

EFFECTS OF SPATIALLY AND TEMPORALLY HETEROGENEOUS DISTURBANCE
ON BENTHIC, NEARSHORE BIOTA IN LARGE, DEEP,
OLIGOTROPHIC LAKES

By

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the requirements for the degree of

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To the Faculty of Washington State University:

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EFFECTS OF SPATIALLY AND TEMPORALLY HETEROGENEOUS DISTURBANCE
ON BENTHIC, NEARSHORE BIOTA IN LARGE, DEEP,
OLIGOTROPHIC LAKES

Abstract

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Ecosystem integrity is threatened by disturbance from human activities. Although disturbances can take many different forms, human disturbances are inherently heterogeneous, as these disturbances tend to occur where and when people are present. This inherent spatio-temporal heterogeneity means that we can investigate how independent and intersecting spatial and temporal components of ecological disturbances can influence biotic communities.

Wastewater released into aquatic systems is an example of a high-impact human disturbance that can reshape aquatic communities. Algal communities, especially in oligotrophic lakes, can rapidly assimilate nutrients, causing increased biomass and altered resources for grazers, which may likewise relocate or adjust behavior and physiology to compensate. Despite seeming straightforward, associating times and locations of nutrient loading with explicit biological responses is challenging, as heterogeneities in nutrient loading and biological succession can obfuscate patterns.

Using Lake Baikal (Siberia) and Flathead Lake (Montana, U.S.) as study systems, this dissertation investigates how human disturbance can alter nearshore communities in large

oligotrophic ecosystems. Despite Baikal being remote and globally the largest lake by volume, low-level sewage pollution is suspected near certain small lakeside settlements. To investigate potential sewage pollution along a 40-km transect, we sampled water column sewage-associated micropollutants (i.e., microplastics and pharmaceuticals and personal care products – PPCPs), algal and macroinvertebrate abundance, as well as fatty acid profiles that provide insights on trophic interactions. Within the U.S. Pacific Northwest, Flathead Lake also is relatively well protected from human influence yet has demonstrated localized signs of sewage pollution. Building on our spatial study at Lake Baikal, we sampled 15 locations in Flathead Lake over four months for PPCPs as well as periphyton composition, stoichiometry, and fatty acids. Together, these studies evaluate how spatio-temporally heterogenous sewage loading can not only influence community composition, but also nutritional content available to the lake's larger food web. For Lake Baikal and Flathead Lake, these results are timely, as both lakes are experiencing increasing tourism and an aging wastewater management infrastructure. For lakes worldwide, this work details how ecosystems can respond to eutrophication, while also providing data and infrastructure for continued study intensively within systems and extensively across scales.

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Dedication

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INTRODUCTION

Ecosystem integrity is threatened by disturbance associated with human activities. The type and intensity of human disturbance can affect ecosystems directly, such as physical alteration of suitable habitat (Turner 1996; Vitousek et al. 1997), as well as indirectly, such as through non-native species introductions (Mack et al. 2000). Together, direct and indirect modifications can affect organismal physiology (Sokolova and Lannig 2008; González-Fernández et al. 2017) and behavior (Brodin et al. 2013), as well as population (Crouse et al. 1987; Hoppe et al. 2012) and community (Rohr et al. 2006; Feijão et al. 2020) structure, food web interactions (Lagesson et al. 2016; Richmond et al. 2018), and even ecosystem function (Rosi-Marshall et al. 2013; Shaw et al. 2015).

Ecosystems adjacent to disturbances, or those located along a system's edge, often experience concentrated disturbances through space and time, as the mutual systems on either side of a boundary exchange energy, nutrients, and even species (Murcia 1995). Consequently, communities along these boundaries can fluctuate with respect to species composition, food web structure, and ecosystem function, thereby creating heterogeneity in the biotic community (Murcia 1995; Harper et al. 2005). For example, spatio-temporal dynamics in human logging can create patch matrices where heterogeneity in disturbance along the edge of a patch can alter species composition of primary producers, consumers, and other inhabiting taxa (Broadbent et al. 2008). Similarly, spatial heterogeneity from the physical alteration of lake shorelines can render some habitats unusable for fish spawning (Scheuerell and Schindler 2004). Regardless of the system, heterogeneous variation in human activity can concentrate disturbances and lead to more extreme environmental stresses (Townsend and Hildrew 1994), and impacts may be magnified if the disturbance co-occurs with sensitive biological processes (Lake 2000; Post et al. 2000).

Likewise, biotic responses to disturbance may vary in magnitude and complexity, as interactions between spatial and temporal disturbance events may amplify effects beyond their additive consequences (Hobbs and Mooney 1995; Higgins and Richardson 1998; Lilleeng et al. 2016; Ren et al. 2021).

Given the potential for spatio-temporally heterogeneous disturbance to create complex biotic responses, conclusively linking those same biological responses to a particular disturbance requires careful methodological planning and a study system where effects can be assigned to a specific cause (Menge and Sutherland 1987; Carpenter et al. 2001). Less urban, large, deep, oligotrophic lakes present unique opportunity to study heterogeneous disturbance due to their capacity to respond strongly to even low levels of human activity. First, less urban environments often have isolated pockets of human activity, creating a system with potentially strong heterogeneity (Moore et al. 2003). Second, large, deep lakes often have a high proportion of biota in the shallow, nearshore area compared to the pelagic, despite the pelagic representing the majority of the overall lake volume (Vadeboncoeur et al. 2008). While lake monitoring efforts historically focus on offshore sampling (Vadeboncoeur et al. 2002), the littoral zone is arguably more susceptible to shoreline alterations because the littoral's close proximity to potential disturbances puts it directly in the path of shoreline disturbances (Rosenberger et al. 2008; Hampton et al. 2011; Vadeboncoeur et al. 2021). However, the detection of nearshore impacts on biological processes can be elusive. For example, in the case the nearshore nutrient addition, benthic primary producers, especially those in oligotrophic lakes, can assimilate dissolved nutrients and other solutes quickly from the water column (e.g., hours; Hadwen and Bunn 2005), such that deviation in typical water concentrations may not be observed, although changes in periphyton (Rosenberger et al. 2008; Hampton et al. 2011; Naranjo et al. 2019; Atkins et al.

2021) and even grazing macroinvertebrate (Fink et al. 2006; Rosenberger et al. 2008; Hampton et al. 2011) community structure may imply otherwise.

Nutrient addition from human sewage into aquatic systems is a widespread, frequently high-impact example of human disturbance that can reshape aquatic food webs (Edmondson 1970; Carpenter et al. 1998). Historically, increased nutrient concentration near areas with adjacent human development have implied wastewater seepage (Edmondson 1970; Carpenter et al. 1998; Moore et al. 2003). However, this conclusion is often circumstantial, as increased nutrients can be associated with disparate non-anthropogenic sources such as agriculture (Powers et al. 2016), forest fires (Hohner et al. 2016) or atmospheric deposition more broadly (Monteith et al. 2007; Elser et al. 2009), melting permafrost (Turetsky et al. 2000), or even changes in terrestrial plant communities (Goldman 1961; Moran et al. 2012). To identify that nutrient pollution is from human sewage rather than other sources, sewage-specific indicators, such as pharmaceuticals and personal care products (PPCPs; Meyer et al. 2019), microplastics (Free et al. 2014), $\delta^{15}\text{N}$ stable isotopes (Gartner et al. 2002), and even microbial biomarkers (Seguel et al. 2001), have been used as sewage-specific indicators capable of defining locations, times, and gradients of sewage pollution. As each of these indicators tend to be frequently, and often consistently, associated with sewage pollution, these indicators likewise have the potential to define heterogeneities in sewage loading. When paired with co-located, contemporaneous biological data, sewage-indicator abundance data can be used to explain how communities may be responding to sewage loading.

Given the potential for sewage-specific indicators to define locations, timepoints, and even gradients of a heterogenous human disturbance, my dissertation's main question is "how does a spatially and temporally heterogeneous disturbance, such as sewage-associated nutrient

addition, cause ecological communities to respond with respect to taxonomic composition and food web structure?" Using a suite of sewage indicators to define the disturbance and large, deep, oligotrophic lakes as a study system, my dissertation focuses on how benthic, littoral periphyton and macroinvertebrate community composition can shift in response to sewage disturbance, and how these changes in community composition can propagate throughout a food web via changing trophic interactions as well as by altering periphyton nutritional content. Beyond this work's contribution to basic ecological questions, my dissertation also helps facilitate future research directions. In particular, my dissertation includes an evidence synthesis of the PPCP literature, which quantitatively showcases both foundations already lain in the PPCP literature and opportunities to address less represented compounds, sewage treatment techniques, and ecosystem types. Additionally, my dissertation includes peer-reviewed data products, multiple scripting routines, and archived code repositories not only to encourage reproducibility and transparency but also to build data and code infrastructures that can allow for syntheses across systems and scales. This work's novelty stems from the integration of limnology, ecology, environmental synthesis, and open science, and it is unusual in that:

- (1) it uses *in situ* emerging contaminants and natural gradients to offer insight into how spatio-temporal heterogeneity in human disturbance can alter ecological communities,
- (2) it emphasizes lakes, whereas historical focus on PPCPs has been on streams, rivers, and groundwater,
- (3) it demonstrates that even voluminous, less urban lakes can contain environmentally relevant PPCP and other sewage indicator concentrations with measurable ecological responses, and

(4) it unifies all four disciplines, while likewise making independent contributions within each.

This dissertation contains four independent, yet connected, studies, all of which are targeted at understanding ecological consequences of sewage pollution in lakes and priming future research directions. Each chapter and its relation to the other chapters are detailed below.

Chapter 1 (Meyer et al. 2019) provides context for the rapid growth in the PPCP literature by quantitatively assessing which compounds, sewage treatment techniques, and ecosystem types have historically been studied over the past 30 years. Using over 6,500 abstracts from Web of Science, this study clearly identifies both strong, historical foundations and opportunities to incorporate less represented topics. In particular, this study highlights lakes as well as decentralized wastewater treatment infrastructures, such as septic systems, as being far less represented in the literature relative to lotic environments and centralized wastewater treatment infrastructures, such as wastewater treatment plants.

Chapter 2 (Meyer et al. Under Review) assesses how spatially heterogeneous sewage pollution along a 40-km stretch of Lake Baikal's southwestern shore can influence periphyton and macroinvertebrate taxonomic composition and trophic interactions. This study uses census data and development indices to compress, into a single metric, information about the density, spatial position, and proximity of human populations, which is then ground-truthed using sewage-specific indicators, such as PPCPs and $\delta^{15}\text{N}$ signatures. Co-located biological data are then compared across this disturbance gradient to assess how communities have diverged at the end of a summer growing period. This study highlights that sewage-associated micropollutants can indeed be used to define a disturbance gradient with periphyton and macroinvertebrate community compositions altering along that gradient. However, food web structure appeared to

be consistent along the spatial gradient, implying that grazers may be compensating through various behavioral or physiological mechanisms.

Chapter 3 (Meyer et al. In Prep) builds on Chapter 2 by including time to evaluate how spatio-temporally heterogeneous sewage pollution can affect periphyton community succession as well as available nutrition to the lake's larger food web. This study uses census data and development indices to create a metric for population through space; further, by scaling spatial human population metrics by relative changes in State Park visitor passes, the metric accommodates changes in human population in time. With local knowledge about wastewater treatment infrastructure, we use this human population metric to ground-truth sewage indicator abundance. Finally, we pair sewage indicator data with co-located, contemporaneous periphyton community abundance, stoichiometry, and fatty acid data to assess how periphyton communities' successional patterns may have deviated throughout a summer. This study highlights how differences in wastewater treatment infrastructure and temporally varying human populations can alter community successional patterns as well as the quality and type of nutrition available to the lake's food web.

Chapter 4 (Meyer et al. Under Revision, 2015, 2020) offers a path forward for future study of ecological responses to sewage pollution by describing, documenting, and archiving the data produced in Chapter 2. As Chapter 1 demonstrated, there has been a rapid increase in the number and diversity of sewage-associated micropollutant study over the past three decades. Aggregating and preserving those data in a reproducible, transparent format is crucial for ensuring reuse. In particular, data collected at Lake Baikal may be useful for filling in gaps in nearshore monitoring data. Beyond Baikal, these data may be useful for synthesis across systems. Regardless of the exact research question, these data are highly uncommon. To the best

of our knowledge, there have not been publicly available nearshore community abundance data for Baikal's endemic amphipods in a machine-readable, peer-reviewed format. By providing both raw and cleaned data as well as all scripting routines, this study not only documents and encourages reuse of an uncommon data source of over 150 variables, but also provides scripted frameworks for harmonizing disparate data sources into a single, analytically-friendly format.

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CHAPTER ONE

AN EVIDENCE SYNTHESIS OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS (PPCPS) IN THE ENVIRONMENT: IMBALANCES AMONG COMPOUNDS, SEWAGE TREATMENT TECHNIQUES, AND ECOSYSTEM TYPES

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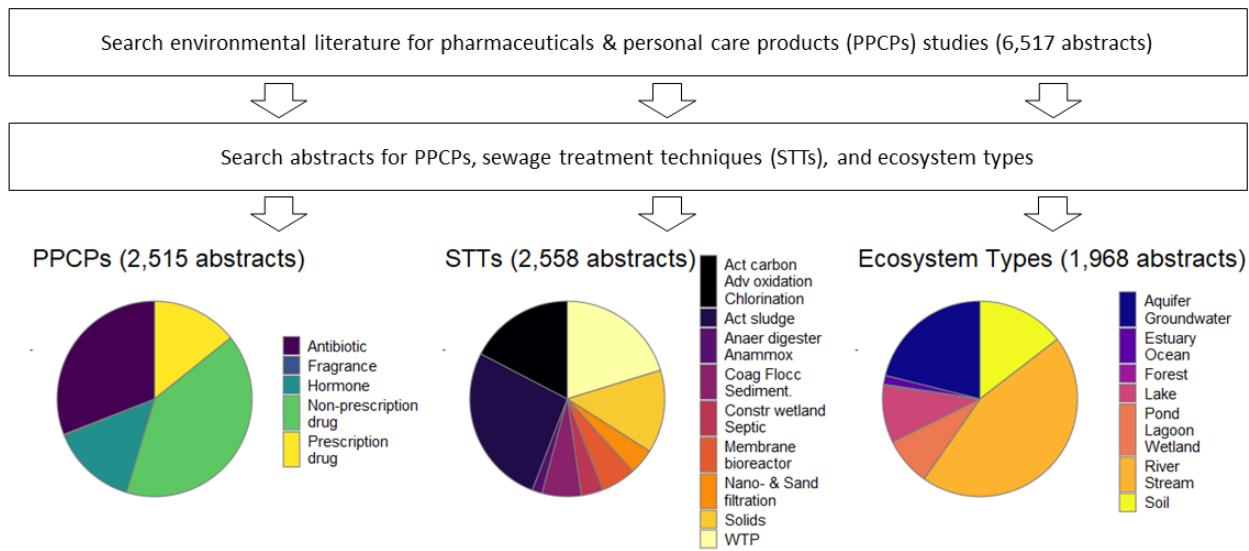
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1.1 Abstract

Pharmaceuticals and personal care products (PPCPs) garner increasing attention globally for both their usefulness as indicators of human waste and their potency as emerging organic toxicants. Three decades of rapid increase in PPCP study combined with an increasing number of PPCPs on the global market have created opportunity (1) to review trends in diversity of compounds, sewage treatment techniques (STTs), and ecosystems investigated as well as (2) to identify knowledge gaps in the literature. We conducted a quantitative evidence synthesis of 6,517 abstracts from primary articles in the environmental PPCP literature by examining relative abundance of specific PPCP classes, STTs, and ecosystem types. Our results demonstrate that non-prescription drugs and antibiotics dominated PPCP abstracts, appearing in 51% and 39% of reviewed abstracts respectively, in comparison to hormones (18%), prescription drugs (18%), fragrances (0.3%), and antioxidants (0.0%), which can all elicit physiological and ecological responses even at low concentrations. References to centralized STTs (e.g., activated sludge - 37%) were more frequent than decentralized STTs (e.g., septic - 2%), despite decentralized STTs being common and frequently high impact sources of sewage pollution worldwide. Freshwater lotic systems (63%) were more prevalent than freshwater lentic (24%) and terrestrial (20%) systems. This discrepancy is notable because the longer residence times of lentic and terrestrial systems may enable PPCPs to concentrate and thus increase risk of biological consequences. These results highlight distinct opportunities to address knowledge gaps in the environmental PPCP literature, including underrepresented compounds (e.g., fragrances), sewage treatment techniques (e.g., septic systems), and ecosystem types (e.g., lakes).

1.2 Graphical Abstract



1.3 Introduction

Pollutants associated with treated and untreated wastewater are pervasive and can threaten ecosystem integrity and human health.¹ While wastewater impact studies have historically focused on increased nutrients, biological oxygen demand (BOD, BOD₅), and chemical oxygen demand (COD) in effluent,^{2,3} recent attention has widened to include various organic micropollutants.⁴ Pharmaceuticals and personal care products (PPCPs), in particular, constitute a growing subgroup of emerging organic micropollutants in research across multiple disciplines for both their usefulness in detecting sewage pollution as well as their potency as toxicants.

Because PPCPs are consistently associated with sewage, even minute concentrations (e.g., ng/L) of PPCPs have garnered attention for their usefulness in detecting presence and measuring magnitude of wastewater pollution in various environments (Figure 1). Research by Kolpin et al.⁵ is often cited (5,126 as of 06 August 2019) as one of the most extensive surveys of

PPCPs in the environment, for which 139 U.S. rivers were surveyed for 95 emerging organic contaminants. At least one PPCP was found in 80% of rivers. PPCPs also have been found in other types of surface waters,⁶ groundwater,⁷ soils,⁸ and biotic tissue.⁹ For both soil and water, wastewater treatment facilities with several potential sewage treatment techniques (STTs) appear to be the main sources of PPCPs with concentrations directly proportional to size of the population served by the STT.¹⁰ Depending on location, the specific cocktail of PPCPs can vary greatly if a wastewater treatment facility receives influent from agricultural,¹¹ medical,¹² or manufacturing¹³ operations. In the case of terrestrial systems, applied biosolids can introduce PPCPs into soils,¹⁴ where compounds can sorb to colloidal materials.¹⁵ In aquatic environments, PPCPs can enter systems directly through wastewater effluent, leaks in the wastewater treatment system, or runoff.^{16,17} Once in the aquatic environment, PPCPs may sorb to organic matter,¹⁸ degrade through physicochemical processes,¹⁹ bioaccumulate¹³ and biomagnify within a food web,²⁰ or be metabolized by the organism itself.²¹

In addition to their role as indicators of human activity, PPCPs can also be toxicants, disrupting biological and ecological processes^{22,23} with physiological,²⁴ developmental,²⁵ population,¹⁸ and ecosystem effects.²⁶ In particular, antibiotics, such as ampicillin,²⁷ erythromycin,²⁸ and tetracycline,²⁹ have been well documented to introduce antibiotic resistance following exposure. At higher levels of biological organization, antibiotics can also alter bacterial community structure and function. For example, exposure to 200 µg/L of ciprofloxacin, a common antibiotic, has been associated with both altered sediment bacterial community composition and decreased capacity to metabolize pyrenes.³⁰ More recently, exposure to antibiotics has demonstrated potential to influence key ecosystem functions, such as the findings

that 50 µg/kg sulfadimethoxine reduces soil nitrification,³¹ and 1 µg/L of enrofloxacin reduces abundance of ammonia-oxidizing Archaea in lake systems.³²

Although the early environmental PPCP literature often focused on antibiotics, a suite of non-prescription and prescription drugs also has been associated with disrupted biological and ecological processes. The mechanisms underlying these associations are often unknown. For example, caffeine is common in aquatic and terrestrial environments, and has been associated with genetic,³³ developmental,³⁴ and behavioral³⁵ responses in invertebrates, without a clear understanding of the physiological mechanism.³⁶ Varied responses following caffeine exposure have also been observed at the community level. In lotic systems, 0.015 M caffeine exposure was associated with decreased biofilm respiration and decreased heterotroph abundance.^{26,37} In contrast, 2.5 mM caffeine exposure was not associated with change in biofilm respiration in lentic systems.³⁸ While these divergent responses may be the product of caffeine concentrations used in each study, the overarching theme of uncertainty in biotic response to PPCPs can be further complicated when considering how various life stages may react to contaminant concentrations, mixtures of co-contaminants, as well as the spatio-temporal heterogeneity of those concentrations.

Given the rapid growth in the PPCP literature, the number of PPCPs on the global market, and the potential consequences for ecological integrity, there is need to evaluate the extent to which chemicals, STTs, and ecosystem types have been studied. Imbalances in the types of PPCPs, STTs, and ecosystem types that appear in the literature could indicate gaps in knowledge. In order to identify potential imbalances within the research examining PPCPs in the environment, we conducted an evidence synthesis to quantitatively evaluate publication trends in existing PPCP research. Evidence syntheses use structured search techniques to holistically

address a literature corpus with the goal of identifying knowledge gaps.³⁹ We compiled 6,517 abstracts pertaining to PPCPs in the environment and noted instances of specific compounds, sewage treatment techniques, and ecosystem types mentioned in abstracts. To our knowledge, no prior reviews have evaluated trends in chemical diversity (i.e., number and types of contaminants considered), sewage treatment techniques, and ecosystem types quantitatively within the environmental PPCP literature. Our review quantitatively examines the available environmental PPCP literature by addressing the following objectives:

- (1) to examine the diversity of PPCPs that have been studied both individually and in mixtures,
- (2) to evaluate which types of PPCP treatment technologies have been investigated, and
- (3) to identify which ecosystem types have been the focus of PPCP research.

1.4 Methods

In order to gather a wide array of environmental PPCP studies that spanned from field- to laboratory-scale and included a diversity of sample types ranging from water to organisms, we performed a Web of Science (WOS) search with the formula “TS = pharmaceutical* AND TS = (sewage OR wastewater)” on 06 August 2019, resulting in 8,803 returned records. The final WOS search formula was selected by iteratively varying the WOS searches (e.g., “TS = (pharmaceutical* OR personal care product*) AND TS = (sewage OR wastewater)”) until returned records largely contained a desired literature corpus of research relevant to the environment. Further refinement by primary articles in English and subject category limited abstracts to environmentally focused topics (e.g., Environmental sciences, Engineering civil; see

Supplemental Methods for a complete list of “Subject Categories”), resulting in 6,517 records from 01 January 1990 to 06 August 2019. In doing so, our process focused on studies that consider presence of PPCPs within an environmental setting, as opposed to studies that may loosely reference environmental consequences of PPCPs. This process is summarized in Figure 2. To assure that these results were relevant to our search, we randomly sampled 250 returned records and assessed the title and abstract for pertinence to an environmental context. This process confirmed that the returned records were within the scope of our environmental PPCP review. We then downloaded full record contents for each of the returned studies. Next, we imported all record contents into the R environment (Supplemental Methods S4-S7),⁴⁰ where subsequent analyses were conducted.⁴¹⁻⁵⁰ More detailed methods regarding data cleaning and wrangling are included in the supplementary information.

1.4.1 PPCPs

To focus on specific PPCPs, we created a list of 70 focal compounds from Kolpin et al.⁵ and Focazio et al.⁵¹ While both Kolpin et al.⁵ and Focazio et al.⁵¹ also include data on various flame retardants and detergents, here we only focus on physiologically active compounds that are ingested and excreted by humans and likely enter wastewater treatment systems. It is worth noting that focusing on PPCPs from Kolpin et al.⁵ and Focazio et al.⁵¹ excludes PPCPs that could not have been detected analytically or were not in existence prior to 2008. However, by selecting pharmaceuticals from these publications, we target compounds that could have been researched for at least eleven years, allowing consideration of trends. Each compound was classified into one of six “usage classes” according to categories used by Kolpin et al.⁵ and Focazio et al.⁵¹: antibiotic, prescription drug, non-prescription drug, hormone, fragrance, and antioxidant. As mentioned in Focazio et al.,⁵¹ delineations of usage classes can vary widely between studies, and

interpretations resulting from these groupings should consider the constituent compounds included within the classification. Table 1 provides a full list of PPCPs considered and their usage classes.

We then searched through the 6,517 abstracts for each of the 70 PPCPs (S7-S10). Among those abstracts, 2,515 abstracts (39% of all abstracts) contained instances of at least one compound, and 605 abstracts contained instances of at least two compounds (S10-S12). To verify that abstracts referencing any of the focal pharmaceuticals were relevant, representative of the entire paper, and detected in the samples collected, we randomly sampled 50 of the 2,515 abstracts referencing at least one PPCP and read the associated articles completely. Among those randomly sampled, 49 of the 50 were within the intended scope of our environmental PPCP review, suggesting the majority of abstracts in the full corpus were relevant. Additionally, to verify that abstracts referencing mixtures of any focal pharmaceuticals were likewise relevant, we randomly sampled 50 of the 605 abstracts referencing at least two PPCP usage classes and read those articles completely. Among those randomly sampled, 49 of the 50 were within the intended scope of our environmental PPCP review, suggesting the majority of abstracts referencing mixtures of multiple PPCP usage classes were relevant.

1.4.2 Sewage Treatment Techniques (STTs)

Next, we examined the original 6,517 abstracts for STTs (S12-S15). We created a list of focal STT terms, including “septic”, “WTP” (e.g., Water Treatment Plant, Wastewater Treatment Plant, Drinking Water Treatment Plant), “activated sludge”, “membrane bioreactor”, “advanced oxidation”, “nanofiltration”, “chlorination”, “solids” (including “biosolids”), “coagulation”, “flocculation”, “constructed wetland”, “sand filtration”, “sedimentation”, “anaerobic digester” (including “anaerobic digestion”), and “anammox”, as identified in Yang et al.⁵² Oulton et al.⁵³

and Onesios et al.'s⁵⁴ reviews of PPCP removal technologies. Our focal terms describe centralized and decentralized STTs, where “septic” and “constructed wetland” refer to a decentralized STT and the remaining focal terms describe centralized STTs. These terms also represent forms of primary and secondary treatment (e.g., “activated sludge”, “membrane bioreactor”) as well as tertiary treatment (e.g., “advanced oxidation”, “nanofiltration”, and “chlorination”). Terms used to refer to similar STTs may vary regionally or through time; we are unaware of such instances in our search terms, but acknowledge that such regional or temporal differences in terminology could affect interpretation. We then searched through 6,517 abstracts for instances of each focal STT. Of the 6,517 abstracts, 2,558 abstracts (39%) contained instances of at least one STT. To assure that our process identified abstracts that were representative of the entire study, we randomly sampled 50 studies of the 2,558 studies referencing at least one STT and read them completely. All were within scope.

1.4.3 Ecosystem Types

Finally, we examined publication trends for particular ecosystem types that have been investigated with respect to PPCPs (S15-S18). We created a list of focal ecosystem types, including “stream”, “river”, “estuary”, “lake”, “pond”, “ocean”, “groundwater”, “aquifer”, “lagoon”, “wetland”, “forest”, and “soil”. These terms were selected by identifying the most frequently used words in all 6,517 abstracts (i.e., those appearing at least 100 times), and then manually selecting ecosystem-specific terms from this list. These terms broadly represented freshwater lentic (“lake”, “pond”, “lagoon”, and “wetland”), freshwater lotic (“stream” and “river”), subsurface (“groundwater” and “aquifer”), marine (“ocean” and “estuary”), as well as terrestrial (“soil” and “forest”) systems. While some of these terms may also refer to a STT (e.g., lagoon), we attempted to remove confounding focal terms where possible, such as excluding

mentions of “polishing pond”, and only searching for complete words. We then searched the 6,517 abstracts for instances of each focal ecosystem type. Of the original 6,517 abstracts, 1,968 (30%) contained reference to at least one ecosystem type. To verify that our process identified abstracts that were representative of the entire study, we randomly sampled 50 studies of the 1,968 referencing at least one ecosystem type and read them completely. All were within scope.

In order to assess study design for each ecosystem type, we collected a stratified subsample of 100 abstracts from the 1,968 abstracts referencing at least one ecosystem type, where strata were weighted by an ecosystem type’s abundance. We then read each study and characterized each as being based on “field observation”, “lab experiment”, “modeling”, or a combination. Because subsampled abstracts were weighted by ecosystem type, this technique enables our analysis of study designs to extend for the entire environmental PPCP literature corpus.

1.4.4 Reported Results

Annual results are reported as the ratio of abstracts referencing a particular PPCP, STT, or ecosystem type to total abstracts referencing at least one PPCP, STT, or ecosystem type in a given year (e.g., $\frac{\text{Number of abstracts referencing septic in 2002}}{\text{Number of abstracts referencing any STT in 2002}}$). Reporting results as proportions rather than raw numbers of studies facilitates comparison of 2019 to successive years, given that the corpus ends on 06 August 2019. Because one abstract may reference more than one term, the sum of proportions across all terms in a given year may be greater than one. For a given focal term, mean frequencies are reported as the average percentage across all years.

1.5 Results

Our selection process identified 6,517 abstracts comprising the literature corpus from 01 January 1990 to 06 August 2019. Research on PPCPs in the environment appears to be accelerating. 6,147 abstract (94% of all abstracts) have been published since 2006, and 5,418 (83%) abstracts have been published since 2010. The highest research production in this field is associated with first authors based in North America, Asia, and Europe (Figure 3).

1.5.1 PPCPs

Our results indicate that antibiotics and non-prescription drugs dominate the PPCP literature compared to other usage classes (Figure 4, Table S2). PPCP publications began to appear in 1990, and references to specific usage classes started appearing in 1997. Of the original 6,517 abstracts, 2,515 (39%) referenced a specific PPCP, with non-prescription drugs appearing first in 1997 and antibiotics appearing in 1998. Non-prescription drug frequencies ranged from 33.3% to 100% of abstracts in a given year, averaging 51%. Similarly, abstracts referencing antibiotics ranged from 0% to 66.7% and averaged 39%. Hormones and prescription drugs were the second most represented compounds beginning shortly after 2000 (Figure 4, Table S2), averaging 18% and 18% respectively. Fragrances were rarely represented, averaging 0.25% of abstracts (Figure 4, Table S2). Antioxidants were completely absent from all abstracts for the entire time series (Figure 4, Table S2). Because these results only include references to the 70 pharmaceuticals in Kolpin et al.⁵ and Focazio et al.,⁵¹ they represent a very small fraction of the over 4,000 pharmaceuticals available on the global market.¹⁶

With respect to viewing PPCPs as components of a mixture, recent years have seen a rise in total number of abstracts with multiple usage classes, but the proportion of abstracts with at least two usage classes remained constant (Figure 5). Among all abstracts mentioning at least

one usage class, 1.9% referenced two or more, 0.3% referenced three or more, and 0.03% referenced four. No abstracts referenced more than four usage classes.

1.5.2 Sewage Treatment Techniques (STTs)

Centralized wastewater treatment techniques, such as wastewater treatment plants, were more frequently mentioned in abstracts than decentralized systems (Figure 6, Table S3). Of the original 6,517 abstracts, 2,392 (39%) referenced a specific STT starting in 1992. While centralized systems entered and remained prevalent since the early 1990s, decentralized systems were less common, such as septic and constructed wetlands averaging 2% and 3% of abstracts respectively (Figure 6, Table S3).

When assessing different techniques in the centralized treatment process, activated sludge systems were most abundant and appeared first, ranging between 0 and 100% of PPCP abstracts (average 37%), followed by membrane bioreactors that ranged from 0% to 20% of abstracts (7%) and anammox averaging 0.2% of abstracts in a given year. With respect to certain components of tertiary treatment, activated carbon appeared first in 1992 (12%), followed by advanced oxidation in 1997 (8%), nanofiltration in 2002 (5%), and chlorination in 2003 (2%). No abstracts referencing a form of tertiary treatment also specified an accompanying form of primary and secondary treatment.

With respect to solid-removal processes and technologies, removal of PPCPs via suspended solids and biosolids was identified in 19% of abstracts. Other forms of solid removal technologies, however, were much less frequent. Coagulation and sedimentation averaged 4% and 3% of abstracts respectively, in addition to flocculation and anaerobic digestion averaging 2% of abstracts each.

1.5.3 Ecosystem Types

Our results indicate that aquatic, and largely lotic freshwater systems, dominate the environmental studies of PPCPs (Figures 7, Table S4). Aquatic systems consistently represent approximately 80% of all PPCP-related studies published within a given year. No abstracts referenced both terrestrial and aquatic environments. When focusing exclusively on aquatic systems, abstracts mentioning river (45%) and stream (18%) systems first appeared in 1994. In contrast to lotic environments, lentic systems such as lakes (13%), wetlands (5%), ponds (4%), and lagoons (2%), are relatively uncommon in the literature. While surface waters were more frequently represented than subsurface systems, in any given year groundwater was still more common in the PPCP literature than non-lotic systems, averaging 18% for groundwater and 11% for aquifers (Figure 7, Table S4). With the least representation, marine systems have appeared within the literature since 2005 and have remained marginally represented with oceans averaging 1% of abstracts and estuaries averaging 1% (Figure 7, Table S4). With respect to system-specific study designs (Table 2), the field-based studies largely included river (29%) and stream (11%) ecosystem types, whereas soil ecosystem types were the most represented laboratory based studies (10%).

1.6 Discussion

Our results demonstrate the worldwide growth of research on PPCPs in the environment, particularly among institutions in North America, Europe, and Asia (Figure 3). Most studies pertaining to PPCPs have been conducted since 2006 (94%), with marked increases in research productivity from institutions in China (Figure 3). Our analysis identified a clear imbalance in research effort for certain usage classes (antibiotics and non-prescription drugs), sources (centralized STTs), and ecosystem types (lotic systems).

Among usage classes, antibiotics and non-prescription drugs dominate the environmental PPCP literature over prescription drugs, hormones, and fragrances (Figure 4), chronologically paralleling the development of mass spectrometry (MS) analytical techniques for PPCP quantification in environmental matrices.⁵⁵⁻⁵⁷ In reviews of analytical challenges for PPCP quantification in environmental matrices, Hao et al.⁵⁵ and Sedlak et al.⁵⁶ highlighted acidic, non-volatile compounds, including many antibiotics and non-prescription drugs, as most amenable for liquid chromatography (LC) and some gas chromatography (GC) MS techniques, whereas neutral and volatile PPCPs, such as many fragrances and hormones, require laborious liquid-liquid extractions prior to GC/MS quantification. This dichotomy in physicochemical properties and associated analytical techniques requires researchers to weigh number of samples processed against variety of usage classes analyzed. In consideration of this analytical dichotomy, our results imply that researchers have likely chosen to process more samples for antibiotic and non-prescription drug quantification over accruing a larger variety of usage classes.

Beyond challenges in analytical detection and quantification of lesser studied substances, imbalances within the environmental PPCP literature likely also skew our understanding of their toxicological properties. Antibiotics were among the first PPCPs demonstrated to significantly alter ecological community compositions in both terrestrial⁵⁸ and aquatic⁵⁹ environments by increasing antibiotic resistant bacterial communities⁶⁰ as well as decreasing prevalence of sensitive taxa.⁶¹ Other PPCPs that are less studied can also have similarly deleterious effects. Toxicological studies have revealed developmental alterations in response to hormones in the environment,⁶² and several studies have reported that hormones were detected in 60 - 80% of US surface waters and constituted >20% of total PPCP concentration.^{5,51} Fragrances likewise occupy only a marginal portion of PPCP literature, despite Focazio et al.⁵¹ having detected fragrances

and flavor-enhancers in over 30% of surface and 10% of subsurface water. Antioxidants were absent from all 6,517 abstracts, which is concordant with their seemingly limited presence in either groundwater or surface water systems.⁵¹

Fragrances' marginal representation in the environmental PPCP literature indicates a distinct knowledge gap, as fragrances are widely used in foods as well as personal care products, can have antimicrobial properties, and can bioaccumulate.^{12,63–65} Our analyses revealed only nine abstracts referencing fragrances; among the nine, seven references were for indole. Indole is a common fragrance found in green teas⁶⁵ and roasted coffees,⁶⁶ originating from natural^{67,68} as well as synthetic sources.⁶⁹ Analyses of biosolids have demonstrated that indole is recalcitrant to degradation in biosolids (6,800 - 21,500 µg/kg) and can bioaccumulate in earthworms (285 – 3,130 µg/kg).⁶³ While indole is common in surface systems,⁶⁴ its toxicological effects are not well understood. In controlled lab settings, 1 mg/L – 400 mg/L indole significantly inhibited bacterial growth.^{12,65} Although such high indole concentrations are not frequently encountered in natural systems, indole has demonstrated time dependent bacterial inhibition, where lower concentrations (e.g., 1 mg/L) inhibited growth earlier but with lesser intensity than higher concentrations (e.g., 100 mg/L).¹² Indole has also demonstrated interactive properties, by which dilute concentrations (e.g., < 1 mg/L) may inhibit bacterial growth only in the presence of other organic contaminants.¹²

Just as some individual PPCP usage classes are not well represented, our results also show that mixtures are not frequently considered in the literature. This trend likely reflects an understanding of PPCPs more as isolated toxicants and less as components of a mixture constituting not only the individual PPCPs but also their transformation products, such as metabolites and decay products. The mixture framework has demonstrated how mixed toxicants

may precipitate non-additive biological responses.³⁷ For example, Lawrence et al.³⁷ experimentally demonstrated that 5 µg/L mixtures of caffeine, acetaminophen, and diclofenac may not reduce biofilm diversity and abundance in a summative manner. Instead, the combination of two or more PPCPs has demonstrated less intense biological responses than single contaminant exposures in both lab and field experiments.^{26,37,38,70} Most of these studies are designed with static mixtures that remain constant in composition of constituents over space and time. As new pharmaceuticals rapidly enter market consumption relative to environmental study of contaminants, we suggest the initiation of new field studies focusing either intensively within individual systems or extensively across landscape scales in order to capture the breadth of PPCP mixtures encountered as well as the associated biological responses.^{71–74} Such studies may lack the experimental control of dose-response studies, but can help reveal the relative composition and diversity of mixtures experienced by organisms in the environment as well as identify regional patterns of PPCP occurrence and concentrations. When combined with co-located biological measurements, PPCP data from field studies could be used to infer relationships between observed PPCP mixtures and biotic responses across multiple spatial scales.

Like PPCP usage classes, certain STTs are more frequently referenced in existing literature. In particular, centralized sewage treatment was more frequently mentioned than decentralized treatment (Figure 6). Centralized STTs may dominate the environmental PPCP literature because more developed nations tend to both produce most environmental PPCP research (Figure 3) and more frequently implement centralized STTs than developing nations.⁷⁵ Abstracts concerning centralized systems tended to focus largely on method development of PPCP removal with references to PPCP effluent concentrations as well as solid byproducts in receiving ecosystems. In a review of PPCP removal techniques, Yang et al.⁵² highlighted primary

and secondary treatment's inadequacies in removing many PPCPs. Extensive tertiary treatment techniques have shown promise in removing PPCPs with >80% efficiencies.⁵² These techniques mostly include membrane filtration,⁷⁶ granular activated carbon,⁷⁷ and advanced oxidation processes.⁷⁸ The inclusion of newer sewage treatment techniques, such as anammox, also suggests that PPCP literature embraces a suite of centralized wastewater treatment systems and has kept pace with technological changes (Figure 5).

Most centralized technologies tend to focus on PPCPs within an aqueous matrix as opposed to a solid matrix. As with diversity of PPCPs studied, this imbalance is likely associated with technological constraints. Detecting and quantifying PPCPs from solids necessitate working with complex matrices, which can obfuscate PPCP signals especially if using GC/MS techniques.⁵⁵ In contrast, HPLC/MS techniques can be customized to mitigate matrix effects, such as through ion addition⁷⁹ or operational changes,⁸⁰ but HPLC/MS's high cost often restricts its adoption.⁵⁶

Despite analytical challenges, solid-phase PPCP removal techniques in general are common within the PPCP literature, although specific techniques such as sedimentation, coagulation, and flocculation are less frequently noted. While solid-phase removal techniques in centralized sewage treatment are effective for certain compounds,⁸¹ questions remain about the fate and concentrations of PPCPs in solid waste, their derivatives, and disposal locations. For example, in agricultural soils, where biosolids have been applied as fertilizer, increased PPCP concentrations have been detected in both the soil itself and biota.^{63,82} Similarly, landfills receiving biosolid waste can leach potent, concentrated doses into groundwater and surface flows.⁸³ Given the potential consequences of PPCP introduction via solids-removal processes,

our results show a distinct knowledge gap in the centralized STT literature for PPCPs associated with solids removal from specific STT processes, such as coagulation and flocculation.

In contrast to centralized systems, PPCPs associated with decentralized STTs, particularly septic systems, are less represented in the environmental PPCP literature, despite their known potential to release sewage pollution when operating ineffectively.^{84,85} While septic systems may not receive influent volumes on the scale of a WTP, septic tanks are frequently used for sewage treatment worldwide.⁸⁶ Septic systems constitute the primary form of wastewater treatment in rural China⁸⁷ and India.⁸⁸ In addition, 25% of the United States,⁸⁹ 26% of Europe,⁹⁰ and 20% of Australia⁹¹ currently use septic systems, with 33% of new US developments implementing septic tanks.⁸⁹ Given that these same countries contribute most markedly to global PPCP research productivity (Figure 3), we suggest that greater attention to septic systems would address a need for understanding water quality in these countries and globally. Although septic systems have demonstrated as much as >90% efficacy at removing PPCPs from influent when operating properly, lack of standard regulations for septic effluent and maintenance heightens concern for their potential to negatively affect water quality via leaks or direct discharge into surface or subsurface systems.⁸⁶ Once released from the septic system, PPCPs can persist either by sorption within the vadose zone or advect through the subsurface. The fate and transport of PPCPs associated with septic systems can be strongly influenced by the surrounding geology and design of the septic system.⁹² Acetaminophen, for example, tends to remain proximal to septic systems in sandy soil, whereas sulfamethoxazole tends to diffuse away from the septic tank in the same sandy soil matrix.⁹³ Together, the combined complexities of septic and other decentralized STTs create a PPCP “hot spot” landscape, where concentrations and compounds may be heterogeneous throughout a clustering of residences.

In addition to differences in PPCP chemical and STT diversity, our results indicate that the environmental fates of PPCPs have been most well researched in lotic systems relative to lentic and terrestrial systems. Several field and lab investigations within lotic systems (Table 2) have identified biological responses to PPCPs, such as changes in microbial community composition and function as well as invertebrate development.^{26,70} For example, Rosi-Marshall et al.⁹⁴ demonstrated that 0.015 M caffeine reduced stream biofilm gross primary production (GPP) and respiration but not composition, whereas 0.015 M diphenhydramine reduced biofilm GPP by changing the composition of the biofilm. Similar experiments have largely been restricted to rivers, and it is not clear whether similar patterns hold true in other aquatic systems. In particular, lentic and marine systems are less well represented in the environmental PPCP literature. Understanding how different hydrologies accumulate PPCPs may be especially important to predict biotic responses. Lentic and marine habitats tend to have longer hydraulic residence times than rivers and streams, which could enhance the opportunity for pollutants to (1) concentrate within the water column or sediment, (2) interact with biota, and (3) introduce new chemical species through transformation and degradation. The low representation of marine systems in the literature is particularly notable since coastal zones are home to a high proportion of the global population.⁹⁵ Additionally, research investigating terrestrial systems also appears to be fairly restricted at a lab-scale, focusing on sorption of PPCPs to organic material as well as biosolid application in agricultural environments,⁵⁸ where toxicants can bioaccumulate in plants and grazing animals.⁵⁹ Like aquatic environments, terrestrial systems may also experience PPCP “hot spots”, such as near landfills, in animal husbandry settings where veterinary medications may be excreted in manure, or agricultural fields where wastewater treatment byproducts may be

disposed. Regardless of the system type, settings where PPCPs can accumulate present opportunity for investigating their potential as legacy contaminants.

1.7 Future Trajectories for PPCP Study

Over the past three decades, environmental PPCPs have been increasingly studied as indicators of sewage pollution and emerging organic toxicants (Figure 3). We conducted an evidence synthesis to quantitatively evaluate frequencies at which certain PPCPs, STTs, and ecosystem types are investigated.³⁹ Our results confirmed the general and sustained increase in PPCP research, with key differences among usage classes, STTs, and ecosystem types. We demonstrate that antibiotics, non-prescription drugs, centralized sewage treatment, and lotic ecosystems were dominant components in this large volume of environmental PPCP literature. With over 4,000 PPCPs on the market worldwide,¹⁶ the research needs are daunting. Sustained monitoring, comparative studies across ecosystem types, and controlled experiments may all prove useful for understanding environmental and biological consequences of PPCPs individually or in mixture. Sustained monitoring may also reveal lag times for pollution transport as new pharmaceuticals become available on the global market, fostering interdisciplinary collaborations that can inform environmental and PPCP policies.

To address knowledge gaps in the environmental PPCP literature, we propose five areas for immediate and long-term research efforts.

1.7.1 Addressing Identified PPCP Knowledge Gaps

Increased study of less represented PPCP usage classes, STTs, and ecosystem types will advance understanding of environmental PPCP fate and transport. Our analyses suggest imbalanced frequencies of study, with prescription drugs, hormones, fragrances, and antioxidants remaining less represented in comparison to antibiotics and non-prescription drugs. Among each

of these usage classes, fragrances may represent the greatest knowledge gap of the PPCP usage classes within this review. Given certain fragrances' potential antimicrobial properties, the paucity of environmental fragrance study relative to their environmental prevalence points to a need to further evaluate how fragrances, such as those common in tea, coffee, and perfume, may disrupt ecological processes. Likewise, our understanding of environmental PPCP distributions is largely based in lotic and subsurface systems. Lentic, marine, as well as some terrestrial systems are less frequently represented, despite PPCPs' potential to accumulate differently among these systems.

In addition to investigating different PPCPs individually, increased study of PPCPs as mixtures may identify synergistic or non-additive effects. Numerous field studies have reported biotic responses to PPCP mixtures through observational studies⁶ and controlled chemical exposure through PPCP diffusing substrates.²⁶ Our results confirm that research has begun to incorporate a mixture framework, which is necessary to assess PPCPs' synergistic effects further. Researchers incorporating a mixture framework should remain cognizant that PPCP transformation products, such as metabolites and their associated decay products, as well as the original PPCP compounds themselves are likely to persist within the system. Supplementing existing knowledge of ecotoxicological effects of PPCPs in addition to the effects of their associated byproducts will offer a more complete picture of the PPCP life-cycle.

1.7.2 STT Technology and PPCP Study

Continued research is needed on the role of sewage treatment technology in PPCP transport and transformation. In general, PPCP literature has embraced STT technological innovations as new STTs, especially membrane bioreactors, activated carbon, and anammox, become more widely available. Additionally, STTs have demonstrated efficacy at removing

certain PPCPs, such as the ability of anammox to remove fragrances.⁶⁰ Inclusion of older technologies, such as activated sludge and septic systems, in new studies is likewise essential to understanding environmental PPCP concentrations, as municipal water treatment plants will take time to fully implement the most recent STTs. As these technologies may leverage biological systems to remove or transform PPCPs, future research could investigate reciprocal relations of PPCPs and biological processes within the wastewater treatment process, recognizing that biological processes responsible for degrading PPCPs also can be altered by PPCP presence, affecting PPCP removal efficiency. Given the relative paucity of PPCP study in decentralized systems, especially septic tanks, relative to the high frequency of decentralized STTs worldwide, we suggest that examining the dynamics and fate of PPCPs released from decentralized STTs as a necessary trajectory for future PPCP research. In addition, while our analyses largely consider septic systems, other forms of decentralized STTs could also be considered, such as constructed wetlands and lagoons,⁷⁵ anaerobic sludge reactors,⁹⁸ passively aerated biological filters,⁹⁹ as well as coupled septic tank-reactor systems.^{100,101}

1.7.3 Sources of PPCPs

Beyond human PPCP consumption and excretion, researchers should remain cognizant of other sources of PPCP release, many of which may introduce source-specific compounds into ecosystems. Veterinary medicine and animal husbandry practices, for example, have demonstrated that the release of veterinary-specific antibiotics, such as kanamycin,¹⁰² can not only accumulate within aquatic and terrestrial systems but also lead to antibiotic resistance within microbial communities.¹¹ Like veterinary sources, wastewater from hospitals can release specialized, potent PPCPs into neighboring environments. For example, the common medical compound iopromide, an x-ray contrast medium,¹⁰³ has been widely used to detect wastewater

pollution from hospitals, yet its toxicological consequences are uncertain.^{104,105} While the presence of such PPCPs may be limited to discrete spatial and temporal scales, they represent a group of micropollutants that is constantly expanding as new pharmaceuticals as well as medical and veterinary practices develop.

