# SPATIO-TEMPORAL VARIATIONS OF THE FUNCTIONAL TRAITS OF BENTHIC MACROINVERTEBRATES IN THE UNIVERSITY OF THE PHILIPPINES DILIMAN CAMPUS WATERWAYS

HERBERT F. MENDOZA

INSTITUTE OF BIOLOGY
College of Science
University of the Philippines
Diliman, Quezon City



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## **Bachelor of Science in Biology**

#### Herbert F. Mendoza

Spatio-temporal Variations of the Functional Traits of Benthic Macroinvertebrates in the University of the Philippines Diliman Campus Waterways

Thesis Adviser:

Francis S. Magbanua, Ph.D.

Institute of Biology

University of the Philippines Diliman

Thesis Examiner:

Christine Jewel C. Uy-Yabut, Ph.D.

Institute of Biology

University of the Philippines Diliman

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Institute of Biology College of Science University of the Philippines Diliman, Quezon City

#### **ENDORSEMENT**

This is to certify that this undergraduate thesis entitled **Spatio-temporal Variations of the Functional Traits of Benthic Macroinvertebrates in the University of the Philippines Diliman Campus Waterways** prepared and submitted by Herbert Franco Mendoza in partial fulfillment of the requirements for the undergraduate degree of Bachelor of Science in Biology, is hereby accepted.

FRANCIS S. MAGBANUA, Ph.D. Thesis Adviser

CHRISTINE JEWEL C. UY-YABUT, Ph.D.
Thesis Examiner

The Institute of Biology endorses acceptance of this undergraduate thesis as partial fulfillment of the requirements for the degree of Bachelor of Science in Biology.

IAN KENDRICH C. FONTANILLA, Ph.D.

Director

Institute of Biology

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#### **ABSTRACT**

Urban streams, including those within the Philippines, are highly vulnerable to pollution and disturbances. The waterways of the University of the Philippines Diliman Campus are no exception. To assess the impact of pollutants and stressors, bioindicators such as benthic macroinvertebrates, which are diverse and ubiquitous, are often utilized. While traditional assessments rely on taxonomic metrics, classification based on functional traits provides additional insights concerning habitat and environmental conditions. This study aims to evaluate the variations in benthic macroinvertebrate functional traits and distributions and their correlation with physicochemical parameters across different land uses and seasons within the University of the Philippines Diliman waterways, to discern the effects of urbanization on water quality. Nine sites were categorized based on three land uses and sampling periods corresponding to the dry and wet seasons of the Philippines. A total of 18,951 benthic macroinvertebrate specimens were included in the study. A two-way PERMANOVA analysis of biotic indices, biological response metrics, and physicochemical parameters revealed a significant difference between seasons (p = 0.0003), but not between land uses (p = 0.1656). Biotic indices indicated the likely presence of severe pollution and disturbance within the campus waterways. Higher values of Average Tolerance Score per Taxa (ATSPT), Simpson's Diversity Index, and Simpson's Evenness observed from April to May compared to November to December indicating a more diverse, evenly distributed, and higher tolerance taxa present during the dry season than the wet season. Common taxa across land uses and seasons include non-biting midges, pond snails from the Physidae and Lymnaeidae families, odonates from Libellulidae and Coenagrionidae families, and oligochaetes, all of which are pollutionand disturbance-tolerant. Four functional traits were identified: Functional feeding groups, mode of respiration, mode of reproduction, and locomotion habit. Functional indices showed no significant differences between seasonality or land use (p =0.7553; p=0.9834). The fourth-corner test indicated relationships of five modalities with six environmental parameters.

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#### INTRODUCTION

Rivers and freshwater bodies harbor a rich array of biotic factors that shape both the aquatic environment and the surrounding landscape. Plants, including submerged, floating, and riparian species, stabilize riverbanks and enhance water quality. Vertebrates such as fish, amphibians, reptiles, and mammals all rely on these waters for breeding, foraging, and shelter. Birds especially species migrating occasionally, nest, and feed across these river lengths. Invertebrates such as insects, mollusks, and crustaceans are ubiquitous within these aquatic environments, serving as food for fish and other organisms as well playing key roles in nutrient cycling and decomposition. Microorganisms play crucial roles in decomposition and water purification. Altogether, they contribute to ecosystem dynamics such as food webs, interspecies interactions, ecosystem resilience, and ecological succession. Equally important is the recognition that these biotic elements are also shaped by the land around them, with interactions between biotic and abiotic factors forming complex freshwater ecosystems essential for biodiversity, ecosystem services, and environmental health. Given this, these bodies of water are especially susceptible to the rapidly changing landscape and circumstances brought about by human urbanization (Unda-Calvo et al., 2020).

