**		0.007* 2.00, 16.00 **Lower**	



R1 vs. R4/1 Comparisons

Gamefish Species Richness

	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	0.64	0.74
Median	0.0	1.0
St. Dev.	0.80	0.79
Range	0 - 4	0 - 3
Wilcoxon test	0.242	
95% CI	-0.30, 0.09	
Direction	**No Change**	

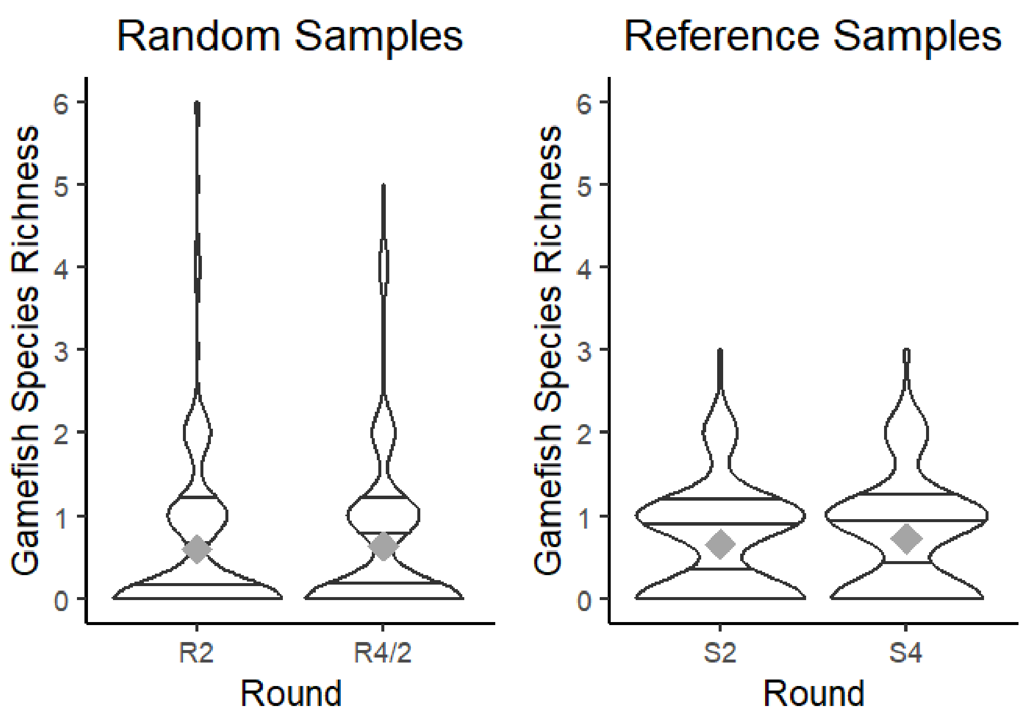


R2 vs. R4/2 & S2 vs. S4 Comparisons

Gamefish Species Richness

Note: * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

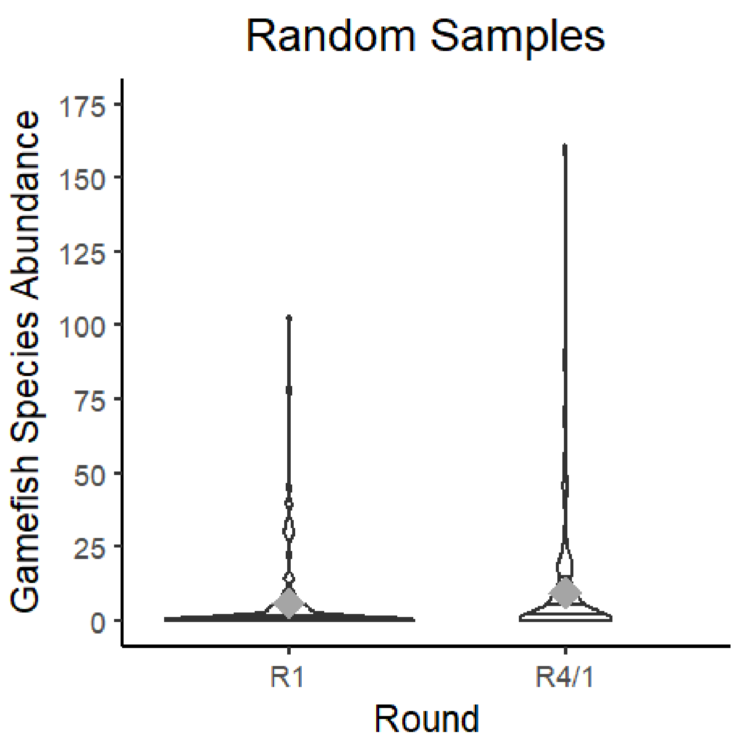
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	0.59	0.63	0.66	0.72
Median	0.0	0.0		
St. Dev.	1.04	0.98	0.68	0.71
Range	0 - 6	0 - 5	0 - 3	0 - 3
Wilcoxon test 95% CI Direction	0.479 -0.12, 0.06 **No Change**		0.309* -0.18, 0.06 **No Change**	



R1 vs. R4/1 Comparisons

Gamefish Species Abundance

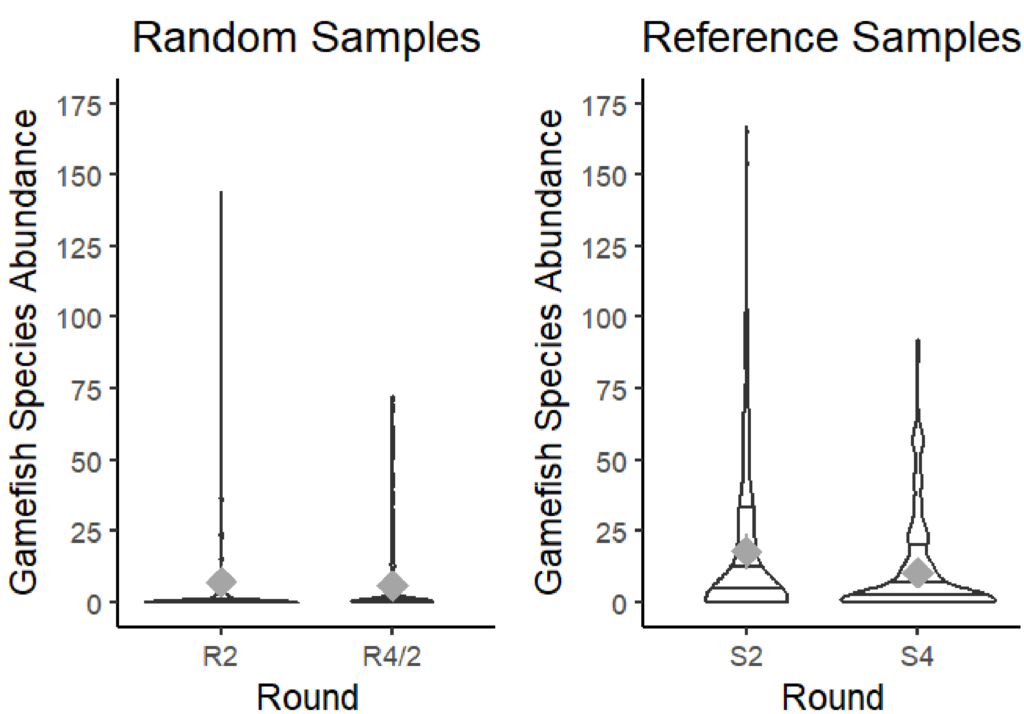
	RANDOM	
	R1	**R4/1**
N (Samples)	119	119
Unique Sites	119	119
Mean	5.84	9.24
Median	0	0
St. Dev.	14.64	20.06
Range	0 - 103	0 - 161
Wilcoxon test	0.004	
95% CI	-7.00, -1.00	
Direction	**Higher**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Gamefish Species Abundance

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	209	209	100	100
Unique Sites	209	209	20	20
Mean	7.21	5.68	17.79	10.38
Median	0	0	2	1
St. Dev.	20.15	12.69	31.73	17.32
Range	0 - 144	0 - 72	0 - 167	0 - 92
Wilcoxon test 95% CI Direction	0.983 -2.00, 2.50 **No Change**		0.034 0.50, 12.00 **No Change**	

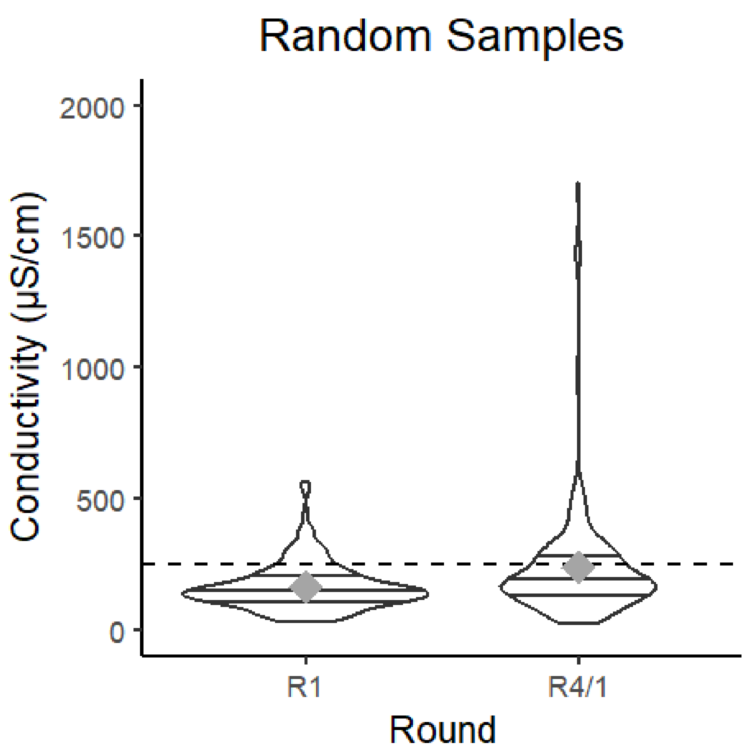


APPENDIX G
Water Chemistry Statistics and Violin Plots

R1 vs. R4/1 Comparisons

Specific Conductivity (µS/cm)

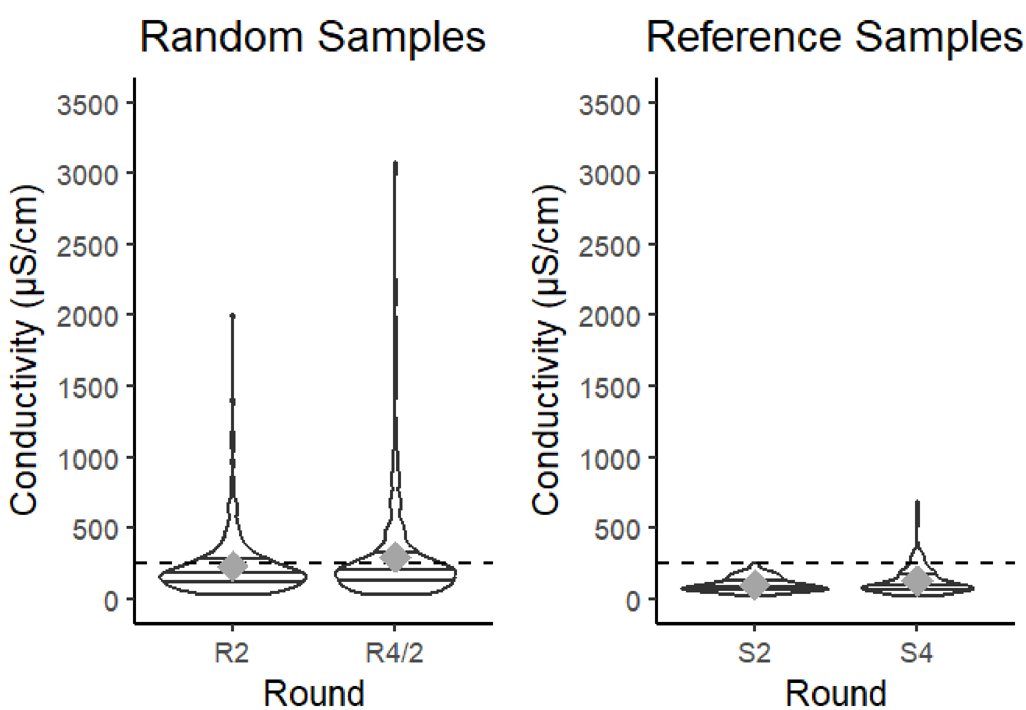
	RANDOM	
	R1	**R4/1**
N (Samples)	147	147
Unique Sites	147	147
Mean	163.28	237.84
Median	146.8	183.6
St. Dev.	90.71	226.02
Range	26.6 - 560.8	21.9 - 1700.2
Wilcoxon test 95% CI Direction	<0.001 -61.40, -37.45 **Higher**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Specific Conductivity (μS/cm)