1.7.4 Intensification, Accumulation, and Legacy of PPCPs

With increasing chemicals and mixtures in circulation, spatial and temporal patterns in PPCP concentrations remain uncertain. Urban centers, where a greater variety of chemical forms of PPCPs occur, likely concentrate PPCPs.⁵ Once introduced to an environment, PPCPs may distribute widely and persist for long durations. Although many PPCPs have relatively short environmental half-lives (e.g., hours to days¹⁶), environmental and biological processes may cause PPCPs to persist much longer (e.g., years, decades). Even in situations where PPCP's rapidly degrade, continued new inputs may still affect organisms. Several studies focused on ecotoxicological aspects of PPCPs have highlighted immediate potential for certain PPCPs to bioaccumulate.^{9,106,107} Long-term accumulation within biological and environmental systems, however, remains uncertain. Legacy accumulation has already been observed for other organic pollutants; organic pollutants such as the insecticide methoxychlor and the herbicide pendimethalin have been observed to accumulate within Arctic ice,⁶¹ and PCBs can accumulate and persist within adipose tissue of organisms.⁶² In such cases, pulses of organic pollutants can occur when pollutants mobilize, such as through ice-melt⁶¹ or high lipid metabolism.⁶² The potential for PPCPs to accumulate and persist within abiotic and biotic environments necessitates increased monitoring of legacy pharmaceuticals as well as recent additions to the global market.

1.7.5 PPCPs and Increasing Global Temperatures

Global change may influence the movement of PPCPs through ecosystems. Increasing temperatures can strongly influence ectothermic organisms in particular by accelerating metabolism, thereby requiring higher consumption and oxygen demand. Certain PPCPs, such as caffeine,³⁶ are well known to likewise increase metabolic rates, potentially demanding even higher resource consumption as well as oxygen demand. The potential for synergistic effects between increasing temperature and PPCP exposure presents opportunity for study, where non-additive effects may exceed an organism's capacity for allostatic response. This model could be studied at multiple biological levels, such as with primary consumers in lakes and streams⁷⁰ as well as microbial communities within STTs.⁵²

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1.9 Supporting Information

The supporting information (PDF) is available on the ACS Publications website. Written in a vignette style, the supporting information details how Web of Science records were imported into as well as manipulated within the R environment. R and package versions are also reported. The document is separated into modules that describe the workflow and R code for identifying, enumerating, and visualizing PPCP usage classes, STTs, ecosystem types, and first author's affiliated country. The supplemental information also contains summary tables of PPCP, STT, and ecosystem type frequencies.

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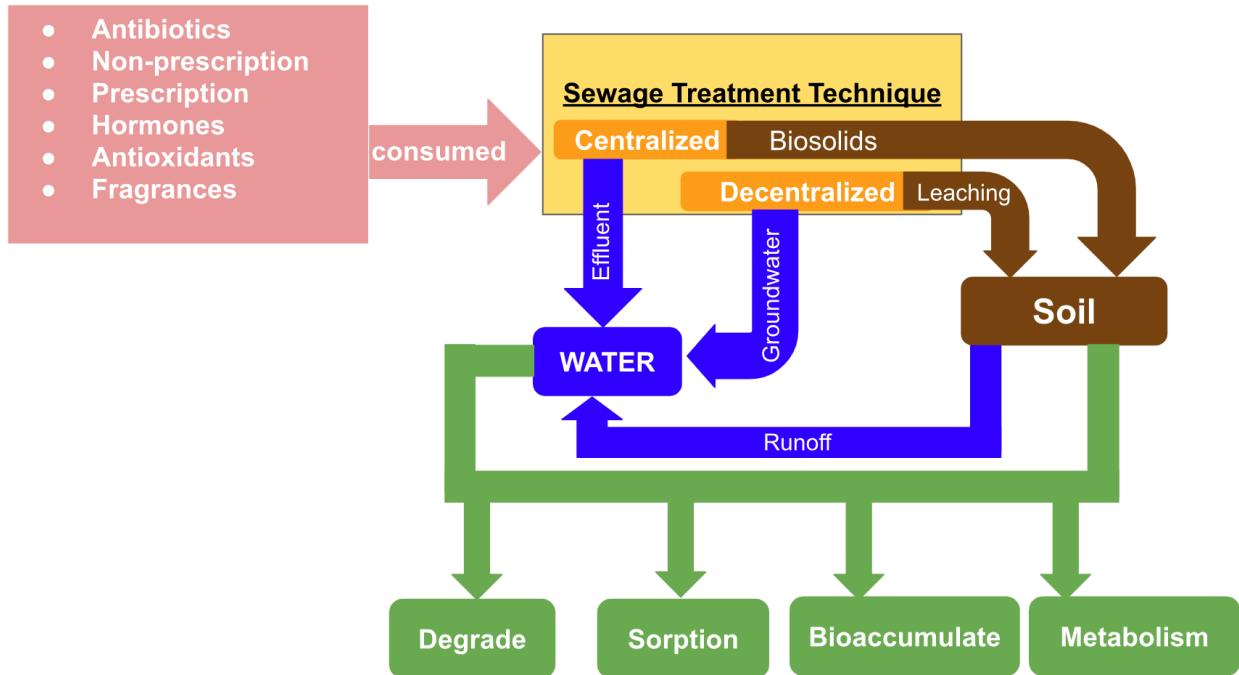


Figure 1: Flow diagram for sources and fates of PPCPs. Humans widely ingest or otherwise use PPCPs. Following disposal or excretion, PPCPs may enter centralized (e.g., wastewater treatment plant) or decentralized (e.g., septic) Sewage Treatment Techniques (STTs). Despite STTs often removing a large proportion of PPCPs from influent, sewage treatment products (e.g., biosolids, effluent, etc.) and inadequacies within STTs can introduce and mobilize PPCPs into both terrestrial and aquatic environments. Within the environment, fates of PPCPs can depend on their physicochemical and biochemical properties. Many PPCPs have short half-lives, causing them to degrade quickly. More hydrophobic compounds may sorb to colloidal material or bioaccumulate in adipose tissue. More hydrophilic compounds may metabolize more easily, and either directly alter organismal physiology or create deleterious metabolites.

8,803 records identified by
Web of Science (WOS) search for
TS = “pharmaceutical” AND
TS = “sewage OR wastewater”

-**2,286** records removed by WOS ‘refine’ function:

- A. -**972**, primary articles only
- B. -**110**, articles in English
- C. -**1,204**, non-environmentally focused studies

6,517 records subsampled for
screening using title and abstract

6,517 studies included in this
evidence synthesis

Figure 2: Flow chart (PRISMA diagram¹¹⁰) of our search and abstract inclusion process.

Table 1: List of PPCPs by usage class as in Kolpin et al. (2002) and Focazio et al. (2008)

| Usage class | Compound |
|-----------------------|--|
| antibiotic | azithromycin, carbodox, chlortetracycline, ciprofloxacin, demeclocycline, doxycycline, enrofloxacin, erythromycin, lincomycin, methotrexate, minocycline, norfloxacin, oxytetracycline, roxithromycin, sarafloxacin, sulfachloropyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfathiazole, tetracycline, trimethoprim, tylosin, virginiamycin |
| prescription drug | albuterol, salbutamol, cimetidine, codeine, dehydronifedipine, digoxin, digoxigenin, diltiazem, diphenhydramine, enalaprilat, fluoxetine, gemfibrozil, metformin, paroxetine, ranitidine, warfarin |
| Non-prescription drug | acetaminophen, paracetamol, caffeine, cotinine, 1,7-dimethylxanthine, ibuprofen |
| fragrance | 1,4-dichlorobenzene, 3-methyl-1(H)-indole, acetyl-hexamethyl-tetrahydro-naphthalene, hexahydrohexamethyl-cyclopentabenzopyran, indole, isoborneole, isoquinoline |
| antioxidant | 2,6-di-tert-butylphenol, 2,6-di-tert-butyl-1,4,-benzoquinone, 3-tert-butyl-4-hydroxy anisole, butylated hydroxy toluene |
| hormone | androsterone, cholesterol, coprostanol, equilenin, equilin, estradiol, estriol, estrone, mestranol, 19-nrethisterone, progesterone, stigmasterol, testosterone |

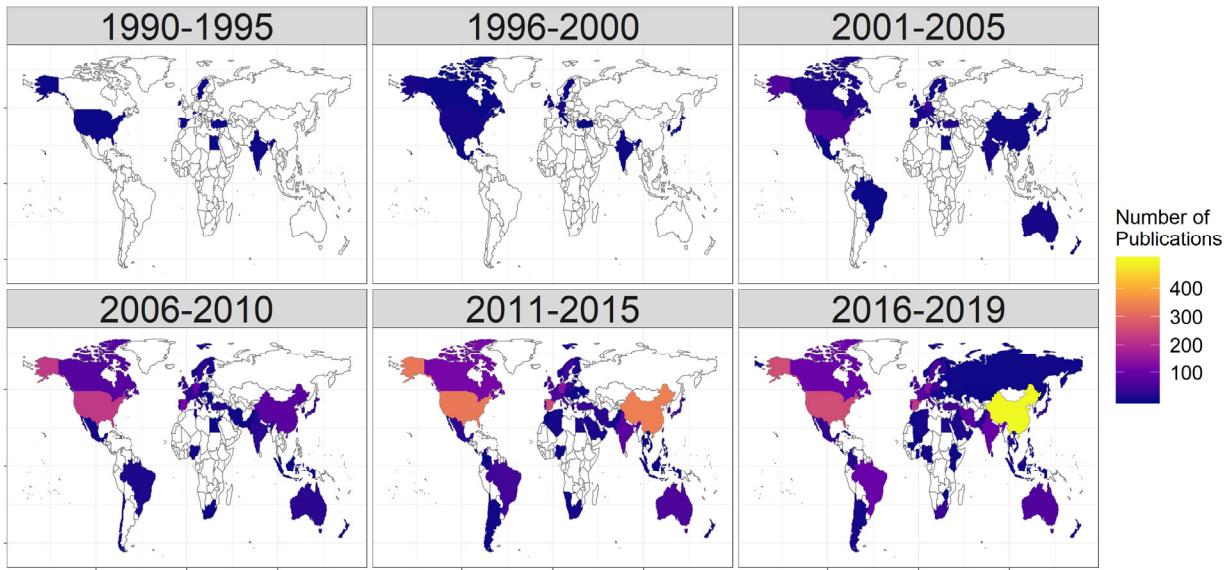


Figure 3: Number of PPCP publications by country of first author affiliation, shown at aggregated 5 year time intervals. While PPCP study has spatially become a global research interest, the majority of this increase has occurred since 2006 (94%).

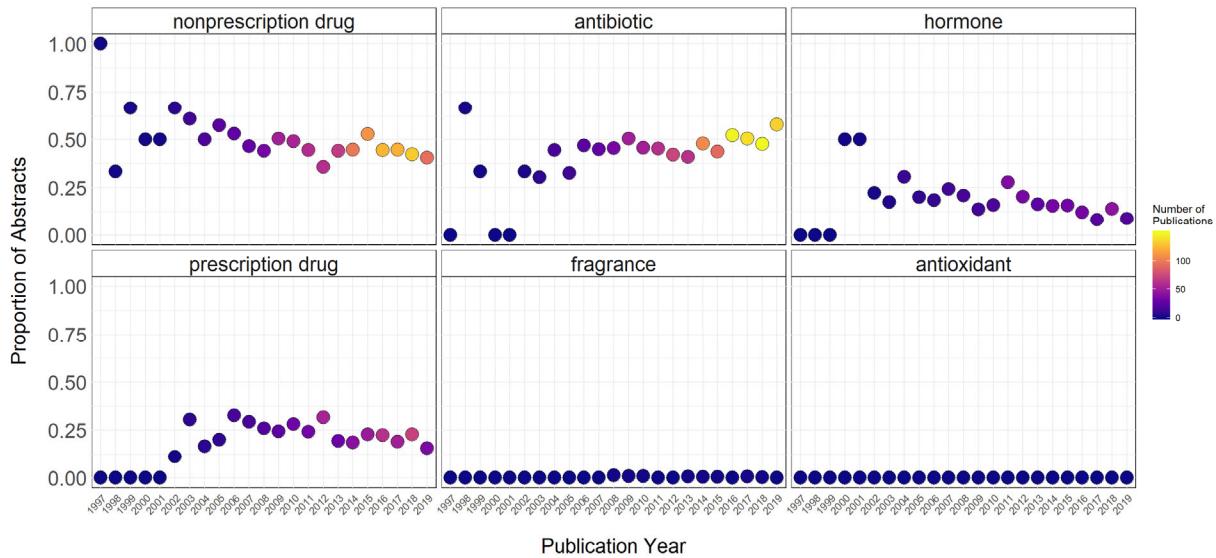


Figure 4: Proportion of abstracts by PPCP usage class ($n=2,515$) in environmental literature from 01 January 1990 to 06 August 2019. Values are reported as the ratio of abstracts referencing a particular PPCP to total abstracts referencing at least one PPCP in a given year. Instances of antioxidants yielded no results for all years. For a given year, proportions across usage classes may sum to greater than one because more than one usage class may be referenced in an abstract.

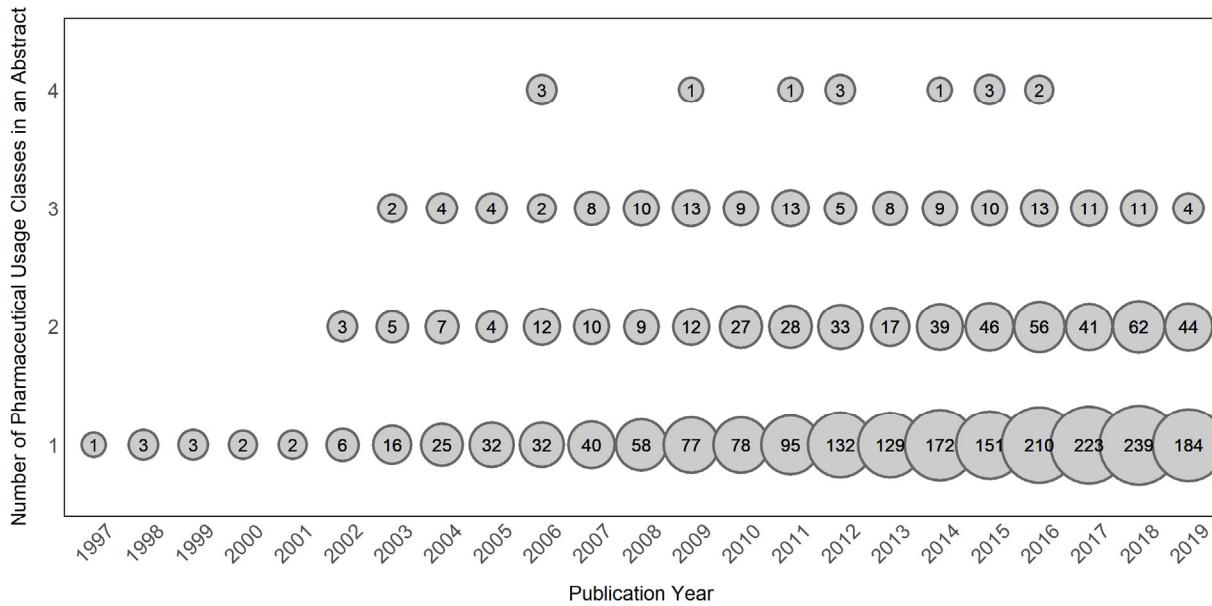


Figure 5: Number of abstracts and PPCP usage classes in literature from 01 January 1990 to 06 August 2019. Numbers inside the circles as well as the circles' size indicate the number of abstracts for that year. No abstracts involved more than four usage classes.

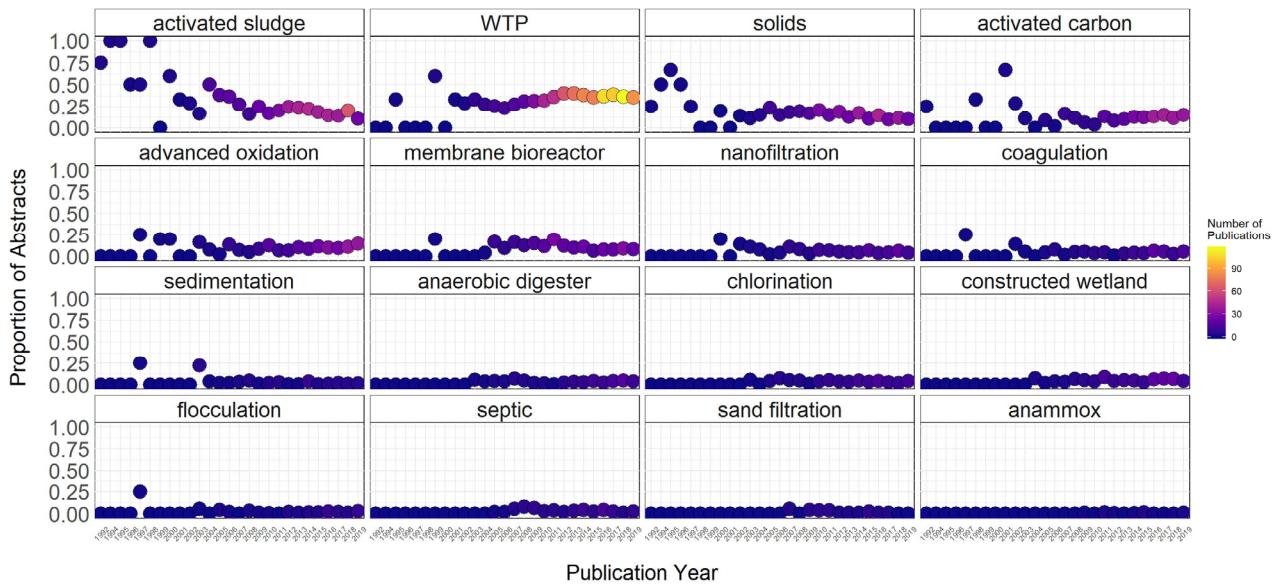


Figure 6: Proportion of abstracts by Sewage Treatment Technique (STT) ($n=2,558$) in environmental literature from 01 January 1990 to 06 August 2019. Values are reported as the ratio of abstracts referencing a particular STT to total abstracts referencing at least one STT in a given year. The year 1993 was omitted because there were no abstracts referencing any STT in that year. For a given year, proportions across STTs may sum to greater than one because more than one STT may be referenced in an abstract.

| | River | Stream | Lake | Soil | Pond | Wetland | Groundwater | Lagoon | Aquifer | Ocean | Estuary | Total |
|-----------------------|-------|--------|------|------|------|---------|-------------|--------|---------|-------|---------|-------|
| Field observation | 29% | 11% | 7% | 5% | 5% | 5% | 3% | 3% | 2% | 1% | 1% | 72% |
| Laboratory experiment | 7% | 4% | 0% | 10% | 1% | 0% | 0% | 0% | 1% | 0% | 0% | 23% |
| Modeling | 1% | 3% | 0% | 3% | 1% | 0% | 0% | 0% | 0% | 1% | 0% | 9% |

Table 2: Percent representation of field observations, laboratory experiments, and modeling techniques employed in PPCP literature that identifies at least one ecosystem type. All percentages were calculated from a stratified, random subsample of PPCP abstracts referencing at least one ecosystem type, for which strata were weighted by prevalence of ecosystem type. A given study could be coded as having multiple experimental designs, and therefore the sum of the total percentages may add to more than 100%.

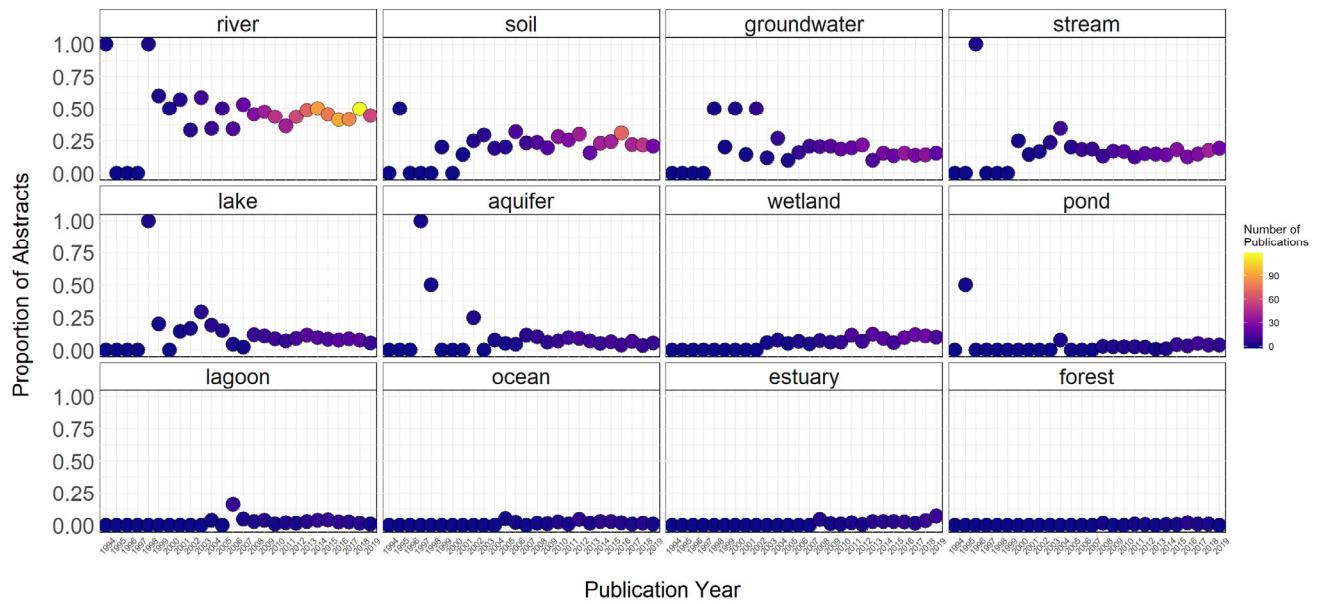


Figure 7: Proportion of abstracts by ecosystem type ($n=1,968$) in environmental PPCP literature from 01 January 1990 to 06 August 2019. Values are reported as the ratio of abstracts referencing a particular ecosystem type to total abstracts referencing at least one ecosystem type in a given year. For a given year, proportions across ecosystem types may sum to greater than one because more than one ecosystem type may be referenced in an abstract.

CHAPTER TWO

EFFECTS OF SPATIALLY HETEROGENEOUS LAKESIDE DEVELOPMENT ON NEARSHORE BIOTIC COMMUNITIES IN A LARGE, DEEP, OLIGOTROPHIC LAKE (LAKE BAIKAL, SIBERIA)

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2.1 Abstract

Sewage released from lakeside development can reshape ecological communities. In particular, nearshore periphyton can rapidly assimilate sewage-associated nutrients, leading to increases of filamentous algal abundance, thus altering both food abundance and quality for grazers. In Lake Baikal, a large, ultra-oligotrophic, remote lake in Siberia, filamentous algal abundance has increased near lakeside developments, and localized sewage input is the suspected cause. These shifts are of particular interest in Lake Baikal, where endemic littoral biodiversity is high, lakeside settlements are mostly small, tourism is relatively high (~1.2 million visitors annually), and settlements are separated by large tracts of undisturbed shoreline, enabling investigation of heterogeneity and gradients of disturbance. We surveyed sites along 40 km of Baikal's southwestern shore for sewage indicators – pharmaceuticals and personal care products (PPCPs) and microplastics – as well as periphyton and macroinvertebrate abundance and indicators of food web structure (stable isotopes and fatty acids). PPCPs, including caffeine and acetaminophen/paracetamol, were spatially related to lakeside development. As predicted, lakeside development was associated with more filamentous algae and lower abundance of sewage-sensitive mollusks. Periphyton and macroinvertebrate stable isotopes and essential fatty acids suggested that food web structure otherwise remained similar across sites; yet, the invariance of amphipod fatty acid composition, relative to periphyton, suggested that grazers adjust behavior or metabolism to compensate for different periphyton assemblages. Our results demonstrate that even low levels of human disturbance can result in spatial heterogeneity of nearshore ecological responses, with potential for changing trophic interactions that propagate through the food web.

2.2 Introduction

The release of treated and untreated wastewater into aquatic ecosystems is a common human disturbance that can introduce pollutants and reshape aquatic ecological communities (Moore et al. 2003). Nitrogen and phosphorus are among the primary pollutants in wastewater and its associated byproducts (Smith et al. 1999), yet these nutrients can also originate from disparate anthropogenic and natural environmental sources, thereby complicating their use as sewage indicators. For example, agriculture (Powers et al. 2016), watershed processes such as melting permafrost (Turetsky et al. 2000), and changes in terrestrial plant communities (Moran et al. 2012) can all increase allochthonous nutrient inputs similar to sewage. Regardless of the nutrients' source, biological processes can further confound sewage detection. Benthic primary producers, especially those in oligotrophic systems (Rosenberger et al. 2008; Hampton et al. 2011; Oleksy et al. 2020; Atkins et al. 2021), can assimilate nutrients quickly from the water column (e.g., hours), such that elevated nutrient concentrations may not be observed (Hadwen and Bunn 2005).

Because nutrients come from numerous non-sewage sources, indicators consistently associated with wastewater pollution, such as enhanced $\delta^{15}\text{N}$ stable isotope signatures (Costanzo et al. 2001; Camilleri and Ozersky 2019), pharmaceuticals and personal care products (PPCPs) (Rosi-Marshall and Royer 2012; Meyer et al. 2019), and microplastics (Barnes et al. 2009), have garnered increasing attention for their usefulness as sewage indicators. Stable isotopes, such as $\delta^{15}\text{N}$, have been frequently used to trace sewage pollution (Gartner et al. 2002), yet their potential to indicate sewage can be obfuscated by complex terrestrial (Craine et al. 2018) and aquatic (Guzzo et al. 2011) processes. In contrast to $\delta^{15}\text{N}$ signatures, PPCPs explicitly originate from human sources (Rosi-Marshall and Royer 2012; Meyer et al. 2019) and generally are not

actively removed from the water column by biota, although certain algal (Bai and Acharya 2017) and animal (Arnnok et al. 2017; Richmond et al. 2018) taxa have been shown to accumulate specific PPCPs from a broader mixture of co-contaminants. Because of PPCPs' tendency to not be actively scoured by biota, PPCP studies from continental (Kolpin et al. 2002; Focazio et al. 2008; Yang et al. 2018) to colloidal pore (Yang et al. 2016) scales, have shown that concentrations tend to be greatest closer to their source. In addition to identifying areas and periods of sewage-specific pollution, PPCPs have also demonstrated robustness in defining gradients of sewage pollution in river systems, with concentrations being directly proportional to population density and inversely proportional to distance from a densely populated area (Bendz et al. 2005). Similar to PPCPs, microplastics (plastic debris up to 5 mm in size) also have been useful to detect sewage pollution (Li et al. 2018) along gradients of increasing human population density (Klein et al. 2015), although they can sometimes originate from non-sewage sources, such as shoreline debris or fishing nets (Free et al. 2014). In contrast to $\delta^{15}\text{N}$ signatures and PPCP concentrations, microplastics are typically resistant to degradation (Barnes et al. 2009), providing a signal over a longer time frame than many PPCPs and nutrients in sewage. As a result of each pollutant's strong association with sewage, co-located $\delta^{15}\text{N}$, PPCP, and microplastic measurements can be used to infer the spatial extent and timing of sewage pollution in an ecosystem.

The effects of sewage pollution are frequently first seen in nearshore benthic communities where increased nutrients alter algal species composition, abundance, nutritional quality, as well as food web trophic structure. Increased filamentous algal abundance, for example, has been frequently observed in areas suspected of sewage pollution (Rosenberger et al. 2008; Hampton et al. 2011), likely due to benthic filamentous algae efficiently removing

nutrients from the water column (Hadwen and Bunn 2005; Andersson and Brunberg 2006; Oleksy et al. 2020). With a changing resource base, grazing macroinvertebrate communities may likewise shift to include more detritivores or species capable of consuming filamentous algae (Rosenberger et al. 2008). In addition to some grazers' physical difficulty consuming filamentous algae (Mazzella and Russo 1989), there also may be changes in algal nutritional quality, as filamentous algae tend to contain a different mixture of essential fatty acids (EFAs) in comparison to diatoms (Kelly and Scheibling 2012), which dominate periphyton communities in unimpacted ecosystems. In particular, the EFAs 18:3 ω 3 and 18:2 ω 6 are commonly associated with green filamentous algae (Taipale et al. 2013), whereas 20:5 ω 3 is more associated with diatoms (Taipale et al. 2013). All EFAs are largely synthesized by primary producers, and each related group produces strongly differentiated multivariate signatures (Taipale et al. 2013; Galloway and Winder 2015). Consumers can acquire fatty acids by grazing (Dalsgaard et al. 2003) or upgrading fatty acids at their own energetic expense (Sargent and Falk-Petersen 1988; Dalsgaard et al. 2003) and often reflect the fatty acid signatures of their diets. Thus, comparing consumer and producer fatty acid compositions can be used to infer how grazing patterns change in response to increasing sewage pollution.

To investigate lake littoral community and food web responses to sewage-associated nutrient pollution, we surveyed 40 km of Lake Baikal's shoreline for indicators of sewage pollution and metrics of benthic community composition and structure. Located in Siberia, Lake Baikal is the oldest, most voluminous, and deepest freshwater lake in the world (Hampton et al. 2018), with the majority of Lake Baikal's biodiversity occurring in the littoral zone (Kozhova and Izmest'eva 1998). While Lake Baikal's pelagic zone is generally ultra-oligotrophic (Yoshida et al. 2003; O'Donnell et al. 2017), nearshore areas abutting lakeside settlements have shown

distinct signs of eutrophication (Timoshkin et al. 2016). Much of Lake Baikal's shoreline lacks human development, and Baikal's watershed is largely roadless and unpopulated (Moore et al. 2009). Despite low levels of development, uncharacteristic filamentous algal blooms have been occurring throughout the lake since 2010 (Kravtsova et al. 2014; Timoshkin et al. 2016; Volkova et al. 2018) and even cyanobacterial blooms in 2019 (Bondarenko et al. 2021). While increased *Ulothrix* spp. abundance has historically occurred in Lake Baikal in late summer (Kozhov 1963; Kozhova and Izmest'eva 1998), recent observations of *Spirogyra* spp. abundance at unprecedented levels are thought to be associated with increased nearshore nutrient concentrations (Volkova et al. 2018; Ozersky et al. 2018). Inadequate wastewater management in lakeside settlements is likely the main driver of these nearshore algal blooms (Timoshkin et al. 2016, 2018), motivating further research to identify the extent to which sewage is altering nearshore communities

Given the growing evidence that Baikal's nearshore periphyton communities are responding to sewage inputs, our goal was to understand how littoral benthic community composition and interactions may be changing near areas of sewage pollution. This overarching goal was divided into three specific objectives:

1. identify areas of wastewater pollution using several complementary sewage indicators,
2. assess the relationship between sewage indicators and littoral periphyton and macroinvertebrate community composition, and
3. evaluate how trophic relationships among littoral benthic community members are impacted by localized sewage pollution.

We hypothesized that (1) sewage indicators, such as PPCP concentrations, $\delta^{15}\text{N}$, and microplastic densities, would increase with increasing population density and proximity of lakeside development; (2) an increasing sewage signal would correlate with increased dominance of filamentous benthic algae; and (3) increasing filamentous algal abundance would result in changes in the abundance of different macroinvertebrate feeding guilds, reflected in community composition and dietary tracers such as carbon and nitrogen stable isotopes and fatty acids.

2.3 Methods

2.3.1 Site description

The vast majority of Lake Baikal's 2,100-km shoreline lacks lakeside development (Moore et al. 2009; Timoshkin et al. 2016). Our study focused on a 40-km section of Baikal's southwestern shoreline, which included three settlements of different sizes (Figure 1; Figure 2). From 19 through 23 August 2015, we sampled 14 littoral and 3 pelagic locations along our 40-km transect. Littoral locations were chosen to capture a range of sites with varying degrees of adjacent shoreline development – from “developed” (along the waterfront of human settlements) to “undeveloped” (no adjacent human settlements and complete forest cover; Figure 1; Figure 2; Table 1). Pelagic sites were located 2 to 5 km offshore from each of the developed sites in water depths of 900 to 1300 m (Figure 1; Table 1). As previous investigations of nearshore algal communities observed increased filamentous algae (Timoshkin et al. 2016, 2018; Volkova et al. 2018) and cyanobacteria (Bondarenko et al. 2021) in mid-to-late summer, the timing of our sampling was intended to coincide with the annual peaks of tourism and summertime community succession, such that gradients of human disturbance and biological response would likely be most apparent relative to environmental noise. All littoral sites were sampled at approximately the same depth (~1.25 m) at a distance of 8.90 to 20.75 m from shore (Table 1), which allowed

us to collect samples without the need for SCUBA but precluded us from sampling deeper littoral environments. At each site, air temperature was measured with a mercury thermometer, and photographs were taken of the substrate and the shoreline. Visual inspection of substrate photographs suggested that substrate was consistent among sites and generally was dominated by pebble to boulder-sized rocks.

Three discrete lakeside settlements were located along our 40-km transect. The largest, Listvyanka, is primarily a tourist town of approximately 2000 permanent residents, although tourism can contribute significantly to the town's population with approximately 1.2 million annual visitors (Interfax-Tourism 2018). The other two settlements are the villages Bolshie Koty and Bolshoe Goloustnoe, which have approximately 80 and 600 permanent residents, respectively. Bolshie Koty is home to two field research stations and several small tourist accommodations. Bolshoe Goloustnoe has several hotels and tourist camps. Although Bolshie Koty and Bolshoe Goloustnoe are built along small streams that empty into Baikal, there are no upstream developed sites, meaning that any observed sewage indicators in Baikal most likely originated either from Bolshie Koty or Bolshoe Goloustnoe. None of these settlements have centralized sewage treatment facilities and most residents rely on unlined cesspools (Timoshkin et al. 2018).

2.3.2 Inverse Distance Weighted (IDW) Population Calculation

We recognized that sewage indicator concentrations at each sampling location may be related to a sampling location's spatial position relative to both the size and proximity of neighboring developed sites. Therefore, we created the inverse distance weighted (IDW) population metric to compress, into a single metric, information about human population size, density, and location along the shoreline as well as distance between developed sites and sampling locations. The

IDW metric reflects the idea that sewage pollution should be positively related to increasing human density and inversely related with distance from densely populated areas (sensu Bendz et al., 2005). Additionally, Timoshkin et al. (2018) noted that sewage enters Baikal's nearshore largely through groundwater, implying that locations with more directly adjacent shoreline development should experience higher sewage concentrations in the lake. Acknowledging that nearshore PPCP concentrations were likely positively proportional to a developed location's shoreline length, we scaled a developed site's population density by its shoreline length. This scaling represents population density that directly interfaces with the lake, thereby capturing the idea that sewage-associated pollutants, such as PPCPs (Karnjanapiboonwong et al. 2010) and nutrients (de Vries 1972), contributed away from the shoreline can be removed via the soil matrix en route to the lake.

Our calculation of IDW population was done in five steps. First, we traced polygons and shorelines from satellite imagery for each developed site in Google Earth. Polygons were traced for the entire area of visible development (Figure 2). Similarly, shoreline traces only reflected shoreline length for which there was visible development (Figure 2). Second, polygon and line geometries were downloaded from Google Earth as a .kml file. Third, the .kml file was imported into the R statistical environment (R Core Team 2019) where, using the sf package (Pebesma, 2018), we calculated shoreline length, polygon area, and centroid location for each developed site. Fourth, we joined point locations of each sampling site with the spatial polygons to calculate the distance from each sampling location to each developed site's centroid. Fifth, we calculated IDW population for each sampling location, using formula (1)

$$(1) \quad Ij = \frac{\frac{P_{LI} * L_{LI}}{A_{LI}}}{D_{j,LI}} + \frac{\frac{P_{BK} * L_{BK}}{A_{BK}}}{D_{j,BK}} + \frac{\frac{P_{BGO} * L_{BGO}}{A_{BGO}}}{D_{j,BGO}}$$

where I is the IDW population at sampling location j , P is the population at each of the three developed sites Listvyanka (LI), Bolshie Koty (BK), Bolshoe Goloustnoe (BGO), A is the area of a developed site in km^2 , L is the shoreline length at a developed site in km , and D is the distance from sampling site j to each developed site's centroid in km . This formulation implies that all sampling locations are influenced by all three developed sites. Thus, the influence of an individual developed site on each sampling location is positively influenced by the size and spatial density of the population and its orientation toward the shoreline, and inversely proportional to a sampling location's distance from each of the three developed sites.

2.3.3 Water Samples

At both pelagic and littoral sites, water samples were collected for nutrient, chlorophyll, microplastic, and PPCP analysis. Samples were collected by hand from 0.75 m depth for each littoral site and with a bucket from aboard the Irkutsk State University “Kozhov” research vessel for pelagic sites. Each water sample collection procedure is described below.

2.3.3.1 Nutrients

Water samples for nutrient analyses were collected in 150 mL glass jars that had been washed with phosphate-free soap and rinsed three times with water from the sampling location. Samples were collected in duplicates and immediately frozen at -20°C until processing at the A.P. Vinogradov Institute of Geochemistry (Siberian Branch of the Russian Academy of Sciences, Irkutsk). Samples were not filtered prior to freezing, meaning that nitrogen and ammonium concentrations may potentially include intracellular nitrogen and overestimate nitrogenous forms in the water column.

For each water sample, nitrate, ammonium, and total phosphorus concentrations were measured. For ammonium (RD:52.24.383-2018 2018) and nitrate (RD:52.24.380-2017 2018) concentrations, samples were analyzed with a spectrophotometer. Total phosphorus concentration was measured with a spectrophotometer following the addition of persulfate (GOST:18309-2014 2016). Further detail on water nutrient sampling methods and handling procedures are provided in Meyer et al. (Under Revision). Concentrations are reported in mg/L.

2.3.3.2 Chlorophyll a

Water samples were collected in 1.5 L plastic bottles from a depth of approximately 0.75 m. Within 12 h of collection, three subsamples (up to 150 mL each) were filtered through 25-mm diameter, 0.2 µm pore size nitrocellulose filters. Filters were then placed in a 35-mm petri dish and frozen in the dark until processing.

Chlorophyll samples were processed in a manner similar to that of Welschmeyer (1994). Nitrocellulose filters were ground in 10 mL of 90% HPLC-grade acetone, in which chlorophyll extraction was allowed to proceed overnight. Chlorophyll extract was then analyzed using a Turner Designs 10-AU fluorometer (Turner Design, Sunnyvale, CA) using an excitation wavelength of 436 nm and emission of 680 nm. 10-AU Secondary Solid Standard (P/N 10-AU-904) was used to calibrate fluorometer prior to samples being processed. Blank samples registered a raw fluorescence of approximately 0.1 FL units. Concentrations were calculated using formula 2

$$(2) \text{Chlorophyll concentration} = (\text{extract reading} - \text{blank reading}) * \frac{\text{mL of extract}}{\text{mL of filtered sample}}.$$

Detection limits are estimated to be approximately 0.02 mg/L. Concentrations are reported as mg/L.

2.3.3.3 PPCPs

Water samples for PPCP analysis were collected in 250 mL amber glass bottles that were rinsed with either methanol or acetone and then three times with sample water prior to collections. Following collection, samples were refrigerated and kept in the dark until solid phase extraction (SPE).

Within 12 h of collection, samples were filtered directly from the amber glass bottle using an in-line Teflon filter holder with glass microfiber GMF (1.0 µm pore size, WhatmanGrad 934-AH) in tandem with a solid phase extraction (SPE) cartridge (200 mg HLB, Waters Corporation, Milford, MA) connected to a 1-liter vacuum flask. Lab personnel wore gloves and face masks to minimize contamination. Prior to filtration, SPE cartridges were primed with at least 5 mL of either methanol or acetone and then washed with at least 5 mL of sample water. Rate of extraction was maintained at approximately 1 drop per second. Extraction proceeded until water could no longer pass through the SPE cartridge or until all collected water was filtered. Cartridges were stored in Whirlpacks at -20°C until analysis for 18 PPCP residues using liquid chromatography tandem mass spectrometry (LC-MS-MS) following methods of Lee et al. (2016) and D'Alessio et al (2018). Concentrations are reported in µg/L.

2.3.3.4 Microplastics

At each location, samples were collected in triplicate using 1.5 L clear plastic bottles that were washed thoroughly with sample water before each collection. Samples were collected by hand for each littoral site and with a metal bucket from aboard the ship for pelagic sites.

For processing, each sample was vacuum filtered on to a 47-mm diameter GF/F filter. During filtration, aluminum foil was used to cover the filtration funnel to prevent contamination from airborne microplastic particles. After filtration, filters were dried under vacuum pressure and then stored in 50-mm petri dishes. Following filtration of all three replicates, the filtrate was collected and then re-filtered through a GF/F filter as a control for contamination from the plastic vacuum funnel or potentially airborne microplastics.

Microplastic counting involved visual inspection of the entire GF/F in a similar manner to methods described in Hanvey et al. (2017). Visual enumeration was conducted under a stereo microscope with ~100x magnification, and microplastics were classified into one of three categories: fibers, fragments, or beads. For all categories, plastics were defined as observed objects with apparent artificial colors, so as to not enumerate plastics potentially contributed from the sampling bottle itself. Fibers were defined as smooth, long plastics with consistent diameters. Fragments were defined as plastics with irregularly sharp or jagged edges. Beads were defined as spherical plastics. Although we did not measure microplastic size, this technique likely allowed us to reliably quantify microplastics as small as ~300 μm (Hanvey et al. 2017). During enumeration, GF/Fs remained covered in the petri dish to minimize potential for contamination from the air. Following enumeration of both experimental and control samples, fibers, fragments, and beads enumerated in the controls were subtracted from the experimental microplastic densities for each plastic type and from each replicate. One location (BK-1) had two control replicates, which were averaged for each plastic type and then subtracted from the experimental samples. Results are reported as the average number of microplastics/L.

2.3.4 Benthic Biological Samples

At each littoral site, periphyton and benthic macroinvertebrates, including amphipods, mollusks, isopods, caddisflies, leeches, and flatworms, were collected for abundance estimates and food web analysis by wading and snorkeling.

2.3.4.1 Benthic Algal Collection

At each littoral site, we haphazardly selected three rocks representative of local substrate. A plastic stencil was used to define a surface area of each rock from which we scraped a standardized 14.5 cm² patch of periphyton. Samples were preserved with Lugol's solution and stored in plastic scintillation vials. Additional periphyton was collected in composite from each site for fatty acid and stable isotope analysis.

Periphyton taxonomic identification and enumeration was performed by subsampling 10 µL aliquots from each preserved sample. For all 10 µL aliquots, cells, filaments, and colonies were counted, for the entire subsample, until at least 300 cells were identified for a given sampling replicate. If the first aliquot contained less than 300 cells, we counted additional subsamples until we reached at least 300 cells in total. In instances when 300 cells were counted before finishing a subsample, we still counted the entire aliquot. Taxa were classified into broad categories consistent with Baikal algal taxonomy (Izhboldina 2007), using coarse groupings to capture general patterns in relative algal abundance. As a result, algal groups consisted of diatoms, *Ulothrix*, *Spirogyra*, and the green algal Order Tetrasporales.

2.3.4.2 Benthic invertebrate collection

At each littoral site, three kick-net samples were collected for assessment of benthic community composition and abundance. Using a D-net, we collected macroinvertebrates by flipping over 1-3 rocks, and then sweeping five times in a left-to-right motion across approximately 1 m. After the

series of sweeps, the catch was rinsed into a plastic bucket. For each replicate, bucket contents were concentrated using a 64- μ m mesh and placed in glass jars with 40% ethanol (vodka; the only preservative available to us at the time) for preservation and refrigerated at 4°C aboard the research vessel. The 40% ethanol preservative was replaced with ~80% ethanol upon return to the lab within 24 to 48 hours, and samples were stored at ~4°C.

Separate collections were conducted for invertebrate fatty acid and stable isotope analyses. Invertebrates were collected using a D-net and by hand. Organisms collected by hand included amphipod species that were observed from the community composition D-net collections but not readily observed in the stable isotope and fatty acids D-net collections. Collected organisms were live-sorted, identified to species, and then frozen at -20°C at the field station. The samples were later transferred to the lab in the U.S. via a Dewar flask with dry ice.

Invertebrate taxonomic identification and enumeration were performed under a stereo microscope. All adult amphipods were identified to species according to Takhteev and Didorenko (2015), whereas juveniles were identified to genus. Mollusks were identified to the family level according to Sitnikova (2012). Leeches were enumerated at the subclass level, but were likely all from the family Glossiphoniidae based on size, depth of sampling locations, and invertebrate communities sampled (Kaygorodova 2012). Caddisflies were enumerated at the order level, although Baikal does contain over 14 species of caddisfly (Valuyskiy et al. 2020). Flatworms were enumerated at the phylum level. All isopods enumerated were from the family Asellidae. Aside from having limited time available to spend with Baikal taxonomists during our field campaign, our choice of taxonomic resolution ultimately was a result of relative abundance for each taxonomic group, where amphipods were the most abundant taxa and flatworms were among the least abundant taxa across all sites (Figure S1). All samples contained oligochaetes

and polychaetes, but due to poor preservation, these taxa were not counted. Six samples of the 42 collected were not well-preserved and were excluded from further analyses, in order to reduce errors in identification. KD-1 and LI-1 were the only sites with 1 sample counted. BK-2 and KD-2 each had two samples counted.

2.3.5 Food Web Characterization

To characterize littoral food webs, we analyzed periphyton and macroinvertebrate carbon and nitrogen stable isotopes as well as fatty acid profiles for periphyton and macroinvertebrates. Due to some samples warming in transit, we only processed samples that were completely frozen upon arrival to the United States. Given the potential for fatty acids to highlight more subtle, multivariate ecological responses along our transect, we prioritized both periphyton and macroinvertebrate fatty acid analyses over stable isotope analyses. The loss of certain samples resulted in our stable isotope analyses focusing solely on amphipod taxa, whereas fatty acids included some mollusks but still largely consisted of amphipods, the most abundant macroinvertebrate taxon in Lake Baikal (Kozhov 1963; Kozhova and Izmest'eva 1998). Prior to isotopic and fatty acid analysis, periphyton and macroinvertebrate samples were lyophilized for ~24 hours, homogenized to powder, and then weighed.

2.3.5.1 Stable Isotope Analysis

Measurements of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were performed on an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS; Finnigan DELTAplus XP, Thermo Scientific) at the Large Lakes Observatory, University of Minnesota Duluth. The EA-IRMS was calibrated against certified reference materials including L-glutamic acid (NIST SRM 8574), low organic soil and sorghum flour (standards B-2153 and B-2159 from Elemental Micro-analysis Ltd., Okehampton, UK) and

in-house standards (acetanilide and caffeine). Replicate analyses of external standards showed a mean standard deviation of 0.06 ‰ and 0.09 ‰, for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

2.3.5.2 Fatty Acid Analysis

Following freeze-drying, samples were transferred to 10 mL glass centrifuge vials, and 2 mL of 100% chloroform was added to each under nitrogen gas. Samples remained in chloroform overnight at -80°C. Fatty acid extractions generally involved three phases: (1) 100% chloroform extraction, (2) chloroform-methanol extraction, and (3) fatty acid methylation. Fatty acid extraction methods were adapted from Schram et al. (2018).

After overnight chloroform extraction, samples underwent a chloroform-methanol extraction three times. To each sample, we added 1 mL cooled 100% methanol, 1 mL chloroform:methanol solution (2:1), and 0.8 mL 0.9% NaCl solution. Samples were inverted three times and sonicated on ice for 10 minutes. Next, samples were vortexed for 1 minute, and centrifuged for 5 minutes (3,000 rpm) at 4°C. Using a double pipette technique, the lower organic layer was removed and kept under nitrogen. After the third extraction, samples were evaporated under nitrogen flow, and resuspended in 1.5 mL chloroform and stored at -20°C overnight.