Urbanization primarily refers to the process by which rural populations are subject to technological and economic growth, resulting in a shift in social, economic, and lifestyle patterns, as well as an increase in population and infrastructure, through which cities are created and grow in size as a result of industrialization and economic development (Chaolin et al., 2012). This also promotes changes that are unique to cities in specialization, labor division, and human behaviors. Urbanization and rising income per capita are closely related in today's emerging nations, just as they were historically, due to high consumption of resources, whether produced or imported, in industrialized cities (Gollin et al., 2015). However, rapid urbanization in developing countries usually causes a lack of infrastructure or employment opportunities, such that a sizable section of people resides in unofficial settlements or slums (Regmi, 2018). Sprawling urbanization as a phenomenon has been frequently cited as the general cause of a variety of environmental issues. Acceleration of deforestation is observed as land use patterns are altered by converting natural landscapes into built environments, leading to the

loss of vegetation and wildlife habitats (Ortiz et al., 2022). Biodiversity loss is apparent in these areas due to unmitigated urbanization and uncareful planning (Simkin et al., 2022). Widespread pollution from the expansion of these cities and infrastructure include industrial emissions, vehicle exhaust, and household waste, which are often carried by runoff during rainfall events, transporting contaminants from urban areas into nearby water bodies, degrading water quality and threatening aquatic ecosystems (Thuzar, 2011).

Water quality in freshwater regions is significantly impacted by anthropogenic factors and activities, with pollution being particularly severe in urban areas (Khatri & Tyagi, 2015). Urbanized regions extract freshwater for household or agricultural use and utilize river flows for disposing of nutrient run-offs from factories and other establishments (Condie et al., 2012). Urban stream syndrome encompasses various environmental issues stemming from human activities within urban areas, including reduced nutrient uptake, resulting in elevated concentrations of nutrients and contaminants in the water. Urbanization often alters stream morphology through channelization, disrupting ecological balance. Improper wastewater disposal and chemical runoffs from urban areas contribute to pollution, exacerbated by inadequate treatment facilities, sewage, and drainage systems. (Walsh et al., 2005). The deterioration of fish habitats, increased water turbidity, declining catches, and loss of aquatic plant diversity have been attributed to both overfishing and the artificial introduction of species (Mendoza et al., 2022). Both point source and diffuse pollution contribute to the degradation of urban streams, leading to phenomena like eutrophication and the formation of anoxic zones. This is evidenced by the transition from oligotrophic algae to eutrophic diatoms (Walsh et al., 2005; Jalilov, 2017). This has been a long running problem, as the continuous and uncontrolled disposal of domestic and industrial wastewater, caused by inadequate treatment facilities and sewerage systems, is a common issue in many rivers and waterways within the National Capital Region (NCR) (Migo et al., 2018).

One area within the NCR that requires early intervention so as not to reach this level of pollution is the waterways of the University of the Philippines Diliman (UPD) Campus. The campus is a 493-hectare area housing students, faculty, staff, and informal settlers. Using high-resolution satellite imaging, Galeon (2008) was able to estimate that 16%, or roughly 79 hectares, of this area may be

labeled as informal settlements. With a lack of dedicated formal funding for facilities and sewerage for these settlements, as well as constant use of water for domestic purposes, downstream areas including the 18 hectares of protected forests and parks may be ecologically degraded. In a previous study, all sampled stream reaches varying by land use on the UPD Campus were found to be of poor to severe pollution conditions (Magbanua et al., 2019). Physicochemical parameters such as the temperature, pH, total dissolved solids (TDS), dissolved oxygen, nitrate, phosphate, ammonia, and chemical oxygen demand (COD) levels in the UP Diliman Lagoon and Academic Oval waterways were also assessed against the standards set by the DENR Administrative Order (DAO) 2016-08 Water Quality Guidelines and General Effluent Standards of 2016 (Doctolero et al., 2016). The results showed that none of these parameters met the required standards, with recorded nutrient concentrations exceeding the maximum allowable levels.