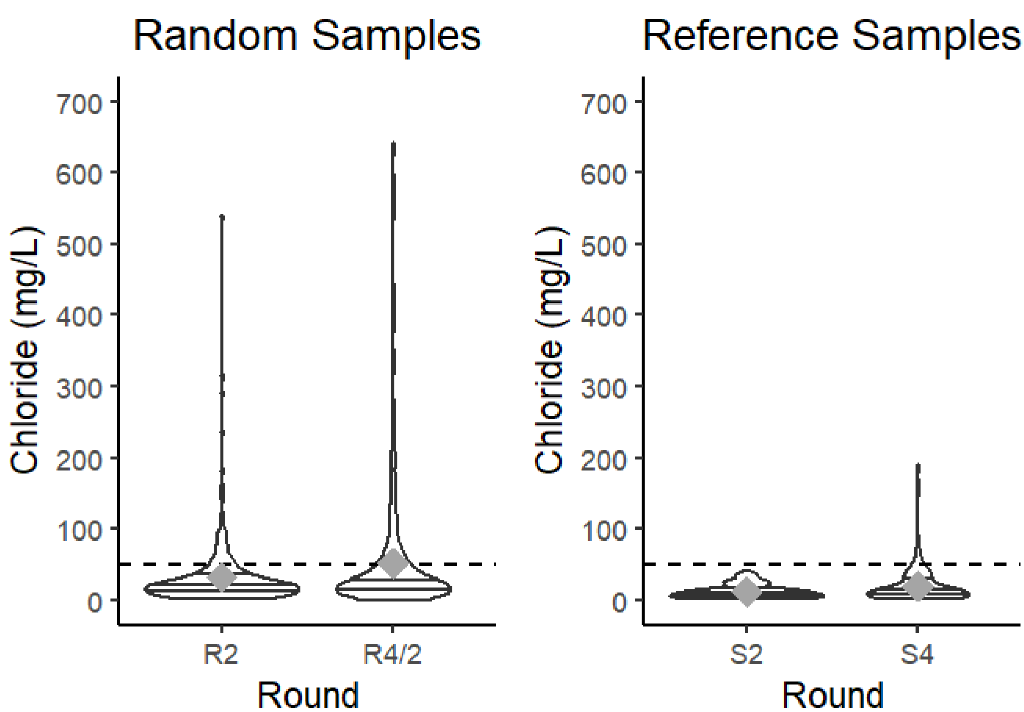
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	231.24	296.82	98.38	125.55
Median	179.6	193.96	88.85	89.56
St. Dev.	220.27	355.56	50.32	101.79
Range	29.9 - 2003.0	26.7 - 3078.0	20.0 - 253.4	19.9 - 686.2
Wilcoxon test	<0.001		<0.001	
95% CI	-33.45, -17.25		-23.20, -6.91	
Direction	**Higher**		**Higher**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Chloride (mg/L)

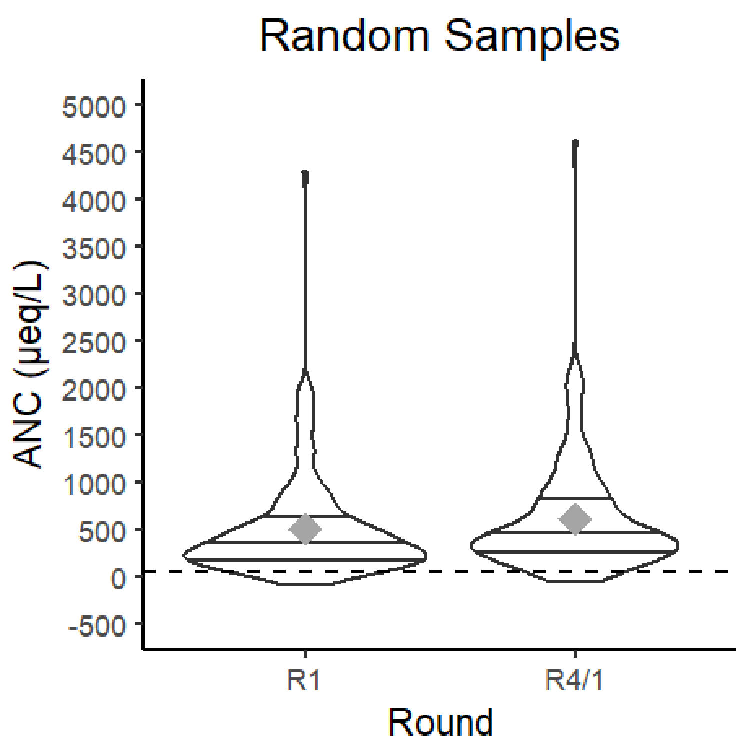
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	32.50	51.59	11.67	20.14
Median	20.27	25.23	9.29	12.90
St. Dev.	50.63	85.11	9.72	27.01
Range	0.77 - 538.20	0.39 - 641.89	0.95 - 39.99	0.60 - 191.09
Wilcoxon test 95% CI Direction	<0.001 -9.46, -5.21 **Higher**		<0.001 -6.63, -2.23 **Higher**	



R1 vs. R4/1 Comparisons

Acid Neutralizing Capacity (µeq/L)

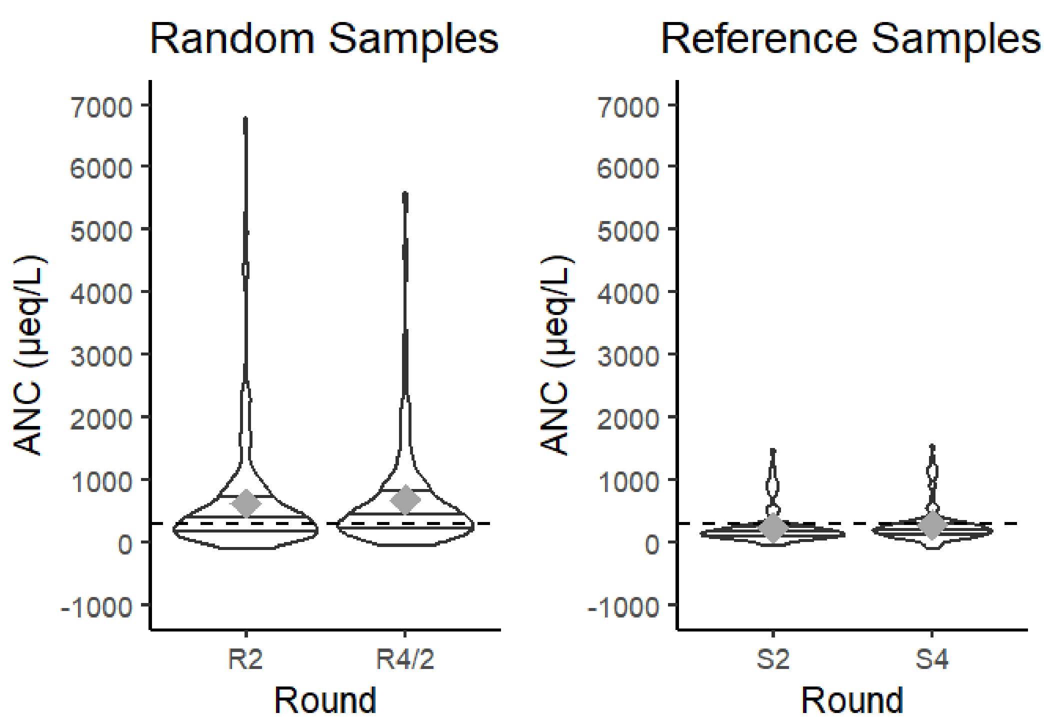
	RANDOM	
	R1	**R4/1**
N (Samples)	147	147
Unique Sites	147	147
Mean	498.18	609.17
Median	335.7	405.6
St. Dev.	546.87	589.71
Range	-92.2 - 4286.0	-62.1 - 4618.1
Wilcoxon test 95% CI Direction	<0.001 -133.30, -81.30 **Higher**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Acid Neutralizing Capacity (µeq/L)

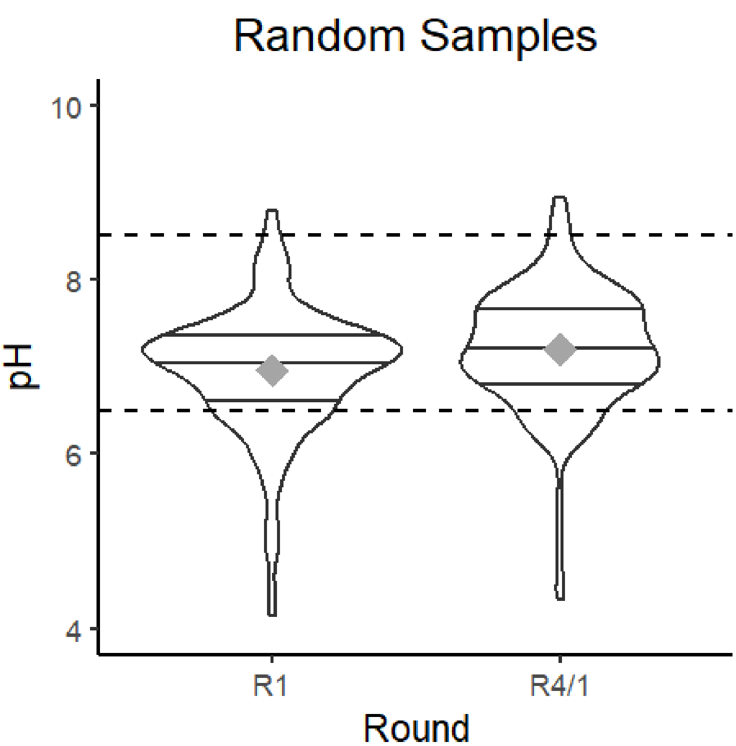
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	620.43	678.22	241.25	282.10
Median	391.2	423.0	155.3	194.85
St. Dev.	873.67	844.58	280.37	308.64
Range	-107.2 - 6788.0	-48.7 - 5573.0	-54.8 - 1477.0	-101.6 - 1544.9
Wilcoxon test	<0.001		<0.001	
95% CI	-77.50, -42.25		-48.50, -18.05	
Direction	**Higher**		**Higher**	