Once resuspended in chloroform, 1 mL of chloroform extract was transferred to a glass centrifuge tube with a glass syringe as well as an internal standard of 4 μL of 19-carbon fatty acid. Samples were then evaporated under nitrogen, and then 1 mL of toluene and 2 mL of 1% sulfuric acid-methanol was added. The vial was closed under nitrogen gas and then incubated in 50°C water bath for 16 hours. After incubation, samples were removed from the bath, allowed to reach room temperature and stored on ice. Next, we performed a potassium carbonate-hexane elution twice. To each sample, we added 2 mL of 2% potassium bicarbonate and 5 mL of 100%

hexane, inverting the capped vial so as to mix the solution. Samples were centrifuged for 3 minutes (1,500 rpm) at 4°C. The upper hexane layer was then removed and placed in a vial to evaporate under nitrogen flow. Once almost evaporated, 1 mL of 100% hexane was added and stored in a glass amber autosampler vial for GC/MS quantification. GC/MS quantification was performed with a Shimadzu QP2020 GC/MS following Schram et al. (2018).

2.3.6 Inferring Food Web Structure

In order to assess food web structure, we compared stable isotopes and fatty acids in periphyton resources with those in amphipods. Periphyton and each amphipod species' stable isotope signatures were each measured in composite for a given sampling location. Because periphyton stable isotope samples were measured for the aggregate community, periphyton could only be used as a single potential resource for inferring amphipod diets. For fatty acids, we constructed a Bayesian mixing model to infer a potential resource's relative abundance in amphipods' diets (Stock et al. 2018b; a). This modelling procedure involved three data inputs:

1. Consumer Fatty Acids: These data were collected from our sampling at Lake Baikal. Because *E. verrucosus* and *E. vittatus* were most abundant along our disturbance gradient, we focused this analysis on those species' fatty acid profiles.
2. Source Fatty Acids: Because our collected fatty acid data considered periphyton species in composite, we used published Baikalian taxon-specific fatty acid profiles to define characteristic diatom (Shishlyannikov et al. 2018) and *Ulothrix* spp. (Osipova et al. 2009) signatures. We used *Draparnaldia* spp. from our collected samples, as *Draparnaldia* spp.'s large cell sizes allowed us to isolate cells individually. We *a priori* assumed that amphipods likely did not consume filamentous algal taxa, such as *Draparnaldia* spp. or *Ulothrix* spp.; yet, we included filamentous fatty acids into our model as potential

resources in the event amphipods were detritivorous on decomposing *Draparnaldia* spp. or *Ulothrix* spp. Therefore, including filamentous taxa as potential resources enabled us to account for nutrition that could be incorporated into the food web by grazers switching from herbivory to detritivory.

3. Trophic Discrimination Factors (TDFs): To the best of our knowledge, there are no published TDFs for Baikal amphipods' fatty acids. Therefore, we used TDFs estimated for Antarctic marine amphipods (Schram et al. 2019), which were fed diets of a single algal resource, as a proxy for Baikal amphipod TDFs. To ensure TDF estimates were conservative across consumers and resources, we averaged each fatty acid's TDF, such that a given fatty acid's TDF was identical for each potential resource.

Each consumer, source, and TDF file was then used as an input to MixSIAR. The model building procedure used uninformed prior distributions for each resource, a chain length of 100,000 with 50,000 burn-in, thin of 50, and 3 Monte Carlo Markov Chains. Because TDFs for this analysis were based on marine taxa, we assessed posterior sensitivity to TDF variation by increasing TDF standard deviations by 5%, 10%, 25%, 50%, and 100%, and then re-running the model. Furthermore, this sensitivity analysis was designed to exceed errors that can arise from differences in mixing model methodologies and prescribed error structures (Happel et al. 2021). Each iteration of the sensitivity analysis produced a similar posterior result as the original TDFs. The accompanying R script “07_foodweb_analysis.R” details the exact data aggregation and model construction procedures and can be accessed from the project's Open Science Framework portal (Meyer et al. 2015).

2.3.7 Statistical Analyses

Total phosphorus, nitrate, ammonium, microplastic abundance and density, and total PPCP concentration were log-transformed and regressed against log-transformed IDW population using a linear model. Analytically, log-transforming made sites comparable, as values spanned three orders of magnitude. Physically, we assumed that these sewage indicators were likely subject to exponential processes (e.g., mixing, diffusion), and log-transforming the data should linearize the relationships between predictor and response variables. In contrast, variables that we *a priori* considered to not likely be influenced by mixing processes – chlorophyll a and $\delta^{15}\text{N}$ values in tissues – were not log-transformed but still regressed against log-transformed IDW population using a linear model. Residuals were assessed for normality and homogeneity of variance.

To increase confidence that our observed sewage indicator patterns were not a product of a limited sample size, we also performed a permutational analysis to compare how our actual results compared to a randomly permuted dataset. This process involved randomly permuting sewage indicator variables, regressing the respective sewage indicator against IDW population, and then extracting the p- and R^2 values for the model. This routine was repeated 5,000 times for each sewage indicator, so as to generate a distribution of p- and R^2 values that could have been possible, given our observed data. We then compared models' p- and R^2 values generated from non-permuted data to those from permuted datasets. If indeed models generated from observed data were describing a non-random process, p- and R^2 values should be located at the tail end of the permuted values' distribution. To summarize our original p- and R^2 values in the context of those from models with permuted datasets, we report the percent of p-values less than and R^2 values greater than those from models generated from non-permuted datasets.

To assess if benthic community composition was associated with increasing sewage indicators, periphyton and macroinvertebrate abundance data were each analyzed with a consistent multivariate workflow. First, replicates were averaged, and taxonomic groups representing less than 1% of the inter-site community were removed from analysis, in order to reduce the influence of rare species on results. Second, community compositions for both periphyton and macroinvertebrates were visualized using non-metric multidimensional scaling (NMDS) with a Bray-Curtis similarity metric. Periphyton community compositions were calculated as relative proportions, whereas invertebrate abundances were grouped at the genus-level for amphipods and at higher taxonomic levels for other macroinvertebrates and then square-root transformed to minimize influence of more abundant taxa. Amphipods were kept at the genus level because their numerical and relative abundance markedly exceeded the abundance of other macroinvertebrates (Figure S1). Visual inspection of the NMDS plot suggested that sites generally tended to separate by increasing PPCP concentrations and IDW population (see Table 2). To test whether sites' benthic communities significantly differed with increasing PPCP concentration and IDW population, we first used k-mediods, also known as Partitioning Around the Mediods (PAM; Kaufman and Rousseeuw 2005), clustering to identify an optimal number of groupings. For this process, we iterated through multiple numbers of clusters (i.e., 1 to 10) and calculated the within-group-sum-of-squares (wss; Figure S2) and average silhouette width (Figure S3). We identified the optimal number of groups when wss decreased most markedly and when silhouette width was greatest (i.e., the elbow method) (Johnson and Wichern 2007). To confirm the optimal number as determined by non-hierarchical PAM clustering, we also used Weighted Pair-Group Centroid Clustering (WPGMC; Figure S4) as a hierarchical approach (Sneath and Sokal 1973), which corrects for clusters that may not be

strongly discriminated regardless of how many samples are assigned to a given cluster (Legendre and Legendre 2012). We then performed two permutational multivariate analyses of variance (PERMANOVA; Anderson 2001) with 999 permutations: the first where community compositions were responses to the groups identified through clustering and the second where community compositions were responses to the continuous IDW population. Unlike traditional multivariate analyses of variance (MANOVA), PERMANOVA does not require assumptions of multivariate normality (Anderson 2001). When significant differences were identified, post-hoc SIMPER analysis (Clarke 1993) was performed following the PERMANOVA to identify which taxonomic groups contributed to 85% of the cumulative variance that most influenced site separation.

To assess whether benthic food webs restructured with increasing sewage indicator concentrations, fatty acid data were analyzed in a manner similar to periphyton and macroinvertebrate abundance data. First, species' fatty acid profiles were visualized by performing NMDS with Bray-Curtis similarity for all organisms' relative fatty acid abundance (Figure S5). This technique broadly demonstrated that, as expected, interspecific variation in fatty acid composition was greater than intraspecific variation. The same pattern was observed for all fatty acids quantified as well as solely essential fatty acids (EFAs; Figure S6). Together, these NMDS plots suggested that periphyton fatty acids differentiated based on sewage indicator concentrations, which was likely a reflection of differences in periphyton community composition (Taipale et al. 2013). Among all taxa and sites, the fatty acids 18:3 ω 3, 18:1 ω 9, and 20:5 ω 3 had among the highest coefficients of variation, enabling comparisons between sites. These fatty acids tend to be associated with filamentous green algae (i.e., 18:3 ω 3 and 18:1 ω 9) and diatoms (i.e., 20:5 ω 3). To increase the robustness of our analysis, we expanded our approach

to include major fatty acids within each taxonomic group, including 18:2 ω 6 (abundant in green algae); 16:1 ω 7 and 14:0 (abundant in diatoms); and 16:0 (abundant in both green algae and diatoms) (Taipale et al. 2013). To evaluate how relative fatty acid abundance may relate to sewage pollution, we assessed patterns among these seven fatty acids with both multivariate and univariate approaches. Within a multivariate framework, we created two NMDS plots with Bray-Curtis similarity, one just with primary producer (Figure S7) and the other with macroinvertebrate (Figure S8) fatty acid profiles. Because multivariate patterns suggested fatty acid profiles may relate to sewage pollution, we regressed a filamentous:diatom fatty acid ratio (Equation 3)

$$(3) \frac{18:3\omega 3\% + 18:1\omega 9\% + 18:2\omega 6\% + 16:0\%}{20:5\omega 3\% + 16:1\omega 7\% + 16:0\% + 14:0\%}$$

against log-transformed PPCP concentrations as well as IDW population using a linear model. Additionally, we evaluated how three essential fatty acids (18:3 ω 3, 18:2 ω 6, and 20:5 ω 3), lipids thought to accumulate in biological systems, may differ in abundance across the sewage gradient. Therefore, we similarly regressed the ratio of $\frac{18:3\omega 3\% + 18:2\omega 6\%}{20:5\omega 3\%}$ against log-transformed PPCP concentrations as well as IDW population using a linear model. As with sewage indicators, we recognized that these regression analyses and the associated interpretations may be compromised by a limited sample size. To ensure the robustness of these trends, we performed a permutational analysis similar to sewage indicators, where p- and R² values for models generated from observed data were compared to models generated from 5,000 permutations.

All analyses were conducted in the R statistical environment (R Core Team 2019), using the tidyverse (Wickham et al. 2019), factoextra (Kassambara and Mundt 2019), cluster (Maechler et al. 2019), pvclust (Suzuki et al. 2019), ggrepel (Slowikowski 2019), viridis (Garnier

2018), fs (Hester and Wickham 2019), spdplyr (Sumner 2019), janitor (Firke 2020), sf (Pebesma 2018), ggpubr (Kassambara 2019), ggtext (Wilke 2020), OpenStreetMap (Fellows and Stotz 2019), cowplot (Wilke 2019), broom (Robinson and Hayes 2019), ggsn (Baquero 2019), MixSIAR (Stock et al. 2018b), and vegan (Oksanen et al. 2019) packages. All data, including .kml files used to calculate IDW metric, are publicly available from the Environmental Data Initiative repository (Meyer et al. 2020), and all R scripts are available from the GitHub repository of this project’s Open Science Framework account (Meyer et al. 2015).

2.4 Results

2.4.1 Water Samples

Nearshore water nitrate ($R^2 = 0.01$, $p = 0.68$), ammonium ($R^2 = 0.17$, $p = 0.11$), total phosphorus ($R^2 = 0.14$, $p = 0.14$), and chlorophyll a ($R^2 = 0.11$, $p = 0.20$) concentrations were not significantly correlated with IDW population (Figure 3). Total PPCP concentrations ($R^2 = 0.26$, $p = 0.04$) and $\delta^{15}\text{N}$ values in macroinvertebrate tissue ($R^2 = 0.33$, $p = 0.02$) were significantly related with IDW population (Figure 3). In the littoral zone, PPCPs detected included caffeine, 1,7-dimethylxanthine/paraxanthine (main human metabolite of caffeine), cotinine (main human metabolite of nicotine), and acetaminophen/paracetamol (Table 3). Other PPCPs, including carbamazepine, diphenhydramine, thiabendazole, amphetamine, methamphetamine, MDA, MDMA, morphine, phenazone, sulfachloropyridazine, sulfamethazine, sulfadimethoxine, sulfamethazole, trimethoprim, and cimetidine, were not detected.

Microplastics were detected in samples from both littoral and pelagic sites. Bead microplastics were only detected near Listvyanka. Fibers (mean = 0.85 microplastics/L, std dev = 1.21 microplastics/L) and fragments (mean = 0.83 microplastics/L, std dev = 1.35 microplastics/L) were the most abundant types of microplastics across all sites, whereas beads

were relatively rare (mean = 0.08 microplastics/L, std dev = 0.31 microplastics/L). Total microplastic densities were not significantly correlated with IDW population ($R^2 = 0.01$, $p = 0.65$; Figure 3), although more types of microplastics were generally observed near areas with higher IDW population values, such as Listvyanka.

Permutational analyses corroborated these findings. Model estimates for total PPCP concentrations and $\delta^{15}\text{N}$ values were both within the tail 5% of values generated from permuted data (Figure S9). Models using total phosphorus, nitrate, ammonium, chlorophyll a, and microplastics tended to have R^2 and p-values similar to randomized datasets (Figure S9).

2.4.2 Benthic Biological Samples

2.4.2.1 Periphyton

Major taxonomic groupings of periphyton consisted of diatoms, Tetrasporales, *Spirogyra* spp., and *Ulothrix* spp. K-medoids (Figures S2a; S3a) and WPGMC (Figure S4a) cluster analyses of periphyton abundance demonstrated two groupings capture most variance, and visual inspection of relative periphyton community abundance NMDS suggested groupings were related to IDW population values (Figure 4). PERMANOVA results demonstrated that periphyton communities were significantly different based on IDW population groupings ($R^2 = 0.52$, $p = 0.001$) and the continuous IDW population ($R^2 = 0.43$, $p = 0.001$). Post-hoc SIMPER results suggested that these differences were primarily associated with sites that had higher *Ulothrix* spp. and *Spirogyra* spp. relative abundance.

2.4.2.2 Macroinvertebrates

Taxonomic groupings included five amphipod genera: *Eulimnogammarus*, *Poekilogammarus*, *Cryptoropus*, *Brandtia* and *Pallasea*; six mollusk families: Planorbidae, Valvatidae, Baicaliidae, Benedictidae, Acroloxidae, and Maackia; flatworms; caddisflies; isopods; and leeches

(summarized in Table S1). K-mediod cluster analysis of macroinvertebrate community composition suggested 2 or 3 major groupings would capture most variance (Figure S2b; S3b), whereas WPGMC analyses suggested 2 groupings would enable all sites except for one to be assigned a cluster (Figure S4b). Because both forms of hierarchical and non-hierarchical clustering suggested two groupings as optimal, we proceeded using two groupings. Visual inspection of NMDS suggested clusters were related to IDW population (Figure 5). PERMANOVA results supported the hypothesis that macroinvertebrate communities significantly differed both among our IDW population groupings ($R^2 = 0.19$, $p = 0.02$) and along our continuous gradient of increasing IDW population ($R^2 = 0.19$, $p = 0.02$). Post-hoc SIMPER analyses suggested that *Poekilogammarus*, *Eulimnogammarus*, Valvatidae, Caddisflies, *Brandtia*, Baicaliidae, Planorbidae, *Cryptoropus*, and flatworms contributed the greatest differences between IDW population groupings (see Table 2).

2.4.3 Food Web Characterization: Stable Isotopes and Fatty Acids

Among periphyton and amphipod samples, $\delta^{13}\text{C}$ values ranged from -19.5 to -9.5 ‰ (Figure 6A). Among periphyton samples, $\delta^{15}\text{N}$ values ranged from 0.77 to 3.76 ‰, whereas amphipod $\delta^{15}\text{N}$ values ranged from 6.42 to 7.92 ‰.

For grazers, $\delta^{15}\text{N}$ values significantly increased with IDW population ($p = 0.01$; Figure 3, Figure 6A). Periphyton $\delta^{15}\text{N}$ signatures did not significantly increase with IDW population ($p = 0.27$). In contrast, $\delta^{13}\text{C}$ values were not related with IDW population for either periphyton or macroinvertebrates.

With respect to fatty acids, macroinvertebrates tended to be characterized by mono-unsaturated fatty acids (MUFAAs) and long-chain (i.e. ≥ 20 -Carbons) polyunsaturated fatty acids (LCPUFAs), whereas periphyton tended to be characterized by short-chain (i.e., 16- and 18-

Carbons) polyunsaturated fatty acids (SCPUFAs) (Table 3). When comparing proportions within taxa across the sewage gradient, periphyton SCPUFA proportions tended to increase (Figure S10) and periphyton SAFA proportions generally decreased. In contrast, benthic macroinvertebrate fatty acid proportions tended to remain consistent across the entire gradient (Figure S10).

With respect to food web structure, stable isotope isospaces suggested that amphipods along our transect likely consumed periphyton (Figure 6A). Results from our Bayesian mixing model further implied that diatom-associated fatty acids constituted approximately 80% of amphipods' diets, whereas *Draparnaldia* spp. and *Ulothrix* spp. fatty acid signatures constituted 12.5% and 6.4%, respectively (Figure 6B).

When assessing how grazing patterns may change over disturbance gradients, our analyses focused mainly on the fatty acids consistently associated with filamentous green algae (i.e., 18:3 ω 3, 18:1 ω 9, 18:2 ω 6, and 16:0) as well as diatoms (i.e., 20:5 ω 3, 16:1 ω 7, 14:0, and 16:0). For periphyton, the ratio of green filamentous:diatom-associated fatty acids significantly increased with an increasing PPCP concentration ($R^2 = 0.62$; $p = 0.04$, Figure 7; S11-12) and to some extent with an increasing IDW population ($p = 0.08$; Figure S13-15). *Eulimnogammarus verrucosus* fatty acid ratios were not significantly related with either increasing IDW population (Figure S13) or increasing PPCP concentrations (Figure 7), but *Eulimnogammaurus vittatus* filamentous:diatom ratios decreased with an increasing IDW population ($p = 0.01$; Figure S13) but not PPCP concentrations (Figure 7). When focusing solely on the essential fatty acids 18:3 ω 3, 18:2 ω 6, and 20:5 ω 3, the same patterns were observed in both periphyton ($R^2 = 0.73$; $p = 0.02$) and amphipods (Figure 7; S13). Permutational analyses for both regression analyses supported these trends. P- and R^2 values estimated for periphyton models were within the 5%

margins in comparison to models produced with a randomized dataset (Figure S11-12; S14-15).

Model estimates for both *E. verrucosus* and *E. vittatus* were more reflective of those observed from randomized datasets (Figure S11-12; S14-15).

2.5 Discussion

Our combined results corroborate previous findings (e.g., Timoshkin et al., 2016; 2018) that sewage pollution is entering Lake Baikal's nearshore area and likely is responsible for changes in nearshore benthic communities. Unlike previous studies, we were able to incorporate highly specific indicators of sewage pollution and food web structure to describe direct, quantitative relationships between human development and ecological responses.

2.5.1 Relating Human Settlements to Sewage Indicator Concentrations

In agreement with our expectations, some sewage pollution indicators in the nearshore of Lake Baikal were associated with size of and distance from human settlements. Total PPCP concentrations, macroinvertebrate $\delta^{15}\text{N}$ values, and, to some degree, total phosphorus as well as ammonium concentrations increased with IDW population. These sewage gradients created by highly localized settlements are noteworthy considering that Baikal's shoreline, including our study area, is largely free of lakeside development (Moore et al. 2009). Furthermore, the use of sewage-associated indicators, such as PPCPs and $\delta^{15}\text{N}$, proved necessary for defining sewage gradients. The use of nutrients as indicators alone would not reveal sewage pollution gradients, since nutrients were not strongly correlated with IDW population and could come from diverse sources. For example, melting permafrost in Lake Baikal's watershed (Anisimov and Reneva 2006) and the Selenga River basin (Tornqvist et al. 2014) as well as climate-driven changes in mixing processes (Swann et al. 2020) have the potential to contribute substantial nutrient loadings to the nearshore. While nutrients also could be contributed by agriculture (Powers et al.

2016) and changing terrestrial plant communities (Moran et al. 2012), these are not currently known to be major sources of elevated nutrients in the Baikal watershed, relative to sewage (Timoshkin et al., 2016, Timoshkin et al., 2018), changing mixing patterns (Swann et al. 2020), and permafrost melt (Anisimov & Reneva, 2006).

This is the first known study to detect PPCPs in Lake Baikal, a voluminous lake in a largely unpopulated watershed. We detected PPCPs nearshore but not at our three offshore sites, suggesting that sewage inputs in Baikal become diluted as pollutants move out of the nearshore area. More generally, these results are important for lake monitoring, as PPCPs are robust indicators of sewage pollution. Beyond Lake Baikal, these data are important for understanding the prevalence of PPCPs in lakes, as lakes have remained less represented in the PPCP literature in comparison to lotic and subsurface systems (Meyer et al. 2019). This literature imbalance creates opportunities to assess how PPCPs, and sewage pollution more broadly, may lead to differing ecological responses in lotic and lentic systems. As lakes tend to have longer hydraulic residence times relative to rivers and streams, pollutants may be more prone to accumulate (Yang et al. 2018; Meyer et al. 2019). In the case of our data, comparing contemporaneous littoral and pelagic PPCP concentrations revealed littoral-pelagic sewage gradients, as PPCPs were degraded, metabolized or accumulated by biota, preserved within sediments, or diluted to undetectable concentrations. In the context of the entire lake, analyses of sediments have shown how PPCPs can remain within lake systems for decades, thereby enabling researchers to reconstruct histories of wastewater pollution in a system (Czekalski et al. 2015; Yang et al. 2018).

Investigating PPCP concentrations across limnic environments could also establish how ecological communities respond not only to sewage but also to the PPCPs themselves. While we

focus on PPCPs as indicators of sewage, previous studies have shown that PPCPs, even at concentrations we observed in Lake Baikal, can elicit biological responses from physiological (e.g., del Rey et al. 2011; Feijão et al. 2020) and behavioral (e.g., Brodin et al. 2013; Dziewczynski et al. 2016) levels to food webs (e.g., Lagesson et al. 2016; Richmond et al. 2018) and ecosystems (e.g., Rosi-Marshall et al. 2013; Richmond et al. 2019; Robson et al. 2020). Although our study was not designed to evaluate the ecotoxicological effects of PPCPs themselves, future studies could potentially address effects of PPCPs on nearshore Baikal biota by using *in situ* sewage gradients as a guide.

In contrast to PPCP concentrations and $\delta^{15}\text{N}$ values, microplastics were not associated with IDW population and may be poor proxies for sewage pollution in Lake Baikal. Additionally, microplastics may originate from non-sewage sources, such as agriculture (Steinmetz et al. 2016) and fishing nets (Eerkes-Medrano et al. 2015). Because of their long degradation time (Brandon et al. 2016), microplastics can indicate accumulated pollution, which likely enables wider distribution (Fischer et al. 2016; Hendrickson et al. 2018). Unlike microplastic concentrations identified in Lake Hovsgol (Free et al. 2014), Lake Superior (Hendrickson et al. 2018), or Lake Erie (Eriksen et al. 2013), microplastic concentrations in Baikal, as quantified by our methods, may be poor proxies for capturing pollution from seasonally varying human populations. It is worth noting that since the time of our field sampling, evidence has accumulated that our methods likely dramatically underestimated microplastic abundance (Wang and Wang 2018; Brandon et al. 2020), and there is potential for the microplastics themselves to cause deleterious ecological responses. While we focus here on microplastics as an indicator of sewage pollution, microplastics are increasingly shown to disrupt food web dynamics by altering grazing patterns (Green 2016) and providing carbon substrate for

microbial growth (Romera-Castillo et al. 2018). Recent investigations of microplastics in Lake Baikal near Bolshie Koty (BK) used analogous methods and measured similarly low concentrations (Karnaukhov et al. 2020). When considering Lake Baikal's large volume, Karnaukhov et al. (2020) noted that the number of plastic pieces may well exceed those observed in other lakes, such as Lake Hovsgol. Together these growing uncertainties suggest that microplastic pollution in Baikal and elsewhere deserves increased attention.

2.5.2 Relating Sewage Indicators with Benthic Algal Communities

Congruent with our hypotheses, increasing sewage indicators tended to be associated with higher relative abundance of filamentous taxa in periphyton. Previous studies investigating Baikal's periphyton composition noted that areas adjacent to human development often had an increased relative abundance of filamentous algae such as *Ulothrix* spp. and *Spirogyra* spp. (Timoshkin et al. 2016, 2018). Lake Baikal's southwestern shore historically experiences short *Ulothrix* spp. blooms in late August (Kozhov 1963), potentially confounding sewage signals with an annually occurring phenomenon. While the potential does exist for both diatoms and filamentous taxa to increase in numerical abundance with increasing sewage pollution, our data are consistent with the results of Timoshkin et al. (2016) and show that relative abundance of filamentous algae is greatest near areas of higher lakeside development.

Even as community composition shifted with increasing sewage indicator concentrations, periphyton $\delta^{15}\text{N}$ values did not differ along our transect. Previous studies in marine (Gartner et al. 2002; Savage and Elmgren 2004; Risk et al. 2009) and freshwater (Wayland and Hobson 2001; Camilleri and Ozersky 2019) systems have highlighted how sewage-associated $\delta^{15}\text{N}$ can increase in algal samples and even throughout the food web. Like PPCPs in our study, $\delta^{15}\text{N}$ values are often most enriched near the source of sewage pollution and can decrease over several

kilometers (Savage and Elmgren 2004), with concentrations varying based on species-specific uptake rates and mixing processes (Gartner et al. 2002). While previous studies using $\delta^{15}\text{N}$ signatures in macroalgae and vascular macrophytes have successfully tracked sewage gradients (Cole et al. 2004), periphyton $\delta^{15}\text{N}$ as a sewage indicator potentially can be confounded by terrestrial $\delta^{15}\text{N}$ contributions such as through agricultural runoff (Chang et al. 2012). In our study, periphyton $\delta^{15}\text{N}$ signatures may be explained by periphyton's typically high cell turnover rates (e.g., days; Swamikannu and Hoagland 1989) dampening isotopic patterns, $\delta^{15}\text{N}$ -accumulating algal taxa being grazed more readily by macroinvertebrates (Rosenberger et al. 2008), or co-limitation dynamics between ammonium and nitrate (York et al. 2007; Piñón-Gimate et al. 2009).

Fatty acid analyses suggested that changes in periphyton community composition altered the nutritional quality of periphyton across the pollution gradient. Periphyton fatty acid profiles from sites with higher sewage pollution had higher cumulative proportions of 18:3 ω 3, 18:1 ω 9, 18:2 ω 6, and 16:0 relative to cumulative 20:5 ω 3, 16:1 ω 7, 16:0, and 14:0 fatty acid proportions. This pattern likely reflects the higher abundance of green algae relative to diatoms (Iverson et al. 2004; Osipova et al. 2009; Taipale et al. 2013; Galloway and Winder 2015; Shishlyannikov et al. 2018), which we observed from our periphyton community composition analysis (Figure 3). Together, our periphyton composition and fatty acid results suggest that Baikal's nearshore periphyton communities near human lakeside developments are more dominated by filamentous green algae, and therefore, have lower nutritional content.

Among the array of fatty acids synthesized in algal communities, essential fatty acids (EFAs) are most likely to be taxonomically associated with, and influenced by, changing community composition. EFAs are a subgroup of polyunsaturated fatty acids (PUFAs) that are

prone to accumulating in organisms (see Kelly & Scheibling, 2012). Among the eight common EFAs (Taipale et al. 2013), 18:3 ω 3, 18:2 ω 6, and 20:5 ω 3 had the highest coefficients of variation between sites. Because these three EFAs demonstrated the greatest variation between sites, our analyses focused on how their relative abundances related to PPCP concentrations and IDW populations. The fatty acids 18:3 ω 3 and 18:2 ω 6 have been previously associated with filamentous algae, such as Baikalian *Ulothrix* spp. (Osipova et al. 2009) and *Draparnaldia* spp., whereas 20:5 ω 3 have previously been associated with Baikalian diatoms (Shishlyannikov et al. 2018). Comparing the ratio of filamentous green algae to diatoms could therefore function as proxy for each algal taxon's relative abundance and potentially offer insights into feeding patterns for the grazers.

2.5.3 Relating Sewage Indicators with Macroinvertebrate Feeding Guilds

In assessing benthic consumer communities' responses to changing periphyton, our data suggest macroinvertebrate guilds reshape with increasing sewage pollution. Our results support the general conclusion of Timoshkin et al. (2016) that Baikalian mollusk abundance tends to decrease with increasing sewage pollution. Decreased mollusk abundance may have several causes, including low tolerance for increased concentrations of PPCPs or other components of sewage (e.g., Hollingsworth et al. 2002, Timoshkin et al. 2016), inability to consume filamentous algae (Mazzella and Russo 1989), or filamentous algae not offering the proper nutrition (Lowe and Hunter 1988). In contrast to mollusks, amphipods were generally prevalent at all littoral sites, regardless of sewage indicator concentrations. *Brandtia* spp. was the only amphipod genus less abundant with sewage indicator signals. This genus tends to be associated with endemic sponges (Taakhteev & Didorenko, 2015), which may also be decreasing in abundance near areas of lakeside development (Timoshkin et al., 2016). *Eulimnogammarus* spp., one of the most

speciose Baikal genera (Takhteev and Didorenko 2015), was prevalent at all sites, and $\delta^{15}\text{N}$ values in its tissue increased slightly but significantly with increasing IDW population. Unlike periphyton, amphipods' increasing $\delta^{15}\text{N}$ values may relate to amphipods having longer cellular turnover rates (e.g., weeks; McIntyre and Flecker 2006) relative to periphyton. Consequently, amphipods' enhanced $\delta^{15}\text{N}$ values suggest that sewage-derived nutrients are being incorporated into the food web. While we did not test amphipod tissues for other sewage indicators such as PPCPs and microplastics, the potential for some PPCPs to bioaccumulate and biomagnify in food webs has been recently demonstrated, with ecological ramifications remaining uncertain (Lagesson et al., 2016; Richmond et al., 2018). These combined results suggest that mollusk abundance and amphipod $\delta^{15}\text{N}$ values may be longer-term indicators of sewage pollution in Baikal.

In contrast to variation in $\delta^{15}\text{N}$ values, amphipod fatty acid profiles did not differ markedly between sites (Figure 7; S11-15). Amphipods from all collected sites expressed consistent 20:5 ω 3 signatures relative to 18:3 ω 3 and 18:2 ω 6. Consumers usually accumulate fatty acids from their food source. Yoshii's (1999) study as well as our own stable isotope data suggest that Baikal's benthic, littoral amphipods are likely a combination of grazers and omnivores. Because fatty acid profiles in amphipods largely reflected fatty acid signatures in periphyton, our data suggest that amphipods likely continue grazing on periphyton, despite the food resource changing in community composition and nutritional content. In particular, results from our mixing model suggest that diatoms constitute a large majority of amphipods' diets (Figure 6B), even though diatoms tended to be less abundant in periphyton communities relative to filamentous taxa along our transect. As a consequence, amphipods may be compensating for the shifting nutritional quality of periphyton through at least three potential mechanisms. First,

amphipods may selectively consume diatoms as opposed to filamentous algae, meaning diatom relative abundance could decrease both from increased grazing and lesser efficiency at taking up nutrients relative to filamentous taxa. Similarly, amphipods may become detritivorous when living diatoms become less abundant. Because amphipods' fatty acid signatures still reflect a predominately diatom-associated diet (Figure 6B; 7; S13) and detrital fatty acids tend to reflect the composite fatty acids of the community (Tenore et al. 1984; Wilson et al. 2001; Vonk et al. 2016), our results imply that even detritivorous amphipods may rely on decomposing diatoms for maintaining consistent nutrition along the disturbance gradient. Second, amphipods themselves (e.g., Desvillettes et al. 1997; Castell et al. 2004) or heterotrophic symbionts (Klein Breteler et al. 1999; Veloza et al. 2006; Hiltunen et al. 2017; Yoshioka et al. 2019) may upgrade fatty acids by investing energy to convert C18 fatty acids to C20 fatty acids. Third, amphipods, especially stenothermic taxa such as *E. verrucosus* (Jakob et al. 2021), may migrate to deep littoral zones (e.g., 10-100 m), where diatoms may be more abundant, but then return to shallow littoral areas where breeding occurs (e.g., < 10 m; Takhteev and Didorenko 2015). Regardless of the exact mechanism, our data suggest that food web interactions would change with increasing sewage pollution and may imply a net energetic cost through amphipods' differential grazing patterns.

2.6 Conclusions

Over the past decade, Lake Baikal has shown signs of nearshore eutrophication, despite the pelagic zone remaining ultra-oligotrophic. While Baikal receives nutrients from multiple sources, sewage-specific indicators used in this study implicate wastewater pollution as one of the sources of nutrients. Our results corroborate work by Timoshkin et al. (2016, 2018) and Bondarenko et al. (2021), demonstrating how patchy hot spots of lakeside development at Baikal can create gradients in sewage concentrations and ecological responses. Unlike previous studies, our approach pairs community abundance data (i.e., periphyton and macroinvertebrate counts) and

nuanced dietary tracers (i.e., fatty acids) to assess benthic community and food web consequences of sewage pollution. While sewage pollution may lead to changing resources for macroinvertebrate grazers, Baikal's amphipods appear to be compensating either (1) by selectively grazing on diatoms, (2) by switching from herbivory to detritivory, or (3) by consuming less desirable food and upgrading fatty acids. In all of these cases, our results suggest shifting community interactions and may imply a net energetic cost for amphipods, as they expend energy either by foraging selectively for diatoms or by catabolizing certain essential fatty acids.

2.7 Future Trajectories: A Call for Increased Nearshore Monitoring

Our results underscore the importance of nearshore monitoring in detecting sewage pollution in large lakes. Lake Baikal is considered ultra-oligotrophic based on pelagic sampling (Yoshida et al. 2003; O'Donnell et al. 2017), but nearshore hot spots of eutrophication are developing throughout the lake (Timoshkin et al. 2016, 2018). While pelagic samples are representative of the lake's overall status, nearshore sampling aids managers in identifying pollution loading before the entire system is affected (Jacoby et al. 1991; Lambert et al. 2008; Hampton et al. 2011). Beyond Baikal, several large, deep, oligotrophic lakes have likewise experienced localized sewage pollution with nearshore biological responses, despite pelagic measurements suggesting oligotrophic status (e.g., Jacoby et al. 1991, Rosenberger et al. 2008; Hampton et al., 2011). Once eutrophication of the open water has occurred, mitigation can involve complex socio-economic factors (Carpenter et al. 1999), require system-specific information (Jeppesen et al. 2005), and necessitate long-term strategies (Tong et al. 2020). Because nutrients may enter systems from numerous sources, incorporating sewage-specific indicators, such as PPCPs, may be necessary. PPCP sampling has the potential to not only identify sewage-associated nutrient pollution but also assess heterogeneities in sewage loading along a shoreline. When PPCP data

are paired with co-located benthic community composition and food web data, managers can take system-specific actions to mitigate ecological consequences before sewage concentrations are detected throughout the lake. Across larger spatial and temporal scales, these paired PPCP-biological samples have potential to offer a synoptic view of the impacts of sewage pollution, enabling regional and local monitoring to coordinate mitigation strategies

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| Site | Latitude | Longitude | Depth (m) | Distance to shore (m) | Air Temperature (°C) | Surface Temperature (°C) | Adjacent Population |
|-------|-----------|-----------|-----------|-----------------------|----------------------|--------------------------|---------------------|
| BK-1 | 51.90316 | 105.07404 | 0.7 | 10 | 18 | 14 | 80 |
| BK-2 | 51.90365 | 105.069 | 0.9 | 17.5 | 19 | 13 | 80 |
| BK-3 | 51.90536 | 105.0957 | 0.8 | 10 | 18 | 14 | 80 |
| BGO-1 | 52.02693 | 105.40102 | 0.9 | 18 | 20 | 13 | 0 |
| BGO-2 | 52.0197 | 105.37707 | 1.1 | 14 | 19 | 14 | 600 |
| BGO-3 | 52.02649 | 105.43577 | 0.7 | 21 | 18 | 16 | 600 |
| OS-1 | 51.98559 | 105.47237 | 900 | NA | 15 | NA | NA |
| KD-1 | 51.92646 | 105.24504 | 0.8 | 20.75 | 23 | NA | 0 |
| KD-2 | 51.91807 | 105.21456 | 0.9 | 14.5 | 23 | 16 | 0 |
| MS-1 | 51.89863 | 105.15017 | 0.6 | 10.5 | 21 | 17 | 0 |
| SM-1 | 51.87152 | 104.98006 | 0.9 | 11.5 | 21 | 15 | 0 |
| LI-1 | 51.86825 | 104.83042 | 0.6 | 8.9 | 19 | 14 | 2000 |
| LI-2 | 51.84626 | 104.87356 | 0.8 | 9.4 | 21 | 15 | 2000 |
| LI-3 | 51.85407 | 104.86216 | 0.7 | 9.25 | 19.5 | 15 | 2000 |
| EM-1 | 51.86005 | 104.93999 | 0.7 | 15.5 | 24.5 | 14 | 0 |
| OS-2 | 51.8553 | 104.8148 | 1300 | NA | 21 | NA | NA |
| OS-3 | 51.859108 | 105.0769 | 1400 | 5000 | NA | 14.5 | NA |

Table 1: Location, depth, temperature and population information for each of the 17 sampling stations. “OS” refers to pelagic locations (i.e., “Offshore”), whereas other site abbreviations refer to littoral sampling locations.

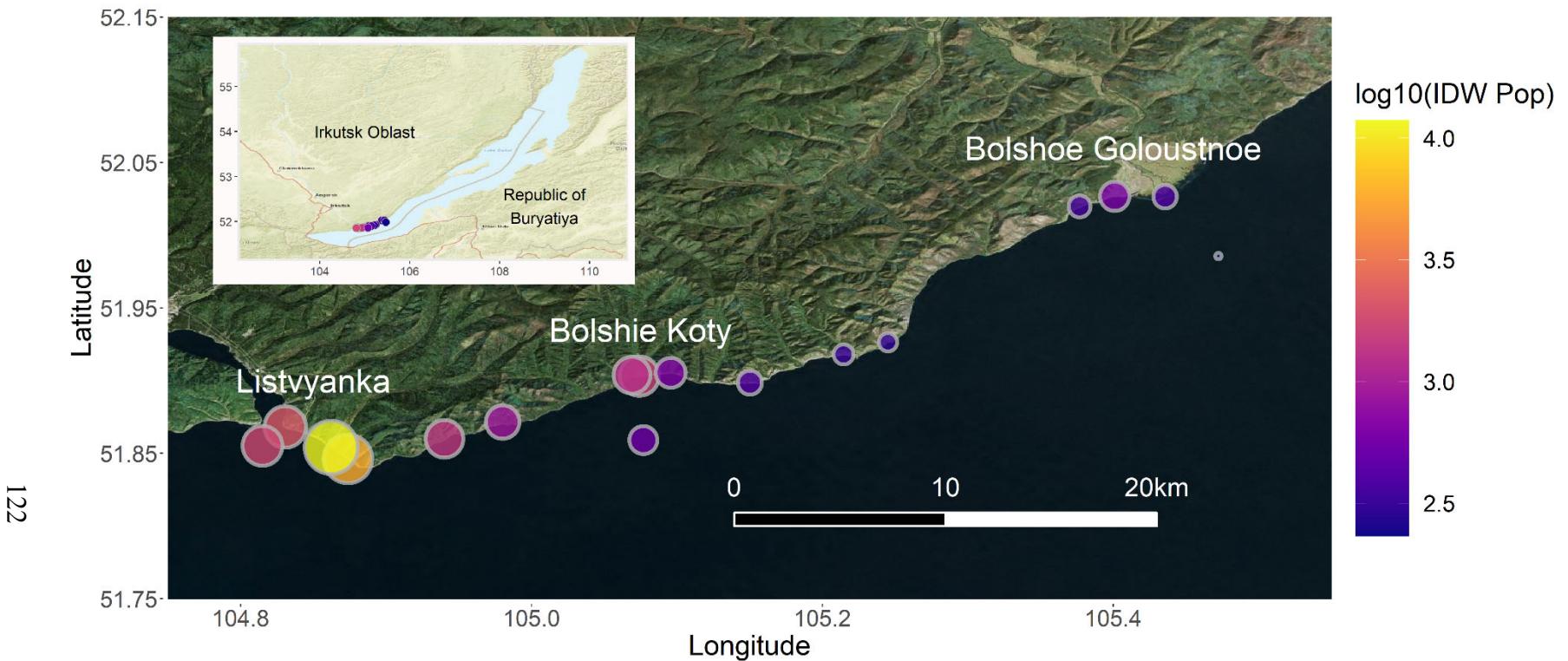


Figure 1: Map of all sampling locations with sites sized and colored by log-transformed IDW population. IDW population was log-transformed so as to make IDW populations across three orders of magnitude more comparable. The entire transect included three developed sites (i.e., Listvyanka, Bolshie Koty, Bolshoe Goloustnoe). Three offshore samples were also collected to compare pelagic sewage signals to those in the littoral. Sampling locations west of Listvyanka are located farther from Listvyanka's centroid, and therefore have lower IDW population values than sites located closer to the centroid. This map was created using the R statistical environment (R Core Team 2019) and the tidyverse (Wickham et al. 2019), OpenStreetMap (Fellows and Stotz 2019), ggpubr (Kassambara 2019), cowplot (Wilke 2019), ggsn (Baquero 2019), and ggrepel (Slowikowski 2019) packages.

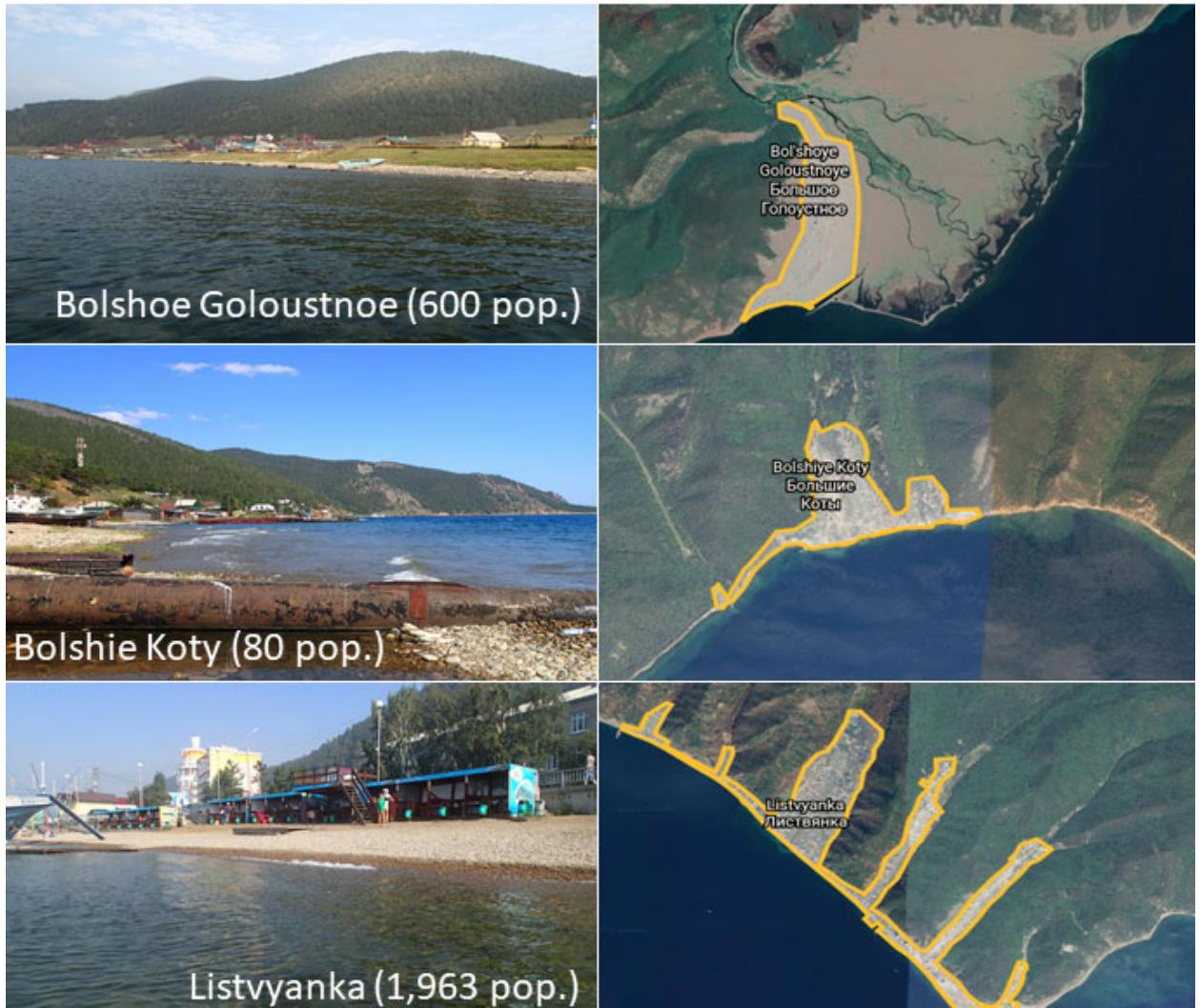
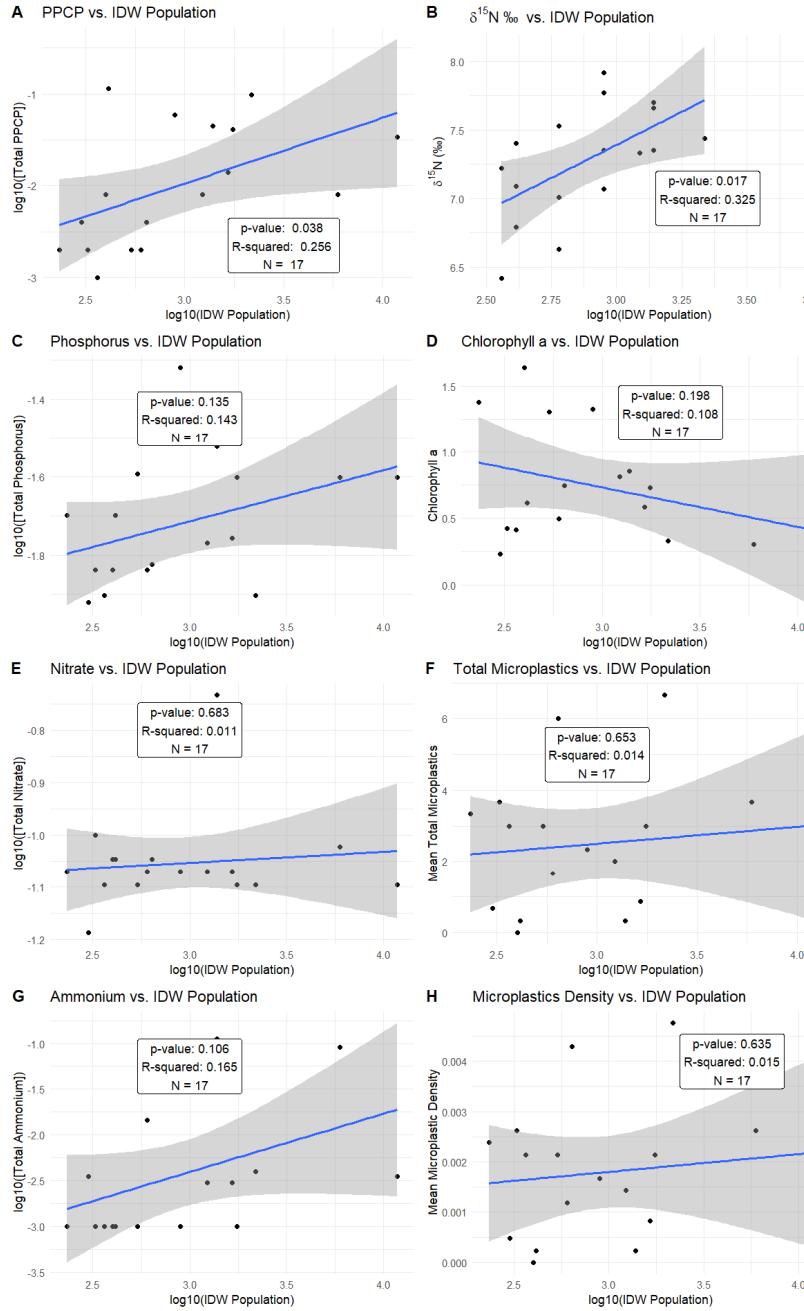


Figure 2: Photographs and Google Earth imagery of each developed area. Photographs were taken by Kara H. Woo and Michael F. Meyer.