As such, to assess the overall quality of water, scientists and researchers must monitor various physicochemical parameters. These parameters include dissolved oxygen (DO), temperature, pH, sulfate content, chloride content, color, hardness, turbidity, and the presence of heavy metals, among others, which determine water safety for potability, domestic, or industrial use (Patil, 2012). Monitoring is typically conducted in accordance with national standards, such as the previously mentioned DAO 2016-08, or Administrative Order (AO) 2017-10 which pertains to the Philippine National Standards for Drinking Water (PNSDW) of 2017. In the Philippine context, evaluations of habitat parameters are also essential to ensure the growth, survival, and reproduction of organisms of interest, such as fish in agriculture or protected species (Newcomb et al., 2006).

The analysis of water quality often involves the utilization of macroinvertebrates as biological indicators. Macroinvertebrates are widely employed for this purpose as they are integral components of river ecosystems and are highly sensitive to anthropogenic pressures (Tampo et al., 2021). Their responsiveness to alterations in freshwater environments renders them valuable for monitoring ecosystem integrity, frequently integrated into indices designed for this objective. Moreover, their discernible size facilitates relatively straightforward sampling procedures, making them easy to collect and identify in the field (Benetti et al., 2012). Due to their ubiquity and sedentary lifestyles,

macroinvertebrates can manifest acute and cumulative responses to pollution across spatial gradients (Jolejole, 2020), enabling the assessment of both short- and long-term impacts of specific parameters within particular sites through taxonomic identification. Additionally, Tampo et al. (2021) underscore the cost-effectiveness of macroinvertebrate sampling as an approach to freshwater ecosystem monitoring.

An emerging approach to macroinvertebrate analysis involves their classification based on functional traits. These traits are selected according to the specific circumstances of the study site, aiming to yield differentiated and non-redundant data while considering potential agricultural or urban stressors (Edegbene et al., 2020). Such traits and ecological preferences encompass various aspects, including but not limited to: body morphology, encompassing diverse shapes and sizes (Dong et al., 2021; Edegbene et al., 2020; Liu et al., 2021); mobility, distinguishing between swimming, crawling, or burrowing behaviors (Dong et al., 2021; Liu et al., 2021); feeding strategy, encompassing filtering or gathering collectors, predators, scrapers, or shredders, as determined by mouthpart morphologies (Dong et al., 2021; Doong et al., 2021); and voltinism, indicating the number of broods per year (Liu et al., 2021). The abundances of macroinvertebrates classified based on these traits serve as a method to characterize habitat conditions (Coccia et al., 2021). This approach is predicated on the principle that these traits exhibit different modalities, with a high functional diversity indicative of a site harboring taxa capable of fully occupying available niches. Functional traits complement species or family-level abundance data by categorizing individuals into distinct ecological roles. The concept of ecological resilience is reinforced through functional redundancy, further justifying the use of functional traits as an additional metric in biodiversity assessment.

Tailoring legislation to align with macroinvertebrate responses carries numerous management and conservation implications. Integrating findings from macroinvertebrate studies into environmental policy frameworks enhances freshwater ecosystem protection. Engaging local communities in conservation efforts fosters stewardship and accountability toward natural resources. Public outreach initiatives highlighting macroinvertebrates' role as bioindicators mobilize support for pollution mitigation, promoting responsible resource management practices. Long-term monitoring enables the

assessment of intervention effectiveness, facilitating adaptive management strategies informed by upto-date data. Meticulous tracking of community dynamics within macroinvertebrate populations informs evidence-based decisions to sustainably manage and preserve freshwater ecosystems in the Philippines.

This study aims to evaluate the variations of benthic macroinvertebrate functional traits and distributions, and their relationship with physicochemical parameters present within the University of the Philippines Diliman waterways across land use and seasonality to determine the effects of urbanization on water quality. Specifically, this study has the following objectives:

#### 1. Assess urbanization effects:

- Evaluate the impact of urbanization on water quality using physicochemical parameters
   and biotic indices derived from benthic macroinvertebrate taxa data.
- 2. Compare seasonal and land use variations:
  - Analyze differences in macroinvertebrate community compositions between the dry and wet seasons.
  - Compare macroinvertebrate community compositions across different land use types.
- 3. Identify influential environmental parameters:
  - Determine which physicochemical or environmental parameters significantly affect the presence and abundance of specific macroinvertebrate taxa.
- 4. Relate functional traits to environmental factors:
  - Establish the relationships between macroinvertebrate functional traits (e.g., feeding habits, mobility, life cycle traits) and environmental parameters to understand their responses to changes in water quality and habitat conditions.