R1 vs. R4/1 Comparisons

pH

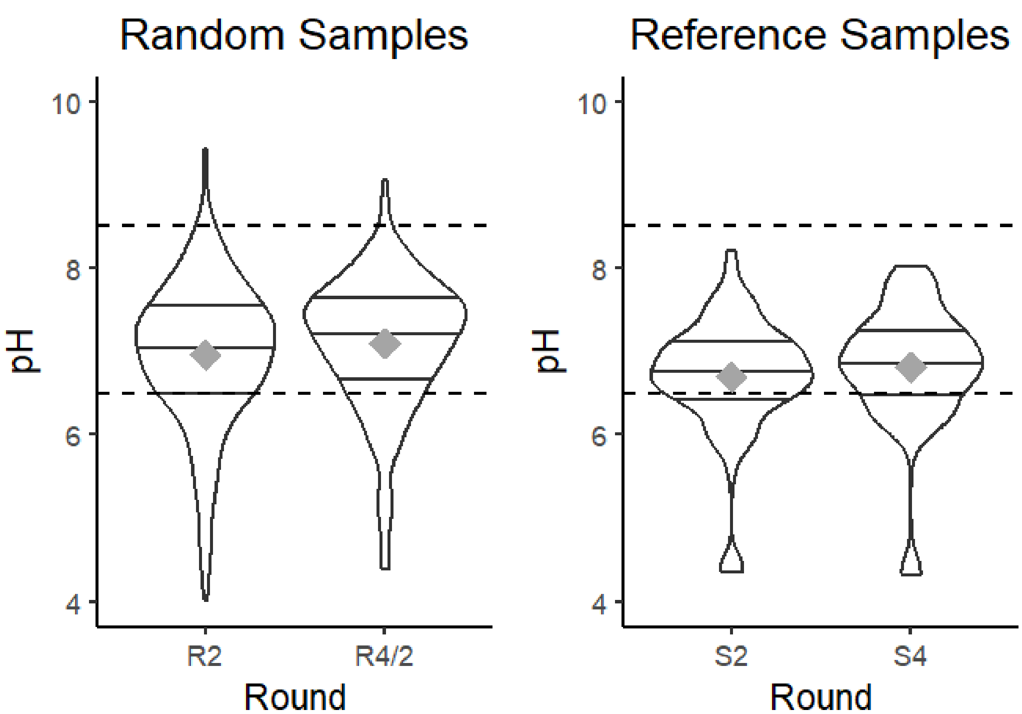
	RANDOM	
	R1	**R4/1**
N (Samples)	147	147
Unique Sites	147	147
Mean	6.96	7.20
Median	7.09	7.22
St. Dev.	0.73	0.70
Range	4.14 - 8.78	4.33 - 8.94
Wilcoxon test	<0.001	
95% CI	-0.29, -0.19	
Direction	**Higher**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

pH

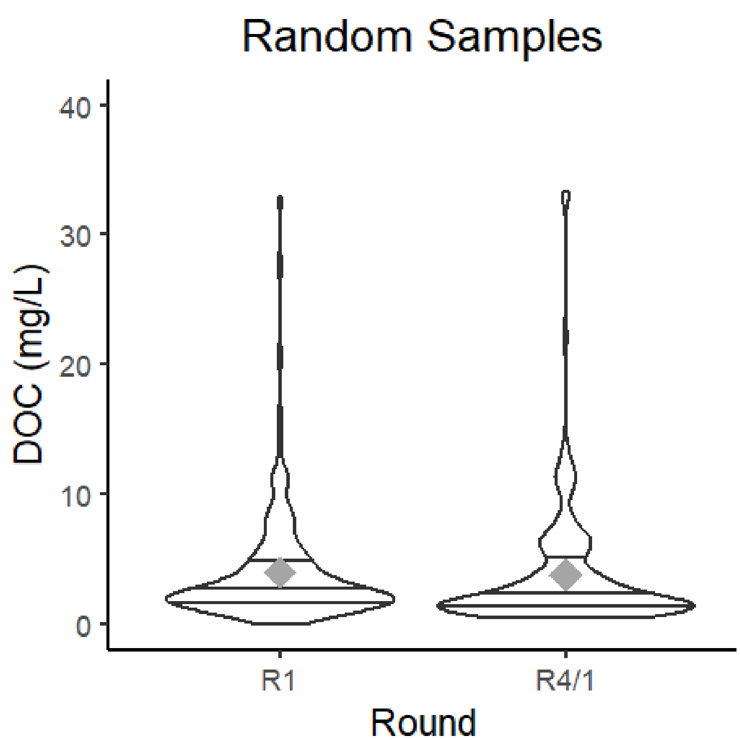
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	6.96	7.10	6.69	6.81
Median	7.04	7.23	6.76	6.86
St. Dev.	0.88	0.79	0.70	0.73
Range	4.01 - 9.42	4.39 - 9.06	4.36 - 8.20	4.31 - 8.02
Wilcoxon test	< 0.001		< 0.001	
95% CI	-0.17, -0.10		-0.17, -0.05	
Direction	**Higher**		**Higher**	



R1 vs. R4/1 Comparisons

Dissolved Organic Carbon (mg/L)

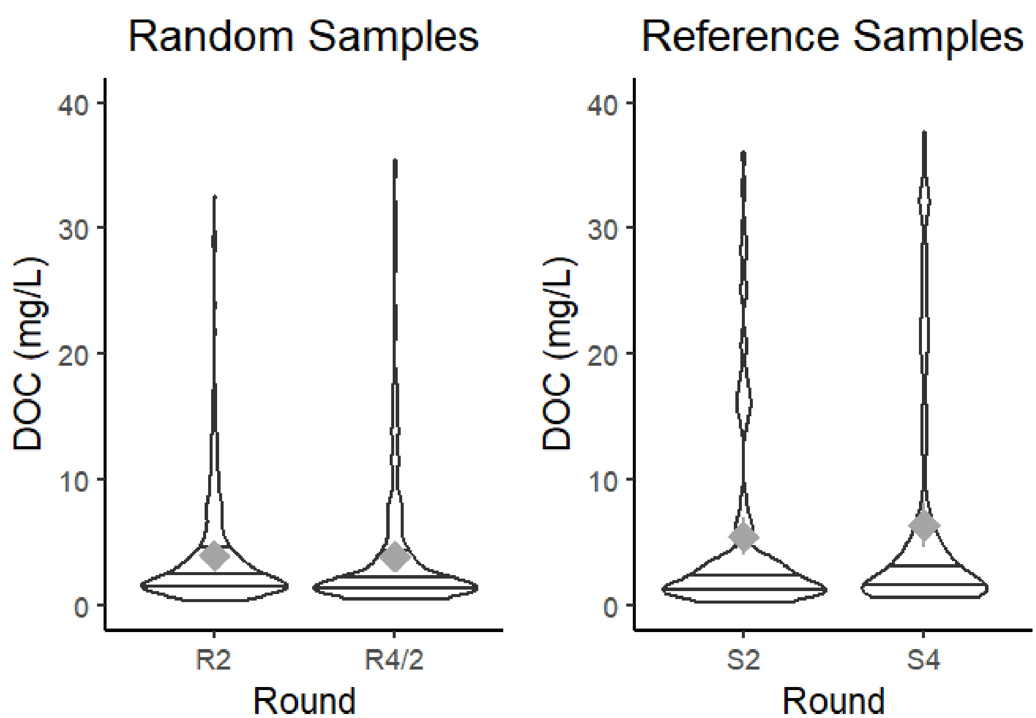
	RANDOM	
	R1	R4/1
N (Samples)	147	147
Unique Sites	147	147
Mean	3.98	3.75
Median	2.40	1.78
St. Dev.	4.48	4.83
Range	0 - 32.9	0.52 - 33.29
Wilcoxon test	0.026	
95% CI	0.03, 0.39	
Direction	**No Change**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Dissolved Organic Carbon (mg/L)

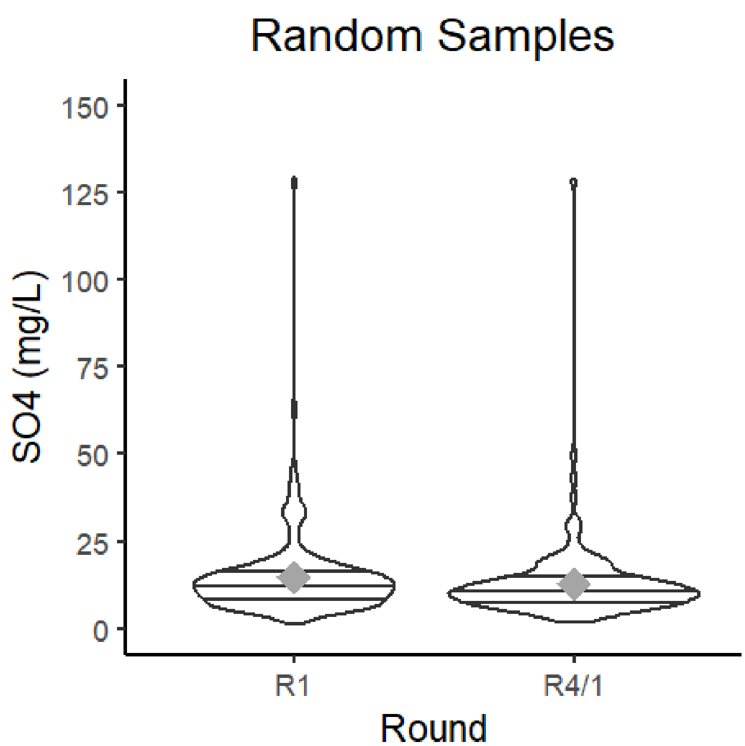
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	3.94	3.90	5.44	6.36
Median	2.40	1.90	2.20	2.16
St. Dev.	4.66	5.18	7.94	9.21
Range	0.4 - 32.6	0.52 - 35.38	0.2 - 36.1	0.56 - 37.71
Wilcoxon test	0.180		0.030	
95% CI	-0.05, 0.43		-0.67, -0.03	
Direction	**No Change**		**No Change**	



R1 vs. R4/1 Comparisons

Sulfate (mg/L)

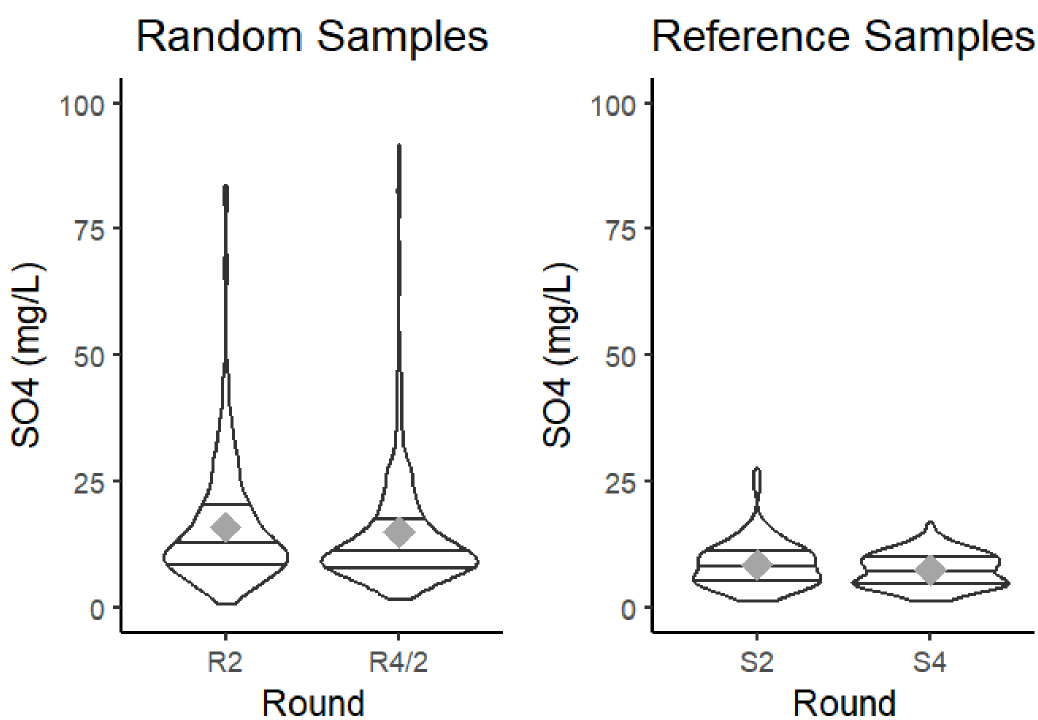
	RANDOM	
	R1	**R4/1**
N (Samples)	147	147
Unique Sites	147	147
Mean	14.82	12.94
Median	12.45	10.77
St. Dev.	13.04	11.96
Range	1.43 - 128.98	1.64 - 128.68
Wilcoxon test	< 0.001	
95% CI	0.98, 1.99	
Direction	**Lower**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Sulfate (mg/L)