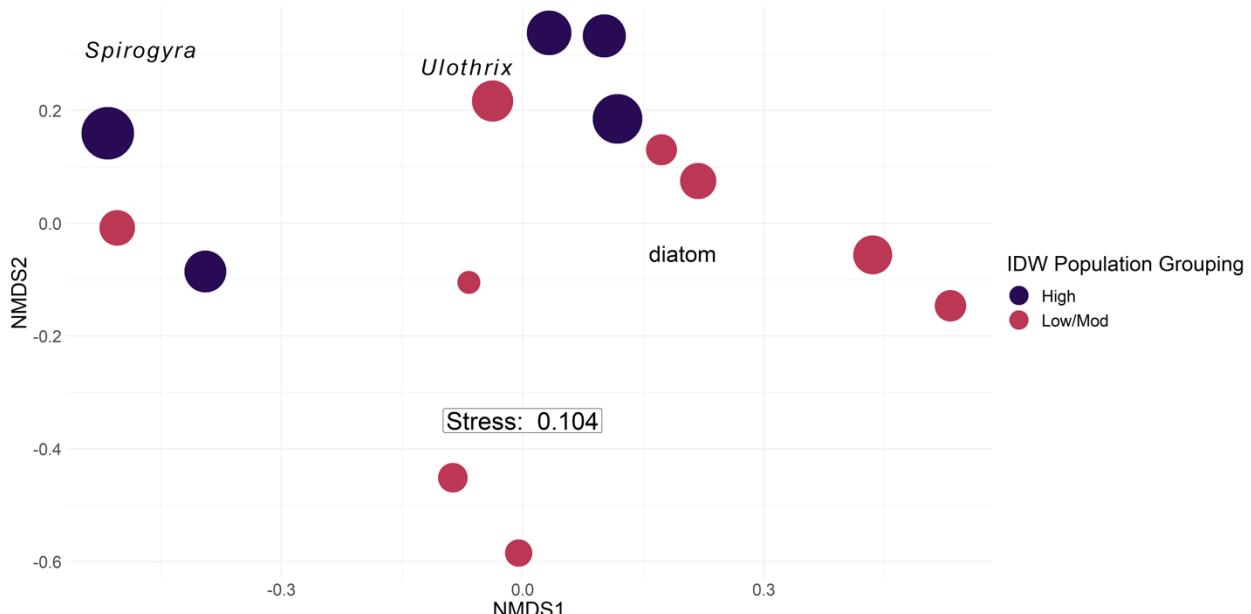
| Site | NH ₄ ⁺ (mg/L) | NO ₃ ⁻ (mg/L) | Total Phosphorus (mg/L) | Caffeine (µg/L) | Acetaminophen (µg/L) | Paraxanthine (µg/L) | Cotinine (µg/L) | Fragment density (MPs/L) | Fiber density (MPs/L) | Bead density (MPs/L) | IDW population | Categorical IDW population |
|-------|--|--|----------------------------|--------------------|-------------------------|------------------------|--------------------|-----------------------------|--------------------------|-------------------------|----------------|----------------------------|
| BK-1 | 0.003 | 0.085 | 0.018 | 0.011 | 0.001 | 0.002 | 0 | 0 | 0.000833 | 0 | 2304.039 | High |
| BK-2 | 0.003 | 0.085 | 0.017 | 0.007 | 0.001 | 0 | 0 | 0.000952 | 0.000476 | 0 | 1891.558 | Mod/Low |
| BK-3 | 0.068 | 0.09 | 0.015 | 0.003 | 0.001 | 0 | 0 | 0.003095 | 0.00119 | 0 | 1231.234 | Mod/Low |
| BGO-1 | 0.0145 | 0.085 | 0.015 | 0 | 0.002 | 0 | 0 | 0.00119 | 0 | 0 | 838.5385 | Mod/Low |
| BGO-2 | 0.001 | 0.08 | 0.013 | 0 | 0.001 | 0 | 0 | 0.000238 | 0.001905 | 0 | 611.91 | Mod/Low |
| BGO-3 | 0.001 | 0.09 | 0.015 | 0.005 | 0.003 | 0 | 0 | 0 | 0 | 0 | 624.455 | Mod/Low |
| OS-1 | 0.001 | 0.085 | 0.020 | 0 | 0.001 | 0 | 0.001 | 0.002381 | 0 | 0 | 455.7733 | Mod/Low |
| KD-1 | 0.0035 | 0.065 | 0.012 | 0.003 | 0.001 | 0 | 0 | 0 | 0.000476 | 0 | 662.4151 | Mod/Low |
| KD-2 | 0.001 | 0.1 | 0.015 | 0.001 | 0.001 | 0 | 0 | 0.000714 | 0.001905 | 0 | 720.5484 | Mod/Low |
| MS-1 | 0.001 | 0.09 | 0.02 | 0.064 | 0.035 | 0.015 | 0 | 0 | 0.000238 | 0 | 903.6733 | Mod/Low |
| SM-1 | 0.001 | 0.085 | 0.048 | 0.042 | 0.012 | 0.005 | 0 | 0 | 0.001667 | 0 | 2146.218 | Mod/Low |
| LI-1 | 0.004 | 0.08 | 0.013 | 0.05 | 0.04 | 0.006 | 0.002 | 0.00381 | 0.000238 | 0.000714 | 5403.209 | High |
| LI-2 | 0.091 | 0.095 | 0.025 | 0.001 | 0.007 | 0 | 0 | 0.001429 | 0.00119 | 0 | 14792.51 | High |
| LI-3 | 0.0035 | 0.08 | 0.025 | 0.027 | 0.002 | 0.002 | 0.003 | 0.000476 | 0 | 0.000714 | 29511.73 | High |
| EM-1 | 0.1125 | 0.185 | 0.030 | 0.029 | 0.014 | 0.002 | 0 | 0 | 0.000238 | 0 | 3389.949 | High |
| OS-2 | 0.001 | 0.08 | 0.025 | 0.033 | 0.001 | 0.004 | 0.003 | 0.000238 | 0.001905 | 0 | 4340 | High |
| OS-3 | 0.001 | 0.08 | 0.026 | 0.001 | 0.001 | 0 | 0 | 0 | 0.002143 | 0 | 1221.424 | Mod/Low |

0 Table 2: Average sewage indicator concentrations and densities per sampling location. Caffeine, acetaminophen/paracetamol,
 1 paraxanthine, and cotinine detection limits are estimated to be 0.001 µg/L based on a 500 mL sample volume.



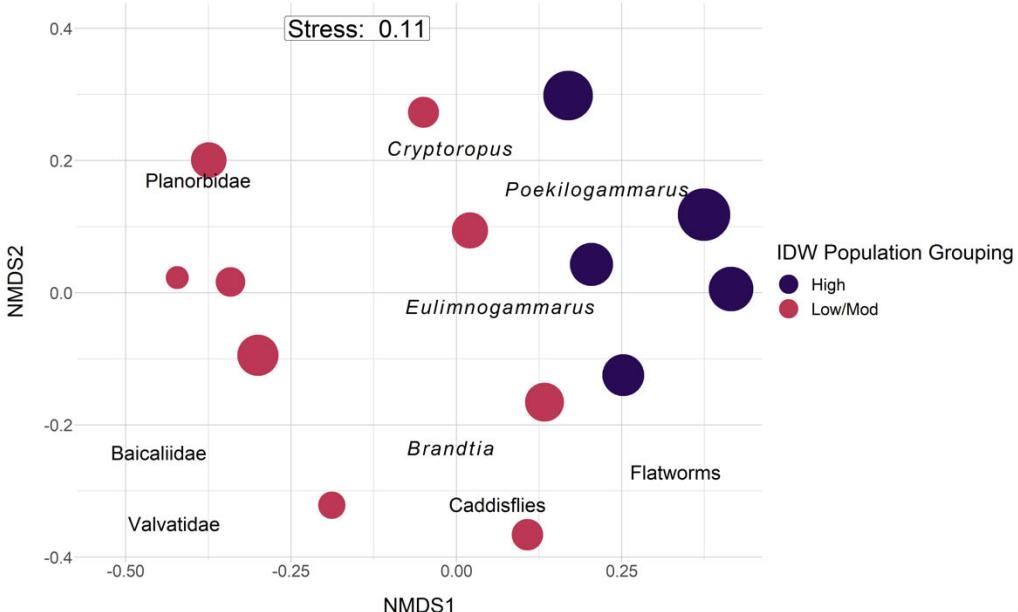
2

3 Figure 3: Linear models of total PPCP concentrations (A), macroinvertebrate $\delta^{15}\text{N}$ (B), total
4 phosphorus (C), chlorophyll a (D), nitrate (E), total microplastics (F), ammonium (G), and
5 microplastic density (H) regressed against log-transformed inverse distance weighted (IDW)
6 population. Total PPCP concentrations (A) and macroinvertebrate $\delta^{15}\text{N}$ values (B) produced
7 significant models. Total phosphorus (C), chlorophyll a (D), nitrate (E), total microplastics (F),
8 ammonium (G), and microplastic density (H) did not produce significant models.



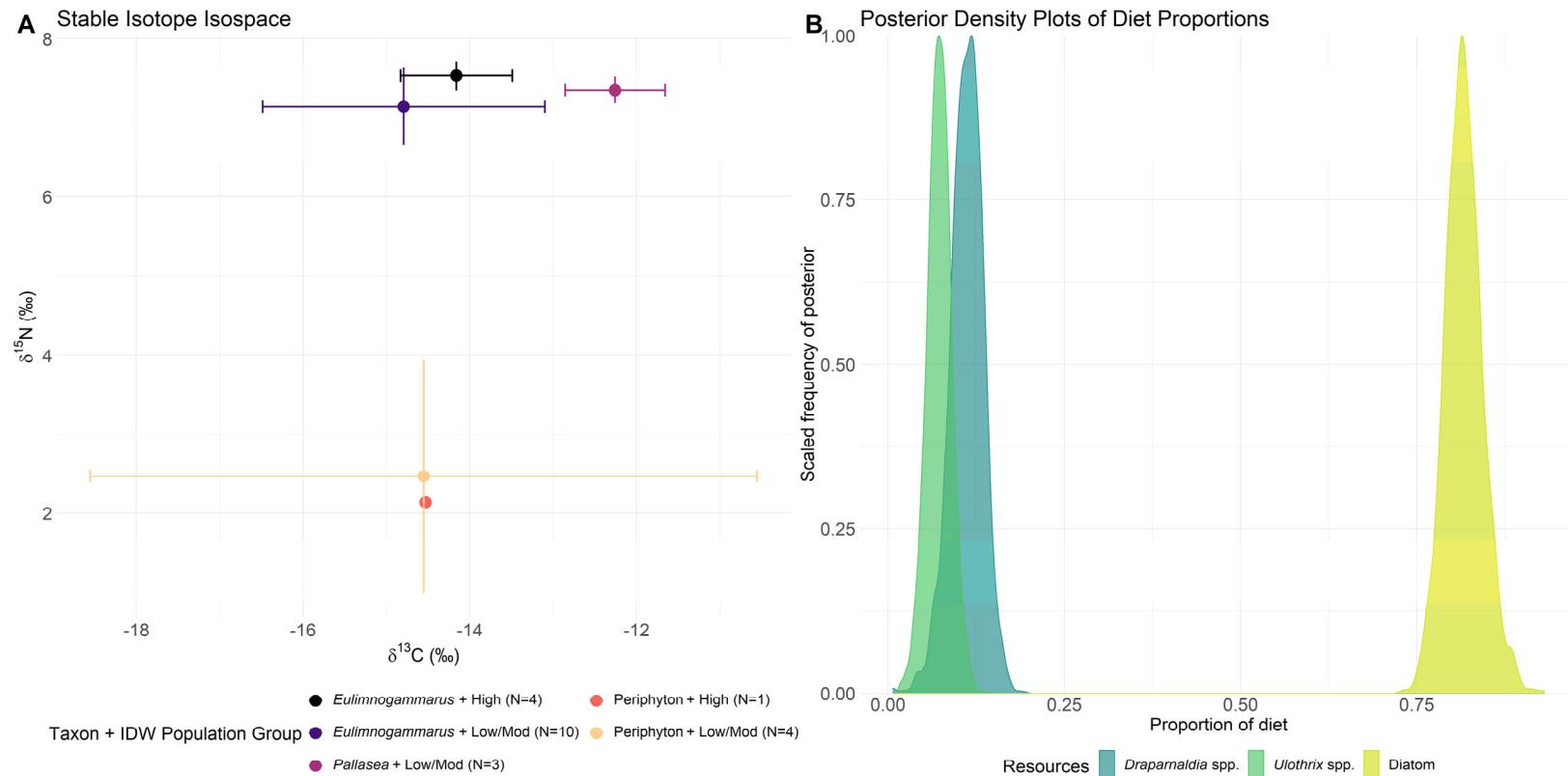
10 Figure 4: Periphyton abundance NMDS with Bray-Curtis dissimilarity. Points are sized by log-
 11 transformed IDW population and colored by grouped IDW population values. Taxonomic labels
 12 represent species scores, which are weighted averages of species contributions from site scores.
 13 For periphyton, PERMANOVA ($p = 0.001$) and post-hoc SIMPER results suggested sites with a
 14 higher IDW population value tended to be more associated with filamentous algal groupings and
 15 separate from sites with moderate and low IDW population values, which were more associated
 16 with diatom abundance.

17



18

19 Figure 5: Macroinvertebrate abundance NMDS with Bray-Curtis dissimilarity. Points are sized
 20 by log-transformed IDW population and colored by grouped IDW population values. Taxonomic
 21 labels represent species scores, which are weighted averages of species contributions from site
 22 scores. For macroinvertebrates, PERMANOVA ($p = 0.02$) and post-hoc SIMPER results
 23 suggested sites with a higher IDW population values tended to be associated with amphipod taxa
 24 (see Table S1), whereas sites with lower and moderate IDW population values were more
 25 associated with increased mollusk abundance (see Table S1).



28 Figure 6: Food web structure analyses as assessed by $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ stable isotopes (A) and fatty acids (B). Mean and standard deviation
 29 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values for littoral amphipods and periphyton are grouped by categorical IDW population (Table 3). In
 30 general, periphyton did not differ in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ signatures with increasing IDW population, whereas amphipods increased in $\delta^{15}\text{N}$
 31 signatures along a continuous gradient of increasing IDW population. *Pallasea* $\delta^{13}\text{C}$ signatures differed from *Eulimnogammarus* most
 32 likely because *Pallasea* tends to remain in the nearshore area, whereas *Eulimnogammarus* will regularly migrate to deeper zones
 33 (Takhteev & Didorenko, 2015). As stable isotopes only considered periphyton as a single, composite resource, the fatty acid analysis
 34 revealed finer trophic interactions, where amphipods likely disproportionately consumed fatty acids reflective of a diatom-based diet.

| Taxon | Number of sites | Branched | LCPUFA | MUFA | SAFA | SCPUFA |
|-----------------------------------|-----------------|----------|--------|-------|-------|--------|
| <i>Draparnaldia</i> spp. | 4 | 0.000 | 0.012 | 0.088 | 0.189 | 0.710 |
| <i>Eulimnogammarus cyaneus</i> | 2 | 0.002 | 0.259 | 0.309 | 0.248 | 0.182 |
| <i>Eulimnogammarus verrucosus</i> | 6 | 0.000 | 0.188 | 0.385 | 0.240 | 0.187 |
| <i>Eulimnogammarus vittatus</i> | 6 | 0.001 | 0.171 | 0.371 | 0.241 | 0.216 |
| <i>Pallasea cancellus</i> | 3 | 0.001 | 0.282 | 0.359 | 0.187 | 0.171 |
| Periphyton | 7 | 0.000 | 0.073 | 0.092 | 0.284 | 0.550 |
| Snail | 3 | 0.002 | 0.470 | 0.123 | 0.194 | 0.211 |

Table 3: Mean inter-site fatty acid proportion of each taxon and fatty acid grouping (as defined in Table S2).

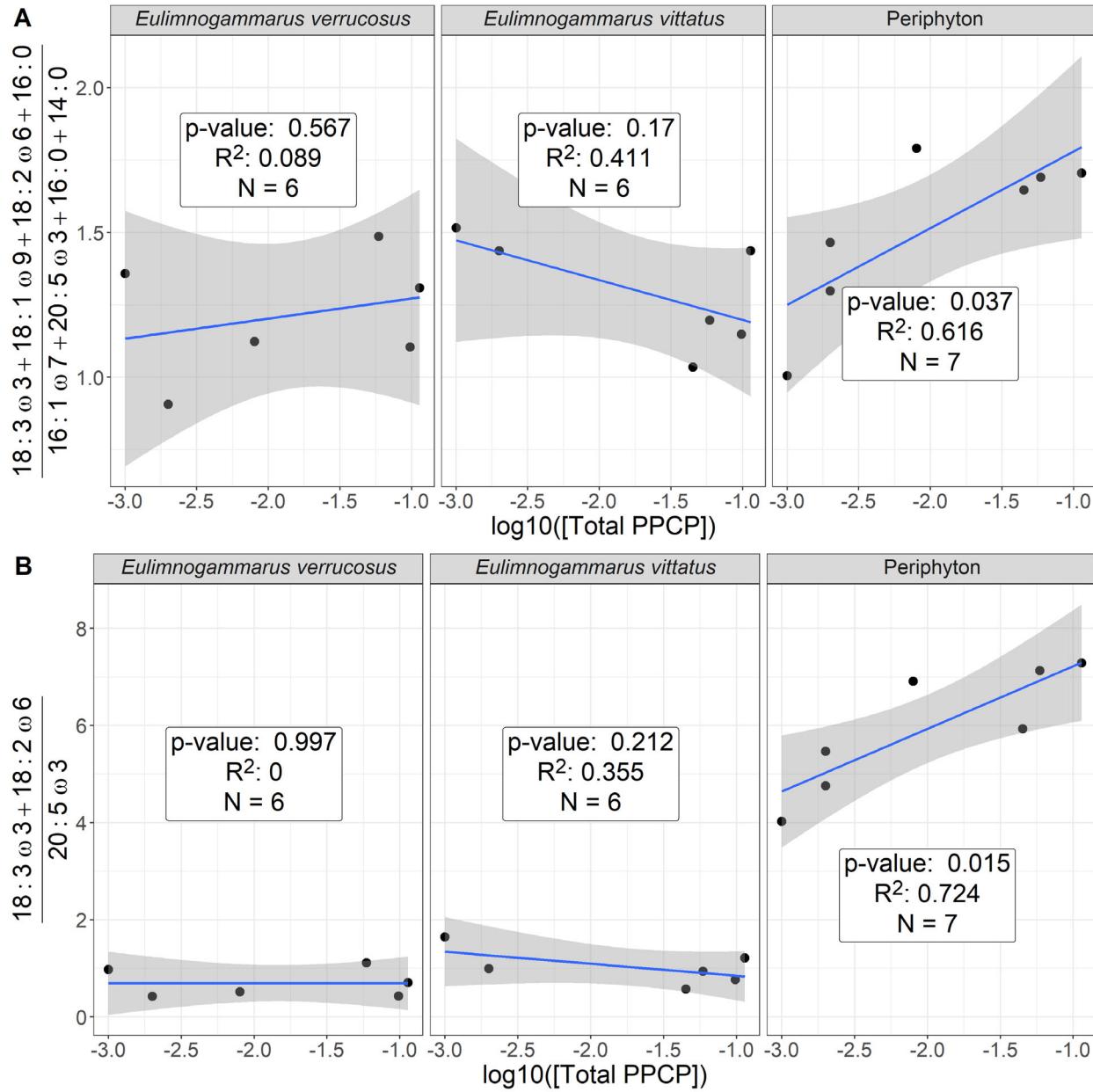


Figure 7: Ratio of filamentous:diatom-associated fatty acids (A) and essential fatty acids (B) across our PPCP gradient. Our first analysis (A) focused solely on green filamentous algal fatty acids (i.e., $18:3\omega 3$, $18:1\omega 9$, $18:2\omega 6$, and $16:0$ relative to diatom fatty acids (i.e., $20:5\omega 3$, $16:1\omega 7$, $16:0$, $14:0$) in relation to increasing PPCP concentrations. This first analysis suggested periphyton reflected an increasing green, filamentous signature relative to diatoms, which corroborates analyses showing community compositional shifts (Figure 4). While periphyton fatty acids changed significantly across our sewage gradient, macroinvertebrate signatures remained consistent. Our second analysis (B) focused solely on the essential fatty acids, which further highlights the trends observed in periphyton and macroinvertebrate grazers. in the

| Amphipoda | Mollusca | Other |
|--|-----------------|--------------|
| <i>Brandtia latissima intermida</i> (Dorogostaiskii 1930) | Acroloxiidae | Asellidae |
| <i>Brandtia latissima lata</i> (Dybowsky 1874) | Baicaliidae | Caddisflies |
| <i>Brandtia latissima latior</i> (Dybowsky 1874) | Benedictidate | Hirudinea |
| <i>Brandtia latissima latissima</i> (Gerstfeldt 1858) | Maackia | Planaria |
| <i>Brandtia parasitica parasitica</i> (Dybowsky 1874) | Planorbidae | |
| <i>Cryptoropuss inflatus</i> (Dybowsky 1874) | Valvatidae | |
| <i>Cryptoropuss pachytus</i> (Dybowsky 1874) | | |
| <i>Cryptoropuss rugosus</i> (Dybowsky 1874) | | |
| <i>Eulimnogammarus capreolus</i> (Dybowsky 1874) | | |
| <i>Eulimnogammarus cruentes</i> (Dorogostaiskii 1930) | | |
| <i>Eulimnogammarus cyaneus</i> (Dybowsky 1874) | | |
| <i>Eulimnogammarus grandimanus</i> (Bazikalova 1945) | | |
| <i>Eulimnogammarus maacki</i> (Gerstfeldt 1858) | | |
| <i>Eulimnogammarus maritui</i> (Bazikalova 1945) | | |
| <i>Eulimnogammarus verucossus</i> (Gerstfeldt 1858) | | |
| <i>Eulimnogammarus viridis viridis</i> (Dybowsky 1874) | | |
| <i>Eulimnogammarus vittatus</i> (Dybowsky 1874) | | |
| <i>Pallasea brandtia brandtia</i> (Dybowsky 1874) | | |
| <i>Pallasea brandtii tenera</i> (Sovinskii 1930) | | |
| <i>Pallasea cancelloides</i> (Gerstfeldt 1858) | | |
| <i>Pallasea cancellus</i> (Pallas 1776) | | |
| <i>Pallasea viridis</i> (Garjajev 1901) | | |
| <i>Poekilogammarus crassimus</i> (Sovinskii 1915) | | |
| <i>Poekilogammarus ephippiatus</i> (Dybowsky 1874) | | |
| <i>Poekilogammarus megonychus perpolitus</i> (Takhteev 2002) | | |
| <i>Poekilogammarus pictus</i> (Dybowsky 1874) | | |

Table S1: Macroinvertebrate taxonomic groupings for abundance estimates. Amphipod taxa were defined as in Takhteev & Didorenko, 2015; mollusk taxa were defined as in Sitnikova, 2012.

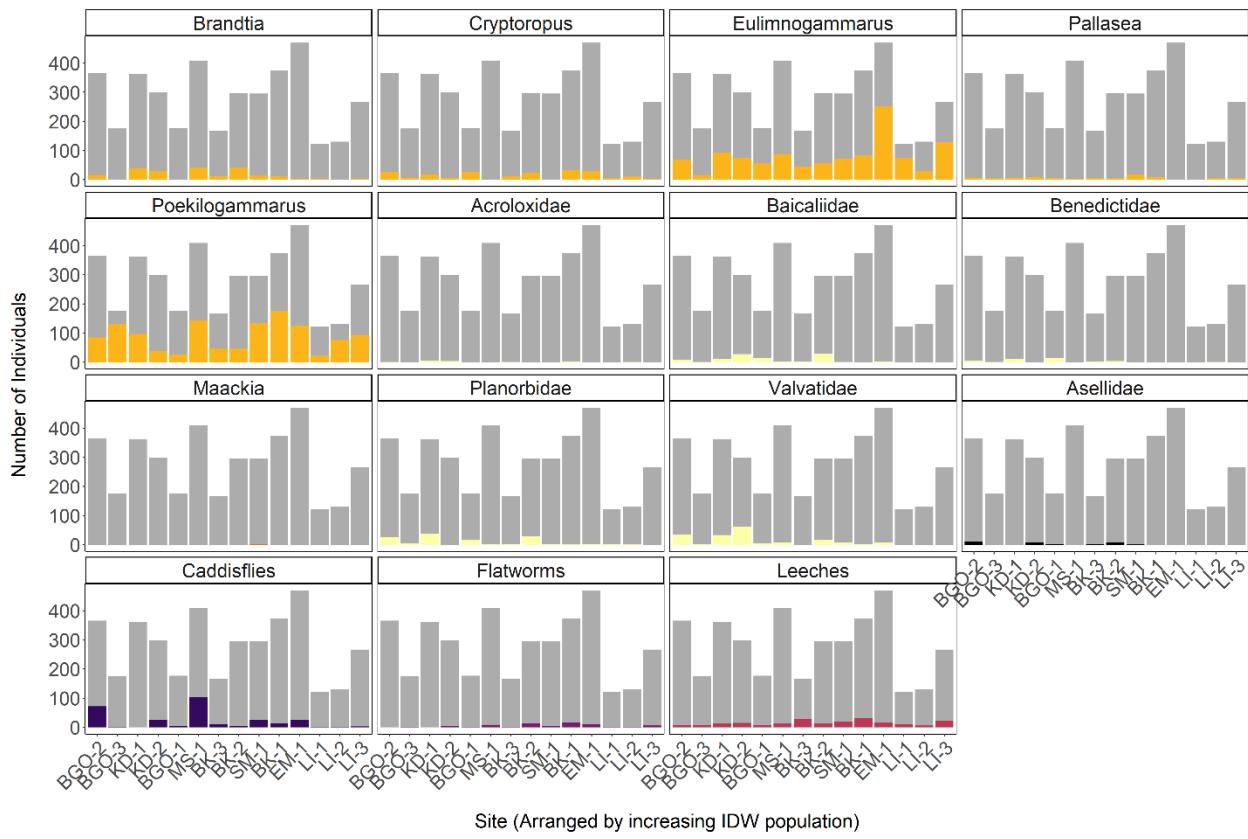


Figure S1: Abundance of main benthic macroinvertebrate taxonomic groups across sampling transect. Sites are ordered left-to-right by increasing inverse distance weighted population. Colored-bars represent the number of individuals counted of a particular taxon, whereas grey bars represent the total number of macroinvertebrates counted at a sampling site. Each distinct color represents a broad taxon ('Orange' = Amphipoda; 'Yellow' = Molluska; 'Black' = Aseillidae; 'Dark Purple' = Caddisflies; 'Magenta' = Flatworms; 'Dark Pink' = Leeches).

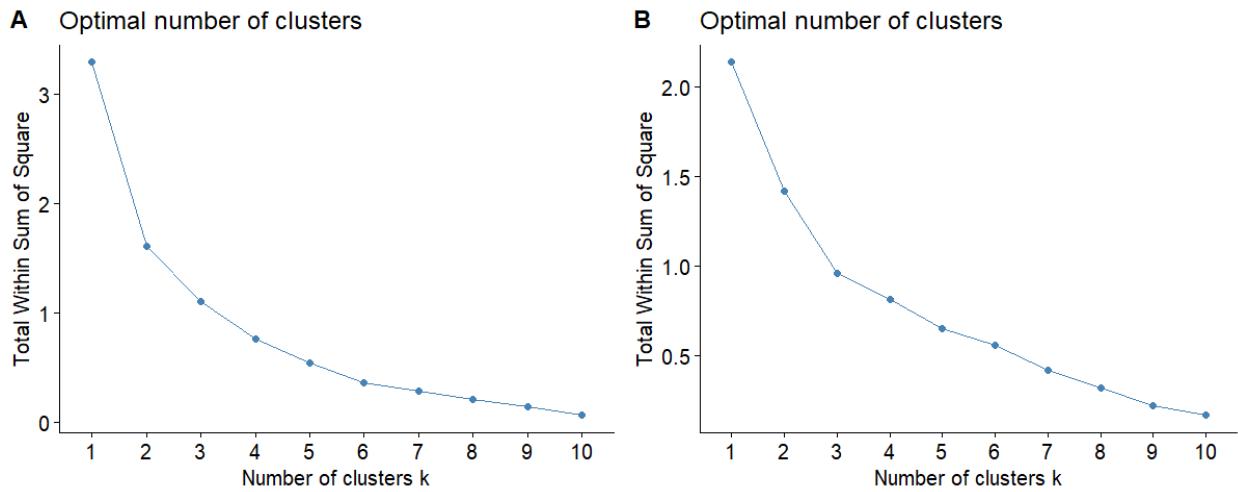


Figure S2: With-group-sum-of-squares (wss) for increasing number of k-mediod clusters for periphyton (A) and invertebrate (B) community data. In the case of periphyton data, wss decreases most markedly with three clusters, whereas invertebrate community abundance is best described by potential two or three clusters.

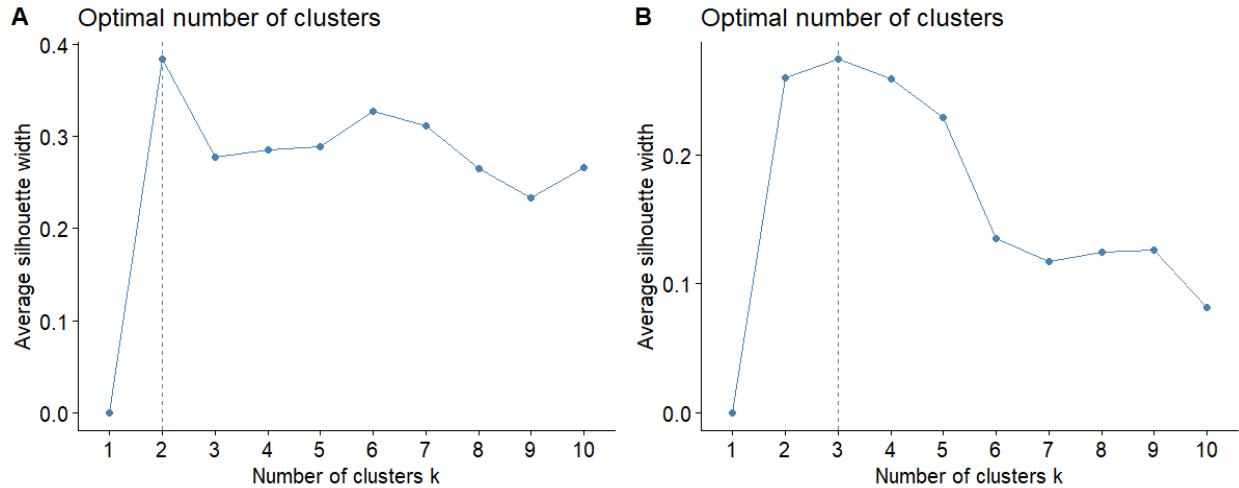


Figure S3: Average silhouette width for increasing number of k-mediod clusters for periphyton (A) and invertebrate (B) community data. In the case of periphyton data, average silhouette width decreases most markedly with three clusters, whereas invertebrate community abundance is best described by two or three clusters as the average silhouette width for both two and three clusters was highest before beginning to decrease.

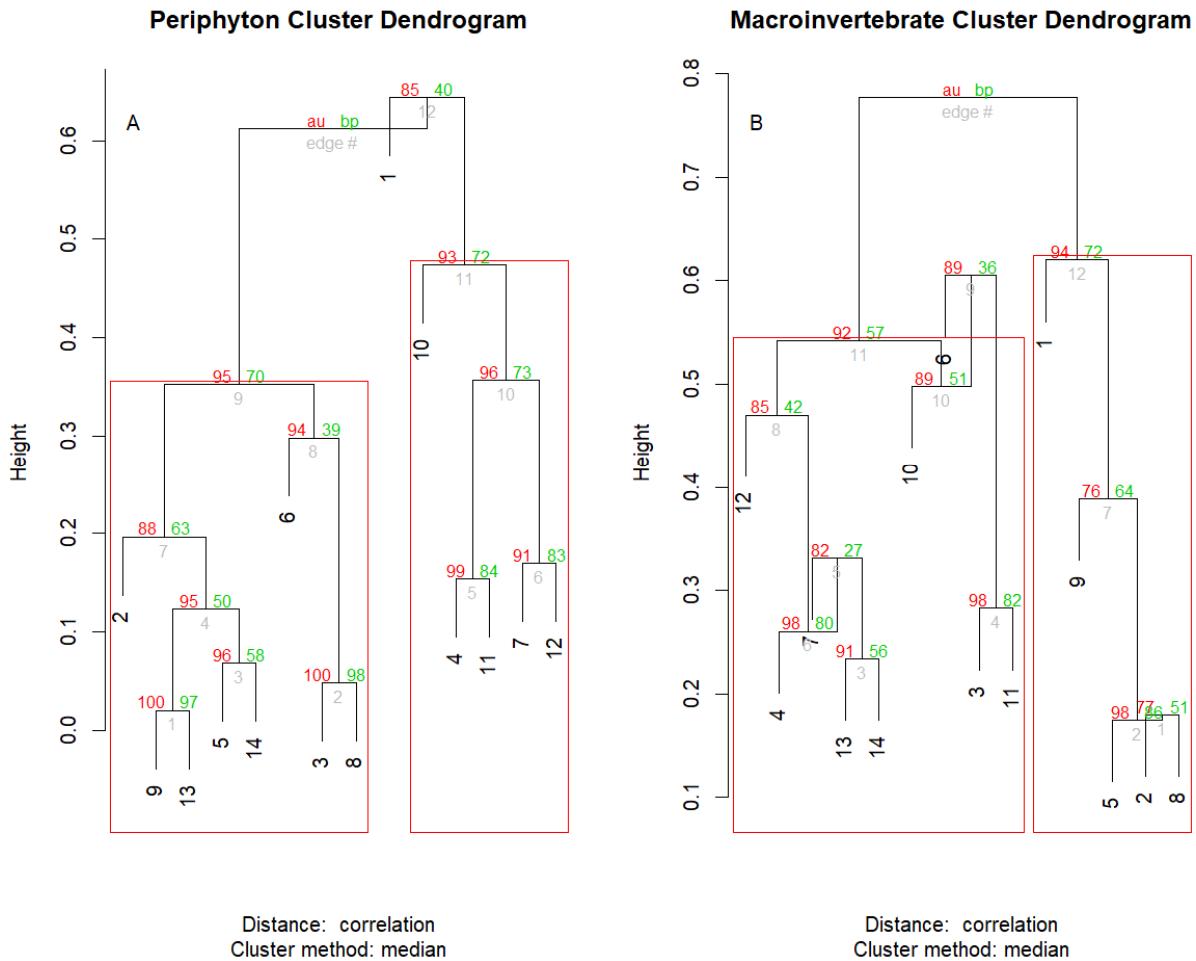


Figure S4: Weighted Pair-Group Centroid Clustering (WPGMC) for periphyton (A) and macroinvertebrate (B) community compositions. Approximately unbiased (au) p-values are computed by multiscale bootstrap resampling, and displayed in red on the left side of each node. Bootstrapped probabilities (bp) are displayed in green on the right side of each node. Unlike k-medoids, WPGMC uses a hierarchical approach to assign clusters, which are bootstrapped in order to generate a probability of group membership. This technique suggested that both periphyton and macroinvertebrates could be grouped in two clusters. Grouping macroinvertebrates into three clusters was possible; however, three clusters resulted in 8 of the 14 sampling locations being assigned to a group. In contrast, two groups enabled 13 of the 14 sampling locations to be assigned to a cluster.

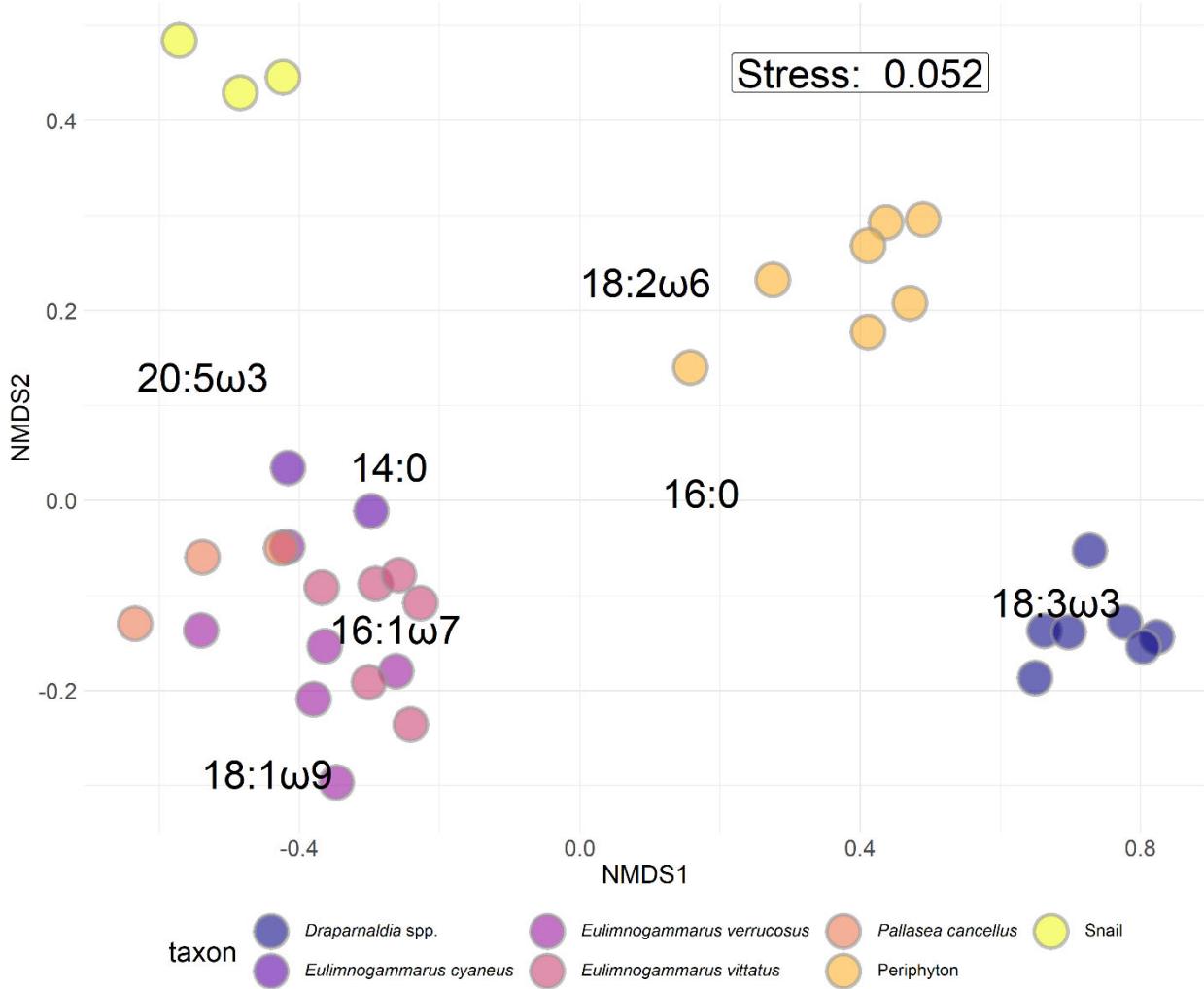


Figure S5: NMDS with Bray-Curtis dissimilarity of proportional fatty acid compositions for each macroinvertebrate and primary producer collected. *Eulimnogammarus* and *Pallasea* are endemic amphipod genera. *Draparnaldia* spp. are endemic filamentous algae that are large and form very dense mats easily collected where it occurs. *Draparnaldia* spp. occurred in large, visible colonies, allowing us to sample and analyze just the *Draparnaldia* spp. fatty acids. Because *Draparnaldia* spp. fatty acids were dominated by 18:3 ω 3 more so than periphyton, they formed their own cluster. Snails were not identified to species prior to fatty acid analysis. Interspecific variation in fatty acid composition tended to be larger than intraspecific variation, implying that fatty acid signatures were largely species-specific and not environmentally driven.

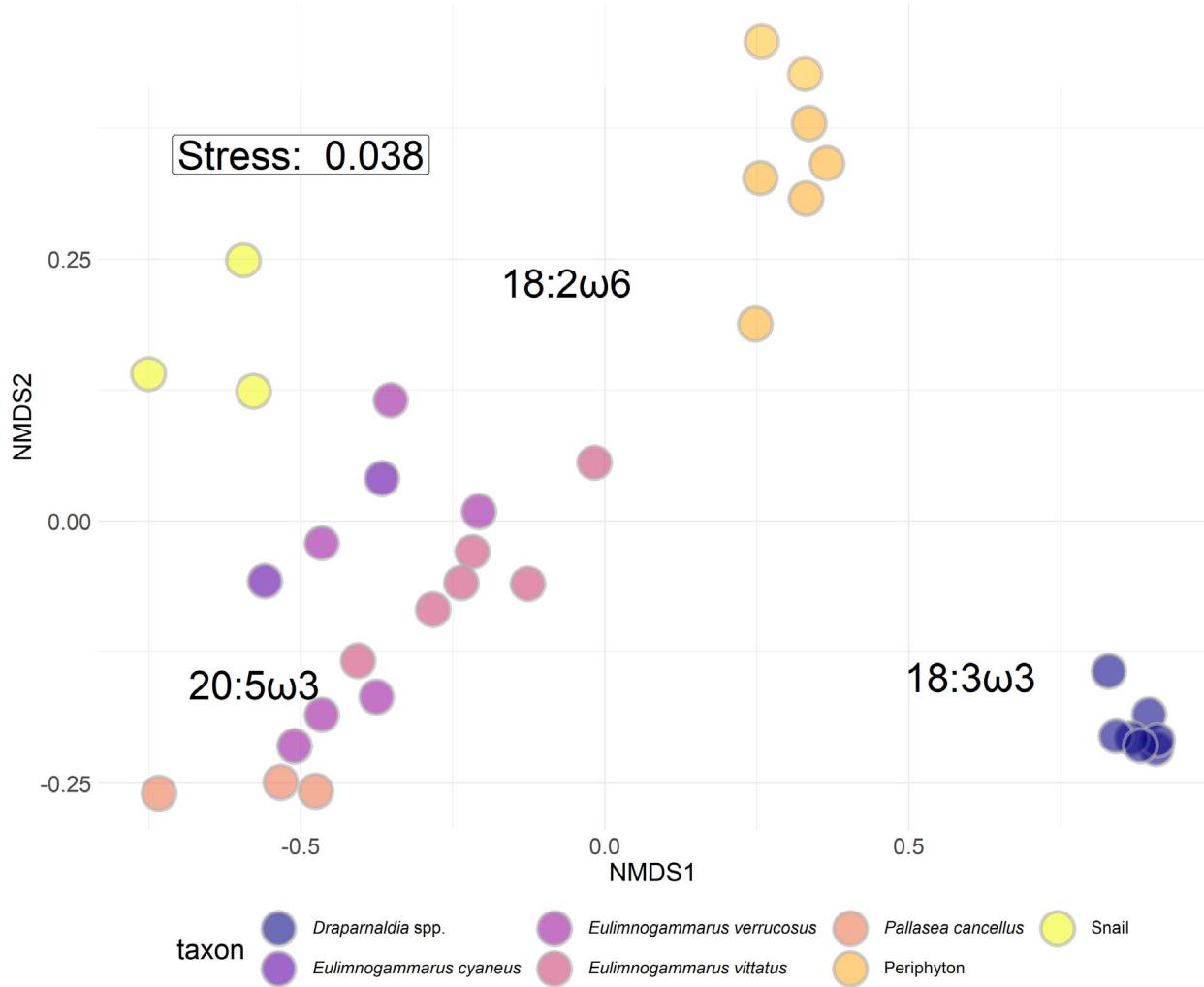


Figure S6: NMDS with Bray-Curtis dissimilarity of proportional biologically essential fatty acid compositions for each macroinvertebrate and primary producer collected. *Eulimnogammarus* and *Pallasea* are endemic amphipod genera. *Draparnaldia* spp. are endemic filamentous algae that are large and form very dense mats easily collected where it occurs. *Draparnaldia* spp. occurred in large, visible colonies, allowing us to sample and analyze just the *Draparnaldia* spp. fatty acids. Because *Draparnaldia* spp. fatty acids were dominated by 18:3 ω 3 more so than periphyton, they formed their own cluster. Snails were not identified to species prior to fatty acid analysis. Interspecific variation in fatty acid composition tended to be larger than intraspecific variation, implying that fatty acid signatures were largely species-specific and not environmentally driven.

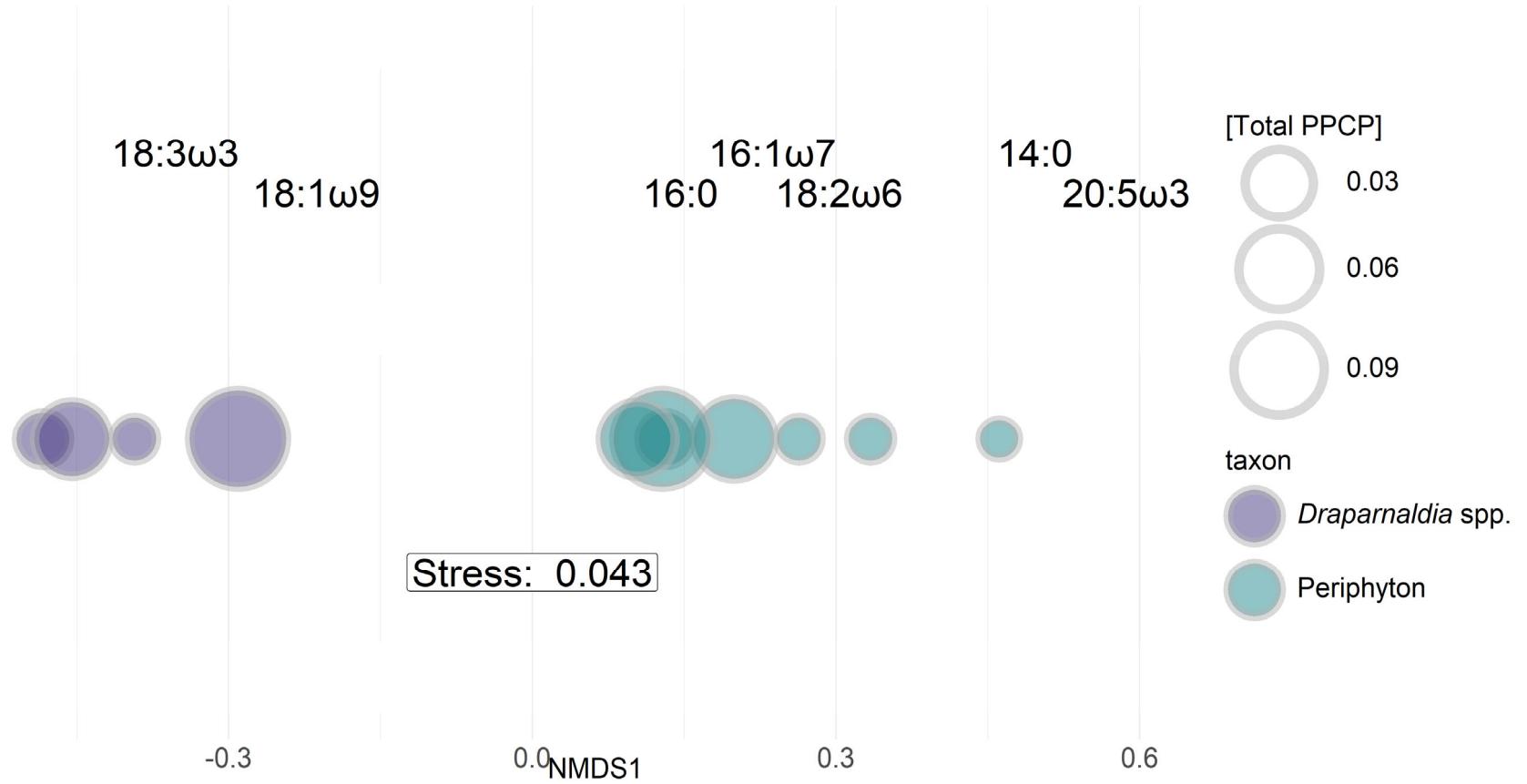
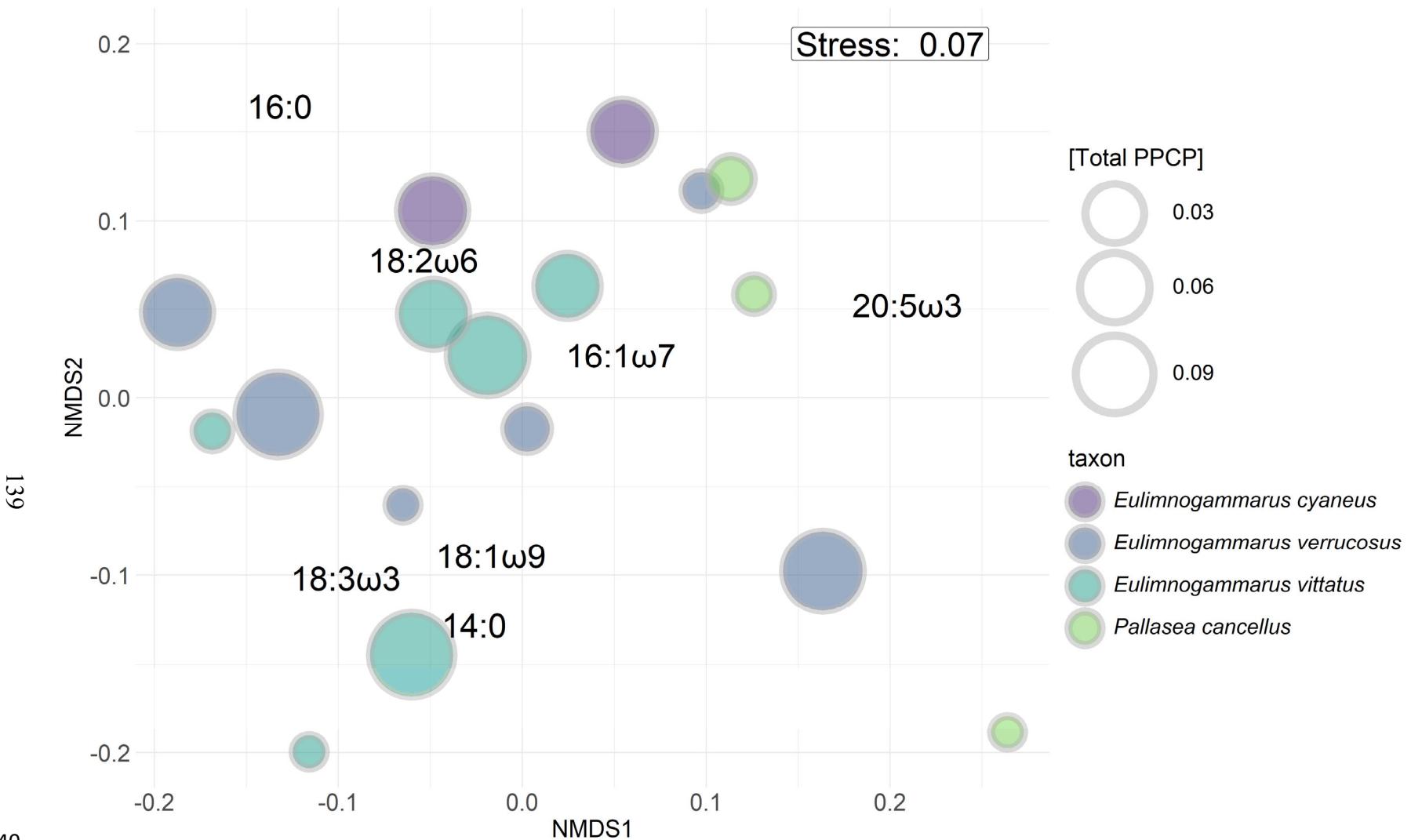


Figure S7: One-dimensional NMDS with Bray-Curtis similarity of seven fatty acids of interest for primary producers. Fatty acid scores are placed above shapes. Shapes are sized by total PPCP concentration. Periphyton (blue-green) tend to increase in size from right-to-left, suggesting that periphyton tend to include more 18:3 ω 3 and 18:1 ω 9 (indicators of green algal taxa) with an increasing sewage signal. In contrast, *Draparnaldia* spp. (purple) fatty acids tend to remain consistent across the sewage gradient.