#### MATERIALS AND METHODS

#### **Study Site**

Sampling sites within the University of the Philippines Diliman Campus were selected based on the land use designations established by Magbanua et al. (2019), with reference to the 2016 sampling period employed in the present study. These designations included Campus Core (CC), Parks and Open Spaces (PO), and Academic Units (AU), which are not necessarily longitudinally continuous. Initially, a total of 19 sampling sites were identified, comprising 8 sites in the Campus Core, 5 sites in Parks and Open Spaces, and 6 sites in Academic Units. However, due to a dry season from late 2015, as well as degradation of samples during storage, most sites were deemed deprecated. Consequently, only 9 sites remained viable for investigation, consisting of 3 sites in the Campus Core (CC), 4 sites in Parks and Open Spaces (PO), and 2 sites in Academic Units (AU). Seasonality was also taken into account, with the dry months of April to May marking the onset of the wet season and the rainy months of November to December signifying the onset of the dry season.

#### **Benthic Macroinvertebrates**

Macroinvertebrate samples were collected from the nine designated sampling sites within the University of the Philippines Diliman Campus for the periods of April to May and November to December of 2016. Following the sampling protocol of de-Jesus & Crespo (2011), macroinvertebrates were collected from major habitats present (leaf packs, marginal vegetation, pools, and riffles) within a 50-m reach for each sampling site. Three collectors handpicked macroinvertebrates for 15 minutes until three replicates from each habitat have been obtained. Samples were treated with 95% ethyl alcohol. Samples were taken to the Wetland and linked Aquatic-Terrestrial Ecosystems Research (WATER) Laboratory, Institute of Biology, University of the Philippines Diliman. They were then washed and elutriated using a 250-µm sieve to separate macroinvertebrates from detritus. Individual identification and enumeration of macroinvertebrates were done up to the genus level under a stereo microscope, based on the keys of Yule & Yong (2004), Mekong River Commission (2006), and Moore (2018). Ambiguous genera are identified according to their tribes or families instead. Exoskeletons, including

empty snail shells, and pupae are considered deprecated specimens. Biotic indices — namely the Hilsenhoff Family Biotic Index (HFBI), SingScore, Stream Invertebrate Grade Number – Average Level (SIGNAL) 2, Biological Monitoring Working Party - Thai version (BMWP<sup>thai</sup>), Average Score per Taxon - Thai version (ASPT<sup>thai</sup>), and Average Tolerance Score per Taxon (ATSPT) — and biological response metrics such as Total Abundance, Total Taxa Richness, EPTC Taxa Richness, Simpson's Diversity Index, and Simpson's Evenness Index were then calculated from the taxa data (Magbanua et al., 2019).

The HFBI index is used to assess organic and nutrient pollution by employing tolerance values of select arthropod families (Hilsenhoff, 1988). BMWP<sup>thai</sup> and ASPT<sup>thai</sup> are modified versions of UK's BMWP and ASPT which were meant to be a quick assessment of water quality as introduced by Metcalfe (1989). The SingScore index was introduced by Blakely et al. (2014) by comparing waterways in urbanized, densely populated areas with those in protected forest reserves within Singapore. Similarly, SIGNAL2 is an index that uses Australian macroinvertebrates as bioindicators to assess possible anthropogenic effects (Chessman, 2001). ATSPT is known as a metric that effectively measures taxa richness as a function of disturbance as rated by assessors at a site (Chessman & Giap, 2010).

Total abundance was calculated to determine the overall quantity of organisms present. Total taxa richness indicated the number of species present per site, while EPTC taxa richness, representing the richness of Ephemeroptera, Plecoptera, Trichoptera, and Coleoptera, was used to measure the diversity within these four orders. Simpson's diversity index was employed to assess the probability of randomly selecting two different taxa from a given pool, providing a measure of diversity. Finally, Simpson's evenness was computed to reflect the proportion of species abundance within sites, indicating how evenly individuals are distributed among the species present.

#### **Physicochemical Parameters**

Water samples were also collected in the same 50-m reaches in three random sites to assess physicochemistry. Parameters such as water temperature (°C) and dissolved oxygen (mg/L) were

measured using the YSI EcoSense DO200A. Conductivity ( $\mu$ S/cm), total dissolved solids (g/L), and salinity (ppm) were measured using the YSI EcoSense 300A. pH levels were observed using the YSI EcoSense pH10A. Other environmental parameters such as bank-full width (m), wetted width (m), water depth (cm), and water velocity (m/s) were also measured onsite.