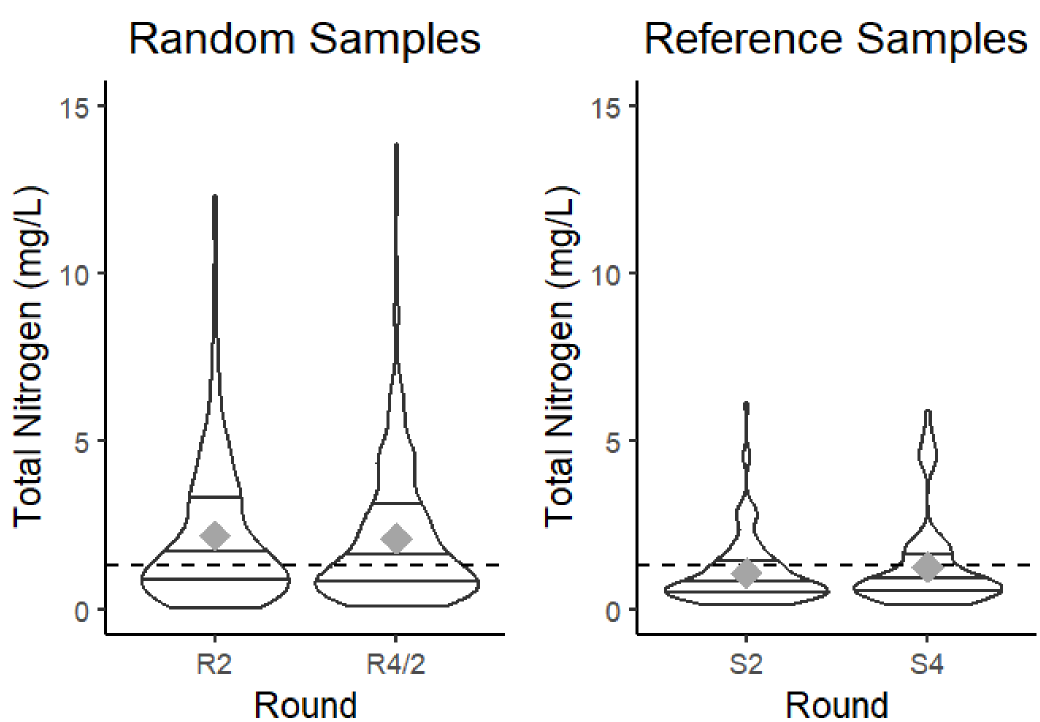
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	15.89	14.81	8.50	7.31
Median	12.15	10.96	8.17	7.32
St. Dev.	11.89	12.54	4.66	3.31
Range	0.55 - 83.44	1.32 - 91.56	1.14 - 27.51	1.29 - 16.75
Wilcoxon test	< 0.001		< 0.001	
95% CI	0.40, 1.24		0.37, 1.09	
Direction	**Lower**		**Lower**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Total Nitrogen (mg/L)

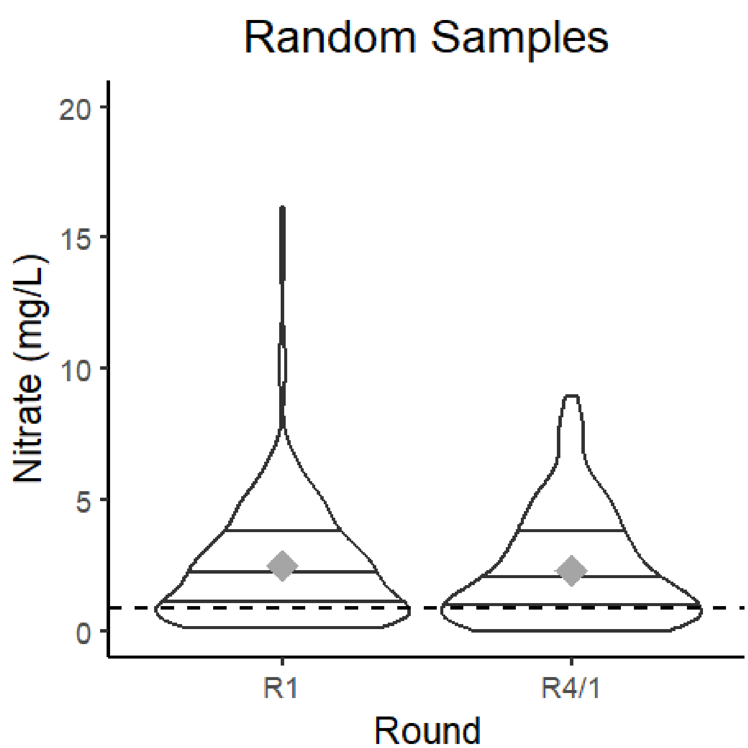
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	2.19	2.11	1.08	1.27
Median	1.43	1.40	0.72	0.79
St. Dev.	2.14	2.05	0.99	1.31
Range	0.038 - 12.31	0.057 - 13.85	0.13 - 6.16	0.14 - 5.92
Wilcoxon test 95% CI Direction	0.483 -0.04, 0.10 **No Change**		0.007 -0.15, -0.02 **Higher**	



R1 vs. R4/1 Comparisons

Nitrate (mg/L)

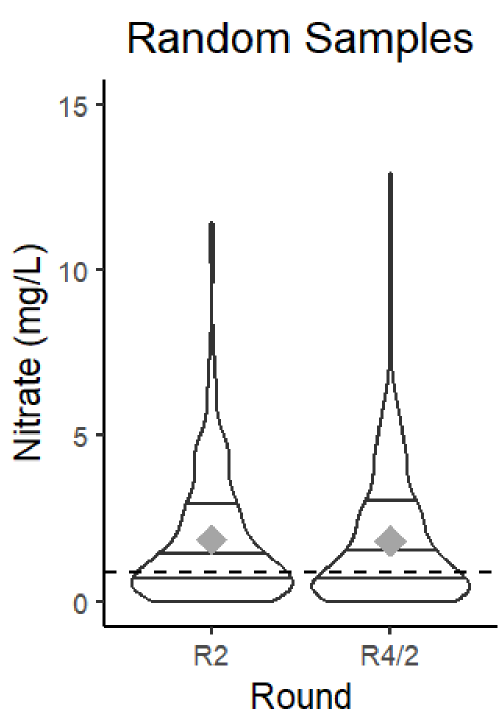
	RANDOM	
	R1	**R4/1**
N (Samples)	147	147
Unique Sites	147	147
Mean	2.49	2.30
Median	1.98	1.65
St. Dev.	2.46	2.12
Range	0.11 - 16.16	0.0062 - 8.9340
Wilcoxon test 95% CI Direction	0.749 -0.08, 0.12 **No Change**	

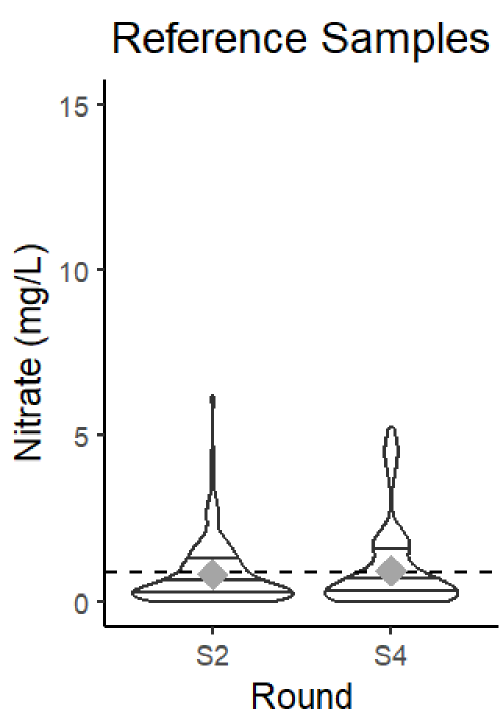


R2 vs. R4/2 & S2 vs. S4 Comparisons

Nitrate (mg/L)

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	1.87	1.83	0.82	0.94
Median	1.18	1.16	0.45	0.49
St. Dev.	2.03	1.99	0.99	1.28
Range	0 - 11.4	0.001 - 12.905	0 - 6.2	0.0015 - 5.3
Wilcoxon test 95% CI Direction	0.732 -0.05, 0.07 **No Change**		0.412 -0.06, 0.02 **No Change**	

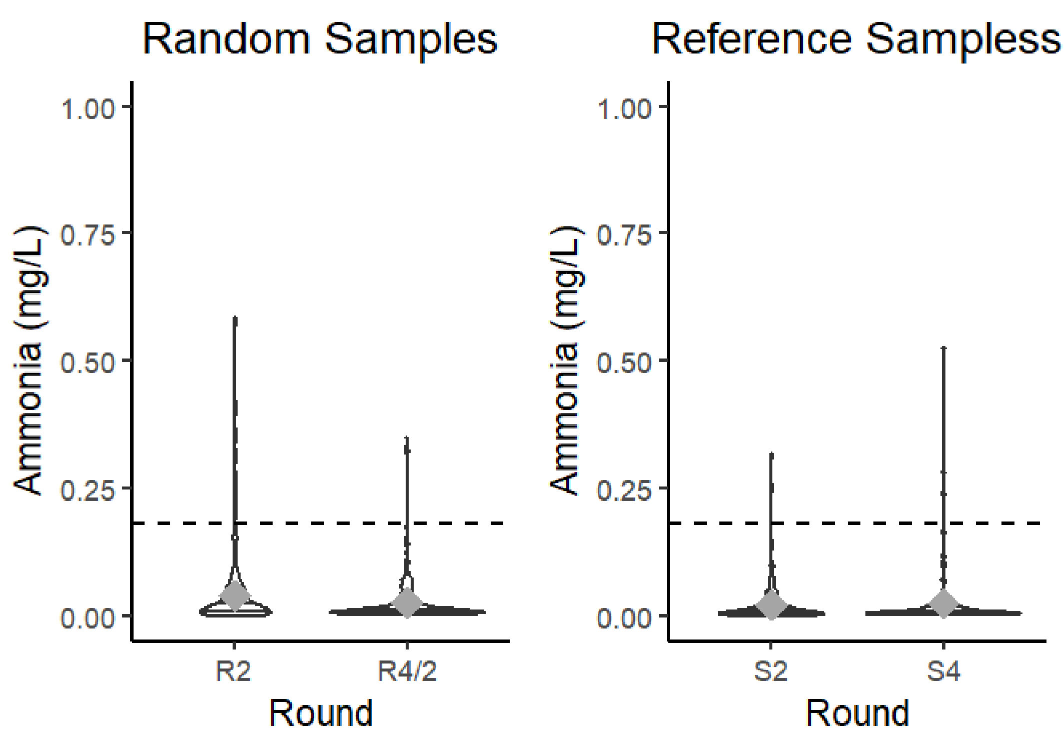




R2 vs. R4/2 & S2 vs. S4 Comparisons

Ammonia (mg/L)