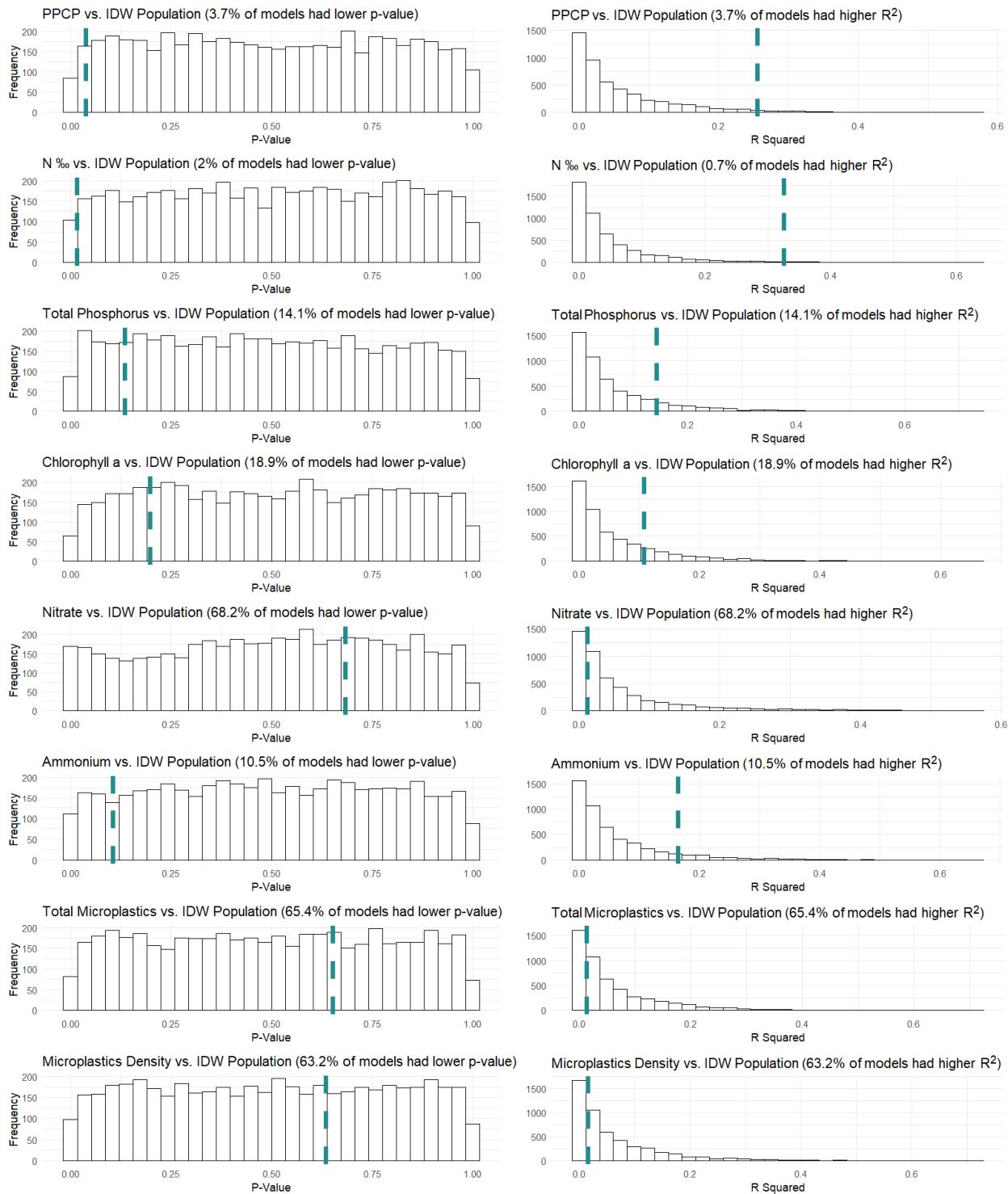


40

41 Figure S8: NMDS with Bray-Curtis similarity of seven targeted fatty acids of interest for primary producers. Points are sized by total

42 PPCP concentration. Visually, there appears to be no distinct separation among or within taxa unlike patterns observed with

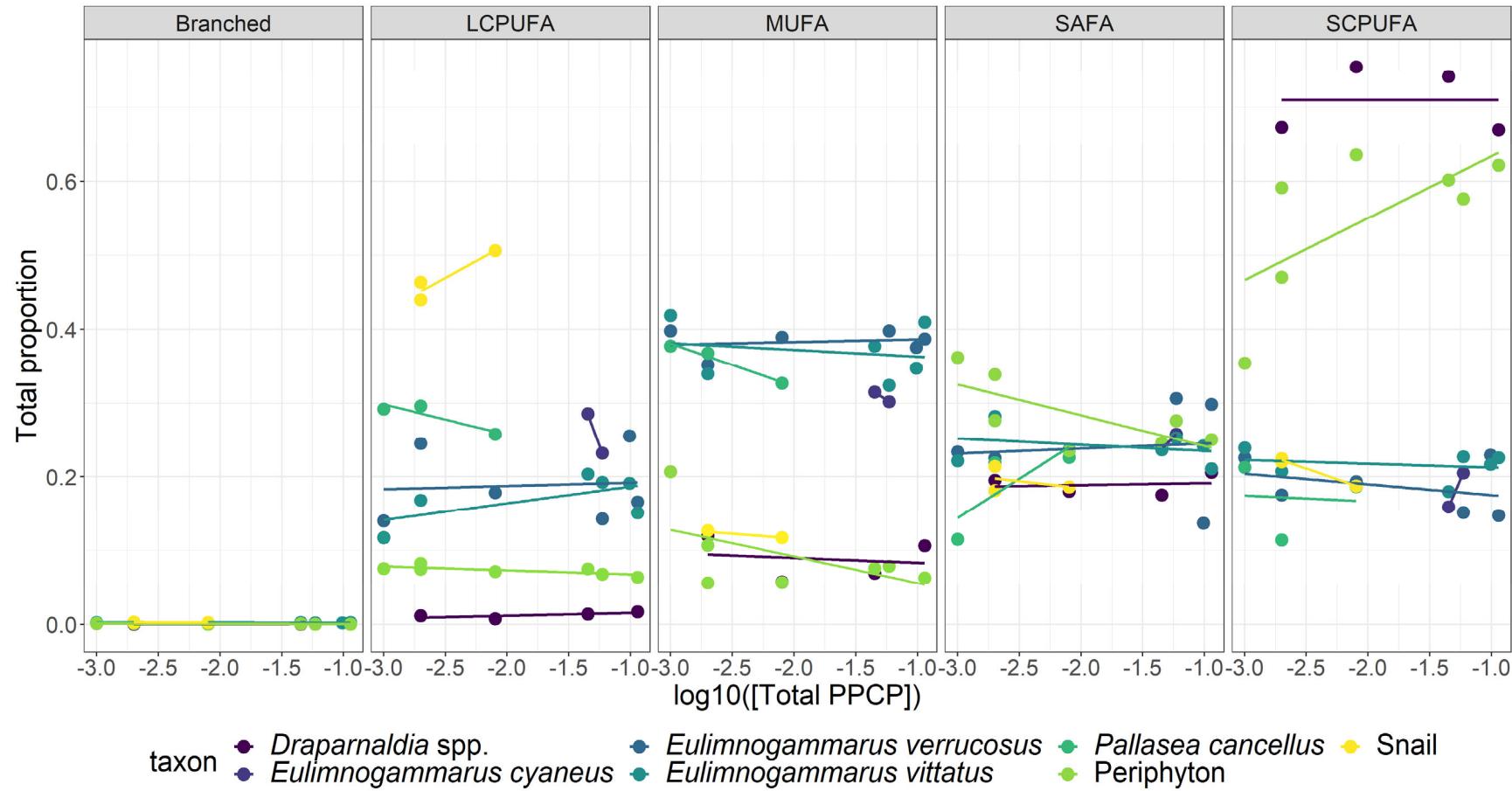
43 periphyton (Figure S7).



44

45 Figure S9: Distributions of p- and R^2 values for sewage indicator values in response to IDW
46 population. Models were generated from 5,000 data permutations. Histograms represent p- and
47 R^2 values estimated from linear models fit with permuted sewage indicator data. The vertical
48 dashed line in each plot represents the p- and R^2 value obtained from the linear model fit with
49 non-permuted data. The percent of p- and R^2 values occurring respectively below and above

50 values estimated from the non-permuted values are listed in the title of each plot. In the case
51 when a model fit is non-random, the dashed green line should be in the lower 5% tail end for p-
52 values and in the upper 5% for R^2 values.



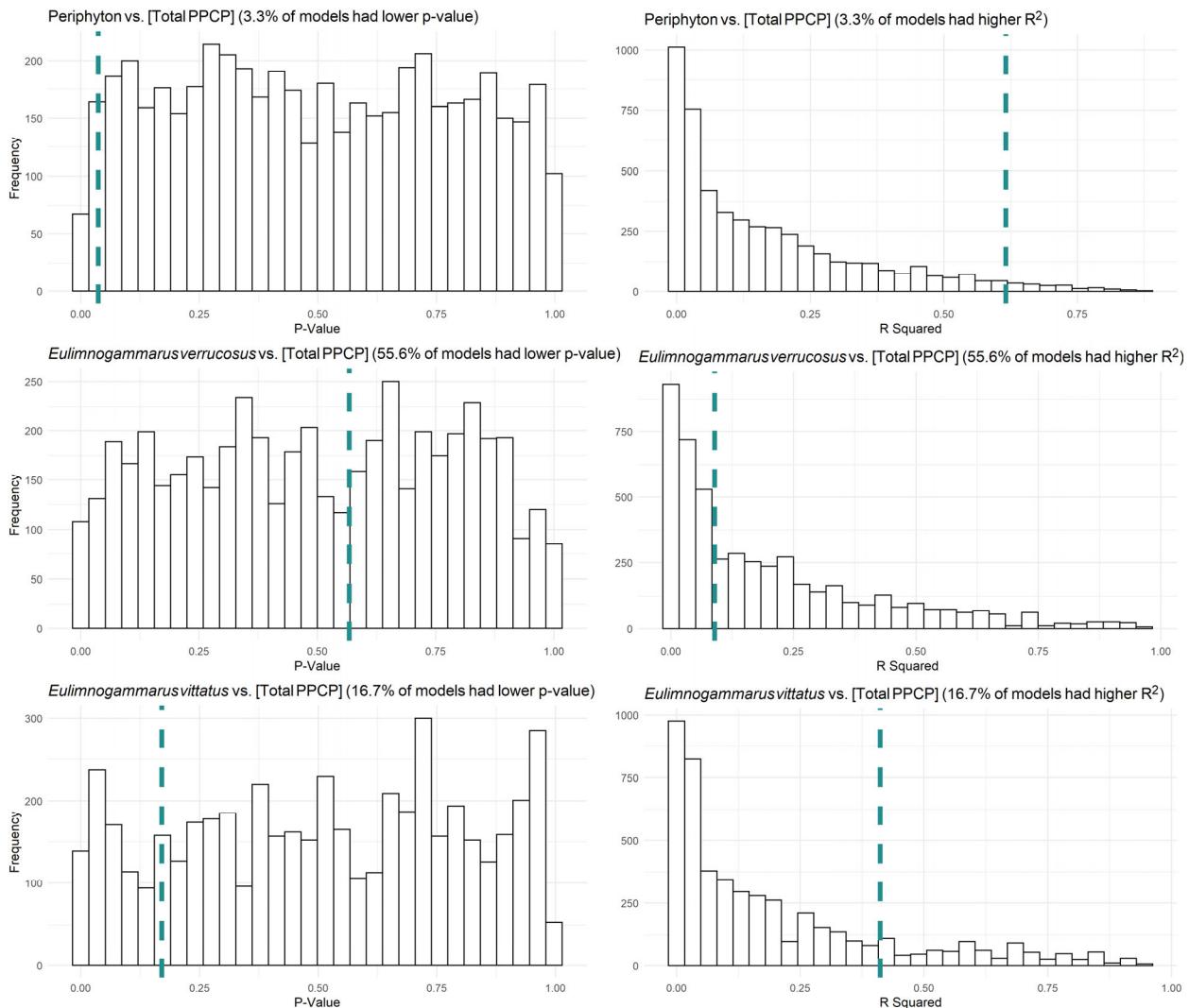


Figure S11: Distributions of p- and R² values for filamentous:diatom fatty acid ratios in response to total PPCP concentrations for permuted data. Models were generated from 5,000 data permutations. Histograms represent p- and R² values estimated from linear models fit with permuted sewage indicator data. The vertical dashed line in each plot represents the p- and R² value obtained from the linear model fit with non-permuted data. The percent of p- and R² values occurring respectively below and above values estimated from the non-permuted values are listed in the title of each plot. In the case when a model fit is non-random, the dashed green line should be in the lower 5% tail end for p-values and in the upper 5% for R² values.

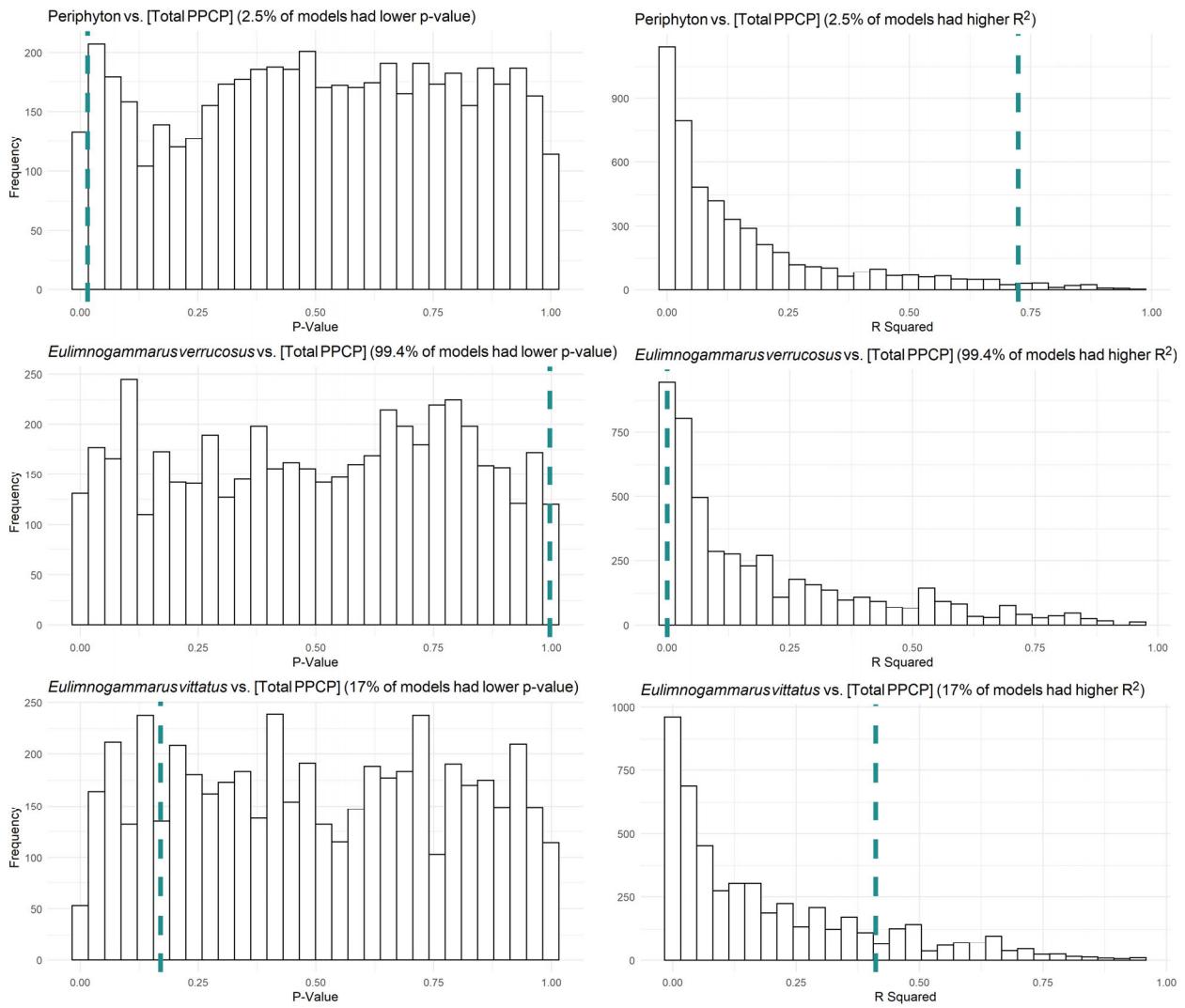


Figure S12: Distributions of p- and R² values for filamentous:diatom essential fatty acid ratios in response to total PPCP concentrations for permuted data. Models were generated from 5,000 data permutations. Histograms represent p- and R² values estimated from linear models fit with permuted sewage indicator data. The vertical dashed line in each plot represents the p- and R² value obtained from the linear model fit with non-permuted data. The percent of p- and R² values occurring respectively below and above values estimated from the non-permuted values are listed in the title of each plot. In the case when a model fit is non-random, the dashed green line should be in the lower 5% tail end for p-values and in the upper 5% for R² values.

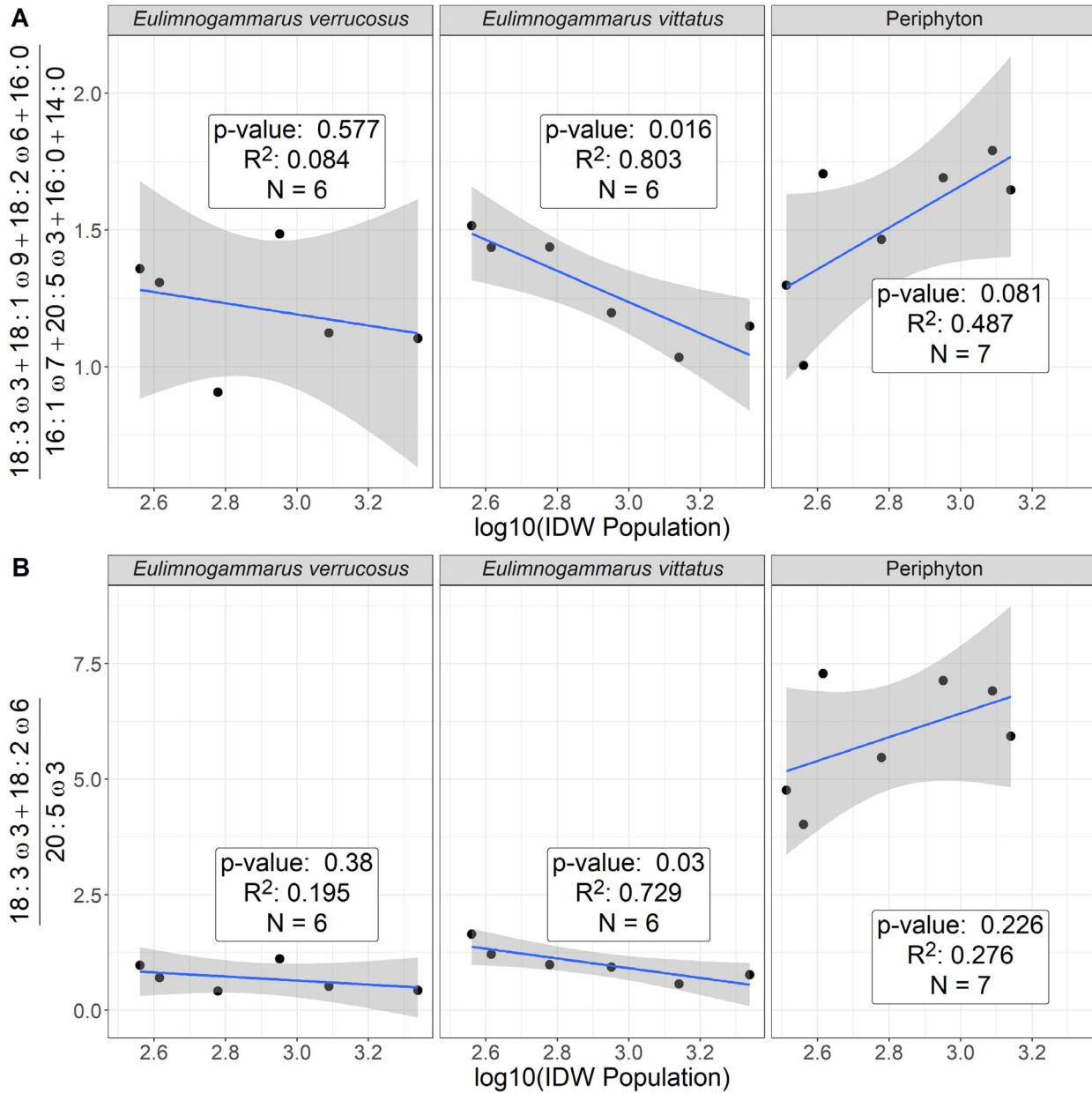


Figure S13: Ratio of filamentous:diatom-associated fatty acids (A) and essential fatty acids (B) across our IDW Population gradient. Our first analysis (A) focused solely on green filamentous algal fatty acids (i.e., $18:3\omega 3$, $18:1\omega 9$, $18:2\omega 6$, and $16:0$ relative to diatom fatty acids (i.e., $20:5\omega 3$, $16:1\omega 7$, $16:0$, $14:0$) in relation to increasing PPCP concentrations. This first analysis suggested periphyton, to some degree, tended to reflect an increasing green, filamentous signature relative to diatoms, which corroborates analyses showing community compositional shifts (Figure 4). Macroinvertebrate signatures generally remained consistent, although *E. vittatus*'s signatures generally reflected an increased diatom signature over the gradient. Our second analysis (B) focused solely on the essential fatty acids. These same general patterns were also observed when using PPCP concentrations in place of IDW population (Figure 7).

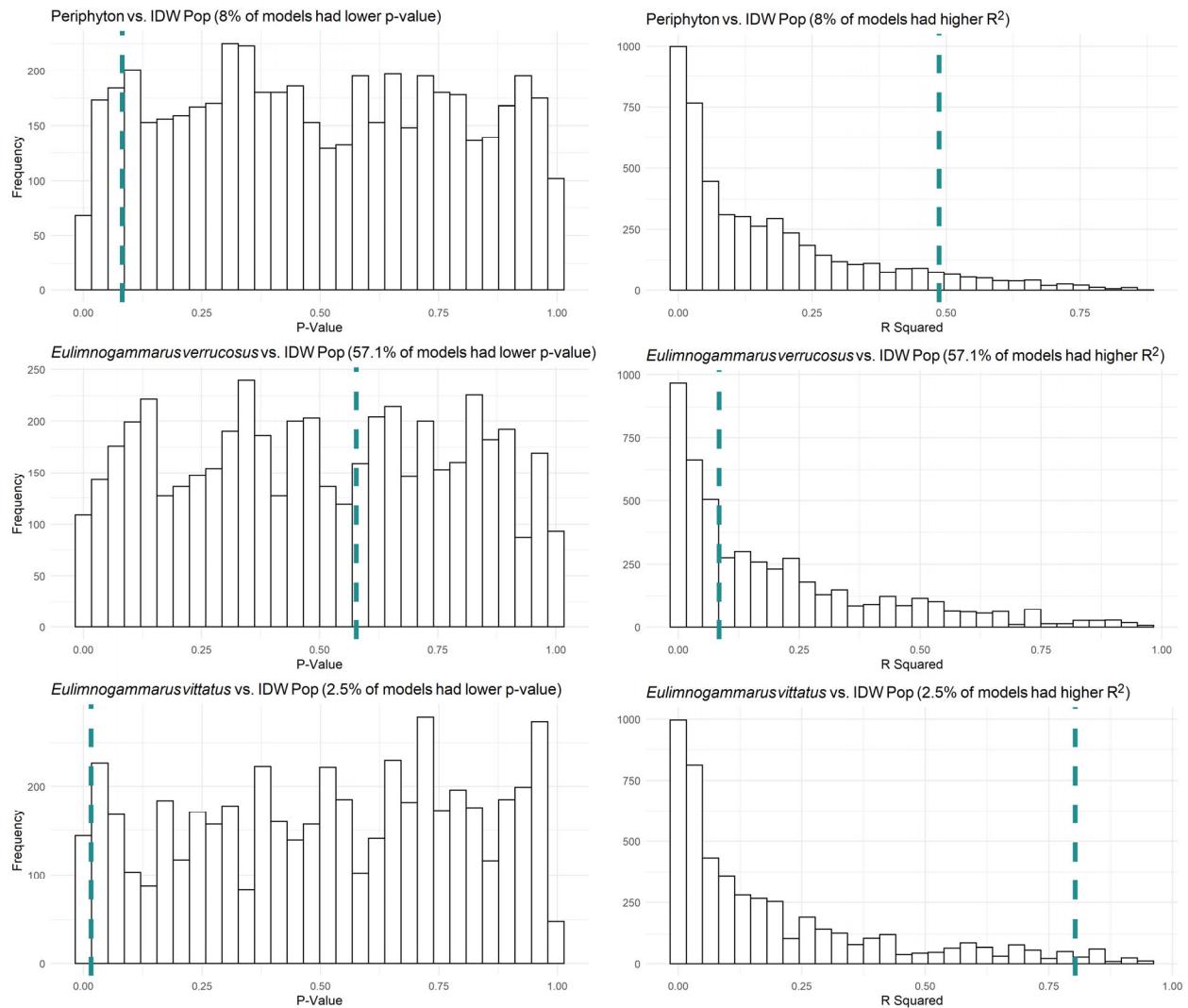


Figure S14: Distributions of p- and R^2 values for filamentous:diatom fatty acid ratios in response to IDW population for permuted data. Models were generated from 5,000 data permutations. Histograms represent p- and R^2 values estimated from linear models fit with permuted sewage indicator data. The vertical dashed line in each plot represents the p- and R^2 value obtained from the linear model fit with non-permuted data. The percent of p- and R^2 values occurring respectively below and above values estimated from the non-permuted values are listed in the title of each plot. In the case when a model fit is non-random, the dashed green line should be in the lower 5% tail end for p-values and in the upper 5% for R^2 values.

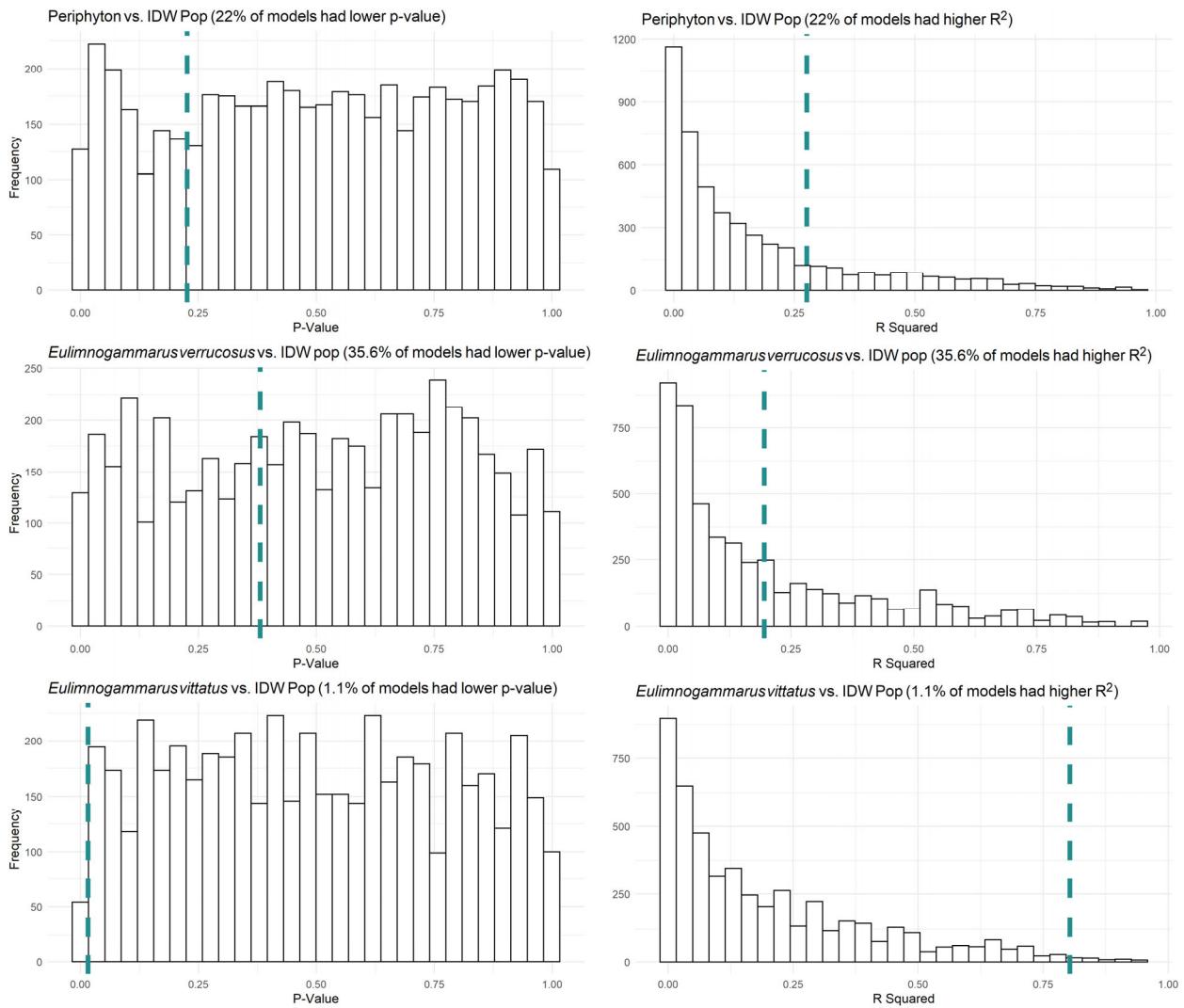


Figure S15: Distributions of p- and R² values for filamentous:diatom essential fatty acid ratios in response to IDW population for permuted data. Models were generated from 5,000 data permutations. Histograms represent p- and R² values estimated from linear models fit with permuted sewage indicator data. The vertical dashed line in each plot represents the p- and R² value obtained from the linear model fit with non-permuted data. The percent of p- and R² values occurring respectively below and above values estimated from the non-permuted values are listed in the title of each plot. In the case when a model fit is non-random, the dashed green line should be in the lower 5% tail end for p-values and in the upper 5% for R² values.

| Fatty Acid Group | Fatty acids considered |
|------------------|---|
| Branched | a-15:0, i-15:0, a-17:0, i-17:0 |
| SAFA | 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0 |
| MUFA | 14:1 ω 5, 15:1 ω 7, 17:1n7, 16:1 ω 5, 16:1 ω 6, 16:1 ω 7, 16:1 ω 8, 16:1 ω 9, 18:1 ω 7, 18:1 ω 9, 20:1 ω 7, 20:1 ω 9, 22:1 ω 7, 22:1 ω 9 |
| SCPUFA | 16:2 ω 4, 16:2 ω 6, 16:2 ω 7, 16:3 ω 3, 16:3 ω 4, 16:3 ω 6, 16:4 ω 1, 16:4 ω 3, 18:2 ω 6, 18:2 ω 6t, 18:3 ω 3, 18:3 ω 6, 18:4 ω 3, 18:4 ω 4, 18:5 ω 3 |
| LCPUFA | 20:2 ω 5(11), 20:2 ω 5(13), 20:2 ω 6, 20:3 ω 3, 20:3 ω 6, 20:4 ω 3, 20:4 ω 6, 20:5 ω 3, 22:2 ω 6, 22:3 ω 3, 22:4 ω 3, 22:4 ω 6, 22:5 ω 3, 22:5 ω 6, 22:6 ω 3 |

Table S2: Fatty acid groupings used in this analysis

CHAPTER THREE

EFFECTS OF SPATIO-TEMPORALLY VARYING LAKESIDE HUMAN DISTURBANCE ON NEARSHORE PERIPHYTON COMMUNITIES IN FLATHEAD LAKE (MONTANA, UNITED STATES)

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Keywords: PPCPs, fatty acids, stoichiometry, food webs

3.1 Abstract

Nutrients from lakeside developments can reshape aquatic ecosystems. Algal communities, especially in oligotrophic lakes, can rapidly remove nutrients from the water column, causing increased biomass and altered resources for grazers. However, associating times and locations of nutrient loading with biological responses can be complicated, as spatio-temporal heterogeneities in nutrient loading and biological succession can obfuscate patterns. To evaluate how variation in nutrient addition may be associated with algal succession and nutritional quality, we sampled 15 littoral sites in Flathead Lake (Montana U.S.) from June through September 2017, when tourism dynamics may create variation in nutrient influx. As indicators of anthropogenic nutrient additions, pharmaceutical and personal care product (PPCPs) concentrations were measured fortnightly. Periphyton community composition, stoichiometry, and fatty acids were assessed monthly. PPCPs, including caffeine, nicotine, and warfarin, were detected, and PPCP concentrations as well as periphyton branched- and odd-chain fatty acid abundance varied with seasonal tourist activity. Sewage indicators near centralized sewage treatment were more temporally consistent throughout the sampling regime in comparison to sites near decentralized treatment. These same locations near centralized treatment were more associated with increased chlorophyte abundance, lower periphyton C:N:P ratios, yet lower quality essential fatty acids. Conversely, locations near decentralized treatment were more associated with temporally transient periphyton community composition, higher periphyton C:N:P ratios, yet higher quality essential fatty acids. Overall, our results suggest that sewage treatment techniques and seasonal human activity can create hot spots and hot moments for nutrient addition, thereby reshaping algal community compositions and available nutrition to the food web.

3.2 Introduction

Pollutants in wastewater released from lakeside developments are a common human disturbance that can alter biological communities and reshape food webs. In particular, nutrients, such as nitrogen and phosphorus, in treated and untreated wastewater can increase biomass and eventually lead to system-wide changes in algal community composition and production (Edmondson 1970) with potential consequences for higher trophic levels (Edmondson 1994; Hampton et al. 2006; Galloway and Winder 2015). Although nutrients in sewage have been shown to drastically alter aquatic ecosystems, explicitly linking increased nutrient concentrations and biological responses to sewage can be challenging, as nutrients can originate from disparate anthropogenic and natural environmental sources. For example, agriculture (Powers et al. 2016), melting permafrost (Turetsky et al. 2000), wildfires (Gould et al. 2016), and even changing terrestrial plant communities (Goldman 1961; Moran et al. 2012) can all contribute nutrients to aquatic systems, potentially obfuscating sewage signals. In addition, biological processes can further confound sewage signals. Benthic primary producers, especially those in oligotrophic systems (Rosenberger et al. 2008; Hampton et al. 2011; Oleksy et al. 2020; Atkins et al. 2021), can remove nutrients quickly from the water column (e.g., hours; Hadwen and Bunn 2005), such that deviations in nutrient concentrations may not be detectable in water samples.

Because nutrients come from numerous non-sewage sources, indicators consistently associated with wastewater pollution, such as pharmaceuticals and personal care products (PPCPs) (Rosi-Marshall and Royer 2012; Meyer et al. 2019) as well as bacterial biomarkers (Seguel et al. 2001) have garnered attention for their usefulness as sewage indicators. PPCPs, such as caffeine, acetaminophen, and sulfamethoxazole, have been used to identify spatially and temporally heterogeneous sewage pollution in surface (Bendz et al. 2005) and subsurface (Yang et al. 2016) aquatic systems. Because biota seem not to actively uptake most PPCPs (Bai and

Acharya 2017), their concentrations within the water column tend to be directly proportionate to increasing human population and inversely proportionate with distance from population centers (Meyer et al.; Bendz et al. 2005). Microbial and bacterial biomarkers, such as branched- and odd-chain fatty acids, also can be useful for defining areas and times of sewage loading (Seguel et al. 2001). In contrast to PPCPs, bacteria-associated fatty acids can be less specific sewage indicators, as bacteria can also originate from numerous non-sewage sources. Thus, tracking the changes in relative bacterial abundance with bacterial biomarkers can be a more reliable indicator of spatial and temporal patterns in sewage loading for both freshwater (Templar et al. 2016; Booncharoen et al. 2018) and marine systems (Abaya et al. 2018; Booncharoen et al. 2018). Together, the combined use of PPCP and bacterial biomarker measurements may provide more robust, multi-proxy information to infer the spatial extent and timing of sewage pollution into an ecosystem.

The effects of wastewater pollution are often first observed among littoral benthic communities, where increased nutrients can alter algal community succession by causing deviations in community composition and abundance (Meyer et al.; Rosenberger et al. 2008; Hampton et al. 2011; Timoshkin et al. 2016; Bondarenko et al. 2021; Atkins et al. 2021). Similar to patterns observed with phytoplankton succession (Sommer et al. 1986, 2012), benthic communities likewise tend to increase in chlorophyte abundance over the course of a growing season and then eventually decrease in abundance relative to diatoms and cyanobacteria (Hoagland et al. 1982; McCormick and Stevenson 1991; Stevenson et al. 1996; Azim et al. 2005). Mechanistically, this transition occurs when nitrogen and phosphorus become limiting for many taxa. In the case of sustained wastewater inputs, benthic chlorophyte taxa can establish and remain dominant within the community (Timoshkin et al. 2016, 2018; Volkova et al. 2018),

potentially due to increased chlorophyte abundance being associated with increased nutrient uptake efficiency (Oleksy et al. 2021). In the case of intermittent wastewater inputs, chlorophyte taxa may increase in relative abundance in response to nutrient inputs, but then become displaced as nutrients become limiting and taxa compete for nutrients, space, and light (McCormick and Stevenson 1991; Marks and Lowe 1993; Stevenson et al. 1996). These two dichotomous disturbance regimes, therefore, present two similar yet diverging mechanisms where nutrient addition into aquatic environments can alter anticipated periphyton successional patterns.

By driving community compositional shifts, nutrients from wastewater can also influence nutritional quality of the periphyton, with consequences for higher trophic levels. Key elements of algal nutritional quality include fatty acid composition (Iverson et al. 2004; Kelly and Scheibling 2012) and C:N:P stoichiometric ratios (Elser et al. 2000), based on fundamental metabolic needs of aquatic grazers. Fatty acids reflect changing nutritional content as a function of altered community composition, where chlorophyte, cyanobacteria, and diatoms each contain taxon-specific multivariate fatty acid signatures (Kelly and Scheibling 2012; Taipale et al. 2013; Galloway and Winder 2015). In particular, the essential fatty acids (EFAs) are where these groups diverge the most, with diatoms being associated with 20:5 ω 3, chlorophytes being associated with 18:3 ω 3 and 18:2 ω 6, and cyanobacteria usually not containing EFAs (Taipale et al. 2013; Galloway and Winder 2015). Similarly, stoichiometric C:N:P ratios can also define nutritional content within periphyton for higher trophic levels (Kahlert et al. 2002; Fink et al. 2006), where lower C:N:P ratios indicate increased nutrition via increased nitrogen and phosphorus content. Unlike fatty acids, stoichiometric analysis does not allow inference of community taxonomic composition. Rather, algal stoichiometric ratios can be plastic (Thrane et al. 2017) and largely reflect environmental conditions such as light and nutrient availability

(Frost and Elser 2002). Even so, these ratios may be modified by taxon-specific traits. Taxa that are associated with high nutrient uptake, such as filamentous chlorophytes, can decrease a community's overall C:N:P ratios (Frost and Elser 2002) and potentially increase food quality for grazers. In the context of sewage-associated nutrient pollution, both periphyton stoichiometric ratios and fatty acid profiles can be useful for inferring taxonomic and environmental aspects of algal nutritional content. Sewage loading that drives successional shifts from diatom-dominated to chlorophyte-dominated communities may be expected to decrease 20-Carbon EFAs and increase less nutritious 18-Carbon EFAs; simultaneously, these communities may become more nutritious (i.e. decreased C:N and C:P ratios) from a stoichiometric perspective, as communities with more chlorophytes exhibit higher nutrient uptake rates (Nydick et al. 2004; Oleksy et al. 2021).

To investigate how sewage-associated nutrient addition can alter nearshore periphyton community composition, and thus standing nutritional resources for higher level consumers, we surveyed 15 littoral locations in Flathead Lake (Montana, United States) throughout the tourism season for indicators of sewage pollution and metrics of periphyton community abundance and nutritional content. Flathead Lake is a large, deep, oligotrophic lake in western Montana (Young 1935; Ellis et al. 2011). With Glacier National Park covering approximately one third of the lake's watershed, Flathead Lake's catchment is largely unpopulated, despite gradual increases in permanent and seasonal human population throughout the previous century (Stanford et al. 1994a). Within Flathead Lake's watershed, areas with intensive development have shown distinct signs of sewage pollution, evidenced by pharmaceutical and personal care product (PPCPs) and volatile organic compounds detected in subsurface waters although not yet in the lake (Tappenbeck and Ellis 2010, 2011). As locations with dense human population are generally

located away from the lake, (e.g., > 20 km), Flathead's pelagic is broadly oligotrophic, likely due to the lake's exceptionally low hydraulic residence time (~3 years; Stanford et al. 1983) relative to its size (Messager et al. 2016). While the human population in Flathead Lake's watershed is low, the littoral areas with adjacent lakeside development have been suspected of localized sewage pollution (Stanford et al. 1994a), evidenced by sustained increases in periphyton biomass since the 1980s. In particular, littoral sites without adjacent centralized wastewater treatment infrastructure (e.g., sewage treatment plants) have demonstrated signs of nonpoint nutrient addition, which could be contributed from decentralized forms of wastewater management such as improperly functioning septic systems (Stanford et al. 1994a; Makepeace and Mladenich 1996; Moore et al. 2003; Withers et al. 2014). These same lakeside developments also experience large tourism activity throughout the summer, where a reliance on decentralized sewage treatment could create moments of increased nutrient loading during the tourism season followed by reduced sewage loading after the tourism season. Similarly, littoral areas near locations with centralized sewage treatment also experience increased tourism during the summer, but as these areas tend to have larger, less transient human populations, these adjacent nearshore locations also likely experience consistent but elevated wastewater-associated nutrient loading relative to nearshore communities with adjacent decentralized infrastructure.

Given prior evidence that Flathead's periphyton communities may be responding to external nutrient loading, our goal was to understand how spatially and temporally heterogeneous nutrient addition from seasonal tourism may alter algal community composition and, thus, the available nutrition to the food web. This goal can be divided into three specific objectives:

1. identify areas and moments of wastewater-associated nutrient loading using sewage-specific indicators,

2. assess the relationship between sewage-specific indicators and periphyton community composition
3. evaluate how periphyton community compositional shifts may correspond with altered nutritional quality.

We hypothesized that (1) sewage-specific indicators, such as PPCP concentration as well as branched- and odd-chain fatty acids characteristic of bacteria, would increase near areas with increased population density and proximity to lakeside development as well as during moments of increased human activity; (2) an elevated sewage signal would correspond with increased abundance of filamentous benthic algae; (3) increased filamentous algal abundance should be associated with increased stoichiometric nutrition but decreased essential fatty acid nutrition of the periphyton community; and (4) sites with consistent sewage indicators (i.e., those near centralized wastewater treatment) would correspond with less temporal fluctuation in periphyton community composition and nutritional content relative to sites with temporally fluctuating sewage indicators (i.e., sites near decentralized wastewater treatment systems).

3.3 Methods

3.3.1 Site Description

Most of Flathead Lake's 260-km shoreline contains some degree of lakeside development, but the vast majority of Flathead's basin is unpopulated and roadless (Stanford et al. 1994a; Makepeace and Mladenich 1996). Located approximately 23 km from the lake, the city of Kalispell is the largest population hub within the basin with approximately 23,000 residents (U.S. Census, 2019). The Flathead River runs through Kalispell and is the main surface input to Flathead Lake. The Flathead River is Flathead Lake's only surface outlet, where the river flows into Flathead Lake in the northeast region near the town of Bigfork with

approximately 4,700 residents and exits in the southwest near the town of Polson with approximately 4,900 residents (U.S. Census, 2019). The Swan River is the second largest surface input into Flathead. Unlike the Flathead River, the Swan River originates within Glacier National Park and does not run through large population hubs.