#### **Data Analyses**

Functional trait data were analyzed using a fuzzy coding matrix following the protocol of Chevenet et al. (1994), rating the affinity of taxa for each modality at 0 to 3, with 3 representing the highest affinity, and making use of available literature. Four functional traits were identified: Functional feeding group (Collector-gatherer [CoGa], Collector-filterer [CoFi], Scrapers [Scrp], Shredders [Shrd], Predators [Pred], Piercers [Prcr], Detritivores/Deposit-feeders [Detr], Absorbers [Absb]), mode of respiration (Tegument [Tgmt], Gills [Gill], Spiracles [Spir], Siphons [Siph], Lungs [Lung]), mode of reproduction (Clustered [Clus], Isolated [Isol], Cemented [Cmtd], Brooding [Brod]), and locomotion habit (Clinger [Clng], Sprawler [Sprl], Swimmer [Swmr], Burrower [Brwr], Climber [Clmb], Skater [Sktr], Crawler [Crwl]). After fuzzy coding, traits were standardized per taxa to accommodate for multiple modalities with varying affinities in a single trait present. Abundances were then recorded per site, and functional indices were later computed.

Biotic and diversity indices as well as physicochemical parameters were tested for normality with Shapiro-Wilk test for normal distribution and Levene's test for homogeneity of variance, which confirmed non-normal distributions of the data population. Analysis for significant differences between groups of land use and seasonality was performed using Two-way PERMANOVA as the non-parametric data was considered. Significantly different individual parameters were later confirmed using pairwise Mann-Whitney U Tests. To further compare the relationship of physicochemical parameters with land use and seasonality, differences in macroinvertebrate taxa across sites were evaluated via non-metric multidimensional scaling (NMDS) ordination technique using a Bray-Curtis similarity matrix after fourth-root transformation of assemblage data to improve rare taxa visibility and diminish common taxa abundance, followed by confirmatory analysis of similarity (ANOSIM) and

similarity percentage (SIMPER) analysis (Magbanua et al., 2019). Following the methodology of Peralta et al. (2019), detrended correspondence analysis (DCA) was first performed on the macroinvertebrates abundance data to determine whether a linear or unimodal ordination method is appropriate for demonstrating the macroinvertebrate response to abiotic factors. DCA revealed that the longest gradient length (DCA axis 1: 7.5254) was more than 3.0, indicating that the unimodal ordination method, canonical correlation analysis (CCA), was better suited for visualization and analysis (Peralta et al., 2019). Before conducting CCA, variables with high multicollinearity were to be removed through inspection of variance inflation factors (VIF < 20) in order to decrease inflation (Peralta et al., 2019; Koudenoukpo et al., 2017). Two-way PERMANOVA was used to determine differences between groups of land use and seasonality for functional indices. Multivariate analysis via RLQ was used to compare environmental variables (R) via Hill-Smith analysis, taxa (L) via correspondence analysis, and traits (Q) via principal component analysis (Dray et al., 2014). The significance of the first two axes was determined using a Monte Carlo permutation test with 9999 permutations. Fourth-corner test was then performed to examine the bivariate relationship between functional traits and physicochemical parameters (Dray et al., 2014). Two-way PERMANOVA, post-hoc tests, ANOSIM, and SIMPER analyses were performed using PAST v4.16c. NMDS, DCA, CCA, RLQ, and fourth-corner tests were conducted using RStudio 2023.06.1+524.

#### **RESULTS**

## **Benthic Macroinvertebrate Composition**

A total of 18,951 benthic macroinvertebrate individuals from 84 genera, belonging to 55 families under 19 orders were sampled across the sampling sites. The general distribution according to season reveals 5,864 specimens collected during April to May, and 13,087 specimens during November to December. For April to May, the top three families were Chironomidae constituting 3,319 specimens (56.60%), Physidae consisting of 417 (7.11%), and Psychodidae with 359 (6.12%). During November to December, the top three families were Chironomidae with 8,192 specimens (62.63%), Oligochaeta with 3,506 (26.79%), and Lymnaea with 201 (1.53%). With respect to land use, 4,095 specimens were sampled from the Campus Core (CC), 8,984 from Parks and Open Spaces (PO), and 5,872 from Academic Units (AU). In the Campus Core (CC), the top three families were Chironomidae with 1,661 specimens (40.56%), Oligochaeta with 971 (23.71%), and Lymnaeidae with 233 (5.69%). In Parks and Open Spaces (PO), the top three families were Chironomidae with 7,272 specimens (80.94%), Physidae with 221 (2.46%), and Psychodidae with 169 (1.88%) specimens. Lastly, in Academic Units (AU), the top three families were Physidae with 157 specimens 2.67%), Chironomidae with 2,405 (0.96%), and Oligochaeta with 2,456 (41.83%).