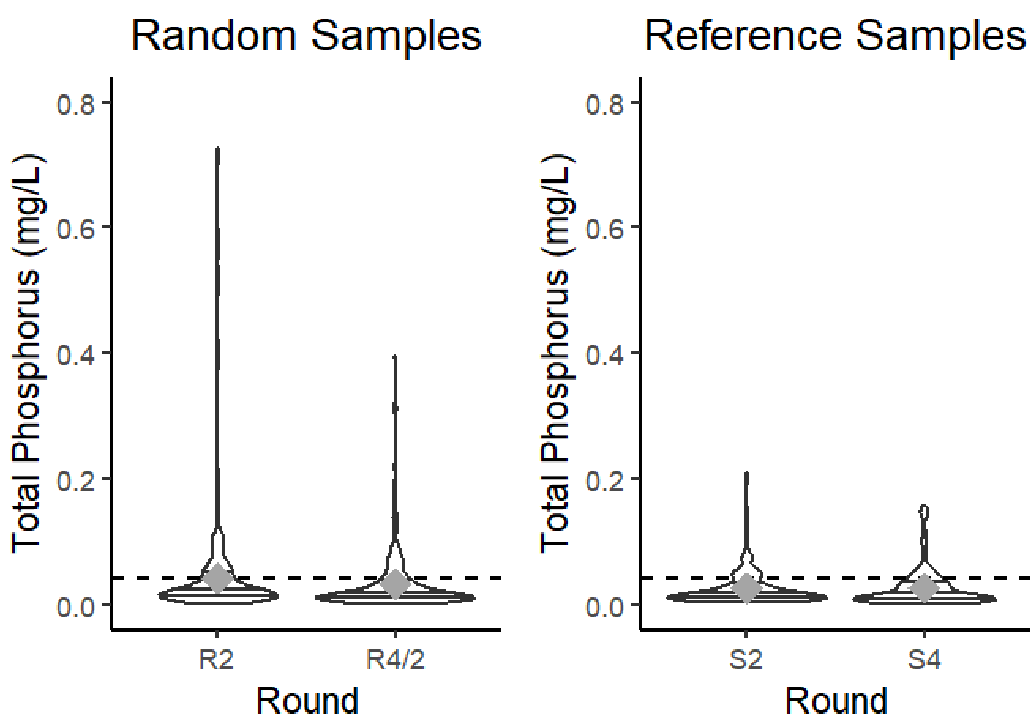
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	0.06	0.03	0.02	0.02
Median	0.02	0.01	0.01	0.01
St. Dev.	0.21	0.14	0.04	0.06
Range	0 - 2.78	0.002 - 2.07	0 - 0.32	0.002- 0.53
Wilcoxon test 95% CI Direction	<0.001 0.003, 0.009 **Lower**		0.380 -0.0009, 0.003 **No Change**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Total Phosphorus (mg/L)

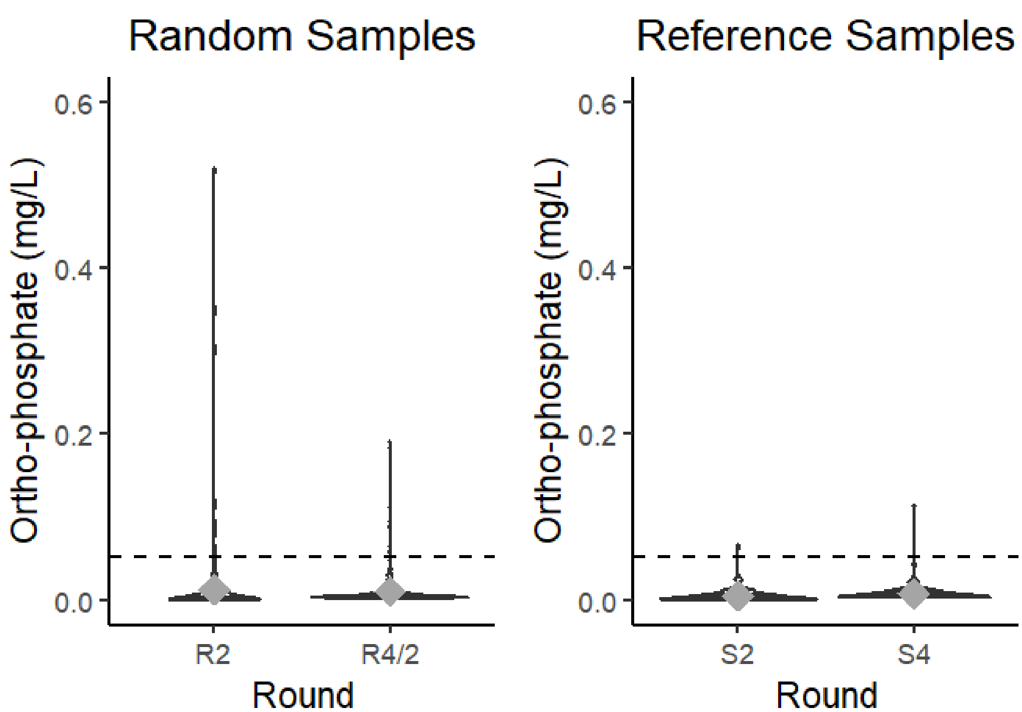
	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	0.04	0.03	0.03	0.03
Median	0.02	0.02	0.02	0.01
St. Dev.	0.07	0.05	0.03	0.03
Range	0.002 - 0.725	0.003 - 0.395	0.004 - 0.211	0.003 - 0.158
Wilcoxon test	<0.001		0.97	
95% CI	0.002, 0.006		-0.002, 0.002	
Direction	**Lower**		**No Change**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Ortho-phosphate (mg/L)

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	251	251	110	110
Unique Sites	251	251	22	22
Mean	0.013	0.011	0.005	0.009
Median	0.0037	0.0046	0.003	0.006
St. Dev.	0.045	0.021	0.008	0.012
Range	0 - 0.520	0.001 - 0.190	0 - 0.066	0.003 - 0.115
Wilcoxon test 95% CI Direction	<0.001 -0.002, -0.001 **Higher**		<0.001 -0.003, -0.002 **Higher**	



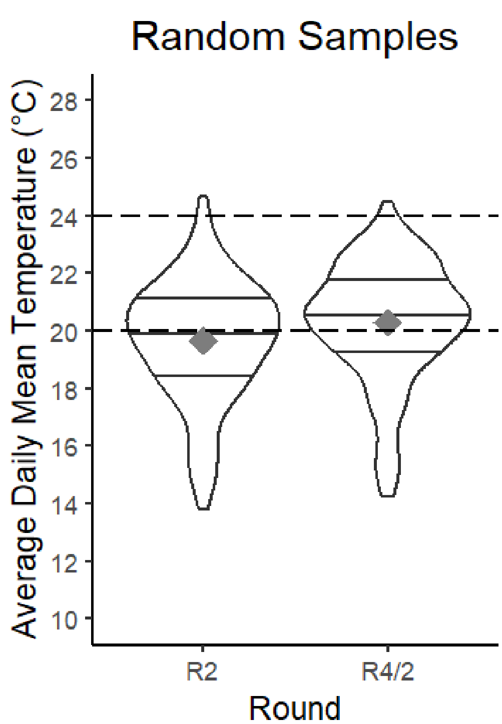
APPENDIX H
Temperature Statistics and Violin Plots

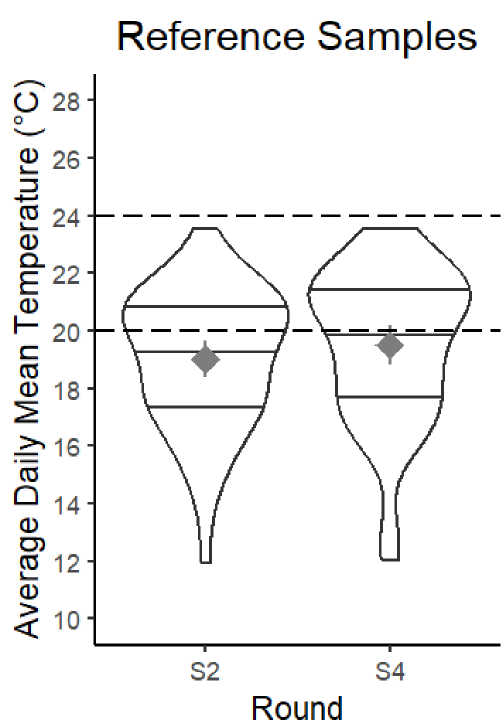
R2 vs. R4/2 & S2 vs. S4 Comparisons

Average Daily Mean Temperature

Note: * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	92	92	58	58
Unique Sites	92	92	24	24
Mean	19.67	20.30	19.03	19.52
Median	19.99	20.54	19.41	20.08
St. Dev.	2.10	2.08	2.39	2.66
Range	13.80 - 24.67	14.26 - 24.49	11.95 - 23.56	12.05 - 23.57
Wilcoxon test 95% CI Direction	<0.001* 0.44, 0.84 **Higher**		<0.001* 0.27, 0.72 **Higher**	





R2 vs. R4/2 & S2 vs. S4 Comparisons

Average Daily Mean Temperature

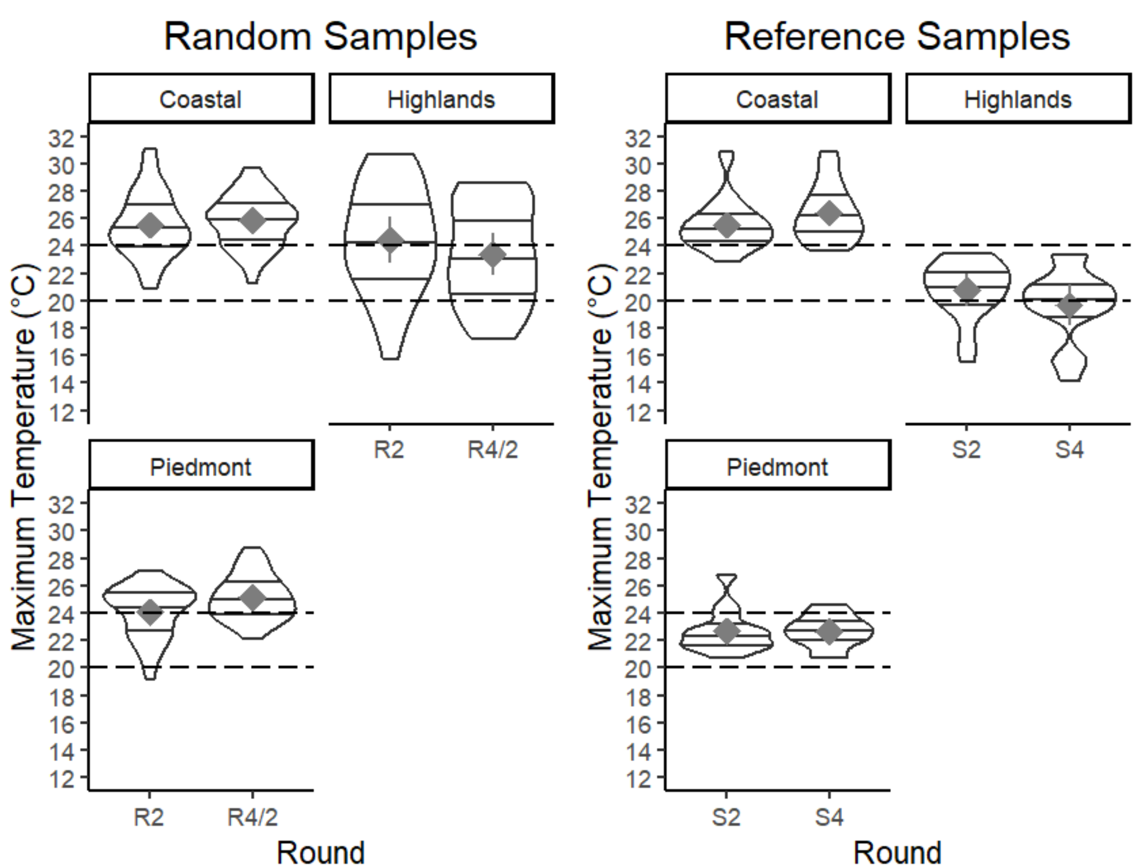
	RANDOM HIGHLANDS		REFERENCE HIGHLANDS	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	24	24	15	15
Unique Sites	24	24	7	7
Mean	18.51	18.74	16.28	16.35
Median	18.61	18.92	16.72	17.04
St. Dev.	2.40	2.58	1.80	2.15
Range	13.80 - 21.52	14.26 - 23.35	11.95 - 18.80	12.05 - 18.89
Wilcoxon test	0.114		0.229	
95% CI	-0.07, 0.59		-0.18, 0.48	
Direction	**No Change**		**No Change**	
	RANDOM PIEDMONT		**REFERENCE PIEDMONT**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	27	27	13	13
Unique Sites	27	27	6	6
Mean	19.16	20.27	17.92	18.41
Median	19.45	20.55	18.12	18.62
St. Dev.	1.78	1.76	1.24	1.23
Range	14.86 - 22.05	14.87 - 22.64	15.31 - 19.93	16.43 - 20.05
Wilcoxon test	<0.001		0.027	
95% CI	0.72, 1.44		0.07, 1.18	
Direction	**Higher**		**No Change**	
	RANDOM COASTAL		**REFERENCE COASTAL**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	41	41	30	30
Unique Sites	41	41	11	11
Mean	20.68	21.23	20.89	21.58
Median	20.44	21.13	20.87	21.49
St. Dev.	1.63	1.30	1.02	0.93
Range	17.63 - 24.67	18.77 - 24.49	19.35 - 23.56	19.70 - 23.57
Wilcoxon test	0.002		<0.001	
95% CI	0.22, 0.89		0.32, 1.03	
Direction	**Higher**		**Higher**	