While larger population hubs are concentrated away from Flathead's shoreline, there are several small lakeside population hubs that host annual summertime tourists. Each of these main tourist hubs have different permanent population sizes and infrastructures for handling human population fluctuations, ranging from centralized wastewater treatment plants with tertiary treatment to septic systems. Previous studies have noted increased nutrient concentrations and periphyton abundance near these lakeside developments (Stanford et al. 1994a) and implicated sewage-associated nutrient loading as the source (Makepeace and Mladenich 1996). To expand on these previous works by specifically identifying sewage-associated nutrient loading and tracking co-located periphyton responses, our study focused on 15 littoral sampling locations throughout the lake from late May through late September 2017. All sampling sites were chosen to represent a range of lakeside development sizes - spanning from sites with centralized sewage treatment and housing for permanent residents to seasonally occupied developments with septic systems. Many sampling locations were located within Montana State Parks, which ensured consistent access to sampling locations throughout the course of a summer. To evaluate potential sewage contributions from Kalispell, one sampling site was located in the Flathead River, upstream of the town of Bigfork. Sampling occurred at a depth of approximately 0.75-1.25 m, depending on wave activity and periphyton accessibility, such that sampling locations ranged 0.5-8.0 m distance from shore. During each sampling event, we recorded air and surface water temperature with an alcohol thermometer, qualitative wave intensity (e.g., mild, moderate, strong

waves), and whether or not tourists were present at time of sampling.

3.3.2 Temporally-Scaled Inverse Distance Weighted (TSIDW) Population Calculation

We recognized that sewage indicator presence was likely spatially related to the human population density of and distance from lakeside developments, as well as temporally related with the tourism season. Therefore, we created a metric that would represent both the human population density at a given sampling site as well as how that population might vary throughout a summer. Additionally, Makepeace and Mladenich (1996) suggested that anthropogenic nutrient loading into Flathead Lake was likely driven by subsurface flows, meaning that the length of development's shoreline (or the size of a development's interface with the lake) was likely directly proportionate to the amount of wastewater that could enter the lake. Building on techniques described in Meyer et al (Under Revision) and concepts expressed in Bendz et al (2005), we created a temporally-scaled inverse distance weighted (TSIDW) population metric, which considers that sewage indicator levels are likely directly related to population density, a lakeside development's shoreline length, and level of tourism as well as inversely related to a sampling location's distance from lakeside developments.

The TSIDW population metric is meant to capture both spatial and temporal variation of human activity at each sampling location. The formulation of TSIDW population can be broken into two main components: (1) the static inverse distance weighted (IDW) population and (2) a temporal scalar of how much the lakeside human population changes through time. First, the static IDW population can be calculated in five main steps (Meyer et al, Under Revision). Municipality boundary and shoreline geometries were drawn using Google Earth and census delineations for five main lakeside municipalities. Shapefiles were then exported from Google Earth as a .kml file, and then imported into the R Statistical Environment (R Core Team 2019),

in which shoreline length as well as municipality area and centroid were calculated using the sf package (Pebesma 2018). Static IDW population was then calculated by summing the distance-weighted, shoreline length-scaled population density to each sampling location relative to each of the five lakeside municipalities. Second, the static IDW population was scaled by the average monthly temporal change in human population. In this case, State Park visitation records were used to track changes in the human population relative to pre-tourism visitation levels. To track how the lakeside human population may change spatially and temporally, we collected data on visitor passes issued at each of the five State Parks around the lake (Figure 1). These data aggregated both day-visitor as well as overnight visitor passes purchased within a month, thereby representing the total number of visitors to each of the five lakeside Parks. To estimate a deviation in human population due to tourism, we normalized each Park's monthly visitors by the number of visitors to the same Park in May, and as such this calculation assumes that the number of visitors in May is characteristic of the pre-tourism season. Because each State Park was not necessarily located within or adjacent to the five main lakeside population hubs, we weighted temporal scalars by the distance of a given sampling location from the centroid of each State Park. Lastly, we averaged monthly temporal scalars across Parks. Averaging the temporal scalars in a given month helped us account for populations that may not be visiting a local Park or correct for populations may have been dispersed when one of the Parks was closed near the end of the tourism season. TSIDW population (I) is expressed by equation 1:

$$I_{i,m} = \frac{1}{5} \sum_{q=1}^{q=5} \frac{n_{m,q}}{n_{May,q}} \times \sum_{j=1}^{j=5} \frac{P_j}{A_j} * \frac{L_j}{D_{i,j}}$$

where the left summation is the temporal scalar formulation and the right summation is the spatial IDW population formulation. The equation represents the TSIDW population I at site i in month m . The IDW formulation considers the population size P , area A , and shoreline length L at development site j and weights the shoreline population by the distance from site i to j . The temporal scalar formulation considers the number of visitor passes sold n in a month m at Park q relative to the number of passes sold in May at Park q and weights this ratio by the distance of sampling site i from Park q . When multiplied, the TSIDW population is meant to capture the temporal change and spatial position of human population around the lake's perimeter, where sites with large populations but low tourism retain a high TSIDW population throughout the summer whereas sites with a smaller static population that experiences waves of tourism increase in TSIDW population at the beginning of the tourism season and then decrease in TSIDW population at the end of the tourism season.

3.3.3 Pharmaceuticals and Personal Care Products (PPCPs)

Water column Pharmaceutical and Personal Care Product (PPCP) samples were collected in duplicate fortnightly at a depth of approximately 0.5 m at each sampling location. Samples were collected in 1-L amber glass bottles that had been rinsed with lake water prior to collection. Samples were kept on ice and out of direct sunlight in a cooler while in the field and then placed in a refrigerator at ~4°C upon return to the lab. While in the field, personnel collecting samples refrained from caffeine, nicotine, and other non-prescription PPCPs on the days of sample collection to avoid potential contamination.

Within 96 h of collection, samples underwent a solid phase extraction (SPE). Samples were filtered directly from the amber glass bottle using an in-line Teflon filter holder with glass microfiber GMF (1.0 μm pore size, WhatmanGrad 934-AH) in tandem with a solid phase

extraction (SPE) cartridge (200 mg HLB, Waters Corporation, Milford, MA) connected to a 4-liter vacuum flask. Lab personnel wore gloves and face masks when handling samples in the lab to minimize potential for contamination. Prior to filtration, SPE cartridges were primed with at least 5 mL of either methanol or acetone and then washed with at least 5 mL of sample water. Rate of extraction was maintained at approximately 1 drop per second. Extraction proceeded until water could no longer pass through the SPE cartridge or until all collected water was filtered. Cartridges were stored in Whirlpacks at -20°C until analysis for 10 PPCP residues using high performance liquid chromatography tandem mass spectrometry (HPLC-MS) following methods of Furlong et al. (2008), Lee et al. (2016), and D'Alessio et al (2018). During the mass spectrometry sample processing, blank samples with methanol as well as a 20 µg/L standard were processed after every tenth sample. This step was added as a quality assurance protocol to flag instances of and correct for cross-sample contamination as well as peak drift when running samples over multiple hours. Regardless of detection thresholds, we manually integrated all PPCP peaks observed in the chromatogram and corrected for cross-sample contamination in post-processing. Samples were processed at the Water Environmental Research Laboratory at Washington State University.

3.3.4 Nutrients

Water column nutrient samples were collected at all 15 sampling locations and contemporaneously with the August and September PPCP sample collection that occurred at the end of the month (i.e., second fortnightly sampling campaign within a month). Samples were collected in 0.5-L plastic Nalgene bottles that had been rinsed with lake water prior to collection. Samples were kept on ice and out of direct sunlight in a cooler while in the field and then placed in a refrigerator at ~4°C upon return to the lab. Prior to freezing, samples intended for

phosphorus analysis were transferred directly to a clear 60-mL HDPE Nalgene bottle. Samples intended for nitrate/nitrite analysis were first filtered through a 0.45 µm nitrocellulose filter to remove intracellular nitrogenous species. Samples were processed using a spectrophotometer. Detection limits were estimated to be 1.5 µg/L-N for nitrogen samples and 0.8 µg/L-P for phosphorus samples. When concentrations were estimated as below detection limits, we replaced values with a random number between 0 and half of the respective minimal detection limit.

3.3.5 Periphyton Sample Collection

Periphyton samples were collected at 13 of the 15 sampling locations and contemporaneously with PPCP sample collection that occurred at the end of the month (i.e., second fortnightly sampling campaign within a month). Two sampling locations (Holt and Ducharme) were largely characterized by clay and silt sediments, making standardized, systematic periphyton collection difficult. The remaining 13 locations were comparable with respect to the substrate, which could be characterized as a mix of large, oblate rocks and gravel. As such, we do not include data from HO and DU in our analysis.

At each of the periphyton sampling locations, nine rocks were haphazardly collected and placed into plastic, Ziplock bags within the field. Within 18 h of collection, a plastic stencil was used to define a surface area of each rock from which we scraped a standardized 1.61 cm² patch of periphyton with a firm bristled toothbrush. Individual samples from three rocks were transferred to 50 mL plastic falcon tubes for abundance estimates. Glutaraldehyde (0.2% final volume) was added to each falcon tube as a preservative. Individual samples from four other rocks were transferred to GF/Fs for ash free dry mass estimates. Following standardized area scrapes, all remaining periphyton on each of the nine rocks was scraped from the rock and into an aluminum tray. The composite periphyton mixture for a given site and sampling time was

then placed into 50 mL plastic falcon tubes, which were centrifuged at ~750 rpm for 20 minutes to concentrate periphyton at the bottom of the tube. Using a 5-mL transfer pipette, visibly compacted periphyton and sediment were removed from falcon tubes and transferred to 1.5-mL Eppendorf tubes until a maximum of 10 Eppendorf tubes were filled with periphyton and sediment. These composite periphyton samples were then placed in a -80°C freezer until processing for stoichiometry and fatty acids.

3.3.6 Benthic Algal Abundance

Periphyton taxonomic identification and enumeration was performed by subsampling 30 µL aliquots from each preserved sample using a compound light microscope. For all 30 µL aliquots, cells and filaments were counted, for the entire subsample, until at least 300 cells were identified for a given sampling replicate. If the first aliquot contained less than 300 cells, we counted additional subsamples until we reached at least 300 cells in total. In instances when 300 cells were counted before finishing a subsample, we still counted the entire aliquot. Taxa were classified into broad categories consistent with periphyton algal taxonomy (Biggs and Kilroy 2000), using coarse groupings to capture general patterns in relative algal abundance. As a result, algal groups consisted of diatoms, chlorophytes, cryptophytes, chrysophytes, and cyanobacteria. For consistency, all samples were processed by one person (MFM) to reduce cross-observer error.

3.3.7 Ash Free Dry Mass

GF/Fs were removed from whirlpacks, placed on aluminum foil, and transferred to a drying oven at ~96°C overnight. Samples were then relocated to a dessicator for at least 24 h. After samples were dry, samples were weighed to the nearest 0.0001 g, and then placed in a muffle furnace at 550°C for ~1.5 h. After cooling for ~30 minutes, samples were massed again,

and pre- and post-ignition were differenced. Samples were processed at the Water Environmental Research Laboratory at Washington State University.

3.3.8 Periphyton Stoichiometry

Two 1.5-mL Eppendorf tubes of concentrated periphyton for each sampling location and time were transferred to a foil pan and allowed to thaw and dry in a drying oven at 60°C overnight. Samples were then homogenized and placed in a desiccator for at least one hour prior to weighing.

Two subsamples of completely dried periphyton were transferred to two glass scintillation vials and covered with foil. Particulate carbon and nitrogen measurements could be processed together and required at least 50 mg of tissue, whereas particulate phosphorus required a separate instrument and no more than 5 mg of tissue for analysis. Samples were combusted at ~500°C for 4.5 h, and once cooled, 10 mL of 0.15 M hydrochloric acid was added. Samples were stored at 4°C until analyzed. Particulate carbon and nitrogen were estimated using a Leco CN628 analyzer. Particulate phosphorus was estimated using an Astoria Pacific, A2 segmented flow analyzer with an Astoria Pacific models 311 XYZ autosampler. The method detection limits were estimated to be 2 µg/L-P. Samples were processed at the Freshwater Research Laboratory at Flathead Lake Biological Station.

3.3.9 Periphyton Fatty Acid Analysis

All collected periphyton samples in 1.5-mL Eppendorf tubes that were not used for stoichiometric assessment were allocated for fatty acids. Samples were allowed to freeze-dry overnight. Following freeze-drying, samples were transferred to 10 mL glass centrifuge vials, and 2 mL of 100% chloroform was added to each under nitrogen gas. Samples remained in chloroform overnight at -80°C. Fatty acid extractions generally involved three phases: (1) 100%

chloroform extraction, (2) chloroform-methanol extraction, and (3) fatty acid methylation. Fatty acid extraction methods were adapted from Schram et al. (2018).

After overnight chloroform extraction, samples underwent a chloroform-methanol extraction three times. To each sample, we added 1 mL cooled 100% methanol, 1 mL chloroform:methanol solution (2:1), and 0.8 mL 0.9% NaCl solution. Samples were inverted three times and sonicated on ice for 10 minutes. Next, samples were vortexed for 1 minute, and centrifuged for 5 minutes (3,000 rpm) at 4°C. Using a double pipette technique, the lower organic layer was removed and kept under nitrogen. After the third extraction, samples were evaporated under nitrogen flow, and resuspended in 1.5 mL chloroform and stored at -20°C overnight.

Once resuspended in chloroform, 1 mL of chloroform extract was transferred to a glass centrifuge tube with a glass syringe as well as an internal standard of 4 µL of 19-carbon fatty acid. Samples were then evaporated under nitrogen, and then 1 mL of toluene and 2 mL of 1% sulfuric acid-methanol was added. The vial was closed under nitrogen gas and then incubated in a 50°C water bath for 16 hours. After incubation, samples were removed from the bath, allowed to reach room temperature and stored on ice. Next, we performed a potassium carbonate-hexane extraction twice. To each sample, we added 2 mL of 2% potassium bicarbonate and 5 mL of 100% hexane, inverting the capped vial to mix the solution. Samples were centrifuged for 3 minutes (1,500 rpm) at 4°C. The upper hexane layer was then removed and placed in a vial to evaporate under nitrogen flow. Once almost evaporated, 1 mL of 100% hexane was added and stored in a glass amber autosampler vial for GC/MS quantification. GC/MS quantification was performed with a Shimadzu QP2020 GC/MS following Schram et al. (2018). Samples were processed at the Community Trophic Ecology Laboratory at the Oregon Institute for Marine

Biology.

3.3.10 Statistical Analysis

To evaluate how lakeside development and tourism season may be associated with human activity and sewage indicator abundance, we tested whether total PPCP concentration, abundance of branched- and odd-chain fatty acids as indicators of bacteria, nitrogen and phosphorus concentrations, as well as ash free dry mass changed across sites and timepoints using ANOVA with sewage treatment technique (i.e., decentralized or centralized) and tourism season (i.e., in-season, out-of-season) and their interaction as predictors. To characterize patterns, we averaged a given site's sewage indicator values observed in and out of the tourism season. Total PPCP and nutrient concentrations were log-transformed prior to analysis, as we assumed these variables were likely subject to non-linear physical processes, such as mixing and diffusion. Branched- and odd-chain fatty acid proportions were arcsine-square root-transformed prior to analysis to meet assumptions of normality. "In Season" samples were defined as samples collected in June, July, or August, and "Out of Season" samples were defined as samples collected in September. Months were classified as "In Season" or "Out of Season" based on the number of visitor passes sold in a particular month relative to the number sold in May, which we assume as being either before or at least at the beginning of the tourism season. Predictors were treated as fixed effects, and because the sampling design was unbalanced, we used a Type II SS formulation (Langsrud 2003). Raw data as well as model residuals were assessed for normality and homogeneity of variance.

To determine how spatial and temporal variation in lakeside development and human activity may influence periphyton community composition, we first performed a non-metric multidimensional scaling (NMDS) with Bray-Curtis similarity, and then visualized the NMDS in

two-dimensional space. Because chrysophytes and cryptophytes were rare (i.e., < 2% of the intersite abundance), we omitted these taxa from analysis and visualization. To test differences among relative abundance of diatoms, chlorophytes, and cyanobacteria, we performed a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) with 4,999 permutations, where sewage treatment technique (i.e., decentralized or centralized) and tourism season (i.e., in-season, out-of-season) were predictors of relative periphyton community abundance. Unlike traditional multivariate analyses of variance (MANOVA), PERMANOVA does not require assumptions of multivariate normality (Anderson 2001). When significant differences were identified, post-hoc SIMPER analysis (Clarke 1993) was performed to identify which taxonomic groups most influenced site separation.

To assess variation in periphyton nutritional content, we performed ANOVAs with sewage treatment technique, tourism season and their interaction as predictors of periphyton stoichiometric ratios and relative fatty acid abundances. For stoichiometry, we assessed Carbon:Nitrogen, Carbon:Phosphorus, and Nitrogen:Phosphorus ratios. For fatty acids, we evaluated relative saturated (SAFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid abundance, where proportions were arcsine-square root-transformed to improve normality.

To understand how sewage treatment technique and the tourism season may influence multivariate essential fatty acid signatures, we performed NMDS with Bray-Curtis similarity of all essential fatty acids constituting more than 1% of intersite proportions, and then visualized the NMDS in two-dimensional space. To test differences between relative essential fatty acid abundance in samples, we performed a PERMANOVA with 4,999 permutations, where sewage treatment technique and tourism season were predictors of the entire essential periphyton fatty

acid profile. When significant differences were identified, post-hoc SIMPER analysis was performed to identify which fatty acids most influenced site separation.

All data aggregation routines and statistical analyses were performed using the R Statistical Environment (R Core Team 2019) with the tidyverse (Wickham et al. 2019), vegan (Oksanen et al. 2019), car (Fox and Weisberg 2019), lubridate (Grolemund and Wickham 2011), stringi (Gagolewski 2020), janitor (Firke 2020), ggpubr (Kassambara 2019), ggrepel (Slowikowski 2019), viridis (Garnier 2018), rnaturalearth (South 2017), ggspatial (Dunnington 2021), ggstar (Xu 2021), cowplot (Wilke 2019), and sf (Pebesma 2018) packages. All R code can be accessed from this project's GitHub Repository (https://github.com/mishafredmeyer/flathead_sewage), and all associated data can be found on this project's Open Science Framework portal (Meyer et al. 2021). All R scripts were written by one person (MFM) and then independently reviewed by another co-author (MRB) to assess scripts for accuracy, reproducibility, and clarity of comments.

3.4 Results

3.4.1 Temporally Scaled Inverse Distance Weighted (TSIDW) Population

TSIDW tended to fluctuate through space and time in a way that reflected static and transient human populations, suggesting that our TSIDW metric effectively captured patterns in human population around Flathead Lake. Spatially, TSIDW population was higher in the northeastern part of the lake, which is likely due to the town of Bigfork being a larger population hub (Figure 2). Temporally, TSIDW population highlighted fluctuations in human population in the southernmost portion of Flathead Lake, near the town of Polson (Figure 2). Generally, TSIDW population values tended to decrease with the tourism season more than with differing sewage treatment technique (Figure 4A).

3.4.2 Water Samples

In general, water column nutrient concentrations did not differ across sites and timepoints. Nitrate/nitrite, total nitrogen, total phosphorous, and, to some extent, soluble reactive phosphorus (SRP) as well as ammonia concentrations were not different based on sewage treatment technique, tourism season, or interaction between site and tourism season (summarized in Table S1). SRP concentrations tended to be higher at sites with centralized sewage treatment relative to those near decentralized wastewater treatment, but neither sewage treatment technique ($F = 3.313, p = 0.081$; Table S1), tourism season ($F = 0.009, p = 0.923$; Table S1), nor the interaction between them ($F = 0.114, p = 0.739$; Table S1) were statistically significant. Ammonia (as nitrogen) concentrations likewise were higher at sites near centralized treatments relative to those near sites with decentralized treatment, although neither sewage treatment ($F = 3.347, p = 0.080$; Table S1), tourism season ($F = 0.454, p = 0.507$; Table S1), nor the interaction between them ($F = 1.040, p = 0.318$; Table S1) were statistically significant. Notably, a large majority of water column SRP concentrations (82% of samples) were below detection limits (<0.8 µg/L). In comparison to SRP, 27% of nitrate/nitrite, 20% of total phosphorus, and 3% of ammonia samples were below detection limits. No total nitrogen samples were below detection limits.

PPCPs detected within the water column included caffeine, paraxanthine/1,7-dimethylxanthine, acetaminophen/paracetamol, cotinine, cimetidine, and warfarin. PPCPs not detected included carbamazepine, codeine, diphenhydramine, sulfamethoxazole, thiabendazole, and trimethoprim. Total PPCP concentrations tended to be higher during the tourism season ($F = 3.915, p = 0.059$), although this result did not meet thresholds for statistical significance (i.e., $p <$

0.05; see Table S2). Neither sewage treatment technique ($F = 0.649$, $p = 0.428$) nor the interaction between sewage treatment and tourism season ($F = 2.085$, $p = 0.161$) were significant predictors of total PPCP concentration (summarized in Table S2).

3.4.3 Periphyton Community Composition

Periphyton community composition largely consisted of chlorophytes, cyanobacteria, and diatoms (Figure 5), with cyanobacteria abundance being higher at sites with decentralized wastewater treatment. NMDS suggested differentiation of chlorophyte and diatom abundance along the horizontal axis that potentially reflects temporal patterns, such as tourism season and community succession. In vertical space on the NMDS, relative cyanobacteria abundance helped differentiate sites as well, potentially reflecting sewage treatment technique, as sites near centralized sewage treatment tended to cluster (Figure 6). PERMANOVA results, however, suggested that groupings based on sewage treatment technique ($F = 1.554$, $p = 0.160$) and tourism season ($F = 1.930$, $p = 0.098$) were not statistically different (summarized in Table S3).

3.4.4 Periphyton Stoichiometry

Periphyton C:N, C:P, and N:P molar ratios varied widely across sites and timepoints. Most values were above stoichiometric ratios for sustained periphyton growth (119:17:1; Hillebrand and Sommer 1999). Neither C:P nor N:P ratios differed based on sewage treatment technique, tourism season, or interactions between tourism season and sewage treatment technique (Figure 7B, 7C; summarized in Table S4). C:N ratios were significantly lower at sites with centralized wastewater treatment in comparison to decentralized wastewater treatment ($F = 5.830$, $p = 0.022$) but did not differ with tourism season ($F = 0.015$, $p = 0.904$) nor interactions between tourism season and sewage treatment technique ($F = 0.051$, $p = 0.823$).

3.4.5 Periphyton Fatty Acids

To characterize benthic bacterial communities, our samples contained 15- and 17-Carbon saturated as well as 15-Carbon branched fatty acids. ANOVA results suggested that relative abundance of branched and odd-chain fatty acids was higher during the tourism season than outside of the tourism season ($F = 4.565$, $p = 0.045$). There was no difference in relative branched and odd-chain fatty acids based on sewage treatment technique ($F = 0.307$, $p = 0.585$) nor the interaction of sewage treatment technique and tourism season ($F = 0.353$, $p = 0.559$).

Other fatty acid groups, such as saturated (SAFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids, also varied across sites and timepoints but not between interactions of sewage treatment and time (Figure 8; Table S5). In particular, PUFA increased in relative abundance following the tourism season ($F = 14.470$, $p = 0.001$) but did not change between types of sewage treatment ($F = 0.160$, $p = 0.692$) nor the interaction of sewage treatment and tourism season ($F = 0.256$, $p = 0.617$). Likewise, SAFAs decreased following the tourism season ($F = 20.529$, $p < 0.001$) and did not differ between types of sewage treatment ($F = 0.070$, $p = 0.793$) nor with interactions of sewage treatment and tourism season ($F = 1.618$, $p = 0.214$). MUFA remained consistent across sewage treatment types ($F = 0.046$, $p = 0.832$), throughout the tourist season ($F = 1.016$, $p = 0.322$), and between interactions of sewage treatment and tourism season ($F = 0.249$, $p = 0.622$).

Deviations in PUFA abundance were largely driven by changes in essential fatty acid (EFA) relative abundance, which were likely reflective of differences in periphyton community composition. The EFAs with the highest coefficients of variation included 20:5 ω 3, 18:2 ω 6, and 18:3 ω 3. Periphyton EFAs at sites with centralized sewage treatment appeared to have more consistent proportions through time, whereas sites with decentralized treatment tended to have

higher proportions of 20-Carbon EFAs, such as 20:5 ω 3. When analyzed in a multivariate framework (Figure 10A), visual inspection of the NMDS suggested that fatty acids more associated with chlorophytes (i.e., 18:2 ω 6, and 18:3 ω 3) and fatty acids more associated with diatoms (i.e., 20:5 ω 3) tended to separate in the horizontal dimension while changes with the tourism season were more apparent along the vertical axis. PERMANOVA results (summarized in Table S3) suggested tourism season as significant in discriminating groups ($F = 2.775$, $p = 0.006$), but sewage treatment technique was not significant ($F = 0.726$, $p = 0.676$). Post-hoc SIMPER analyses suggested 20:5 ω 3 as the most influential discriminating fatty acid (Cumulative Variance = 37.6%, $p = 0.04$). Together, 20:5 ω 3, 18:2 ω 6, and 18:3 ω 3 accounted for more than 85% of the total variance.

3.5 Discussion

Our combined results corroborate previous findings (e.g., Makepeace and Mladenich 1996) that sewage pollution is entering Flathead Lake's nearshore area and likely is responsible for differences in nearshore periphyton community composition and nutritional content. Unlike previous studies in Flathead Lake (Stanford et al. 1994a; Makepeace and Mladenich 1996), we incorporated highly specific indicators of sewage pollution as well as periphyton nutritional content to describe how sewage pollution entering Flathead may influence periphyton stoichiometry and fatty acid composition. Thus, our results provide direct, quantitative evidence that sewage entering Flathead may alter nutrition available to the lake's larger food web.

3.5.1 Relating Human Population with Sewage Indicators

In agreement with our expectations, sewage-specific indicators tended to increase during the tourism season, when lakeside human populations were larger. These patterns are noteworthy as Flathead's watershed is largely unpopulated, but permanent and seasonal human populations

have been growing since the 1950s (U.S. Census, 2020). In particular, many lakeside developments around Flathead Lake rely upon septic systems for wastewater treatment (Stanford et al. 1994a), and our data suggest that during the tourism season, sites with decentralized sewage treatment tended to have PPCP concentrations comparable to those near centralized treatment. In contrast, sites with centralized sewage treatment tended to have more temporally consistent sewage indicators throughout the summer. Furthermore, spatial and temporal patterns in sewage indicator concentrations mirrored patterns observed in TSIDW population, suggesting that our sampling successfully captured heterogeneous sewage loadings into Flathead's nearshore.

Patterns in nutrient concentrations alone were not fully reflective of patterns observed with more specific sewage indicators such as PPCPs (Figure 3). These incongruencies are likely due to nutrients originating from non-sewage sources as well as nutrients being actively removed from and released into the water column through physicochemical and biochemical processes. For example, allochthonous nutrients can originate from fertilizers applied in directly adjacent lakeside orchards (Stanford et al. 1994a), pollen from conifers (Graham et al. 2006), and even forest fires (Gould et al. 2016) or atmospheric deposition more broadly (Elser et al. 2009). Throughout the course of our sampling campaign, wildfires, significant pollen deposition, and lakeside agriculture all occurred and likely contributed nutrients to Flathead's nearshore communities, but these are currently not known to contribute significant nutrient loadings in Flathead Lake relative to sewage.

To our knowledge, this is the first study to detect PPCPs in Flathead Lake, a high-volume lake with a short hydraulic residence time relative to its size (Messager et al. 2016), and located in a largely unpopulated and undeveloped watershed (Stanford et al. 1994a). Previous

investigations of sewage pollution into groundwater near Kalispell, Montana (the watershed's most populous city with ~23,000 people; U.S. Census) have highlighted the potential for PPCPs to occur within Flathead's watershed but from distal developments (Tappenbeck and Ellis 2010, 2011). Furthermore, unknown factors include: (1) how subsurface PPCP concentrations within Flathead's basin may enter surface flows through groundwater-surface water connections or (2) how PPCPs contributed from Kalispell may affect subsurface, hyporheic, and potentially the nearshore biota such as stonefly larvae (Stanford et al. 1994b; Stewart 2002; Malison et al. 2020). Our sampling scheme included one location within the Flathead River (HO; Figure 1), which runs through Kalispell and is the main surface input into Flathead Lake. Although we detected PPCPs at this site, concentrations were lower than those observed near the lakeside development of Bigfork, implying that concentrations observed in Flathead Lake likely originated from adjacent lakeside settlements and were not contributed from distal sources. More broadly, our results suggest that wastewater infrastructure as well as the size and temporal variation of human populations at adjacent lakeside developments can contribute spatially and temporally varying sewage inputs, thereby creating hot spots as well as hot moments of sewage pulses into the nearshore.

Beyond Flathead Lake, these data and results are important for understanding PPCP prevalence in lakes and sewage contributions from decentralized wastewater treatment. Despite rapid worldwide growth in the PPCP literature over the past three decades, both lakes and forms of decentralized sewage treatment have remained far less represented in the literature relative to lotic environments and forms of centralized wastewater treatment (Meyer et al., 2019). These imbalances may be important for shaping our understanding of PPCPs in the environment. Lakes tend to have longer hydraulic residence times relative to lotic systems, meaning that pollutants

within sewage may concentrate and elicit stronger biological responses. Likewise, decentralized wastewater treatment can contribute significant nutrient (Moore et al. 2003; Rosenberger et al. 2008; Hampton et al. 2011; Withers et al. 2014) and micropollutant (Meyer et al.; Bendz et al. 2005) loadings to proximal aquatic systems. Adding to this complexity, PPCPs contributed from septic systems have demonstrated complex transport dynamics that may influence their residence time, where some PPCPs, such as caffeine, can pulse through subsurface systems and enter into surface flows, yet others, such as acetaminophen, can sorb to proximal colloidal materials, thereby creating PPCP hotspots (Yang et al. 2016). Considering that more than half of the world's population utilizes some form of decentralized wastewater treatment (Withers et al. 2014), the current uncertainties around PPCP prevalence, PPCPs' potential to accumulate and create pollution hot spots, and PPCPs' often uncertain biological effects, our data underscore the potential for PPCP concentrations to vary spatially and temporally in lakes and highlight the need for continued study of the biophysical processes influencing their abundance.

While our study focused on using PPCPs as indicators of sewage, PPCPs themselves can cause deleterious ecological consequences, even at concentrations we observed in Flathead (e.g., < 10 ng/L; Feijão et al. 2020). Biotic responses can include physiological (del Rey et al. 2011) and behavioral (Brodin et al. 2013) consequences as well as food web (Medor et al. 2016; Lagesson et al. 2016; Richmond et al. 2018) and ecosystem (Rosi-Marshall et al. 2013) alterations. Although our study was not designed to evaluate ecotoxicological effects of PPCPs on periphyton communities nor higher trophic levels, future studies could build on this study as well as the empirical results of others to address how wastewater treatment infrastructure and variation in human population can create diverse mixture of PPCPs that affect biological communities differently across various spatial and temporal scales.

3.5.2 Relating Human Population with Periphyton Community Composition

Congruent with our hypotheses, areas and timepoints with increased human population and sewage indicator abundance tended to be associated with increased filamentous chlorophyte taxa. These same patterns have been noticed in the nearshore of other large, deep, oligotrophic lakes, such as Lake Baikal (Meyer et al.; Timoshkin et al. 2016, 2018; Volkova et al. 2018), Lake Crescent (Rosenberger et al. 2008; Hampton et al. 2011), Lake Pend Oreille and Priest Lake (Rosenberger et al., 2008), and Lake Tahoe (Naranjo et al. 2019; Atkins et al. 2021). In addition to sewage inputs providing increased nutrients, filamentous chlorophyte abundance in Flathead may also be responding to other nutrient co-limitation dynamics (Marks and Lowe 1993), seasonal shifts in light availability (Marks and Lowe 1993), and increasing surface temperatures (Thrane et al. 2017; Pilla et al. 2020). While much understanding of algal successional patterns is based on phytoplankton dynamics (Sommer et al. 1986, 2012), periphyton appear to undergo similar phenological shifts from spring through autumn (McCormick and Stevenson 1991; Stevenson et al. 1996; Azim et al. 2005). Some complications in understanding periphyton community succession patterns, relative to those of the pelagic algae, may emerge from complex algal life histories and adaptation such as diatom mucilage and stalk development (Roemer et al. 1984) or variation in mat thickness (Dodds et al. 1999). Recent syntheses have suggested that increased allochthonous nutrients into oligotrophic lakes' littoral zones may be responsible for increased filamentous algal abundance worldwide and eventually contribute to nuisance filamentous algal blooms (Vadeboncoeur et al. 2021). In addition, other mechanisms may contribute to these patterns. For example, seasonal wave action can transport hypolimnetic nutrients to the littoral zone and stimulate increased filamentous algal production

(reviewed in Vadeboncoeur et al. 2021). Likewise, shifting environmental conditions, such as temperature or dissolved oxygen, may limit the capacity for macroinvertebrates to graze, allowing for quickly growing, filamentous algae to outcompete diatom taxa (reviewed in Vadeboncoeur et al. 2021). Regardless of the exact mechanism occurring in Flathead Lake, our algal abundance data are consistent with other nearshore sewage-associated eutrophication patterns observed in other, large, deep, oligotrophic systems (e.g., Meyer et al.; Timoshkin et al. 2016, Rosenberger et al. 2008; Hampton et al. 2011). By mirroring patterns in sewage-specific indicator abundance, our data further suggest that relative abundance of filamentous taxa tends to be greatest near areas and during times of increased human activity at lakeside developments.

Beyond algal community composition, relative abundance of branched- and odd-chain fatty acids, which are highly specific indicators of bacterial communities, mirrored patterns observed in total PPCP concentrations and algal taxonomic abundances. While bacteria are core constituents of biofilm communities (Azim et al. 2005), their relative abundance can be useful for inferring sewage pollution (Seguel et al. 2001; Rocchetta et al. 2014). Similar studies at Lake Baikal measured *E. coli* and *Enterococcus* abundances near areas suspected of increased sewage pollution and noted that these areas tended to have higher fecal indicator bacteria abundances (Timoshkin et al. 2016). While branched- and odd-chain fatty acids can suggest bacterial abundance (Seguel et al. 2001; Rocchetta et al. 2014), the diversity of branched- and odd-chain fatty acids observed in our data could not identify specific taxonomic groups. These differences in heterotroph abundance may be consequential for autotrophic and heterotrophic productivity. Rosi-Marshall et al. (2013), for example, demonstrated how caffeine can act as generalist toxicant and reduce community respiration by reducing abundance of the entire community, whereas diphenhydramine can act as a specialist toxicant and reduce community respiration by

reducing certain taxa's relative abundance. In the context of our study, patterns in periphyton branched- and odd-chain fatty acids underscore the potential for heterogeneities in sewage loading to influence benthic bacterial communities. Our data, in addition to the uncertainties in how sewage may influence heterotroph composition and productivity in lotic and lentic systems (e.g., Rosi-Marshall et al. 2013; Shaw et al. 2015), suggest that non-autotrophic responses to sewage pollution warrant increased research emphasis.

3.5.3 Relating Human Population with Periphyton Nutritional Content

Fatty acid analyses suggested that changes in periphyton community composition altered periphyton nutritional quality across sites and timepoints. Periphyton fatty acids at sites with centralized sewage treatment tended to have consistent fatty acid ratios through time, although one site following the tourism season did have increased PUFA proportions (YB; Figure 1). In contrast, periphyton fatty acid profiles at sites with decentralized sewage treatment tended to show a tradeoff of SFAs and PUAs throughout the course of the summer, where SFAs decreased and PUAs increased in relative abundance (Figure 8). These patterns are likely reflective of changes in the periphyton community composition, as algal taxa tend to have strongly differentiated multivariate fatty acid signatures (Taipale et al. 2013; Galloway and Winder 2015).

Among all PUAs detected, increased PUFA abundance at decentralized sites following the tourism season was driven by increases in diatom-associated essential fatty acids (EFA). EFAs comprise a subgroup of PUAs that accumulate in organisms, are mainly synthesized by primary producers, and can provide vital nutrition to higher trophic levels (Iverson et al. 2004; Kelly and Scheibling 2012). Among the eight common essential fatty acids, 18:3ω3, 18:2ω6, and 20:5ω3 had the highest coefficients of variation across sites and timepoints. Both 18:3ω3 and

18:2 ω 6 are associated with chlorophytes; 20:5 ω 3 is consistently associated with diatoms (Taipale et al. 2013; Galloway and Winder 2015). Comparing taxonomic compositions with fatty acid profiles, the increased diatom presence relative to chlorophytes at decentralized sites following the tourism season was likely responsible for increased 20:5 ω 3 abundance (Figure 9). Together, our analysis of sewage indicators, periphyton composition, and fatty acid results suggest that Flathead's nearshore periphyton communities near centralized sewage treatment may experience more temporally consistent sewage loading that likely supports a consistent assemblage of chlorophytes and diatoms, and therefore these sites maintain a more temporally consistent fatty acid signature. In contrast, these same results suggest that Flathead's nearshore periphyton communities near decentralized sewage treatment tend to experience more inconsistent sewage loading that likely allows for tradeoffs in chlorophyte, cyanobacteria, and diatom relative abundance. Therefore, these sites may maintain a temporally inconsistent fatty acid signature. In particular, sites with decentralized treatment tended to experience increased PUFA abundance, especially 20:5 ω 3, following the tourism season, which could provide necessary nutrition to higher trophic levels as they prepare to overwinter.

In contrast to fatty acids, periphyton stoichiometric ratios tended to be lower at sites with centralized treatment, implying that sites with centralized treatment have more nutritious periphyton. Unlike fatty acids, periphyton stoichiometric ratios do not map to community composition, and furthermore, periphyton stoichiometric ratios may be influenced by ambient water column nutrient concentrations (Frost and Elser 2002), production of extracellular matrices or mucilage tubes (Frost et al. 2005), and variable nutrient uptake rates (Oleksy et al. 2021). Among each of the stoichiometric ratios considered, C:N ratios were the only ones that differed across locations and timepoints. The lack of differences between sites and timepoints for C:P and

N:P ratios is not necessarily surprising, as phosphorus is usually a limiting element in oligotrophic Flathead (Axler and Reuter 1996). Conversely, C:N ratios were significantly lower at sites with centralized treatment than those at sites with decentralized treatment (Figure 7A). Mechanistically, the difference in C:N potentially stems from chlorophytes' increased abundance at sites with centralized treatment, as periphyton assemblages containing chlorophytes have been associated with increased nitrogen uptake (Nydick et al. 2004; Hogan et al. 2014; Oleksy et al. 2021). Temporally, stoichiometric ratios did not differ. Thus, the combined results of our sewage indicator, periphyton community composition, and stoichiometric data suggest that algal communities near centralized wastewater treatment, can create temporally consistent, stoichiometrically nutritious periphyton assemblages. In contrast, the inconsistent sewage loading at sites with decentralized treatment may create stoichiometrically less nutritious periphyton assemblages.

Although differences in periphyton fatty acids and stoichiometries appear to tell diverging narratives about periphyton nutritional content in response to spatio-temporally heterogeneous sewage loading, each interpretation can be valid when considering the grazer guilds that might be consuming the periphyton. Through a stoichiometric lens, our results suggest that stoichiometric measures of nutrition vary more in space than in time. Sites near centralized wastewater treatment tended to have temporally consistent, low C:N periphyton that largely contained chlorophytes and diatoms. This periphyton assemblage may favor generalist grazers capable of consuming filamentous taxa as well as detritivores capable of consuming decomposing filamentous strands. Because these low C:N ratios and periphyton community composition appear to remain consistent throughout a growing season, these resources are likely to be reliable for consumer guilds throughout a summer. Through a fatty acid lens, our results

suggest that fatty acid markers of nutrition vary more in time than in space. Following the tourism season, increased EFA abundance, particularly increased 20:5ω3 abundance at sites near decentralized wastewater treatment, was likely associated with increased diatom and decreased chlorophyte abundance. This periphyton assemblage may support grazers that require higher order PUFAs, which can be crucial for maintaining membrane fluidity (Nichols et al. 1993) as grazers prepare to overwinter. Because increased diatoms and 20:5ω3 tended to co-occur with decreased sewage indicator abundance, these resources may be temporally transient and potentially vulnerable to prolonged or enhanced sewage loading that could arise through an extended tourism season or increased lakeside development.

3.6 Conclusions

Over the past century, Flathead Lake's watershed has experienced consistent growth in its permanent and seasonal human population (U.S. Census 2019). Although Flathead's depth, volume, and short hydraulic residence time have likely aided in keeping the pelagic waters oligotrophic, there are distinct signs of eutrophication in the nearshore, particularly near areas with larger human populations. While Flathead receives nutrients from multiple sources, our repeated sampling of sewage specific indicators and co-located biological data throughout the lake implicate wastewater pollution as one of the nutrient sources. Our results corroborate previous work by Makepeace and Mladenich (1996), demonstrating how hot spots and hot moments of sewage pollution can occur within Flathead Lake's nearshore. Unlike previous studies, our work pairs human population, highly specific sewage indicator, periphyton community composition, fatty acid, and stoichiometric data to understand how differences in human disturbance are associated with differences in algal community structure as well as nutrition available to the lake's larger food web. While our data do not include higher trophic levels' response to changing resources, our data do suggest that grazing macroinvertebrate

communities would need to respond in some substantial way, either by migrating to where nutritious resources may be more abundant or altering community composition entirely to include more generalist or detritivorous grazers. In either event, our results suggest grazers requiring nutrition from diatom assemblages may be more vulnerable to increasing sewage pollution, especially in cases when tourism seasons may be extended or lakeside developments with decentralized sewage treatment increase in size.

3.7 Beyond Flathead: A Case for Increased Nearshore Monitoring

Our results stress the importance of nearshore monitoring for detecting sewage inputs in large lakes. Flathead Lake's pelagic is considered oligotrophic (Ellis et al. 2011), but increased tourism and development along the shoreline and within the watershed may increase potential for eutrophication hot spots or hot moments within the nearshore. While pelagic samples are useful for describing the lake's overall status, nearshore sampling can aid managers in identifying pollution sources before the entire system is affected. Flathead's large volume, short hydraulic residence time, and general lack of intense development likely buffer the system from abrupt perturbations. However, once eutrophication of the pelagic has started, mitigation and restoration can be complex (Jeppesen et al. 2005) and require system-specific interventions (Jacoby et al. 1991) with long term strategies (Tong et al. 2020). Several other large, deep, oligotrophic lakes, such as Baikal (Timoshkin et al 2016; 2018; Bondarenko et al. 2021; Meyer et al, Under Revision), Tahoe (Njora et al., 2019; Atkins et al., 2021), and Superior (Camillieri & Ozerky, 2019), have likewise experienced localized eutrophication in the nearshore. Many of these cases implicated sewage as the source of increased nutrient concentrations and filamentous algal abundance. However, incorporating sewage specific indicators, such as PPCPs as well as branched- and odd-chain fatty acids, may be necessary to directly associate ecological consequences with sewage pollution. As observed in Flathead Lake, repeated PPCP sampling

throughout the lake has the potential to define areas and times of greatest concern, especially in instances where wastewater treatment infrastructure and temporal swings in human population may vary. By pairing human population and sewage indicator data with co-located, contemporaneous biological data, managers can better contextualize and mitigate ecological consequences before they affect the entire lake. When compared across lakes, these same data can be useful tools to synthesize ramifications of sewage pollution, thereby enabling coordination between local, regional, and national management efforts.

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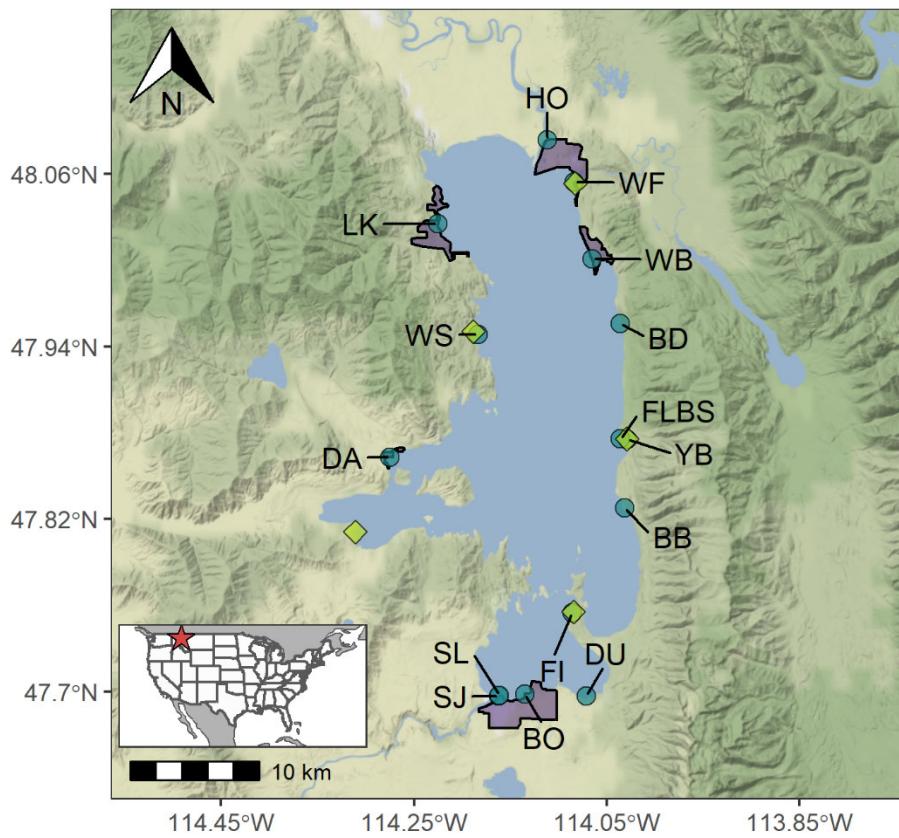


Figure 1: Map of sampling locations, State Parks, and major lakeside developments. Sampling locations are denoted by the blue circles and are labeled with the site name. HO and DU were considered reference sites, where HO was sampled within the Flathead River before it enters Flathead Lake and DU was sampled where there was no adjacent lakeside development. Green diamonds correspond to locations of five State Parks, from which we gather monthly data on visitor passes sold. Many sampling locations (blue circles) and State Parks (green diamonds) are co-located, and their respective symbols may overlap. Purple polygons refer to area delineated for five major lakeside developments (Bigfork, Lakeside, Wood's Bay, Dayton, and Polson).

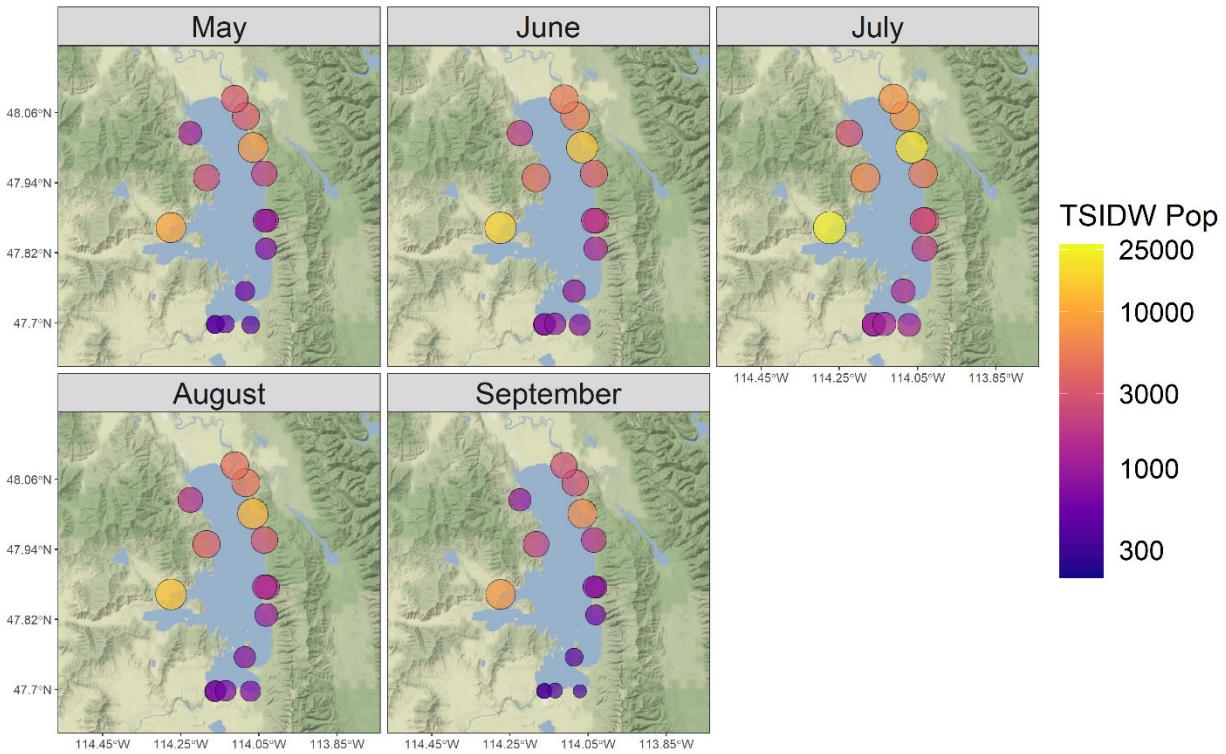


Figure 2: Temporally scaled inverse distance weighted (TSIDW) population for each sampling location and month. Larger, yellow points indicate sites with high TSIDW populations. Smaller, more purple points represent sites with lower TSIDW populations. Spatially, TSIDW population tended to be highest in the northeast portion of the lake, near the towns of Bigfork and Wood's Bay. Temporally, TSIDW population tended to be highest in July. TSIDW population values have been log-transformed to make sites more comparable across multiple orders of magnitude.