#### **Environmental Indicators**

Two-way PERMANOVA of biotic indices, biological response metrics, and physicochemical parameters showed a significant difference between seasonality (p = 0.0003), but not land use (p=0.1656).

Mann-Whitney U tests revealed significant differences in several parameters between the sampling periods of April to May and November to December (Table 1). Among the biotic indices, ATSPT showed significant differences, with April to May having a higher mean than November to December, indicating taxa with higher tolerance to disturbed habitats in the former period. Biological response metrics showed significant differences in both Simpson's Diversity and Evenness Indices, where April to May had higher values despite having lower total abundance. Significant physical

parameters for seasonality included a higher wetted width for November to December, while water velocity and temperature were observed to be higher for April to May. Significant chemical parameters included total dissolved solids and dissolved oxygen, both of which were higher for November to December.

**Table 1.** Two-way PERMANOVA results for temporal (seasonality) groups showing mean values and standard errors in parentheses. Significant p-values are shown in bold. Rankings according to group means are also displayed.

Parameter		Apr-May	Nov-Dec	P-value	Ranking
Biotic Indices	HFBI	7.95 (0.21)	8.12 (0.09)	0.9296	
	SingScore	47.54 (3.42)	46.12 (3.23)	0.7239	
	SIGNAL2	5.98 (0.35)	6.59 (0.22)	0.3536	
	BMWPthai	12.15 (1.25)	16.33 (1.88)	0.3091	
	ASPTthai	3.87 (0.34)	3.15 (0.20)	0.2002	
	ATSPT	28.18 (0.95)	21.65 (1.13)	0.0036	Apr-May>Nov-Dec
Biological response	Total Abundance	217.19 (49.98)	484.70 (89.06)	0.0701	
metrics	Total Taxa Richness	6.63 (0.59)	8.63 (0.82)	0.2685	
	EPTC Taxa Richness	0.56 (0.11)	0.56 (0.09)	0.9258	
	Simpson's Diversity Index	0.51 (0.05)	0.32 (0.04)	0.0423	Apr-May>Nov-Dec
	Simpson's Evenness	0.45 (0.04)	0.26 (0.04)	0.0171	Apr-May>Nov-Dec
Physicochem	Bank-full Width (m)	3.79 (0.24)	4.03 (0.18)	0.3800	
	Wetted Width (m)	1.53 (0.31)	2.15 (0.18)	0.0273	Nov-Dec>Apr-May
	Water velocity (cm/s)	53.08 (5.38)	8.36 (2.23)	0.0004	Apr-May>Nov-Dec
	Depth (cm)	9.54 (0.91)	10.02 (0.68)	0.9296	
	Temperature (°C)	28.30 (0.27)	26.45 (0.15)	0.0081	Apr-May>Nov-Dec
	Conductivity (µS/cm)	463.32 (16.92)	381.82 (16.13)	0.0637	
	TDS (mg/L)	124.52 (8.73)	242.54 (9.93)	0.0027	Nov-Dec>Apr-May
	Salinity (ppm)	0.23 (0.01)	0.24 (0.03)	0.5608	
	DO (mg/L)	0.92 (0.12)	1.96 (0.23)	0.0341	Nov-Dec>Apr-May
	pH	7.81 (0.28)	7.66 (0.03)	0.7238	

The analysis revealed a significant difference in pH levels between Campus Core (CC) and Parks and Open Spaces (PO) sites (p=0.0053) (Table 2). CC sites had a mean pH of 8.67, which is more alkaline compared to the circumneutral mean pH of 6.93 in PO sites.

**Table 2.** Two-way PERMANOVA results for spatial (land use) groups showing mean values and standard errors in parentheses. Significant p-values are shown in bold. Rankings according to group means are also displayed.