R2 vs. R4/2 & S2 vs. S4 Comparisons

Maximum Temperature

Note: * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	92	92	58	58
Unique Sites	92	92	24	24
Mean	24.84	25.00	23.68	23.84
Median	25.01	25.09	23.81	24.13
St. Dev.	2.82	2.61	2.76	3.55
Range	15.77 - 31.12	17.20 - 29.74	15.58 - 30.91	14.15 - 30.87
Wilcoxon test 95% CI Direction	0.529* -0.29, 0.58 **No Change**		0.626* -0.38, 0.65 **No Change**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Maximum Temperature

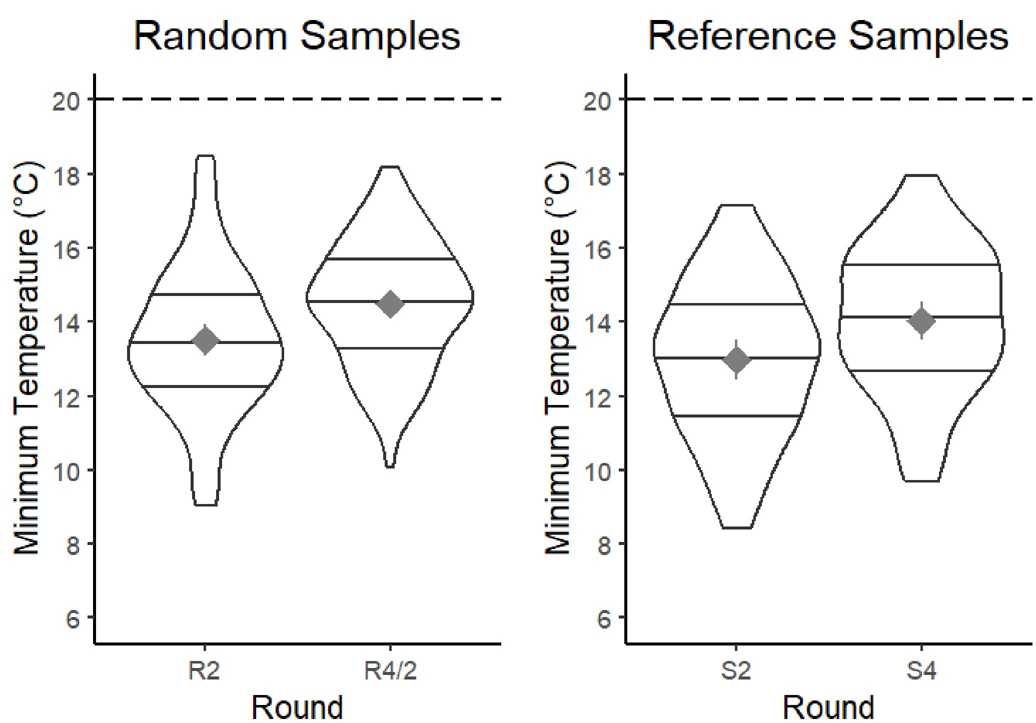
	RANDOM HIGHLANDS		REFERENCE HIGHLANDS	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	24	24	15	15
Unique Sites	24	24	7	7
Mean	24.49	23.40	20.81	19.68
Median	24.93	23.26	21.08	20.25
St. Dev.	3.98	3.67	2.17	2.68
Range	15.77 - 30.73	17.20 - 28.62	15.58 - 23.45	14.15 - 23.35
Wilcoxon test 95% CI Direction	0.004 -1.88, -0.40 **Lower**		0.015 -1.78, -0.26 **No Change**	
	RANDOM PIEDMONT		**REFERENCE PIEDMONT**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	27	27	13	13
Unique Sites	27	27	6	6
Mean	24.07	25.15	22.69	22.67
Median	24.82	24.85	22.51	22.71
St. Dev.	1.92	1.65	1.60	1.15
Range	19.16 - 27.07	22.13 - 28.74	20.76 - 26.77	20.76 - 24.56
Wilcoxon test 95% CI Direction	0.004 0.35, 1.74 **Higher**		0.588 -1.18, 1.26 **No Change**	
	RANDOM COASTAL		**REFERENCE COASTAL**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	41	41	30	30
Unique Sites	41	41	11	11
Mean	25.54	25.85	25.54	26.43
Median	25.21	26.01	25.34	25.99
St. Dev.	2.37	1.91	1.82	2.05
Range	20.90 - 31.12	21.29 - 29.74	22.87 - 30.91	23.64 - 30.87
Wilcoxon test 95% CI Direction	0.404 -0.36, 0.94 **No Change**		0.058 -0.04, 1.56 **No Change**	

R2 vs. R4/2 & S2 vs. S4 Comparisons

Minimum Temperature

Note: * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	92	92	58	58
Unique Sites	92	92	24	24
Mean	13.51	14.50	12.98	14.03
Median	13.37	14.54	13.15	14.01
St. Dev.	1.98	1.67	2.09	1.98
Range	9.03 - 18.50	10.08 - 18.20	8.42 - 17.15	9.68 - 17.94
Wilcoxon test 95% CI Direction	<0.001* 0.68, 1.38 **Higher**		<0.001* 0.73, 1.44 **Higher**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Minimum Temperature

Note: * indicates when a p-value or confidence interval was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

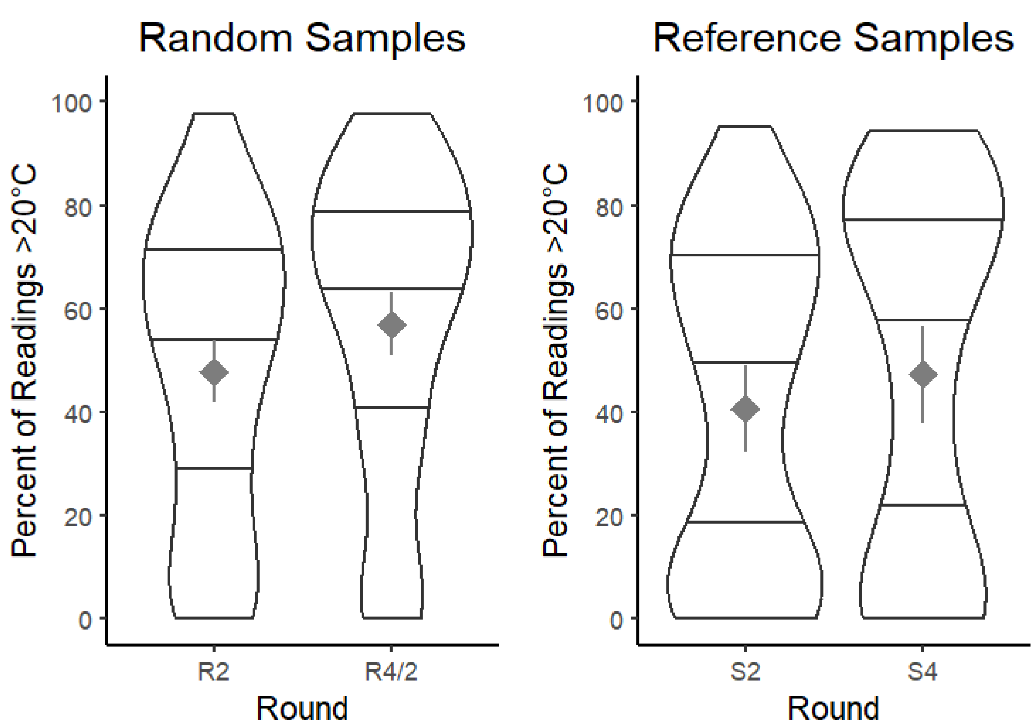
	RANDOM HIGHLANDS		REFERENCE HIGHLANDS	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	24	24	15	15
Unique Sites	24	24	7	7
Mean	12.55	12.96	10.72	11.75
Median	12.63	12.57	10.19	12.07
St. Dev.	2.27	1.61	1.52	1.17
Range	9.03 - 16.36	10.08 - 16.44	8.42 - 13.56	9.68 - 13.38
Wilcoxon test	0.214*		0.035	
95% CI	-0.52, 1.38*		0.09, 1.99	
Direction	**No Change**		**No Change**	
	RANDOM PIEDMONT		**REFERENCE PIEDMONT**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	27	27	13	13
Unique Sites	27	27	6	6
Mean	13.32	14.24	12.34	13.16
Median	13.28	14.43	11.74	13.52
St. Dev.	1.66	1.15	1.23	0.96
Range	10.91 - 18.24	12.15 - 16.82	10.92 - 14.74	10.55 - 14.29
Wilcoxon test	0.002		0.094	
95% CI	0.38, 1.52		-0.27, 1.90	
Direction	**Higher**		**No Change**	
	RANDOM COASTAL		**REFERENCE COASTAL**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	41	41	30	30
Unique Sites	41	41	11	11
Mean	14.20	15.57	14.38	15.56
Median	13.86	15.61	14.35	15.57
St. Dev.	1.75	1.14	1.42	1.09
Range	11.57 - 18.50	13.45 - 18.20	11.99 - 17.15	13.19 - 17.94
Wilcoxon test	<0.001*		<0.001	
95% CI	0.91, 1.93*		0.77, 1.58	
Direction	**Higher**		**Higher**	

R2 vs. R4/2 & S2 vs. S4 Comparisons

Percentage of temperature readings above 20°C

Note: * indicates when a p-value was determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	92	92	58	58
Unique Sites	92	92	24	24
Mean	47.89	57.06	40.67	47.27
Median	54.73	65.58	48.59	55.09
St. Dev.	29.15	29.74	31.75	35.74
Range	0 - 97.60	0 - 97.72	0 - 95.26	0 - 94.43
Wilcoxon test 95% CI Direction	<0.001* 5.78, 11.94 **Higher**		<0.001* 2.55, 9.34 **Higher**	



R2 vs. R4/2 & S2 vs. S4 Comparisons

Percentage of temperature readings above 20°C

Note: * indicates when a p-value and confidence interval determined by a Wilcoxon signed rank test were approximated with a continuity correction.