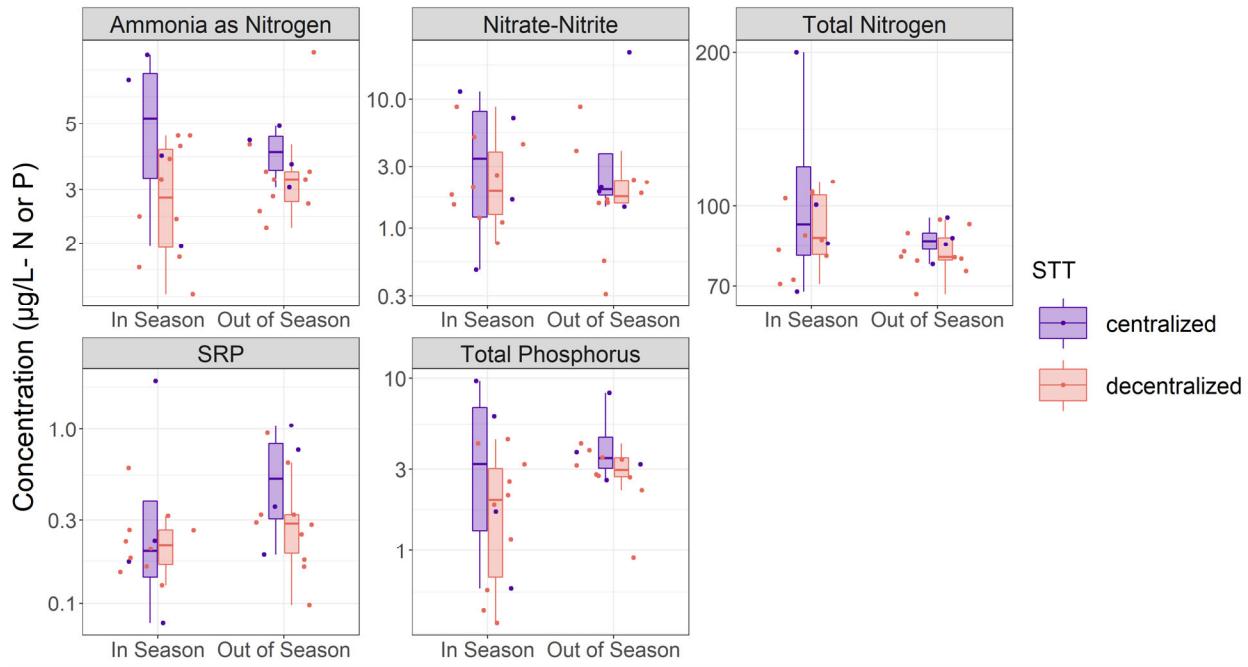


Figure 3: Littoral water column nutrient concentrations observed at each of the sampling locations. Concentrations are visualized on a log-scaled y-axis. While most nutrient concentrations were consistent between sewage treatment techniques and timepoints, SRP and Ammonia were often higher near sites with centralized sewage treatment in comparison to decentralized sewage treatment, although this may be a product of detection limits. When nutrient concentrations were below minimal detection limits (1.5 $\mu\text{g/L}$ for ammonia, nitrate/nitrite, and total phosphorus; 25 $\mu\text{g/L}$ for total nitrogen; and 0.8 $\mu\text{g/L}$ for SRP), we assigned a random value between zero and half of the minimal detection limit.

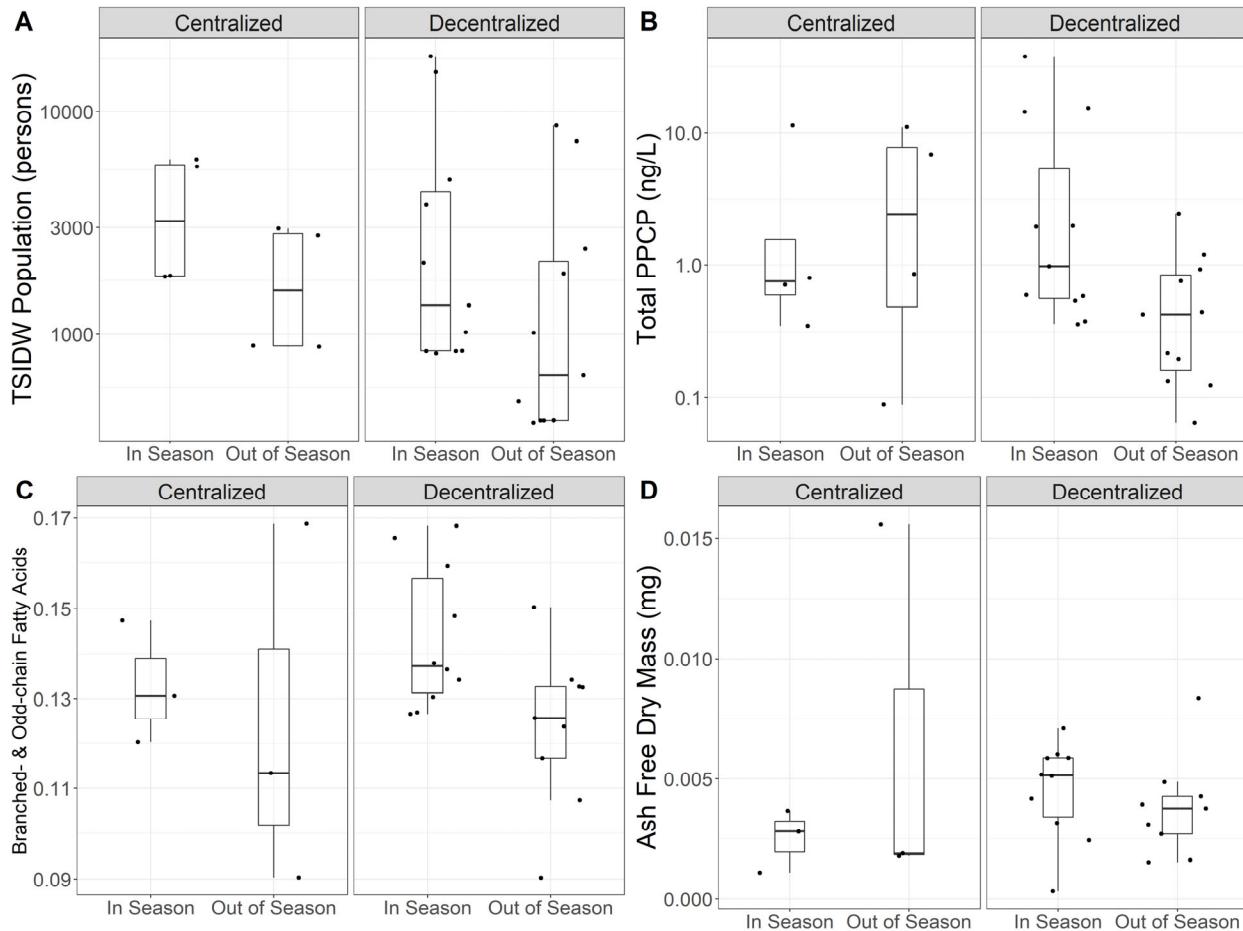


Figure 4: Boxplots of TSIDW population (A), total PPCP concentration (B), relative abundance of branched- and odd-chain fatty acids (C), and Ash Free Dry Mass (D) in response to tourism season (x-axis) and sewage treatment technique (faceted axis). To characterize overarching patterns, values are averaged within a sewage treatment technique and season for a given site. TSIDW Population (A) generally was higher at sites with centralized wastewater treatment and also decreased following the tourism season both for sites with centralized and decentralized wastewater treatment. Total PPCP concentrations (B) were often higher at sites with decentralized wastewater treatment during the tourism season than at those same sites following the tourism season. Periphyton branched- and odd-chain fatty acids (C) were higher during tourism season both for sites with centralized and decentralized wastewater treatment. These fatty acids are indicative of bacterial communities, which would be expected to be more abundant during times and at locations of increased wastewater loading. Ash Free Dry Mass (D), a proxy for periphyton productivity and detritus biomass, was consistent between sites in relation to sewage treatment technique and tourism season.

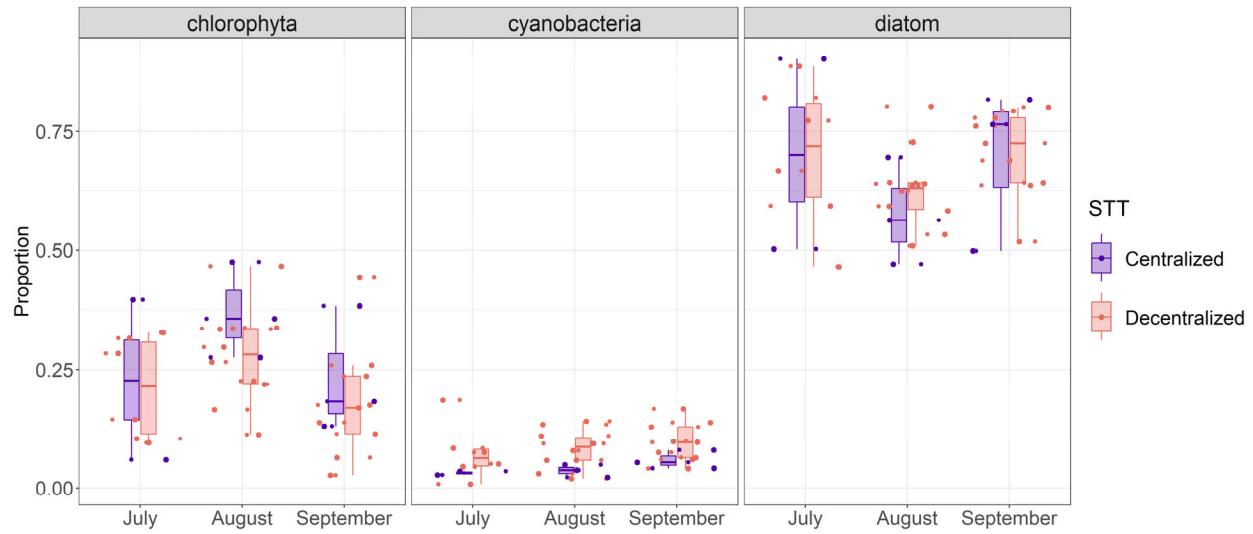


Figure 5: Boxplots of algal taxonomic group proportional abundance throughout the course of a summer. Sites with centralized wastewater treatment were generally dominated by chlorophyte and diatoms, whereas sites with decentralized treatment tended to have a more marked cyanobacteria abundance that gradually increased over the course of a summer.



Figure 6: NMDS with Bray-Curtis similarity of relative periphyton abundance. The NMDS biplot suggested that sites did not separate based on multivariate patterns in algal community composition. Rather, sites with centralized wastewater treatment tended to cluster at the lower end of NMDS2, which likely was driven by these sites having lower relative cyanobacterial abundance. This same pattern was observed in the univariate analysis of each taxon's relative abundance (Figure 5).

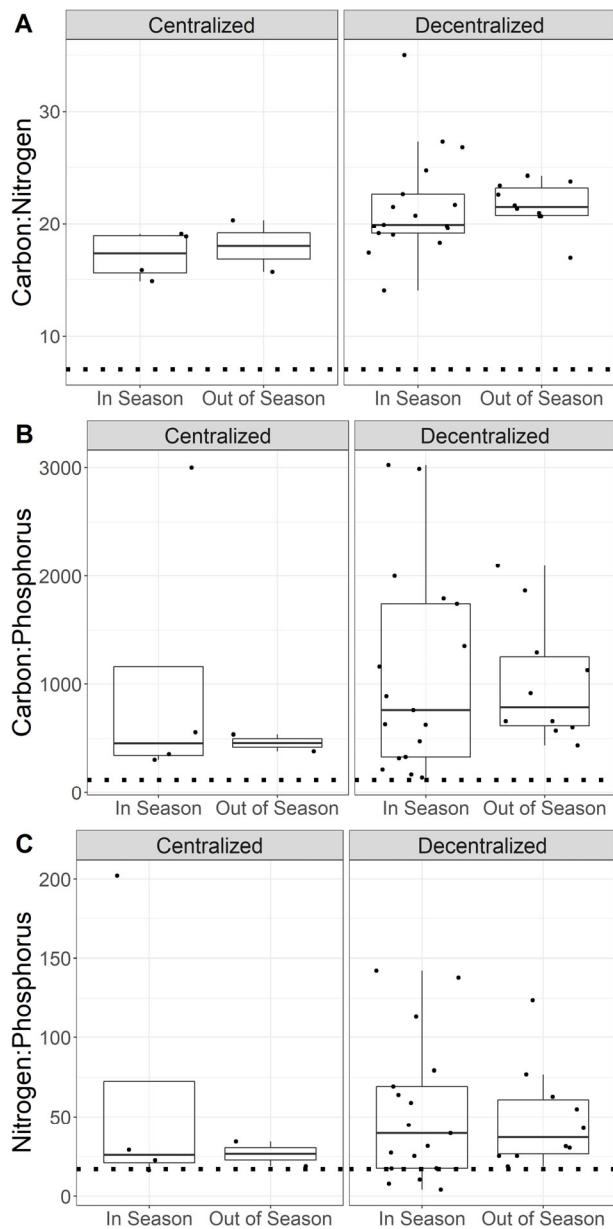


Figure 7: Molar Carbon:Nitrogen (A), Carbon:Phosphorus (B), and Nitrogen:Phosphorus (C) ratios for periphyton with samples groups by whether sites were located near centralized or decentralized wastewater treatment, or samples were collected during or following the tourism season. “In Season” denotes samples collected during June, July, or August, whereas “Out of Season” denotes samples collected in September. Dotted lines in each plot reflect the empirically derived stoichiometric ratios for non-nutrient limited growth of periphyton communities (i.e., 119:17:1; Hillebrand and Sommer 1999).

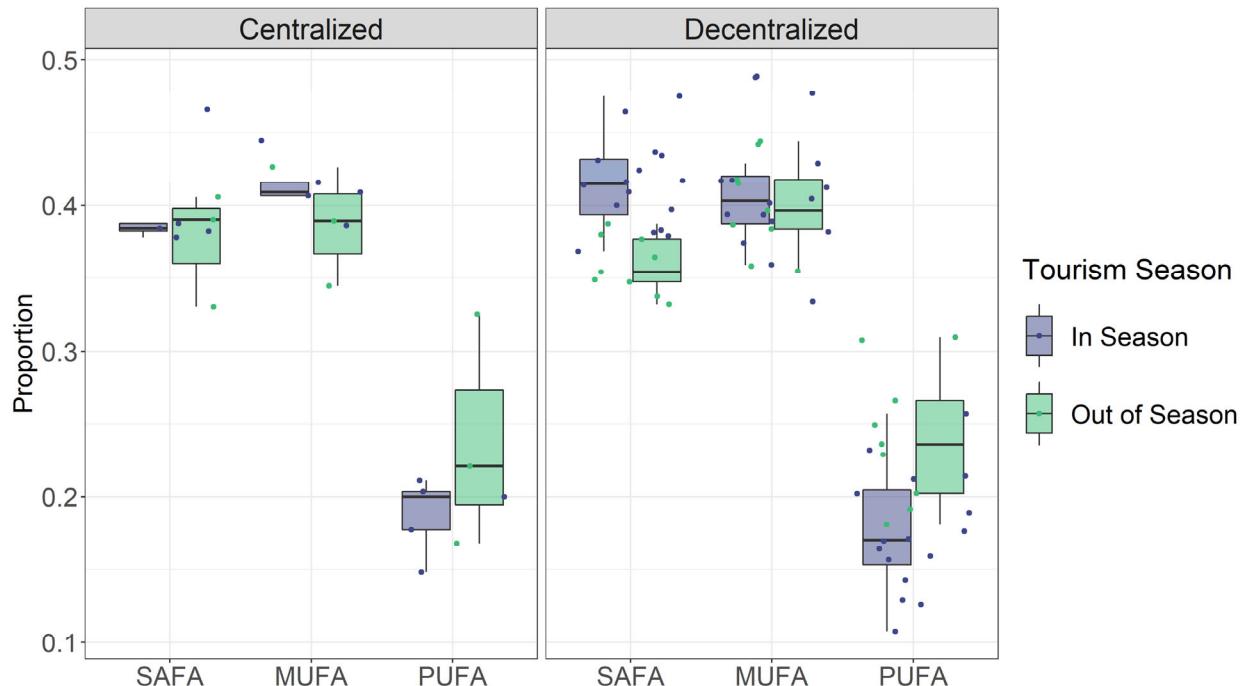


Figure 8: Relative concentrations of saturated (SAFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in periphyton with samples groups by whether sites were located near centralized or decentralized wastewater treatment or samples were collected during or following the tourism season. “In Season” denotes samples collected during June, July, or August, whereas “Out of Season” denotes samples collected in September.

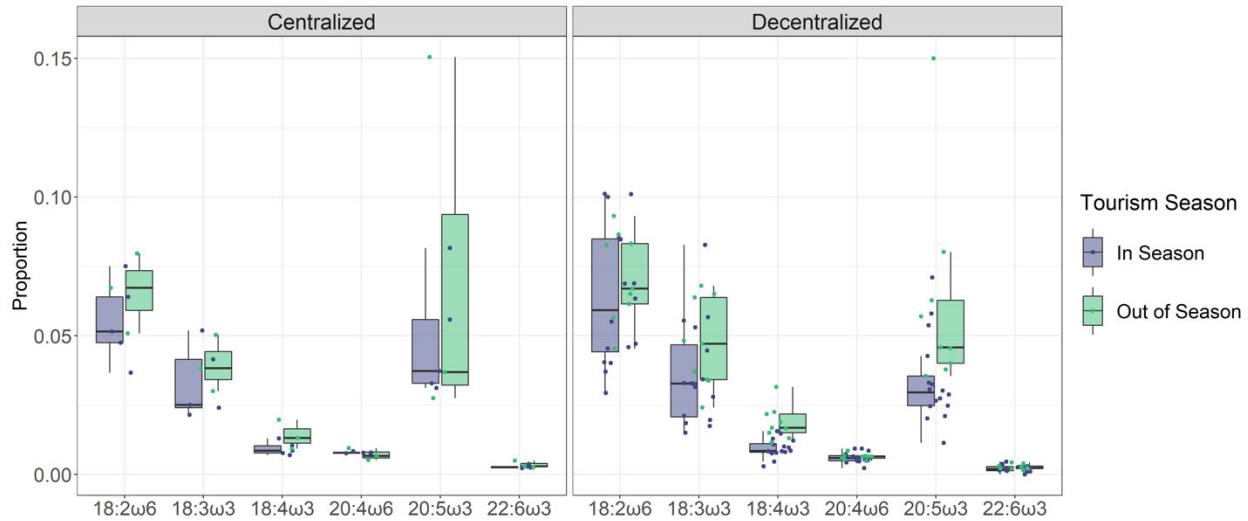


Figure 9: Relative concentrations of essential fatty acids (EFAs) in periphyton with samples groups by whether sites were located near centralized or decentralized wastewater treatment or samples were collected during or following the tourism season. “In Season” denotes samples collected during June, July, or August, whereas “Out of Season” denotes samples collected in September. EFAs are considered highly nutritious fatty acids for higher trophic levels and are primarily synthesized by primary producers. Greatest differences in EFAs between locations and timepoints occurred at sites with decentralized sewage treatment, which contained increased 20:5 ω 3 following the tourism season. Because fatty acids tend to map to algal community compositions, this increased 20:5 ω 3 signature was likely related with increased diatom abundance (Figure 5).

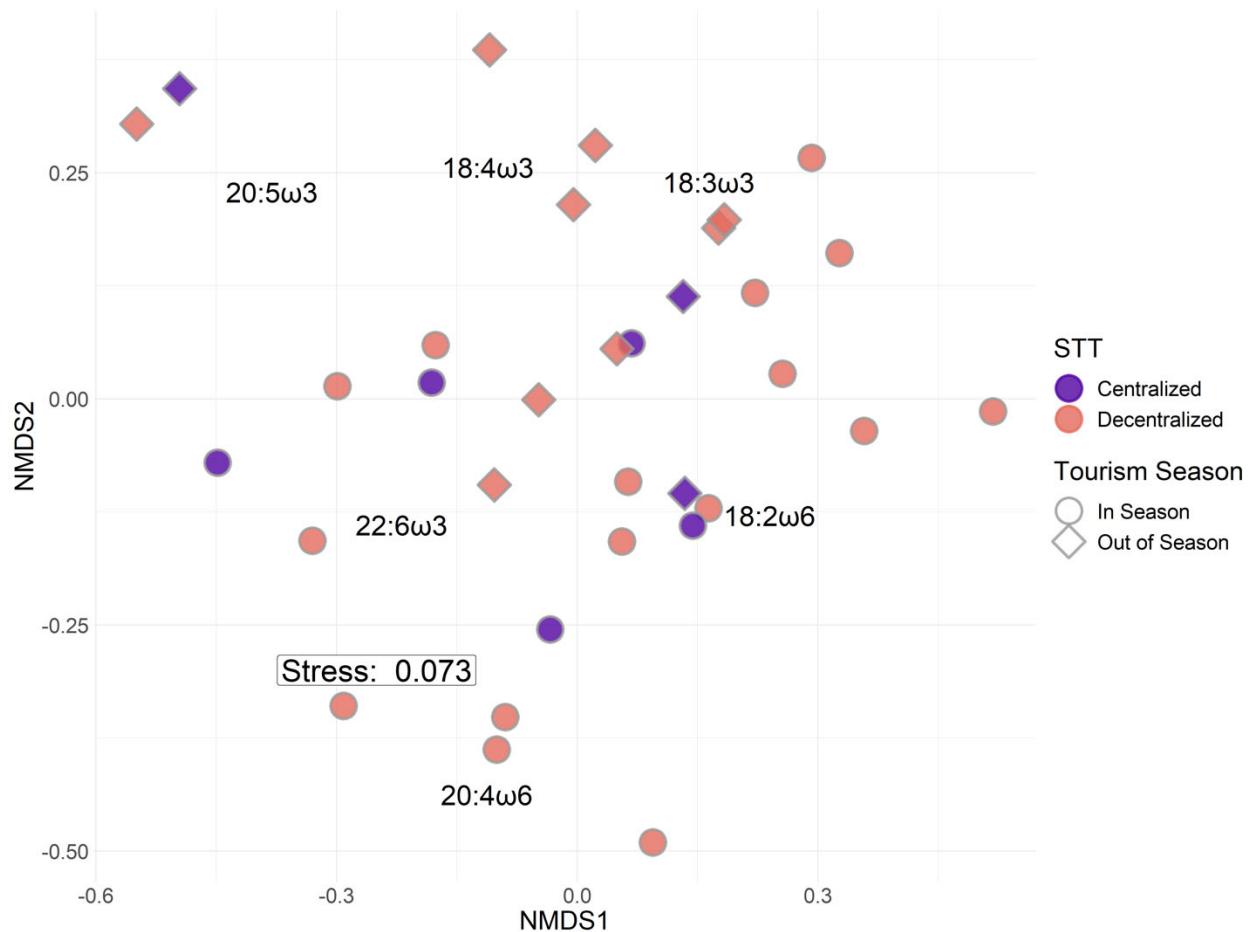


Figure 10: NMDS with Bray-Curtis similarity based on EFA relative abundance. The NMDS biplot suggested that sites separated by differences in tourism season (circles and diamonds). Post hoc SIMPER analyses suggested $20:5\omega 3$ relative abundance as the most influential EFA in driving point separation, which was likewise supported by the univariate analysis of EFA abundance (Figure 9).

| (A) Ammonia | | | | | |
|-----------------------------|----------------|--------------------|---------|---------|--|
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | 0.121 | 1 | 3.347 | 0.080 | |
| Tourist Season | 0.016 | 1 | 0.454 | 0.507 | |
| STT:Tourist Season | 0.038 | 1 | 1.040 | 0.318 | |
| Residuals | 0.866 | 24 | | | |
| (B) Nitrate/Nitrite | | | | | |
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | 0.255 | 1 | 1.058 | 0.314 | |
| Tourist Season | 0.059 | 1 | 0.243 | 0.627 | |
| STT:Tourist Season | 0.125 | 1 | 0.517 | 0.479 | |
| Residuals | 5.793 | 24 | | | |
| (C) SRP | | | | | |
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | 0.378 | 1 | 3.313 | 0.081 | |
| Tourist Season | 0.001 | 1 | 0.009 | 0.923 | |
| STT:Tourist Season | 0.013 | 1 | 0.114 | 0.739 | |
| Residuals | 2.741 | 24 | | | |
| (D) Total Nitrogen | | | | | |
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | 0.011 | 1 | 1.374 | 0.253 | |
| Tourist Season | 0.020 | 1 | 2.554 | 0.123 | |
| STT:Tourist Season | 0.003 | 1 | 0.316 | 0.579 | |
| Residuals | 0.191 | 24 | | | |
| (E) Total Phosphorus | | | | | |
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | 0.256 | 1 | 2.101 | 0.160 | |
| Tourist Season | 0.400 | 1 | 3.282 | 0.083 | |
| STT:Tourist Season | 0.018 | 1 | 0.148 | 0.704 | |
| Residuals | 2.922 | 24 | | | |

Table S1: ANOVA tables for nutrient concentrations in response to sewage treatment technique (STT) and tourist season.

| (A) PPCPs | | | | | |
|--------------------|----------------|--------------------|---------|---------|--|
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | 0.290 | 1 | 0.649 | 0.428 | |
| Tourist Season | 1.750 | 1 | 3.915 | 0.059 | |
| STT:Tourist Season | 0.932 | 1 | 2.085 | 0.161 | |
| Residuals | 11.624 | 26 | | | |

| (B) Branched- and Odd-Chain Fatty Acids | | | | | |
|--|----------------|--------------------|---------|---------|--|
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | < 0.001 | 1 | 0.307 | 0.585 | |
| Tourist Season | 0.002 | 1 | 4.565 | 0.045 | |
| STT:Tourist Season | < 0.01 | 1 | 0.353 | 0.559 | |
| Residuals | 0.008 | 21 | | | |

| (C) Ash Free Dry Mass | | | | | |
|------------------------------|----------------|--------------------|---------|---------|--|
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | < 0.001 | 1 | 0.039 | 0.845 | |
| Tourist Season | < 0.001 | 1 | 0.096 | 0.760 | |
| STT:Tourist Season | < 0.001 | 1 | 2.558 | 0.125 | |
| Residuals | < 0.001 | 21 | | | |

1 Table S2: ANOVA tables for sewage indicator values in response to sewage treatment technique
 2 (STT) and tourist season.

| (A) Periphyton Community Composition | | | | |
|---|----------------|--------------------|---------|---------|
| | Sum of Squares | Degrees of Freedom | F value | P value |
| STT | 0.127 | 1 | 0.127 | 0.127 |
| Tourist Season | 0.158 | 1 | 0.158 | 0.158 |
| Residuals | 2.459 | 30 | | |

| (B) Fatty Acids | | | | |
|------------------------|----------------|--------------------|---------|---------|
| | Sum of Squares | Degrees of Freedom | F value | P value |
| STT | 0.082 | 1 | 0.726 | 0.667 |
| Tourist Season | 0.311 | 1 | 2.775 | 0.008 |
| Residuals | 3.367 | 30 | | |

Table S3: PERMANOVA tables for periphyton community and multivariate fatty profiles in response to sewage treatment technique (STT) and tourist season.

| (A) Carbon:Nitrogen | | | | | |
|----------------------------|----------------|--------------------|---------|---------|--|
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | 85.606 | 1 | 5.830 | 0.022 | |
| Tourist Season | 0.215 | 1 | 0.015 | 0.904 | |
| STT:Tourist Season | 0.752 | 1 | 0.051 | 0.823 | |
| Residuals | 425.863 | 29 | | | |

| (B) Carbon:Phosphorus | | | | | |
|------------------------------|----------------|--------------------|---------|---------|--|
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | 233756.454 | 1 | 0.310 | 0.582 | |
| Tourist Season | 204497.198 | 1 | 0.271 | 0.606 | |
| STT:Tourist Season | 300369.615 | 1 | 0.399 | 0.533 | |
| Residuals | 21848264.461 | 29 | | | |

| (C) Nitrogen:Phosphorus | | | | | |
|--------------------------------|----------------|--------------------|---------|---------|--|
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | 25.980 | 1 | 0.012 | 0.915 | |
| Tourist Season | 728.571 | 1 | 0.328 | 0.571 | |
| STT:Tourist Season | 1570.836 | 1 | 0.707 | 0.407 | |
| Residuals | 64403.453 | 29 | | | |

Table S4: ANOVA tables for periphyton stoichiometric ratios in response to sewage treatment technique (STT) and tourist season.

| (A) PUFA | | | | | |
|--------------------|----------------|--------------------|---------|---------|--|
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | < 0.001 | 1 | 0.160 | 0.692 | |
| Tourist Season | 0.044 | 1 | 14.470 | 0.001 | |
| STT:Tourist Season | 0.001 | 1 | 0.256 | 0.617 | |
| Residuals | 0.089 | 29 | | | |
| (B) SAFA | | | | | |
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | < 0.001 | 1 | 0.070 | 0.793 | |
| Tourist Season | 0.019 | 1 | 20.519 | < 0.001 | |
| STT:Tourist Season | 0.001 | 1 | 1.618 | 0.213 | |
| Residuals | 0.026 | 29 | | | |
| (C) MUFA | | | | | |
| | Sum of Squares | Degrees of Freedom | F value | P value | |
| STT | < 0.001 | 1 | 0.046 | 0.832 | |
| Tourist Season | 0.002 | 1 | 1.016 | 0.322 | |
| STT:Tourist Season | < 0.001 | 1 | 0.249 | 0.622 | |
| Residuals | 0.044 | 29 | | | |

Table S5: ANOVA tables for periphyton relative fatty acid abundance in response to sewage treatment technique (STT) and tourist season. Prior to analysis, values were arcsine-square root transformed to normalize proportional data.

CHAPTER FOUR

A UNIFIED DATASET OF CO-LOCATED SEWAGE POLLUTION, PERIPHYTON,

AND BENTHIC MACROINVERTEBRATE COMMUNITY AND FOOD

WEB STRUCTURE FROM LAKE BAIKAL (SIBERIA)

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Key Words: pharmaceuticals, microplastics, fatty acids, stables isotopes, amphipod, mollusk, diatom, spirogyra

4.1 Abstract

Sewage released from lakeside development can introduce nutrients and micropollutants that can restructure aquatic ecosystems. Lake Baikal, the world's most ancient, biodiverse, and voluminous freshwater lake, has been experiencing localized sewage pollution from lakeside settlements. Nearby increasing filamentous algal abundance suggests benthic communities are responding to localized pollution. We surveyed 40-km of Lake Baikal's southwestern shoreline 19-23 August 2015 for sewage indicators, including pharmaceuticals, personal care products, and microplastics, with co-located periphyton, macroinvertebrate, stable isotope, and fatty acid samplings. The data are structured in a tidy format (a tabular arrangement familiar to limnologists) to encourage reuse. Unique identifiers corresponding to sampling locations are retained throughout all data files to facilitate interoperability among the dataset's 150+ variables. For Lake Baikal studies, these data can support continued monitoring and research efforts. For global studies of lakes, these data can help characterize sewage prevalence and ecological consequences of anthropogenic disturbance across spatial scales.

4.2 Background and Motivation

Globally, sewage pollution is a common and often concentrated source of nitrogen and phosphorus inputs that can reshape aquatic ecosystems. Sewage inputs are often associated with increased primary production (Edmondson 1970; Moore et al. 2003), which can eventually lead to nuisance algal blooms (Hall et al. 1999; Lapointe et al. 2015). Even in instances where sewage pollution is mitigated, restoring systems can be complicated and necessitate system-specific (Jeppesen et al. 2005), long-term mitigation strategies (Hall et al. 1999; Tong et al. 2020). As such, effective sewage monitoring can require merging a suite of chemical, biological, and ecological data to synthesize locations and timing of inputs with associated shifts in ecological communities (Rosenberger et al. 2008; Hampton et al. 2011).

Definitively identifying sewage as the source of excess nutrients in a system can be challenging. Nutrients can originate from multiple sources, such as agriculture (Powers et al. 2016) or melting permafrost (Turetsky et al. 2000; Anisimov and Reneva 2006; Moore et al. 2009), which can obfuscate wastewater signals. Unlike nutrients, sewage-specific indicators, such as enhanced $\delta^{15}\text{N}$ stable isotope signatures (Costanzo et al. 2001; Camilleri and Ozersky 2019), pharmaceuticals and personal care products (PPCPs) (Bendz et al. 2005; Rosi-Marshall and Royer 2012; Meyer et al. 2019) and microplastics (Barnes et al. 2009), can be highly specific to human wastewater. Accordingly, sewage-associated micropollutants have garnered global attention for their usefulness in identifying presence and quantifying magnitude of wastewater inputs. While indicators may accumulate differentially in certain taxa (Gartner et al. 2002; Green 2016; Vendel et al. 2017; Richmond et al. 2018), acutely dangerous concentrations are not common in most aquatic systems (Kolpin et al. 2002; Focazio et al. 2008; Yang et al. 2018). However, chronic exposure to microplastics and PPCPs at even minute concentrations (e.g., $\mu\text{g/L}$) can still disrupt ecological processes (Richmond et al. 2017). For example, oxazepam can increase feeding rate and decrease sociability of river perch (Brodin et al. 2013), and microplastics can release dissolved organic carbon, thereby altering microbial communities (Romera-Castillo et al. 2018). The pervasiveness and diversity of sewage-associated micropollutants in tandem with their potency as ecologically disrupting compounds necessitates investigation within and across systems, thereby enabling synthesis of how micropollutants alter ecosystems.

When assessing biological responses to increased nutrient loading, littoral benthic algal, and macroinvertebrate communities often respond most markedly, as their physical proximity to the shoreline puts them in the path of sewage pollution entering the lake (Rosenberger et al. 2008; Hampton et al. 2011). Filamentous algae, for example, can quickly increase in abundance near

sewage sources (Rosenberger et al. 2008; Hampton et al. 2011). As algal communities change, food webs can also restructure. For example, change in algal communities can alter the nutritional value of primary producers or cause changes in the relative abundance of different feeding groups (e.g., increased representation of detritivores). Among the suite of food quality metrics, availability of essential fatty acids (EFAs) offers a nuanced understanding of food quality as primary producers usually maintain consistent EFA signatures (Taipale et al. 2013) and consumers acquire EFAs by grazing (Dalsgaard et al. 2003) or trophic upgrading (Sargent and Falk-Petersen 1988; Dalsgaard et al. 2003).

Together, food web structure, community composition, and sewage indicator data can be powerful tools to assess biological impacts of sewage pollution. Despite their utility, these data are not often available for many limnological systems. PPCPs, for example, have historically been less measured in lake environments (Meyer et al. 2019). In instances where data are available, efficiently merging disparate data into a single, analytically-friendly format can be challenging and sometimes require complex, computationally intensive workflows (Meyer et al. 2020).

To offer a template for harmonizing sewage indicator and biological data, we present a unified data product, which contains disparate data collected from 14 littoral and 3 pelagic sites at Lake Baikal from 19 through 23 August 2015 (Figure 1). Located in Siberia, Lake Baikal is the oldest, most voluminous, and deepest freshwater lake in the world (Hampton et al. 2018). Lake Baikal also has the global distinction of being the most biodiverse lake, with the highest endemism (Moore et al. 2009). The lake is experiencing rapid warming associated with climate change, including decrease in ice cover duration (Moore et al. 2009), and it exhibits offshore plankton community changes associated with warming (Hampton et al. 2008; Katz et al. 2015; Izmest'eva et al. 2016). Less is known of the change occurring in the nearshore of Lake Baikal, where not only

climatic changes (Swann et al. 2020) but also human activity (Timoshkin et al. 2018) may introduce nutrients that alter the environment. Nearshore change is particularly important to understand in Lake Baikal, since the majority of the lake's biodiversity and endemic species occur in the littoral zone (Kozhova and Izmest'eva 1998). While Lake Baikal's pelagic zone is generally ultra-oligotrophic (Yoshida et al. 2003; O'Donnell et al. 2017), littoral areas abutting lakeside settlements have recently shown distinct signs of eutrophication, such as increased filamentous green algae abundance (Timoshkin et al. 2016; Volkova et al. 2018) as well as cyanobacteria (Bondarenko et al. 2021).

As a means of identifying sewage from small, concentrated lakeside towns and the associated ecological responses, we assembled a dataset consisting of over 150 variables collected at 14 littoral and 3 pelagic sampling sites. We structured the dataset in a tidy format, where each row is a sample, each column is a variable, and each CSV file is an observable unit, where more similar variables are contained within an individual file (Wickham 2014). Independent CSV files can be merged using unique locational identifiers as relational keys, enabling future researchers to customize analyses around a particular suite of variables. As a result of the dataset's interoperability, reproducibility, and extensive variable content, it is well poised for future reuse as supporting evidence of sewage pollution in Lake Baikal. Additionally, the data's flexibility and consistent structure enable it to be merged with similar datasets, so as to synthesize biological responses to sewage across systems and scales.

To our knowledge, no raw data on Lake Baikal macroinvertebrates, periphyton, or nearshore water quality are public in a machine-readable format, for any variable (i.e., abundance, fatty acid content, stable isotopes, nutrient and pollutant concentration), and no georeferenced data on pharmaceuticals and personal care products or microplastics appear to be publicly available for any

boreal, subarctic, or arctic lakes or rivers in Siberia. Thus, the dataset fills a substantial gap for future studies, providing a window into nearshore biotic assemblages and water quality in a unique, ancient ecosystem that holds 20% of the world's liquid surface water (Moore et al. 2009).

4.3 Data Description

The final, replicate-level data products are available on the Environmental Data Initiative (EDI), where they can be freely accessed without potential barriers such as paywalls or account registrations (Meyer et al. 2021). The final data are provided as 11 separate CSV files, each structured in a tabular format and containing a “site” column that can be used to merge tables. The repository also contains a compressed folder of R scripts (scripts.tar.gz), which were used in the main analysis of the dataset (Meyer et al., Under Review).

site_information.csv

This file contains metadata for each of the pelagic and littoral sampling locations. Missing data are assigned as NA.

year

Year sampling occurred.

month

Month sampling occurred.

day

Day of month sampling occurred.

time

Time sampling occurred as Hours:Minutes.

site

Unique alphanumeric identifier for a sampling location.

lat

Latitude of sampling location in decimal degrees.

long

Longitude of sampling location in decimal degrees.

site_description

Researchers' description of sampling location at the time of sampling.

distance_to_shore_m

Distance from *in situ* sampled location to the shoreline in meters.

depth_m

Maximum depth at sampling location in meters.

air_temp_celsius

Temperature of air at sampling location in Celsius.

surface_temp_celsius

Temperature of water's surface at sampling location in Celsius.

mid_temp_celsius

Temperature of water midway (i.e., $\text{depth_m}/2$) between surface and bottom at sampling location in Celsius.

bottom_temp_celsius

Temperature of water near sediment at sampling location in Celsius.

comments

Notes in the field describing sampling conditions.

shore_photo

Whether or not photos of the shoreline were taken. Photos are available on the project's Open Science Framework portal (Meyer et al. 2015).

substrate_photo

Whether or not photos of the substrate were taken.

sponges

Whether or not sponges were present at a sampling location.

brandtia

Whether or not *Brandtia* spp. (endemic amphipod species) were present at a sampling location.

distance_weighted_population_metrics.csv

This file contains inverse distance weighted, census-based human population data for each sampled location. Although the majority of sites do not have adjacent shoreline human developments, we calculated inverse distance weighted (IDW) population for each sampling location. IDW population is a generalized representation of the size of and proximity to a sampling location's neighboring human settlements. As these population estimates are based on census data, they reflect static populations and do not account for seasonal population deviations from tourism. A full description

of the methods used to calculate IDW population can be found in the companion manuscript Meyer et al. (Under Review).

site

Unique alphanumeric identifier for a sampling location.

distance_weighted_population

Inverse distance weighted population for a given sampling location and estimated as number of people. Because this interpolation process is a function of the size of and proximity to neighboring developed sites, values can contain decimal values.

[nutrients.csv](#)

This file contains nutrient concentrations for each of the associated sampling locations. Samples were collected at a depth of 0.75 m. Nutrient samples were not filtered prior to analysis, meaning that nitrogen concentrations have the potential to include intracellular nitrogen. Therefore, nitrogenous species' concentrations may be spurious. Minimal detection limits were estimated as 0.01 mg/L for nitrate, 0.005 mg/L for ammonium, and 0.04 mg/L for phosphorus.

site

Unique alphanumeric identifier for a sampling location.

replicate

Replicate for a given sampling location.

nh4_mg_dm3

Ammonium concentration in milligrams of ammonium per cubic decimeter.

no3_mg_dm3

Nitrate concentration in milligrams of nitrate per cubic decimeter

tp_mg_dm3

Total phosphorus concentration in milligrams of phosphorus per cubic decimeter.

tpo43_mg_dm3

Total phosphate concentration as phosphate in milligrams per cubic decimeter.

chlorophylla.csv

This file contains chlorophyll a concentrations in the water column as well as fluorometric corrections for each littoral and pelagic sampling location. Minimal detection limits were estimated to be 0.02 mg/L.

site

Unique alphanumeric identifier for a sampling location.

replicate

Replicate number.

filtered_volume_ml

Lake water volume filtered in milliliters for a given replicate.

sample_volume_ml

Sample volume filtered for chlorophyll a extraction.

raw_fluo

Raw, uncorrected fluorometric reading for chlorophyll analysis.

adjusted_raw

Corrected fluorometric reading for chlorophyll analysis.

chl_conc

Chlorophyll a concentration in milligrams per liter.

ppcp.csv

This file contains Pharmaceutical and Personal Care Product (PPCP) concentrations in the water column at each littoral and pelagic sampling location. Detection limits are estimated to be 0.001 µg/L based on a 500 mL sample volume.

site

Unique alphanumeric identifier for a sampling location.

paraxanthine

Concentration of paraxanthine, also known as 1,7-dimethylxanthine, in micrograms per liter.

Paraxanthine is the main human metabolite of caffeine.

acetaminophen

Concentration of acetaminophen, also known as paracetamol, in micrograms per liter.

amphetamine

Concentration of amphetamine in micrograms per liter.

caffeine

Concentration of caffeine in micrograms per liter.

carbamazepine

Concentration of carbamazepine in micrograms per liter.

cimetidine

Concentration of cimetidine in micrograms per liter.

cotinine

Concentration of cotinine, which is the main human metabolite of nicotine, in micrograms per liter.

diphenhydramine

Concentration of diphenhydramine in micrograms per liter.

mda

Concentration of methylenedioxymethamphetamine in micrograms per liter.

mdma

Concentration of methylenedioxymethamphetamine in micrograms per liter.

methamphetamine

Concentration of methamphetamine in micrograms per liter.

morphine

Concentration of morphine in micrograms per liter.

phenazone

Concentration of phenazone in micrograms per liter.

sulfachloropyridazine

Concentration of sulfachloropyridazine in micrograms per liter.

sulfamethazine

Concentration of *sulfamethazine* in micrograms per liter.

sulfamethoxazole

Concentration of sulfamethoxazole in micrograms per liter.

thiabendazole

Concentration of thiabendazole in micrograms per liter.

trimethoprim

Concentration of trimethoprim in micrograms per liter.

collection_year

Year sample was collected in the field.

collection_month

Month sample was collected in the field.

collection_day

Day of month sample was collected in the field.

analysis_year

Year sample was analyzed.

analysis_month

Month sample was analyzed.

analysis_day

Day of month sample was analyzed.

microplastics.csv

This file contains suspended microplastics counts for each of the pelagic and littoral sampling locations. Although we did not measure microplastic size, our enumeration techniques likely allowed us to reliably quantify microplastics as small as ~300 µm (Hanvey et al. 2017).

site

Unique alphanumeric identifier for a sampling location.

replicate

Replicate for a given sampling location. Replicate values of “C” indicate a control.

fragments

Number of microplastic fragments observed.

fibers

Number of microplastic fibers observed.

beads

Number of microplastic beads observed.

comments

Observer comments while enumerating microplastics.

volume_filtered_ml

Volume in milliliters for a given replicate filtered.

periphyton.csv

This file contains periphyton abundance data, collected from rocks at each of the sampled littoral locations. For poorly preserved samples, counts are listed as NA for each taxonomic grouping, and a note in the “comments” column is provided.

site

Unique alphanumeric identifier for a sampling location.

replicate

Replicate number for a given sampling site.

subsamples_counted

Number of 10 microliter subsamples counted for a given replicate.

diatom

Number of diatom cells counted for a given replicate.

spirogyra

Number of *Spirogyra* spp. cells counted for a given replicate.

spirogyra_filament

Number of *Spirogyra* spp. filaments counted for a given replicate.

ulothrix

Number of *Ulothrix* spp. cells counted for a given replicate.

ulothrix_filament

Number of *Ulothrix* spp. filaments counted for a given replicate.

tetrasporales

Number of Tetrasporales cells counted for a given replicate.

pediastrum

Number of *Pediastrum* spp. cells counted for a given replicate.

desmidales

Number of *Desmidales* spp. cells counted for a given replicate.

comments

Notes from the observer.

[invertebrates.csv](#)

This file contains abundance for benthic macroinvertebrates collected at each of the 14 littoral sampling locations. Only amphipod taxa were identified to species.

site

Unique alphanumeric identifier for a sampling location.

replicate

Replicate for sampling location. While three replicates were collected in the field, some samples were poorly preserved, and invertebrates were not enumerated so as to prevent potential errors.

Acroloxiidae

Mollusk family.

Asellidae

Isopod family.

Baicaliidae

Mollusk family.

Benedictidae

Mollusk family.

Brandtia_latissima

Endemic amphipod species. Three subspecies exist, but samples were not identified to subspecies to reduce potential errors.

Brandtia_parasitica_parasitica

Endemic amphipod species.

Caddisflies

General grouping; specimens were not identified to species.

Cryptoropus_inflatus

Endemic amphipod species.

Cryptoropus_pachytus

Endemic amphipod species.

Cryptoropus_rugosus

Endemic amphipod species.

Eulimnogammarus_capreolus

Endemic amphipod species.

Eulimnogammarus_cruentes

Endemic amphipod species.

Eulimnogammarus_cyaneus

Endemic amphipod species.

Eulimnogammarus_grandimanus

Endemic amphipod species.

Eulimnogammarus_juveniles

Endemic amphipod genus. Identification kept at genus level so as to prevent misclassification.

Eulimnogammarus_maackii

Endemic amphipod species.

Eulimnogammarus_maritiji

Endemic amphipod species.

Eulimnogammarus_verucossus

Endemic amphipod species.

Eulimnogammarus_viridis_viridis

Endemic amphipod species.

Eulimnogammarus_vittatus

Endemic amphipod species.

Flatworms

Not identified beyond phylum.

Leeches

Not identified beyond order, although 12 endemic species occur in Lake Baikal.

Maackia

Mollusk family.

Pallasea_brandtia_brandtia

Endemic amphipod species.

Pallasea_brandtii_tenera

Endemic amphipod species.

Pallasea_cancelloides

Endemic amphipod species.

Pallasea cancellus

Endemic amphipod species.

Pallasea viridis

Endemic amphipod species.

Planorbidae

Mollusk family.

Poekilogammarus crassimus

Endemic amphipod species.

Poekilogammarus ephippiatus

Endemic amphipod species.

Poekilogammarus juveniles

Endemic amphipod genus. Identification kept at genus level so as to prevent misclassification.

Poekilogammarus megonychus perpolitus

Endemic amphipod species.

Poekilogammarus pictus

Endemic amphipod species.

Valvatidae

Mollusk family.

stable_isotopes.csv

This file contains carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) values for various benthic macroinvertebrate genera and periphyton collected from the 14 littoral sampling locations.

site

Unique alphanumeric identifier for a sampling location.

Genus

Genus of the analyzed organism.