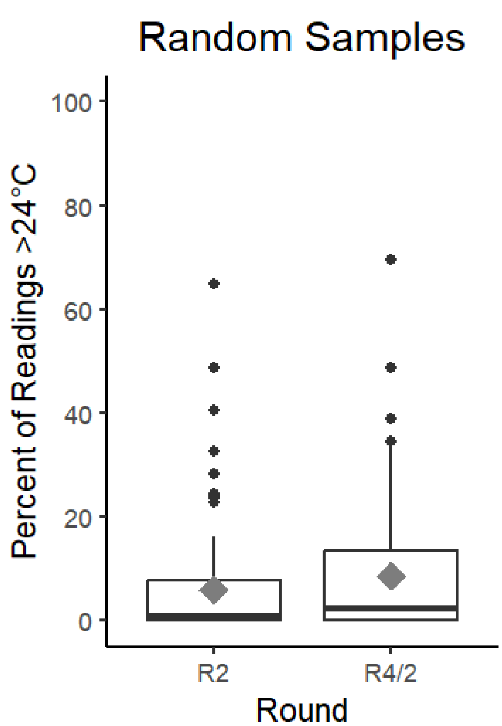
	RANDOM HIGHLANDS		REFERENCE HIGHLANDS	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	24	24	15	15
Unique Sites	24	24	7	7
Mean	32.82	33.51	7.01	6.49
Median	27.73	30.84	2.04	0.80
St. Dev.	28.80	33.93	9.94	12.24
Range	0.00 - 75.27	0.00 - 95.46	0.00 - 35.00	0.00 - 40.11
Wilcoxon test	0.751*		0.541*	
95% CI	-5.07, 6.47*		-4.87, 3.58*	
Direction	**No Change**		**No Change**	
	RANDOM PIEDMONT		**REFERENCE PIEDMONT**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	27	27	13	13
Unique Sites	27	27	6	6
Mean	40.83	56.44	16.37	22.36
Median	40.28	63.04	11.51	17.21
St. Dev.	27.21	25.64	16.25	20.50
Range	0.00 - 89.26	0.11 - 86.49	0.08 - 52.70	0.69 - 54.57
Wilcoxon test	<0.001		0.376	
95% CI	8.33, 22.07		-3.21, 21.00	
Direction	**Higher**		**No Change**	
	RANDOM COASTAL		**REFERENCE COASTAL**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	41	41	30	30
Unique Sites	41	41	11	11
Mean	61.36	71.25	68.04	78.45
Median	69.05	73.64	70.54	79.99
St. Dev.	24.88	19.60	14.09	11.00
Range	0.42 - 97.60	16.65 - 97.72	31.11 - 95.26	43.15 - 94.43
Wilcoxon test	0.001		<0.001	
95% CI	4.35, 13.93		5.31, 13.40	
Direction	**Higher**		**Higher**	

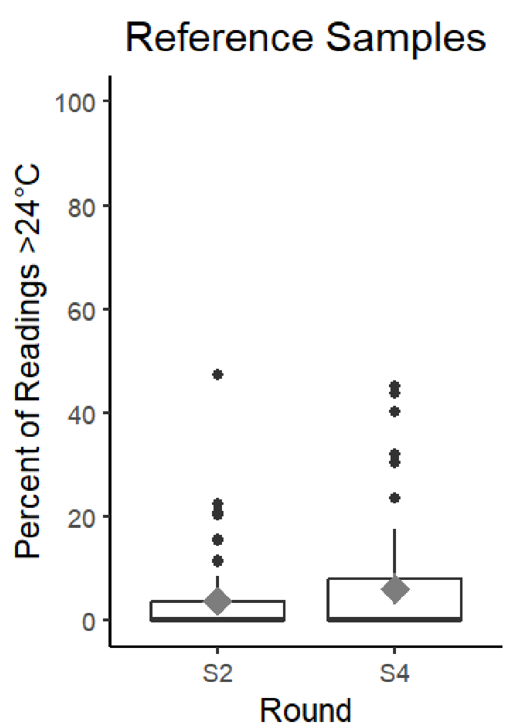
R2 vs. R4/2 & S2 vs. S4 Comparisons

Percentage of temperature readings above 24°C

Note: * indicates when a p-value and confidence interval were determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction.

	RANDOM		REFERENCE	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	92	92	58	58
Unique Sites	92	92	24	24
Mean	5.97	8.59	3.73	6.24
Median	0.82	2.19	0.00	0.17
St. Dev.	11.15	13.00	8.15	11.43
Range	0 - 64.85	0 - 69.57	0 - 47.34	0 - 45.20
Wilcoxon test	0.023*		0.019*	
95% CI	0.39, 5.42		0.28, 6.71*	
Direction	**No Change**		**No Change**	





R2 vs. R4/2 & S2 vs. S4 Comparisons

Percentage of temperature readings above 24°C

Note: * indicates when a p-value and confidence interval were determined by a Wilcoxon signed rank test that used a normal approximation with a continuity correction due to the presence of zeros. ** indicates when a Wilcoxon signed rank test was limited to an 80% confidence interval with a continuity correction.

	RANDOM HIGHLANDS		**REFERENCE HIGHLANDS**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	24	24	15	15
Unique Sites	24	24	7	7
Mean	4.03	6.10	0.00	0.00
Median	0.11	0.00	0.00	0.00
St. Dev.	6.45	11.07	0.00	0.00
Range	0.00 - 24.45	0.00 - 38.96	—	—
Wilcoxon test	0.490*		—	
95% CI	-2.67, 9.94*		—	
Direction	**No Change**		**No Change**	
	RANDOM PIEDMONT		**REFERENCE PIEDMONT**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	27	27	13	13
Unique Sites	27	27	6	6
Mean	2.14	6.77	0.34	0.05
Median	0.70	1.40	0.00	0.00
St. Dev.	3.10	10.08	1.08	0.11
Range	0.00 - 10.83	0.00 - 28.87	0.00 - 3.90	0.00 - 0.32
Wilcoxon test	0.032*		0.584*	
95% CI	0.38, 10.78*		-3.90, 0.32**	
Direction	**No Change**		**No Change**	
	RANDOM COASTAL		**REFERENCE COASTAL**	
	R2	**R4/2**	**S2**	**S4**
N (Samples)	41	41	30	30
Unique Sites	41	41	11	11
Mean	9.63	11.25	7.07	12.05
Median	1.66	4.80	3.00	7.96
St. Dev.	15.07	15.32	10.31	13.59
Range	0.00 - 64.85	0.00 - 69.57	0.00 - 47.34	0.00 - 45.20
Wilcoxon test	0.346*		0.013*	
95% CI	-1.72, 4.60*		0.74, 8.00*	
Direction	**No Change**		**No Change**	

APPENDIX I
Site Specific Temperature Differences Between Rounds

Table I.1. Changes in temperature metrics from Round Two to Round Four in random and reference samples by region. R^2 values indicate the strength of the relationship between air and water temperature for each sample in Round Four; weaker relationships might indicate influence on stream temperatures from other factors such as groundwater. Regression line slopes indicate thermal sensitivity of a stream to changing air temperatures in Round Four. For average daily mean, maximum, and minimum temperature, changes are highlighted in the following categories: +0.01 to +0.50 (light pink), +0.51 to +2.00 (pink), greater than +2.00 (red), -0.01 to -0.50 (light blue), -0.51 to -2.00 (blue), and greater than -2.00 (dark blue). For percent of readings greater than 20°C and 24°C, percentage point changes are highlighted in the following categories: +0.01 to +5.00 (light pink), +5.01 to +10.00 (pink), greater than +10.00 (red), -0.01 to -5.00 (light blue), -5.01 to -10.00 (blue), and greater than -10.00 (dark blue). △ indicates change from Round Two to Round Four.

RANDOM HIGHLANDS SAMPLES							
Sample	**Slope**	**R^2**	**Avg Daily △**	**Max △**	**Min △**	**Percent >20°C △**	**Percent >24°C △**
CASS-111-R-2014	0.14	0.08	+0.47	+1.43	+0.35	0	0
UMON-229-R-2014	0.3	0.31	-0.30	-1.46	+0.50	-7.49	0
PRUN-104-R-2015	0.3	0.41	+0.54	-0.40	+2.50	0	0
YOUG-107-R-2015	0.32	0.25	+0.45	-3.83	+2.75	-2.64	0
MARS-210-R-2014	0.34	0.61	+0.36	-0.81	+0.82	-3.84	0
FIMI-202-R-2014	0.4	0.51	-0.55	-1.19	0	-8.14	0
CASS-307-R-2014	0.44	0.32	+0.63	+0.45	+1.04	+10.76	+0.68
PRWA-104-R-2014	0.45	0.45	+0.53	-2.58	-1.12	+7.73	-0.05
PRAL-208-R-2015	0.5	0.72	+0.27	-2.60	+3.35	-4.47	-0.17
YOUG-123-R-2015	0.54	0.53	0	-2.32	+1.77	-2.14	0
PRUN-205-R-2015	0.54	0.74	+0.81	+0.15	+2.90	0	0
FIMI-109-R-2014	0.55	0.56	-0.09	-0.71	0	-0.94	0
MARS-224-R-2014	0.58	0.74	-0.04	-2.27	+1.30	-2.54	-1.03
PRMO-110-R-2016	0.61	0.71	+0.50	-2.80	-1.66	+10.62	+5.31
CONO-101-R-2016	0.63	0.73	-2.15	-2.68	+0.04	-24.40	-19.90
CONO-222-R-2016	0.65	0.79	-1.57	-2.96	-2.20	-22.61	-13.65
DOUB-119-R-2016	0.67	0.79	+0.68	+2.24	-2.68	+13.84	+0.53
CONO-312-R-2016	0.68	0.81	-0.85	-1.00	-2.43	-14.71	-2.08
SAVA-105-R-2016	0.69	0.58	-0.24	-1.18	+0.69	0	0
SENE-211-R-2015	0.69	0.8	+1.22	-0.43	+2.74	+14.45	+14.57
DOUB-218-R-2016	0.7	0.79	+1.17	+2.61	-1.78	+13.41	+14.26
DOUB-221-R-2016	0.71	0.83	+0.15	-3.52	-1.86	+3.21	-0.03
SENE-210-R-2015	0.71	0.84	+1.31	0.31	+2.74	+13.88	+17.13
PRMO-222-R-2016	0.76	0.79	+2.22	-0.73	+0.08	+22.65	+34.01

Table I.2.

REFERENCE HIGHLANDS SAMPLES							
Sample	**Slope**	**R^2**	**Avg Daily △**	**Max △**	**Min △**	**Percent >20°C △**	**Percent >24°C △**
SAVA-276-S-2014	0.18	0.3	-2.53	-5.56	+0.32	0	—
SAVA-276-S-2017	0.26	0.41	+0.57	+0.03	+1.73	0	—
UMON-288-S-2014	0.3	0.14	+0.21	-0.44	+0.76	0	—
ANTI-101-S-2014	0.3	0.29	+0.33	-1.53	+1.40	-2.04	—
SAVA-276-S-2016	0.34	0.56	-0.70	-1.42	-0.34	0	—
ANTI-101-S-2015	0.41	0.53	+0.41	-1.47	+3.67	-5.32	—
YOUG-432-S-2014	0.42	0.57	+0.50	-0.50	+0.47	0	—
PRLN-626-S-2014	0.43	0.45	+0.35	-2.59	+1.07	-2.31	—
YOUG-432-S-2015	0.48	0.68	-0.28	-1.68	+2.02	-1.87	—
PRLN-626-S-2015	0.5	0.49	-0.01	-2.08	+2.53	-5.74	—
PRLN-626-S-2017	0.55	0.56	+1.02	-0.45	+2.67	+2.85	—
YOUG-432-S-2016	0.62	0.7	-0.34	-1.41	-1.22	-7.70	—
SAVA-204-S-2017	0.64	0.77	+0.89	-0.07	+2.63	-0.02	—
PRLN-626-S-2016	0.71	0.64	+0.09	+0.69	-1.17	+5.11	—
UMON-119-S-2016	0.73	0.78	+0.47	+1.63	-1.22	+9.20	—

Table I.3.

RANDOM PIEDMONT SAMPLES							
Sample	**Slope**	**R^2**	**Avg Daily △**	**Max △**	**Min △**	**Percent >20°C △**	**Percent >24°C △**
LIGU-108-R-2017	0.15	0.61	+2.22	+1.46	+1.75	+39.44	0
JONE-213-R-2016	0.38	0.51	+1.34	+3.04	-0.40	+5.16	0
BROA-104-R-2017	0.39	0.57	+1.78	+2.05	+1.24	+9.56	0
BELK-110-R-2017	0.42	0.5	+0.24	-0.18	-1.75	-4.82	+11.31
BYNU-117-R-2018	0.47	0.52	+0.26	-0.03	-0.24	+6.18	-0.83
LOGU-305-R-2016	0.47	0.75	+0.41	-0.76	-0.80	+7.26	-1.04
DEER-117-R-2015	0.49	0.61	+0.01	+2.97	+1.72	+0.11	0
DEER-101-R-2015	0.52	0.85	-0.03	-1.10	+1.66	-4.54	-1.33
NEAS-109-R-2015	0.55	0.76	+0.77	-0.48	+2.50	+14.89	-0.73
BROA-312-R-2017	0.55	0.8	+2.47	+4.17	+2.46	+39.28	+8.94
GWYN-102-R-2018	0.56	0.76	+0.69	-1.04	+0.93	+14.68	-1.77
ROCK-106-R-2017	0.57	0.65	+1.93	+2.64	+1.31	+45.94	+2.20
BIRD-107-R-2016	0.57	0.78	+1.43	+1.54	+0.44	+20.28	+19.35
RKGR-405-R-2016	0.59	0.5	+1.05	+1.18	+1.49	+6.20	0
BYNU-112-R-2018	0.59	0.69	+1.09	-1.35	-0.44	+25.20	-0.49
RKGR-107-R-2016	0.6	0.6	+0.18	-0.58	-0.27	+6.41	-2.59
OCTO-213-R-2018	0.61	0.75	+0.87	+0.67	+0.31	+21.07	0
BROA-318-R-2017	0.63	0.8	+2.26	+1.43	+2.32	+36.75	+4.62
NEAS-103-R-2015	0.64	0.8	+0.33	-0.82	+2.79	+4.91	-2.05
ANAC-313-R-2018	0.65	0.56	+1.19	+0.89	+1.55	+8.50	+19.13
LOCH-112-R-2016	0.67	0.73	+0.53	+0.26	-0.31	+12.81	0
CABJ-102-R-2017	0.67	0.74	+1.86	+1.67	+2.09	+16.19	+25.44
LIEL-318-R-2017	0.67	0.8	+1.39	+2.66	+3.13	+9.72	+18.79
LOCH-114-R-2016	0.7	0.8	+2.63	+3.82	-0.23	+48.68	+3.31
LIGU-307-R-2017	0.71	0.8	+2.37	+3.11	+2.12	+22.99	+22.06
RKGR-101-R-2016	0.73	0.78	+0.75	+1.96	-1.49	+11.74	+3.28
NEAS-312-R-2015	0.79	0.78	+0.03	-0.02	+0.97	-3.23	-2.54

Table I.4.

REFERENCE PIEDMONT SAMPLES							
Sample	**Slope**	**R^2**	**Avg Daily △**	**Max △**	**Min △**	**Percent >20°C △**	**Percent >24°C △**
LIBE-102-S-2015	0.36	0.31	+0.03	-1.18	+1.88	-1.78	0
JONE-109-S-2016	0.45	0.71	+1.62	+2.38	-0.70	+1.10	0
LOCH-120-S-2018	0.46	0.62	-0.01	-0.48	+0.41	-6.55	0
JONE-315-S-2015	0.47	0.67	+0.24	+0.81	+0.58	+3.35	0
RKGR-119-S-2016	0.51	0.74	+0.12	+0.72	-1.22	+1.87	+0.27
LOCH-120-S-2017	0.55	0.65	+1.71	+1.21	+2.12	+28.31	0
RKGR-119-S-2017	0.62	0.76	+2.33	+2.77	+1.92	+49.48	+0.32
FURN-101-S-2018	0.65	0.73	+1.44	+1.50	+0.15	+39.04	0
FURN-101-S-2015	0.7	0.74	+0.15	-2.57	+2.41	-7.48	-0.53

Table I.5.

RANDOM COASTAL SAMPLES							
Sample	**Slope**	**R^2**	**Avg Daily △**	**Max △**	**Min △**	**Percent >20°C △**	**Percent >24°C △**
ZEKI-103-R-2015	0.28	0.31	+0.56	+1.86	+2.05	-0.43	0
UPPC-216-R-2015	0.29	0.41	-1.27	-1.97	+2.43	-19.46	-7.78
NANJ-104-R-2014	0.3	0.27	-0.09	-0.26	+0.45	-12.11	0
CHIN-119-R-2015	0.31	0.44	-0.77	-4.55	+3.06	-20.81	-0.88
PISC-115-R-2015	0.37	0.33	+1.16	-1.75	+3.06	+24.42	-0.03
BODK-101-R-2015	0.38	0.54	+2.36	+4.77	+2.07	+57.35	+0.74
PRMT-110-R-2018	0.39	0.67	-1.46	+0.08	-1.40	-13.48	-12.26
WEBR-105-R-2015	0.43	0.62	+0.46	+1.47	+3.13	+13.08	-0.48
SASS-120-R-2015	0.46	0.77	-0.49	+2.22	-0.66	-26.57	+3.37
GILB-112-R-2015	0.47	0.54	+0.89	+0.03	+2.59	+23.58	0
TUCK-203-R-2017	0.47	0.64	+0.54	+0.25	+0.55	+11.80	0
MATT-210-R-2014	0.48	0.49	+0.36	+0.01	+1.36	+5.09	+2.35
SEVE-203-R-2017	0.48	0.54	+1.41	+2.22	+2.55	+35.64	+0.26
GILB-108-R-2015	0.48	0.55	+0.89	+2.15	+2.74	+13.10	+4.41
BALT-113-R-2015	0.5	0.62	+0.45	-1.88	+2.41	+10.15	-0.11
PAXL-124-R-2018	0.5	0.66	+2.78	+2.91	+1.27	+63.77	0
TRAN-211-R-2018	0.51	0.63	+0.33	-0.43	+0.07	+3.18	+4.03
MANO-119-R-2017	0.51	0.67	+0.70	+4.33	-0.92	+4.20	+15.17
LOCK-126-R-2017	0.51	0.71	+0.78	+1.04	+1.14	+7.44	+3.59
PRUT-107-R-2015	0.52	0.7	-0.02	-1.54	+2.59	+8.46	-6.81
PTOB-108-R-2017	0.54	0.7	+1.94	-0.08	+3.81	+18.85	+36.92
PAXM-122-R-2015	0.54	0.75	+0.38	-0.51	+2.54	+6.70	-0.73
OXON-101-R-2015	0.54	0.77	+0.19	-2.38	+2.33	+7.79	-6.27
MICR-208-R-2016	0.55	0.57	+0.97	+1.63	-0.39	+17.18	+4.68
GILB-101-R-2015	0.55	0.71	-0.17	-2.37	+2.09	+3.84	-5.10
PISC-104-R-2015	0.56	0.57	+0.90	-0.44	+3.11	+18.46	+1.57
ZEKI-117-R-2015	0.57	0.58	-1.92	-3.25	-0.99	-6.64	-40.41
BACK-111-R-2016	0.58	0.56	-0.12	-1.19	-0.77	-1.30	-0.93
UELK-308-R-2017	0.6	0.73	+2.12	+4.05	+2.20	+19.42	+33.55
PAXL-120-R-2018	0.61	0.7	+0.78	+0.07	+0.25	+16.69	0
MATT-320-R-2014	0.63	0.69	-0.84	-0.80	+0.92	+0.90	-21.24
WYER-216-R-2017	0.63	0.74	+1.18	+1.02	+0.36	+13.61	+6.02

NANT-109-R-2016	0.63	0.75	-0.71	+0.41	+1.29	-7.20	-10.56
SOUT-101-R-2016	0.63	0.75	+1.05	+0.95	-0.33	19.26	+4.16
PRMT-206-R-2018	0.65	0.77	-0.46	-1.03	+0.25	-0.48	-11.06
PTOB-104-R-2017	0.65	0.82	+1.44	+0.04	+2.99	+16.34	+20.26
WCHE-105-R-2017	0.67	0.79	+1.86	+0.81	+1.88	+28.35	+4.32
BRET-101-R-2016	0.74	0.73	+0.21	+1.85	-0.04	-2.19	+8.66
PTOB-103-R-2017	0.74	0.84	+2.21	+2.85	+2.14	+27.87	+8.93
ZEKI-312-R-2015	0.8	0.84	+1.26	+0.86	+2.06	+11.04	+21.00

Table I.6.

REFERENCE COASTAL SAMPLES							
Sample	**Slope**	**R^2**	**Avg Daily △**	**Max △**	**Min △**	**Percent >20°C △**	**Percent >24°C △**
UPCK-113-S-2015	0.22	0.23	-0.46	-0.45	+2.48	-3.89	+0.57
WIRH-220-S-2017	0.41	0.45	+1.40	+2.09	+0.79	+22.52	+4.63
LOCR-102-S-2017	0.42	0.29	-1.16	-0.74	+0.27	+2.75	-30.12
UPCK-113-S-2017	0.42	0.39	+2.21	+3.95	+2.85	+31.99	+8.00
PTOB-002-S-2014	0.44	0.49	-0.01	-1.28	+0.69	+0.52	-2.25
STCL-051-S-2014	0.44	0.51	-0.04	-0.11	+0.47	-4.34	0
WIRH-220-S-2016	0.46	0.56	+2.36	+3.36	+1.14	+53.63	+10.34
PTOB-002-S-2017	0.48	0.54	+0.26	-0.65	+2.25	+3.02	-7.40
CORS-102-S-2015	0.5	0.7	+0.13	-3.69	+2.30	+11.13	-4.52
PTOB-002-S-2015	0.52	0.66	+0.01	-0.99	+2.40	+8.34	-5.01
NANJ-331-S-2014	0.54	0.58	+0.41	+0.31	+0.29	+11.65	+1.55
STCL-051-S-2015	0.54	0.77	+0.90	-0.07	+2.96	+21.57	-0.06
NASS-108-S-2017	0.57	0.5	-0.01	+5.56	-0.48	-5.24	+4.35
PAXL-294-S-2014	0.57	0.59	-0.01	-0.58	+0.23	+2.48	+0.03
LOCR-102-S-2015	0.59	0.48	+0.08	+0.92	+0.19	-6.43	+7.97
UPCK-113-S-2016	0.59	0.63	+0.25	+6.28	-0.18	+4.40	+2.65
CORS-102-S-2017	0.59	0.65	+0.67	+1.12	+1.26	+6.50	+4.05
LOCR-102-S-2018	0.6	0.41	+1.22	-0.86	+0.90	+7.57	+24.44
NASS-108-S-2016	0.6	0.72	+0.49	-0.77	+1.99	+13.96	-3.18
MATT-033-S-2017	0.61	0.61	+0.69	+1.71	+1.71	+8.78	+11.72
STCL-051-S-2017	0.62	0.67	+1.04	+0.46	+1.77	+11.84	+0.29
STCL-051-S-2018	0.62	0.69	+1.03	+0.97	+0.86	+16.81	+0.08
MATT-033-S-2015	0.65	0.55	+0.55	+1.2	+1.64	+3.69	+15.90
NANJ-331-S-2015	0.66	0.71	+0.65	+0.41	+2.18	+7.05	+6.02
PTOB-002-S-2018	0.67	0.75	-0.06	-0.81	-0.48	-0.36	-1.18
NASS-302-S-2017	0.67	0.78	+1.98	+3.51	-0.10	+11.87	+40.06
NANJ-331-S-2017	0.7	0.81	+1.49	+1.93	+2.14	+14.97	+15.16
PAXL-294-S-2017	0.71	0.8	+2.08	+2.35	+1.91	+26.62	+9.75
NASS-302-S-2016	0.73	0.8	+2.06	+2.27	+0.97	+21.50	+32.33
STCL-051-S-2016	0.76	0.73	+0.52	-0.62	-0.22	+7.63	+3.08