Species

Species of the analyzed organism. When an organism was identified solely to genus, the Species value is NA.

C13

Carbon ($\delta^{13}\text{C}$) stable isotope values in parts per thousand.

N15

Nitrogen ($\delta^{15}\text{N}$) stable isotope values in parts per thousand.

comments

Quality flag column where $\delta^{13}\text{C}$ samples were outside of the range of standards.

fatty_acid.csv

This file contains fatty acid concentrations for various benthic macroinvertebrate genera, periphyton, and endemic *Draparnaldia* spp. benthic algae collected from the 14 littoral sampling locations.

site

Unique alphanumeric identifier for a sampling location.

Genus

Genus of the analyzed organism.

Species

Species of the analyzed organism. When an organism was identified solely to genus, the Species value is NA.

c12_0

Concentration of 12:0 fatty acid as micrograms of fatty acid per milligram of tissue.

i_14_0

Concentration of i-14:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c14_0

Concentration of 14:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c14_1w5

Concentration of 14:1 ω 5 fatty acid as micrograms of fatty acid per milligram of tissue.

i_15_0

Concentration of i-15:0 fatty acid as micrograms of fatty acid per milligram of tissue.

a_15_0

Concentration of a-15:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c15_0

Concentration of 15:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c15_1w7

Concentration of 15:1 ω 7 fatty acid as micrograms of fatty acid per milligram of tissue.

i_16_0

Concentration of i-16:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_0

Concentration of 16:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_1w9

Concentration of 16:1 ω 9 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_1w8

Concentration of 16:1 ω 8 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_1w7

Concentration of 16:1 ω 7 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_1w6

Concentration of 16:1 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_1w5

Concentration of 16:1 ω 5 fatty acid as micrograms of fatty acid per milligram of tissue.

i_17_0

Concentration of i-17:0 fatty acid as micrograms of fatty acid per milligram of tissue.

a_17_0

Concentration of a-17:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c17_0

Concentration of 17:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c17_1w7

Concentration of 17:1 ω 7 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_2w7

Concentration of 16:2 ω 7 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_2w6

Concentration of 16:2 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_2w4

Concentration of 16:2 ω 4 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_3w6

Concentration of 16:3 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_3w4

Concentration of 16:3 ω 4 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_3w3

Concentration of 16:3 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_4w3

Concentration of 16:4 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c16_4w1

Concentration of 16:4 ω 1 fatty acid as micrograms of fatty acid per milligram of tissue.

c18_0

Concentration of 18:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c18_1w9

Concentration of 18:1 ω 9 fatty acid as micrograms of fatty acid per milligram of tissue.

c18_1w7

Concentration of 18:1 ω 7 fatty acid as micrograms of fatty acid per milligram of tissue.

c18_2w6t

Concentration of 18:2 ω 6t fatty acid as micrograms of fatty acid per milligram of tissue.

c18_2w6

Concentration of 18:2 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c18_3w6

Concentration of 18:3 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c18_3w3

Concentration of 18:3 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c18_4w4

Concentration of 18:4 ω 4 fatty acid as micrograms of fatty acid per milligram of tissue.

c18_4w3

Concentration of 18:4 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c18_5w3

Concentration of 18:5 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_0

Concentration of 20:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_1w9

Concentration of 20:1 ω 9 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_1w7

Concentration of 20:1 ω 7 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_2w5_11

Concentration of 20:2 ω 5-11 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_2w5_13

Concentration of 20:2 ω 5-13 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_2w6

Concentration of 20:2 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_3w6

Concentration of 20:3 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_4w6

Concentration of 20:4 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_3w3

Concentration of 20:3 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_4w3

Concentration of 20:4 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c20_5w3

Concentration of 20:5 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_0

Concentration of 22:0 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_1w9

Concentration of 22:1 ω 9 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_1w7

Concentration of 22:1 ω 7 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_2w6

Concentration of 22:2 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_4w6

Concentration of 22:4 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_5w6

Concentration of 22:5 ω 6 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_3w3

Concentration of 22:3 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_4w3

Concentration of 22:4 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_5w3

Concentration of 22:5 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c22_6w3

Concentration of 22:6 ω 3 fatty acid as micrograms of fatty acid per milligram of tissue.

c24_0

Concentration of 24:0 fatty acid as micrograms of fatty acid per milligram of tissue.

comments

Quality flag column. Two samples spilled during fatty acid extraction. These samples are flagged as such. Although concentrations are lower than other samples, proportions between fatty acids are consistent.

total_lipid.csv

This file contains gravimetry data for each fatty acid sample.

site

Unique alphanumeric identifier for a sampling location.

Genus

Genus of the analyzed organism.

Species

Species of the analyzed organism. When organism was identified solely to genus, the Species value is NA.

total_lipid_mg_per_g

Total amount of lipids in a sample in milligrams of lipid per gram of tissue.

deviation

Samples were weighed three times and standard deviation in measurement was calculated. All values are reported in milligrams of lipid per gram of tissue.

comments

Quality flag column. Two samples spilled during fatty acid extraction. These samples are flagged as such.

4.4 Data Availability

Data are available at the replicate level at the Environmental Data Initiative

(doi.org/10.6073/pasta/9554b7f19ddd4a614e854f18be978dca).

4.5 Methods

4.5.1 Site Information

The vast majority of Lake Baikal's 2,100-km shoreline lacks lakeside development (Moore et al. 2009; Timoshkin et al. 2016). Our sample collection focused on a 40-km section of Lake Baikal's southwestern shoreline, which included three settlements of different sizes (Figure 1) during a time of the year when tourism and summertime biological succession were likely at their annual peaks. Littoral locations were chosen to capture a range of sites with varying degrees of adjacent shoreline development – from “developed” (along the waterfront of human settlements) to “undeveloped” (no adjacent human settlements and complete forest cover; Figure 1). The largest, Listvyanka, is primarily a tourist town of approximately 2000 permanent residents, although tourism can contribute significantly to the town's population with approximately 1.2 million annual visitors (Interfax-Tourism 2018). The other two settlements are the villages Bolshie Koty and Bolshoe Goloustnoe, which have approximately 80 and 600 permanent residents, respectively. Bolshie Koty is home to two field research stations and several small tourist accommodations. Bolshoe Goloustnoe has several hotels and tourist camps.

To assess disturbance gradients and ecological responses from littoral-to-pelagic zones and laterally along the shoreline, our transect consisted of 17 sampling sites that were meant to

characterize differences along these gradients. Pelagic sites were located 2 to 5 km offshore from each of the developed sites in water depths of 900 to 1300 m (Figure 1; Table 1). All littoral sites were sampled at approximately the same depth (max depth of ~1.25 m) at a distance of 8.90 to 20.75 m from shore (Table 1), which allowed us to collect samples without the need for SCUBA but precluded us from sampling deeper littoral environments. Due to this constraint, only littoral sites contain macroinvertebrate and algal samples. Otherwise, data are available for both littoral and pelagic sites. At each site, air temperature was measured with a mercury thermometer, and photographs were taken of the substrate and the shoreline. Visual inspection of substrate photographs suggested that littoral sites' substrate was consistent among sites and generally was characterized by large, oblate rocks and gravel.

4.5.2 Inverse Distance Weighted (IDW) Population Calculation for Each Sampling Location

We recognized that sewage indicator concentrations at each sampling location may be related to a sampling location's spatial position relative to both the size and proximity of neighboring developed sites. Therefore, we created the inverse distance weighted (IDW) population metric to compress, into a single metric, information about human population size, density, and location along the shoreline as well as distance between developed sites and sampling locations.

Our workflow for calculating IDW population required five steps. First, we traced polygons of each lakeside development's perimeter and line geometries of each development's shorelines from satellite imagery for each developed site in Google Earth. Polygons were traced for the entire area of visible development. Similarly, shoreline traces only reflected shoreline length for which there was visible development. Second, polygon and line geometries were downloaded from Google Earth as a .kml file. Third, the .kml file was imported into the R statistical environment (R Core Team 2019), where using the sf package (Pebesma 2018) we calculated shoreline length,

polygon area, and centroid location for each developed site. Fourth, we joined point locations of each sampling site with the spatial polygons to calculate the distance from each sampling location to each developed site's centroid. Fifth, we calculated IDW population for each sampling location, using formula (1)

$$(1) \quad I_j = \frac{P_{LI} * L_{LI}}{A_{LI}} + \frac{P_{BK} * L_{BK}}{A_{BK}} + \frac{P_{BGO} * L_{BGO}}{A_{BGO}}$$

where I is the IDW population at sampling location j , P is the population at each of the three developed sites Listvyanka (LI), Bolshie Koty (BK), Bolshoe Goloustnoe (BGO), A is the area of a developed site in km^2 , L is the shoreline length at a developed site in km, and D is the distance from developed site j to each developed site's centroid in km. As these population estimates are based on census data, they reflect current, static populations and do not account for seasonal population swings from tourism.

4.5.3 Nutrients

Water samples for nutrient analyses were collected in 150 mL glass jars that had been washed with phosphate-free soap and rinsed three times with water from the sampling location. Samples were collected at a depth of approximately 0.75 m in duplicates and immediately frozen at -20°C until processing at the A. P. Vinogradov Institute of Geochemistry (Siberian Branch of the Russian Academy of Sciences, Irkutsk). Samples were not filtered prior to freezing, meaning that nitrogen and ammonium concentrations may include intracellular nitrogen and overestimate dissolved nitrogenous forms in the water column.

For ammonium (RD:52.24.383-2018 2018) and nitrate (RD:52.24.380-2017 2018) concentrations, samples were analyzed with a spectrophotometer (SF-26). GSO 7258-96 and 7259-96 standards of 1 g/L stock concentration were used to calibrate nitrate and ammonium

measurements, respectively. When nitrate and ammonium analyses could be performed within 24 h after thawing, samples were kept at 2-8°C without addition of preservative agents. When nitrate analyses were performed between 24-48 h after thawing, samples were kept at 3-5°C and chloroform was added as a preservative at a ratio of 2-4 mL per 1 L of sample volume. When ammonium analyses were performed within 24-96 h after thawing, samples were kept at 3-5°C and ~10% sulfuric acid solution was added as a preservative. Phosphorus concentration was measured with a spectrophotometer (SF-46) following the addition of persulfate (GOST:18309-2014 2016). When possible, samples were analyzed within three hours of thawing. When analyses could not be performed within three hours, samples were kept at 3-5°C and chloroform was added as a preservative at a ratio of 2-4 mL per 1 L of sample volume. Minimal detection limits were estimated as 0.01 mg/L for nitrate, 0.005 mg/L for ammonium, and 0.04 mg/L for phosphorus. Concentrations are reported in mg/L of each analyte.

For comparable methods in English, we recommend data users consult International Standards Organization (ISO) (1984) and ISO (2004) as analogs. Copies of the Russian-language methods are included in the Open Science Framework portal within the directory “Nearshore _ sampling/methods”.

4.5.4 Chlorophyll a

Water samples were collected in 1.5 L plastic bottles from a depth of approximately 0.75 m. Although we did not note the plastic bottles’ materials within the field, all bottles for chlorophyll a measurement were cleaned, beverage bottles and likely made of polyethylene terephthalate. Within 12 h of collection, three subsamples (up to 150 mL each) were filtered through 25-mm diameter, 0.2 µm pore size nitrocellulose filters. Filters were then placed in a 35 mm petri dish, which was wrapped with aluminum foil to prevent light exposure, and frozen in the dark until processing.

Chlorophyll samples were processed in a manner similar to that of Welschmeyer (1994). Nitrocellulose filters were ground in 10 mL of 90% HPLC-grade acetone, in which chlorophyll extraction was allowed to proceed overnight. Chlorophyll extract was then analyzed using a Turner Designs 10-AU fluorometer (Turner Design, Sunnyvale, CA) using an excitation wavelength of 436 nm and emission of 680 nm. 10-AU Secondary Solid Standard (P/N 10-AU-904) was used to calibrate fluorometer prior to samples being processed. Blank samples registered a raw fluorescence of approximately 0.1 FL units. Concentrations were calculated using formula 2

$$(2) \text{Chlorophyll concentration} = (\text{extract reading} - \text{blank reading}) * \frac{\text{mL of extract}}{\text{mL of filtered sample}}.$$

Detection limits are estimated to be approximately 0.02 mg/L. Concentrations are reported as mg/L.

4.5.5 Pharmaceuticals and Personal Care Products (PPCPs)

Water samples for PPCP analysis were collected in 250 mL amber glass bottles that were rinsed with either methanol or acetone and then three times with sample water prior to collections. Following collection, samples were refrigerated and kept in the dark until solid phase extraction (SPE).

Within 12 h of collection, samples were filtered directly from the amber glass bottle using an in-line Teflon filter holder with glass microfiber GMF (1.0 μm pore size, WhatmanGrad 934-AH) in tandem with a solid phase extraction (SPE) cartridge (200 mg HLB, Waters Corporation, Milford, MA) connected to a 1-liter vacuum flask. Lab personnel wore gloves and face masks to minimize contamination. Prior to filtration, SPE cartridges were primed with at least 5 mL of either methanol or acetone and then washed with at least 5 mL of sample water. Rate of extraction was maintained at approximately 1 drop per second. Extraction proceeded until water could no longer pass through the SPE cartridge or until all collected water was filtered. Cartridges were stored in Whirlpacks at -20°C until analysis for 18 PPCP residues using liquid chromatography tandem mass

spectrometry (LC-MS-MS) following methods of Lee et al. (2016) and D’Alessio et al (2018) with labeled internal standards (¹³C₃-caffeine, methamphetamine-d8, MDMAd8, morphine-d3, and ¹³C₆-sulfamethazine). Detection limits are estimated to be 0.001 µg/L based on a 500 mL sample volume. Concentrations are reported in µg/L.

4.5.6 Microplastics

At each location, samples were collected at a depth of approximately 0.75 m in triplicate using 1.5 L clear plastic bottles that were washed thoroughly with sample water before each collection. Samples were collected by hand for each littoral site and with a metal bucket from aboard the ship for pelagic sites.

For processing, each sample was vacuum filtered on to a 47-mm diameter GF/F filter. During filtration, aluminum foil was used to cover the filtration funnel to prevent contamination from airborne microplastic particles. After filtration, filters were dried under vacuum pressure and then stored in 50-mm petri dishes. Following filtration of all three replicates, the filtrate was collected and then re-filtered through a GF/F filter as a control for contamination from the plastic vacuum funnel or potentially airborne microplastics.

Microplastic counting involved visual inspection of the entire GF/F in a similar manner to methods described in Hanvey et al. (2017). Visual enumeration was conducted under a stereo microscope with ~100x magnification, and microplastics were classified into one of three categories: fibers, fragments, or beads. For all categories, plastics were defined as observed objects with apparent artificial colors, so as to not enumerate plastics potentially contributed from the sampling bottle itself. Fibers were defined as smooth, long plastics with consistent diameters. Fragments were defined as plastics with irregularly sharp or jagged edges. Beads were defined as spherical plastics. Although we did not measure microplastic size, this technique likely allowed us

to reliably quantify microplastics as small as ~300 µm (Hanvey et al. 2017). During enumeration, GF/Fs remained covered in the petri dish to minimize potential for contamination from the air.

It is worth noting that since the time of our field sampling, evidence has accumulated that our methods likely dramatically underestimated microplastic abundance (Wang and Wang 2018; Brandon et al. 2020). Recent investigations of microplastics in Lake Baikal near Bolshie Koty (BK) used analogous methods and measured similar microplastic concentrations (Karnaughov et al. 2020). Future studies aiming to use these data for comparison or supplementing potential data gaps should consider the minimum microplastic size that could be reliably detected by the method, so as to ensure data are comparable across methods.

4.5.7 Periphyton collection and Abundance Estimates

At each littoral site, we haphazardly selected three rocks representative of local substrate. A plastic stencil was used to define a surface area of each rock from which we scraped a standardized 14.5 cm² patch of periphyton. Samples were preserved with Lugol's solution and stored in plastic scintillation vials. Additional periphyton was collected in composite from each site for fatty acid and stable isotope analysis.

Periphyton taxonomic identification and enumeration was performed by subsampling 10 µL aliquots from each preserved sample, containing approximately 10-15 mL of preserved periphyton. For all 10 µL aliquots, cells, filaments, and colonies were counted, for the entire subsample, until at least 300 cells were identified for a given sampling replicate. If the first aliquot contained less than 300 cells, we counted additional subsamples until we reached at least 300 cells in total. In instances when 300 cells were counted before finishing a subsample, we still counted the entire aliquot. Taxa were classified into broad categories consistent with Baikal algal taxonomy (Izboldina 2007),

using coarse groupings to capture general patterns in relative algal abundance. As a result, algal groups consisted of diatoms, *Ulothrix* spp., *Spirogyra* spp., and the green algal Order Tetrasporales.

Separate periphyton samples for stable isotope and fatty acid analyses were also collected. Instead of preserving samples in Lugol's solution, these samples were immediately frozen at -20°C at the field station. The samples were later transferred to the lab in the U.S. via a Dewar flask with dry ice.

4.5.8 Benthic Macroinvertebrate Collection and Abundance Estimates

Three kick-net samples were collected for assessment of benthic community composition and abundance. Using a D-net, we collected macroinvertebrates by flipping over 1-3 rocks, and then sweeping five times in a left-to-right motion across approximately 1 m. After the series of sweeps, the catch was rinsed into a plastic bucket. For each replicate, bucket contents were concentrated using a 64-µm mesh and placed in glass jars with 40% ethanol (vodka; the only preservative available to us at the time) for preservation and refrigerated at 4°C aboard the research vessel. The 40% ethanol preservative was replaced with ~80% ethanol upon return to the lab within 24 to 48 hours, and samples were stored at ~4°C.

Invertebrate taxonomic identification and enumeration were performed under a stereo microscope. All adult amphipods were identified to species according to Takhteev and Didorenko (2015), whereas juveniles were identified to genus. Mollusks were identified to the family level according to Sitnikova (2012). Leeches were enumerated at the subclass level, but were likely all from the family Glossiphoniidae based on size, depth of sampling locations, and invertebrate communities sampled (Kaygorodova 2012). Like mollusks, caddisflies were also enumerated at the order level, although Baikal does contain over 14 species of caddisfly (Valuyskiy et al. 2020). Flatworms were enumerated at the phylum level. All isopods enumerated were from the family

Asellidae. Aside from having limited time available to spend with Baikal taxonomists during our field campaign, our choice of taxonomic resolution ultimately was a result of relative abundance for each taxonomic group, where amphipods were the most abundant taxa and flatworms were among the least abundant taxa across all sites. All samples contained oligochaetes and polychaetes, but due to poor preservation, these taxa were not counted. Six samples of the 42 collected were not well-preserved and were excluded from further analyses, in order to reduce errors in identification. KD-1 and LI-1 were the only sites with 1 sample counted. BK-2 and KD-2 each had two samples counted.

Separate collections were conducted for invertebrate fatty acid and stable isotope analyses. Invertebrates were collected using a D-net and by hand. Organisms collected by hand included amphipod species that were observed from the community composition D-net collections but not readily observed in the stable isotope and fatty acid D-net collections. Collected organisms were live-sorted, identified to species, and then frozen at -20°C at the field station. The samples were later transferred to the lab in the U.S. via a Dewar flask with dry ice.

Due to some samples warming in transit, we only processed samples that were completely frozen upon arrival to the United States. Given the potential for fatty acids to highlight more subtle, multivariate ecological responses along our transect than stable isotopes, we prioritized both periphyton and macroinvertebrate fatty acid analyses over stable isotope analyses. As such, there is an imbalance across species' abundance, stable isotope, and fatty acid data. Dominant taxa, such as *E. veruccosus* and *E. vittatus*, though have paired data throughout the transect, whereas less common taxa, such as *Brandtia* spp., only have abundance estimates. Table 2 summarizes data available for each variable and taxonomic group.

4.5.9 Stable Isotope Analysis

Following freeze-drying, measurements of periphyton and macroinvertebrate $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were performed on an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS; Finnigan DELTAplus XP, Thermo Scientific) at the Large Lakes Observatory, University of Minnesota Duluth. Stable isotope values were calibrated against certified reference materials including L-glutamic acid (NIST SRM 8574), low organic soil and sorghum flour (standards B-2153 and B-2159 from Elemental Micro-analysis Ltd., Okehampton, UK) and in-house standards (acetanilide and caffeine).

4.5.10 Fatty Acid Analysis

Following freeze-drying, samples were transferred to 10 mL glass centrifuge vials, and 2 mL of 100% chloroform was added to each under nitrogen gas. Samples were allowed to sit in chloroform overnight at -80°C. Fatty acid extractions generally involved three phases: (1) 100% chloroform extraction, (2) chloroform-methanol extraction, and (3) fatty acid methylation. Fatty acid extraction methods were adapted from Schram et al. (2018).

After overnight chloroform extraction, samples underwent a chloroform-methanol extraction three times. To each sample, we added 1 mL cooled 100% methanol, 1 mL chloroform:methanol solution (2:1), and 0.8 mL 0.9% NaCl solution. Samples were inverted three times and sonicated on ice for 10 minutes. Next, samples were vortexed for 1 minute, and centrifuged for 5 minutes (3,000 rpm) at 4°C. Using a double pipette technique, the lower organic layer was removed and kept under nitrogen. After the third extraction, samples were evaporated under nitrogen flow, and resuspended in 1.5 mL chloroform and stored at -20°C overnight.

Once resuspended in chloroform, 1 mL of chloroform extract was transferred to a glass centrifuge tube with a glass syringe as well as an internal standard of 4 μL of 19-carbon fatty acid.

Samples were then evaporated under nitrogen, and then 1 mL of toluene and 2 mL of 1% sulfuric acid-methanol was added. The vial was closed under nitrogen gas and then incubated in 50°C water bath for 16 hours. After incubation, samples were removed from the bath, allowed to reach room temperature and stored on ice. Next, we performed a potassium carbonate-hexane extraction twice. To each sample, we added 2 mL of 2% potassium bicarbonate and 5 mL of 100% hexane, inverting the capped vial so as to mix the solution. Samples were centrifuged for 3 minutes (1,500 rpm) at 4°C. The upper hexane layer was then removed and placed in a vial to evaporate under nitrogen flow. Once almost evaporated, 1 mL of 100% hexane was added and stored in a glass amber autosampler vial for GC/MS quantification. GC/MS quantification was performed with a Shimadzu QP2020 GC/MS following Schram et al. (2018). As part of our peak quantification protocol, we quantified and identified every lipid compound that showed up in the chromatogram. Each sample contained peaks that were associated with known fatty acids, and among the 59 fatty acids contained in our dataset, few fatty acids were completely absent from a sample. Consequently, it is difficult for us to definitively ascribe a minimal detection limit to this analysis, but based on standards used, we estimate that this procedure had a minimal detection limit of 1 ng/mL.

Following methylation, remaining extracts were assessed for total lipid masses. Remaining sample extracts (~0.5 mL) were allow to evaporate to dryness under a fume hood overnight. Dried samples were then left in a weigh room to acclimatize for 30-60 mins and then massed within the scintillation vials. To calculate an average lipid mass, samples were massed three times, so as also to assess deviation in measurements. Lipid gravimetry is reported as the mg of lipids per g of dry-weight tissue.

4.6 Technical Validation

The dataset had three main validation procedures: taxonomic, analytical, and reproducible.

For taxonomic validation, all phylogenetic groupings were based off most recent identification keys. Amphipods were identified according to Takhteev & Didorenko (2015). Mollusks were identified according to Sitnikova (2012). Algal taxa were identified according to Izhboldina (2007). For consistency, all taxa were identified by one person (Michael F. Meyer), who was trained by experts in Baikal algal and macroinvertebrate taxonomy.

For analytical validation, internal standards were used for all mass-spectroscopy analyses. PPCP analyses involved labeled internal standards ($^{13}\text{C}_3$ -caffeine, methamphetamine-d8, MDMA-d8, morphine-d3, and $^{13}\text{C}_6$ -sulfamethazine). Stable isotope values were calibrated against certified reference materials including L-glutamic acid (NIST SRM 8574), low organic soil and sorghum flour (standards B-2153 and B-2159 from Elemental Micro-analysis Ltd., Okehampton, UK) and in-house standards (acetanilide and caffeine). Replicate analyses of external standards showed a mean standard deviation of 0.06 ‰ and 0.09 ‰, for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Finally, fatty acid estimations used an internal 19:0 standard to assess oxidation of fatty acids during extraction, methylation, and quantification.

For data reproducibility, data aggregation and harmonization procedures were conducted in the R statistical environment (R Core Team 2019), using the tidyverse (Wickham et al. 2019) packages. As part of the data aggregation, an initial cleaning script (00_disaggregated_data_cleaning.R) removed incorrect spellings, erroneous data values, and inconsistent column names from raw data. This step created the standardized CSV files detailed above, which are available on the EDI repository (Meyer et al. 2021). Raw data files are available on the project's Open Science Framework portal (Meyer et al. 2015) but are not included in the EDI repository to prevent confusion or incorrect usage. Data hosted on EDI are at the replicate-level but can be aggregated to the sampling-site-level using script "01_data_cleaning.R". In addition to

aggregation scripts, six R scripts used for analyses in Meyer et al. (*Under Review*) are also available on the EDI repository within the compressed entity “scripts.tar.gz”. All R code for data aggregation was written by one person (Michael F. Meyer) and then independently reviewed by two others (Matthew R. Brousil and Kara H. Woo) to confirm that code performed as intended, was well documented, and annotations were complete.

A commitment to FAIR and TRUST principles

Throughout the dataset’s development, we strove to incorporate both FAIR (Findable, Accessible, Interoperable, and Reproducible) and TRUST (Transparency, Responsibility, User Focus, Sustainability, and Technology) principles where applicable.

With respect to FAIR principles (Wilkinson et al. 2016), the data are openly accessible in a standardized, replicate-level format on the EDI portal. The 11 CSV files contained within the dataset are entirely interoperable using the “site” column, enabling all variables to efficiently be merged together. Finally, all analytical and some data wrangling scripts are available on the EDI portal in a compressed format, such that future users can reproduce data manipulation and analyses described in Meyer et al. (*Under Review*).

With respect to TRUST principles (Lin et al. 2020), we strove to document additional metadata and data-cleaning practices in a public Open Science Framework (OSF) repository (Meyer et al. 2015). These steps are not necessarily critical to the core EDI dataset, but provide increased transparency for future users wishing recreate the dataset de novo. All “raw” data are provided in the OSF portal, including an initial cleaning script (00_disaggregated_data_cleaning.R) to remove incorrect spellings, erroneous data values, and inconsistent column names. This repository also includes photographs of both field notes as well as photographs of shoreline and substrate from

sampling locations. To empower and expedite future reuse, all directories are accompanied with documentation that details directory contents, and all associated scripts are documented and annotated. While many of the files are redundant from the EDI repository, the OSF repository is meant to supplement the EDI repository, so as to enable sustainable, user-focused transparency of how data were collected and cleaned from their raw formats.

4.7 Data Use and Recommendations for Reuse

Recognizing the potential for continued low-level, sewage pollution at Lake Baikal (Timoshkin et al. 2016, 2018; Volkova et al. 2018) and lakes worldwide (Yang et al. 2018; Meyer et al. 2019), the final dataset can be applied to a suite of research questions pertaining to ecological responses to human disturbance. We highlight two main areas for immediate application.

First, the final data products can be harmonized with other littoral sampling efforts throughout Lake Baikal, so as to enhance spatial coverage and data diversity. Since 2010, Lake Baikal has experienced increasing filamentous algal abundance, especially near larger lakeside developments (Kravtsova et al. 2014; Timoshkin et al. 2016, 2018; Volkova et al. 2018). Recent benthic algal surveys throughout Lake Baikal's entirety, but especially near our sampling locations, have suggested that cosmopolitan filamentous algae, such as *Spirogyra* spp., tend to be more abundant near larger lakeside developments (Timoshkin et al. 2016; Volkova et al. 2018). For example, Listvyanka is a small town located at the beginning of the Angara River, Lake Baikal's only surface outflow. While Listvyanka's permanent population is approximately 2,000 persons, the town is a growing tourism hub, and hosts over 1.2 million tourists per year (Interfax-Tourism 2018). Surveys conducted near Listvyanka have suggested increased *Spirogyra* spp. abundance is associated with wastewater release (Timoshkin et al. 2016). Although wastewater inputs are likely low and are diluted to negligible concentrations offshore (Meyer et al., Under Review), combining

monitoring efforts across spatial and temporal scales are necessary to evaluate the spatial and temporal extent of wastewater entering Lake Baikal. As such, our data could complement previous, current, and future monitoring efforts, where observations may be missing.

Second, the final data products are useful to expanding freshwater PPCP, microplastic, and associated biological responses across large spatial scales. Recent syntheses of the PPCP literature have reported that studies involving lakes are less abundant relative to those focused on lotic systems (Meyer et al. 2019). Likewise, microplastic studies have noted that freshwater environments are less represented in the literature relative to marine ecosystems (Horton et al. 2017). For both PPCPs and microplastics, toxic responses to even minute concentrations can be uncertain and differ between ecosystem types (e.g., Rosi-Marshall et al. 2013 for lotic and Shaw et al. 2015 for lentic). As a result of PPCPs and microplastics garnering increasing attention worldwide, sampling of PPCPs and microplastics with co-located biological data across multiple spatial and temporal scales would be necessary to synthesize biotic responses to micropollutants across systems. Although our data constitute a limited sample number of PPCP and microplastic data that exist globally, our final data products are highly structured and flexible for merging with similar datasets. Additionally, our dataset's sequential harmonization workflow could be adopted by similar monitoring efforts, thereby facilitating data interoperability. Through integration with similar monitoring efforts, our dataset can contribute to global synthesis of emerging contaminant consequences, especially in a region of the world that is often not easily accessible to many researchers.

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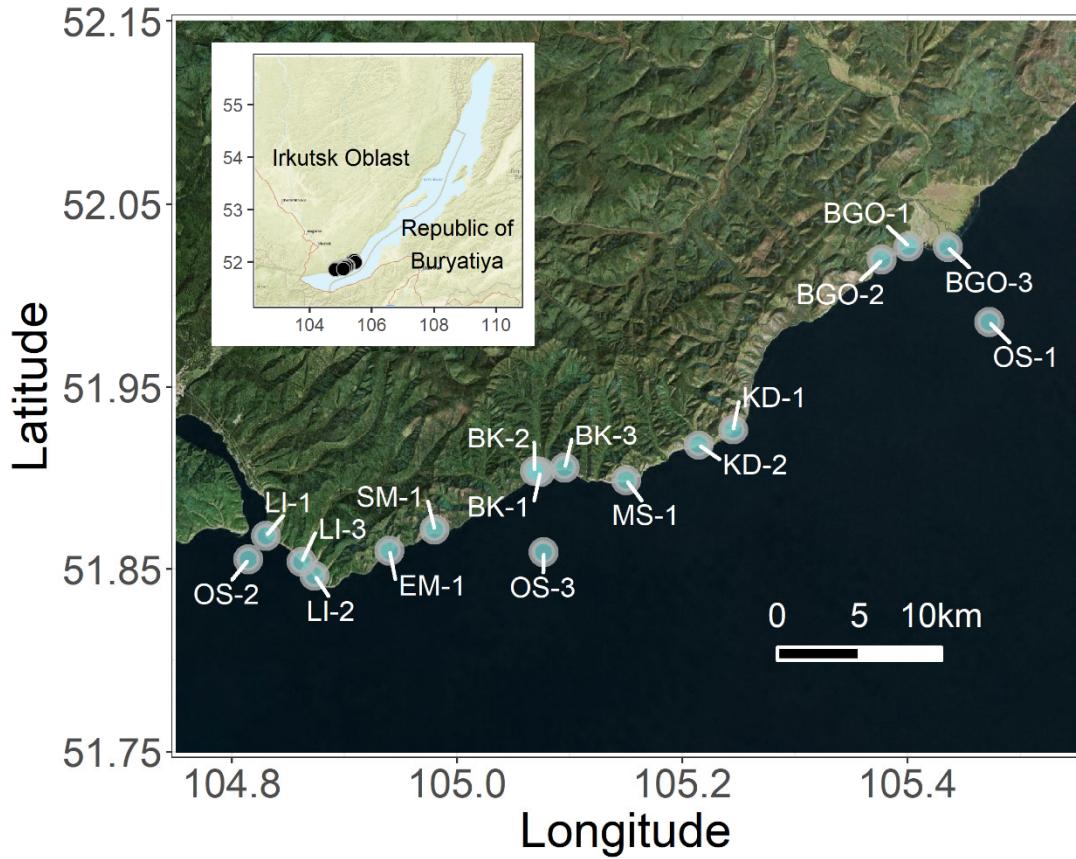


Figure 1: Map of all sampling locations with sites labeled with unique alphanumeric code. The entire transect included three developed sites (i.e., Listvyanka (LI), Bolshie Koty (BK), Bolshoe Goloustnoe (BGO)). Three offshore sites (OS) were also sampled to compare pelagic sewage signals to those in the littoral. Sites without adjacent lakeside development included Emelyanikha Bay (EM), Maloe Kadiilnoe (KD), Mys Soboliny (MS), Sredny Mys (SM). Littoral sampling locations were all 8.90–20.75 m from shore and at a depth approximately of 0.75 m, whereas pelagic sites were approximately 2–5 km from shore and ranged in depth from 900 to 1300 m. This map was created using the R statistical environment (R Core Team 2019) and the tidyverse (Wickham et al. 2019), OpenStreetMap (Fellows and Stotz 2019), ggpubr (Kassambara 2019), cowplot (Wilke 2019), ggsn (Baquero 2019), and ggrepel (Slowikowski 2019) packages. This map was produced using data from © OpenStreetMap contributors (<https://www.openstreetmap.org/copyright>), which is licensed under the Open Data Commons Open Database License (ODbL) by the OpenStreetMap Foundation (OSMF). Base map and data from OpenStreetMap and OSMF were created using the © ESRI (inset map) and © 2021 Microsoft Corporation Earthstar Geographics SIO “bing” (zoomed-in map) tiles.

| Site | Latitude | Longitude | Depth (m) | Distance to shore (m) |
|-------------|-----------------|------------------|------------------|------------------------------|
| BK-1 | 51.90316 | 105.074 | 0.7 | 10 |
| BK-2 | 51.90365 | 105.069 | 0.9 | 17.5 |
| BK-3 | 51.90536 | 105.0957 | 0.8 | 10 |
| BGO-1 | 52.02693 | 105.401 | 0.9 | 18 |
| BGO-2 | 52.0197 | 105.3771 | 1.1 | 14 |
| BGO-3 | 52.02649 | 105.4358 | 0.7 | 21 |
| OS-1 | 51.98559 | 105.4724 | 900 | NA |
| KD-1 | 51.92646 | 105.245 | 0.8 | 20.75 |
| KD-2 | 51.91807 | 105.2146 | 0.9 | 14.5 |
| MS-1 | 51.89863 | 105.1502 | 0.6 | 10.5 |
| SM-1 | 51.87152 | 104.9801 | 0.9 | 11.5 |
| LI-1 | 51.86825 | 104.8304 | 0.6 | 8.9 |
| LI-2 | 51.84626 | 104.8736 | 0.8 | 9.4 |
| LI-3 | 51.85407 | 104.8622 | 0.7 | 9.25 |
| EM-1 | 51.86005 | 104.94 | 0.7 | 15.5 |
| OS-2 | 51.8553 | 104.8148 | 1300 | NA |
| OS-3 | 51.85911 | 105.0769 | 1400 | 5000 |

Table 1: Locational information for each of the 17 sampling stations. “OS” refers to pelagic locations (i.e., “Offshore”), whereas other site abbreviations refer to littoral sampling locations.

| Variable | Course Taxonomic Grouping | Finest Taxonomic Group in Dataset |
|---------------------|---------------------------|--|
| Abundance Estimates | Amphipoda | <i>Brandtia latissima</i> subspp. (Dorogostaiskii 1930; Dybowsky 1874) <i>Brandtia parasitica parasitica</i> (Dybowsky 1874) <i>Cryptoropus inflatus</i> (Dybowsky 1874) <i>Cryptoropus pachytus</i> (Dybowsky 1874) <i>Cryptoropus rugosus</i> (Dybowsky 1874) <i>Eulimnogammarus capreolus</i> (Dybowsky 1874) <i>Eulimnogammarus cruentes</i> (Dorogostaiskii 1930) <i>Eulimnogammarus cyaneus</i> (Dybowsky 1874) <i>Eulimnogammarus grandimanus</i> (Bazikalova 1945) <i>Eulimnogammarus maacki</i> (Gerstfeldt 1858) <i>Eulimnogammarus maritui</i> (Bazikalova 1945) <i>Eulimnogammarus verucossus</i> (Gerstfeldt 1858) <i>Eulimnogammarus viridis viridis</i> (Dybowsky 1874) <i>Eulimnogammarus vittatus</i> (Dybowsky 1874) <i>Pallasea brandtia brandtia</i> (Dybowsky 1874) <i>Pallasea brandtii tenera</i> (Sovinskii 1930) <i>Pallasea cancelloides</i> (Gerstfeldt 1858) <i>Pallasea cancellus</i> (Pallas 1776) <i>Pallasea viridis</i> (Garjajev 1901) <i>Poekilogammarus crassimus</i> (Sovinskii 1915) <i>Poekilogammarus ephippiatus</i> (Dybowsky 1874) <i>Poekilogammarus megonychus perpolitus</i> (Takhteev 2002) <i>Poekilogammarus pictus</i> (Dybowsky 1874) |
| | Molluska | Acroloxidae Baicaliidae Benedictidate Maackia Planorbidae Valvatidae |
| | Other Macroinvertebrates | Asellidae Caddisflies |

| | | |
|-----------------|---------------|---|
| | | Hirudinea Planaria |
| | Benthic Algae | Diatom <i>Ulothrix</i> spp. <i>Spirogyra</i> spp. Tetrasporales |
| Stable Isotopes | Amphipoda | <i>Eulimnogammarus cyaneus</i> (Dybowsky 1874) <i>Eulimnogammarus verucossus</i> (Gerstfeldt 1858) <i>Eulimnogammarus vittatus</i> (Dybowsky 1874) <i>Pallasea cancellus</i> (Pallas 1776) |
| | Benthic Algae | Periphyton |
| Fatty Acids | Amphipoda | <i>Eulimnogammarus cyaneus</i> (Dybowsky 1874) <i>Eulimnogammarus verucossus</i> (Gerstfeldt 1858) <i>Eulimnogammarus vittatus</i> (Dybowsky 1874) <i>Hyalella</i> spp. <i>Pallasea cancellus</i> (Pallas 1776) |
| | Molluska | Processed in composite and not identified to family. |
| | Benthic Algae | Periphyton <i>Draparnaldia</i> spp. |

Table 2: Summary table of algal and macroinvertebrate data within the dataset. Although fatty acids contain data on *Hyalella* spp., these specimens were likely misidentified in the field before processing. For consistency and detailing the breadth of fatty acid profiles among Baikal's littoral amphipods, we have included them in the dataset, but caution should be taken when considering these fatty acids explicitly as those representative of *Hyalella* spp.

CONCLUSIONS

Ecosystem integrity is threatened by disturbance associated with human activities. Nutrient and other micropollutant additions from human sewage into aquatic systems is a widespread, frequently high-impact example of human disturbance that can reshape aquatic food webs (Edmondson 1970; Carpenter et al. 1998). However, definitively linking the human disturbance with the ecological response is complex and challenging. Defining sewage pollution as a disturbance is a challenge because (1) nutrients can originate from non-sewage sources (Turetsky et al. 2000; Anisimov and Reneva 2006; Moran et al. 2012; Powers et al. 2016; Gould et al. 2016), (2) primary producers can quickly remove nutrients from the water column (e.g., hours; Hadwen and Bunn 2005) and dampen sewage signals, and (3) the micropollutant literature is still new relative to the eutrophication literature and has historically focused on certain pollutant and ecosystem types (e.g., Meyer et al. 2019). Defining ecological responses to sewage likewise is a challenge, and biota may respond in uncertain, less visible ways. Together, spatial and temporal heterogeneities in nutrient addition and coupled biological processes can additionally obfuscate patterns, thereby necessitating specific indicators for evaluating both the disturbance and biological response.

Further, as numerous studies have previously demonstrated, understanding the heterogeneity of human disturbance in environmental systems is difficult. This dissertation is no exception. However, the combined works in this dissertation both individually and synthetically contribute to a larger disturbance narrative by using emerging contaminants, nuanced food web metrics, and data-intensive approaches to synthesize how heterogeneous nutrient addition can cause ecological communities to restructure (summarized in Table 1).

Chapter 1 synthesizes 30 years of rapid growth in the PPCP literature and identifies this literature's areas of focus and potential blind spots. Our evidence synthesis suggested that lakes and forms of decentralized wastewater treatment were historically far less represented in the literature relative to centralized wastewater treatment and lotic environments. We identified both distinct knowledge gaps, such as specific ecosystem and sewage treatment types, and also historical research foci. The previous works included in our evidence synthesis were dominated by a large literature from centralized sewage treatment in lotic systems and demonstrated the pervasiveness and potential for PPCPs to track sewage pollution in these systems. Because of these foundations in lotic environments, we were able to extend these ideas into lentic systems, especially in areas where decentralized sewage treatment has been suspected of contributing significant nutrient loading.

Chapter 2 evaluates how littoral benthic community composition and food web interactions may respond to sewage pollution across a spatial gradient. Our data suggest that in Lake Baikal's nearshore, sewage-associated nutrient addition along a 40-km transect has contributed to benthic communities restructuring. Periphyton communities near larger human populations were characterized by higher chlorophyte relative abundance and decreased diatom relative abundance. Along this transect, macroinvertebrate communities were more characterized by decreased mollusk relative abundance. Despite such compositional shifts, trophic biomarkers suggested that amphipods acquired consistent nutrition along the transect, implying that amphipods were able to compensate along this disturbance gradient either physiologically or behaviorally. Together, these data suggest that spatially heterogeneous disturbances can restructure community composition, but food web consequences may not be immediately apparent, as metabolic demands require organisms to compensate.

Chapter 3 addresses how spatially and temporally heterogeneous nutrient addition from transient human populations may alter algal community composition and, thus, the available nutrition to the food web. Our data suggest that in Flathead Lake's nearshore, periphyton communities near areas with consistent human disturbance tend to remain taxonomically consistent. At these same locations, the nutrition available to higher trophic levels was lower quality than those near inconsistent human disturbance but was temporally less variable. In contrast, periphyton communities near temporally inconsistent disturbance underwent more compositional change and constituted a resource of higher nutritional quality than those near consistent human disturbance. Together, these results suggest that consistent disturbances can create reliable resources and stable environments for biota, whereas inconsistent disturbances can create transient but high-quality resources that might promote more diverse communities.

Chapter 4 is predicated on the idea that an important pathway to scaling our understanding of ecological responses to heterogeneous disturbance is to practice open data sharing and code publication. Thus, this chapter documents and archives all data, scripting routines, and metadata to facilitate future reuse in a peer reviewed open access format to increase its value in the scientific endeavor at large. By preserving these data and methods in an indexed, machine-readable, and reproducible format, these data can be repurposed to further understanding of spatially and temporally heterogeneous disturbances both intensively within individual systems and extensively across spatial scales. For Lake Baikal, these data can be used to fill gaps in monitoring efforts or even to provide a code infrastructure for continued harmonization of disparate data sources. Beyond Lake Baikal, these data may be useful for synthesis across systems, enabling for quantitative investigations of general trends in heterogeneous sewage loading and associated biological consequences.

Beyond contributing to basic ecological science, each study in this dissertation employed open data and computational methods to not only increase transparency of the work but also to build scripting infrastructures that will allow others to tailor these techniques for their own research questions (Table 1). **Chapter 1** includes a companion tutorial made available on GitHub, where users can walk through guided R code that details how the evidence synthesis was performed (see supplement in Meyer et al. (2019) or https://github.com/mishafredmeyer/esa_inspire_talk/blob/master/analysis_20200717.md). Likewise, **Chapters 2, 3, and 4** contain tracked and archived script repositories that can serve as templates for others looking to harmonize disparate sewage indicator and ecological response data across various spatial and temporal scales (Meyer et al. 2015, 2020, 2021).

When considering this entire dissertation as one narrative, our work at Lake Baikal and Flathead Lake suggests that heterogeneous, bottom-up disturbances can more immediately influence sessile, lower trophic levels, but mobile, higher trophic levels may experience muted or lagged responses (*sensu* Menge and Sutherland 1987). In both lakes, sewage-associated nutrient addition corresponded with changes in algal community composition, thereby altering nutrition available to higher trophic levels. Amphipod fatty acids, however, in Lake Baikal suggested that Baikal's amphipods did not visibly respond to sewage pollution. Rather, amphipods appeared to maintain consistent nutrition, which could be explained by amphipods' grazing more intensively on diatoms, migrating to where diatoms are more abundant, or investing their own energy to upgrade fatty acids. In contrast, mollusk abundance decreased significantly along the disturbance gradient. Mollusk fatty acid profiles suggested even higher diatom-associated diets than amphipods, meaning that less-mobile mollusks may be decreasing in abundance or entirely relocating to locations with more temporally consistent resources and less competition with other

grazers. In any event, these implications are consistent with those from Flathead, where we would expect consistent disturbance to support macroinvertebrate assemblages that can compensate by grazing selectively, migrating, or upgrading their own fatty acids. Temporally inconsistent disturbances, however, may encourage more diverse macroinvertebrate guilds, especially if disturbances do not co-occur with sensitive biota that require specific diets to maintain metabolic demands.

Returning to the dissertation's main goal of understanding how spatially and temporally heterogeneous nutrient addition can restructure community composition and food webs, our results suggest that ecological responses depend both on the intensity of the disturbance and the capacity for the community to compensate. In instances when community members may be more sensitive, such as periphyton assemblages, heterogeneous responses can alter communities more immediately, whereas less sensitive taxa may offset a disturbance by behavioral or physiological modifications. Beyond Lake Baikal and Flathead Lake, this same conceptual model is pertinent to aquatic and terrestrial systems alike. Conceptually, Ginn et al. (2007) envisioned a modeling framework where individuals, populations, or even communities with various age structures could respond to different toxicant doses and their corresponding exposure times to a toxicant could influence behavior, body burden, and reproductive success. In a terrestrial context, Craine et al. (2018) demonstrated how foliar oligotrophication brought about by increased growing seasons or increasing temperatures can disproportionately limit nitrogen content in plants with arbuscular mycorrhizal associations, which could have implications for long-term grazer protein content (Craine et al. 2017), soil C:N content available to plant and subsurface microbial communities (Grüneberg et al. 2014; Ren et al. 2021), and even aquatic nitrogen concentrations

(Bernal et al. 2012). Regardless of the exact system or mechanisms, the main concept of bottom-up disturbances more immediately influencing sensitive taxa still applies.

The work contained within this dissertation contributes evidence to how ecological communities respond to heterogeneous disturbances, both human and otherwise “natural”. By offering both insights and infrastructure for evaluating heterogeneous disturbance, this work provides opportunities for future investigations to build directly on its advances. Yet, the work is far from finished. In times of a changing climate and human landscape alteration, ecosystems are constantly responding to disparate, co-occurring disturbances. These increasingly complex disturbances may reveal unexpected adaptations or emergent properties that allow systems to compensate. Simultaneously, these disturbances will likely become more complex in the future, thereby necessitating continued inquiry and discussion. This work is one piece of that larger conversation. But by providing flexible tools and generalizable insights, this work empowers current and future scientists to further the larger disturbance narrative, both intensively within individual systems and extensively across scales and ecosystem types.

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| | Chapter 1 | Chapter 2 | Chapter 3 | Chapter 4 |
|--|---|--|---|--|
| Tool or Dataset Development | R script and tutorial for extracting and analyzing Web of Science records | Scripted routine for harmonizing complex, disparate datasets over space | Scripted routine for harmonizing complex, disparate datasets over space and time | Unified data product with more than 150 co-located variables |
| Scientific Advancement | Identifying knowledge gaps within PPCP literature for future research | Understanding how disturbance can propagate through community composition shifts and food web interactions | Understanding how disturbances can alter algal community composition and available nutritional content to the larger food web | Offers open, machine readable data for Lake Baikal's nearshore communities, including endemic amphipod species abundance |
| Information for Decision-making | Awareness of imbalances within PPCP literature for policy and management | Emphasizes the importance of nearshore monitoring | Emphasizes the importance of nearshore monitoring | Can aid synthesis and comparison across systems suspected of sewage pollution |

Table 1: Summary of advancement in basic science, tools and dataset development, and information for decision makers contained within this dissertation. This table was inspired by the work of Reyes (2017).