Parameter		C	С	P	0		AU	P-value	Ranking
Biotic Indices	HFBI	8.51	(0.20)	7.89	(0.12)	7.63	(0.33)	0.07943	
	SingScore	46.69	(3.11)	46.21	(3.20)	48.28	(7.83)	0.9214	
	SIGNAL2	6.15	(0.43)	6.11	(0.30)	6.82	(0.40)	0.5367	
	BMWPthai	11.72	(2.13)	14.96	(1.61)	16.58	(2.85)	0.5188	
	ASPTthai	2.84	(0.29)	4.03	(0.35)	3.48	(0.36)	0.2358	
	ATSPT	28.66	(1.74)	23.21	(88.0)	22.71	(1.91)	0.1729	
Biological response	Total Abundance	227.50	(51.87)	374.33	(78.79)	489.33	(175.88)	0.4226	
metrics	Total Taxa Richness	7.89	(0.92)	7.21	(0.83)	8.08	(1.18)	0.866	
	EPTC Taxa Richness	0.56	(0.12)	0.63	(0.12)	0.42	(0.15)	0.7022	
	Simpson's Diversity Index	0.47	(0.06)	0.34	(0.05)	0.48	(0.05)	0.2003	
	Simpson's Evenness	0.40	(0.07)	0.33	(0.04)	0.34	(0.06)	0.9613	
Physicochem	Bank-full Width (m)	3.89	(0.22)	4.07	(0.27)	3.65	(0.27)	0.7432	
	Wetted Width (m)	1.54	(0.13)	1.80	(0.23)	2.30	(0.58)	0.7547	
	Water velocity (cm/s)	22.45	(5.42)	40.58	(7.64)	24.47	(6.77)	0.4076	
	Depth (cm)	7.54	(0.62)	11.05	(0.77)	10.46	(1.48)	0.1362	
	Temperature (°C)	26.99	(0.32)	27.45	(0.27)	27.75	(0.49)	0.6607	
	Conductivity (µS/cm)	473.34	(22.46)	389.13	(20.33)	414.61	(16.51)	0.1651	
	TDS (mg/L)	215.75	(16.44)	173.42	(13.93)	159.43	(24.38)	0.2813	
	Salinity (ppm)	0.24	(0.01)	0.24	(0.03)	0.21	(0.01)	0.2152	
	DO (mg/L)	1.14	(0.19)	1.55	(0.23)	1.64	(0.33)	0.6964	
	pH	8.67	(0.21)	6.93	(0.16)	7.93	(0.13)	0.005288	CC > PO

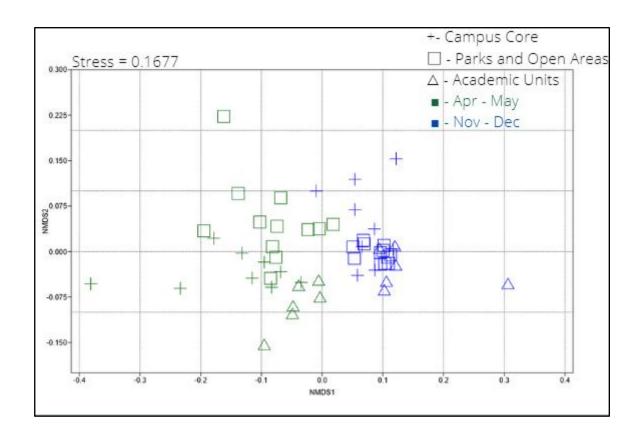
#### **Analysis of Biotic Index Values**

Mean values of 7.26 in the HFBI index or higher are indicative of very poor water quality, with a likely severe degree of organic pollution present (Hilsenhoff, 1988). All values across land uses and seasonality fall within this margin, which indicates the presence of high organic and nutrient pollution within the University of the Philippines Diliman waterways. The SingScore Index scales up to 200 points, and water quality below 79 is indicative of poor water quality and stream health with reference to Singaporean streams and datasets obtained by Blakely et al., 2014. All scores across land uses and seasonality fall well below this boundary, which mirrors the negative effects of urbanization to Singaporean streams upon the UP Diliman waterways. A higher SIGNAL2 value corresponds to elevated dissolved oxygen (DO) levels, as well as lower levels of salinity, turbidity, and nutrients like nitrogen and phosphorus. Values scale according to richness of families and designated tolerance values

(Chessman, 2001). A value closer to 10, scaling from 0, indicates the presence of more dissolved oxygen and less nutrient pollution. However, the values obtained across both land use and seasonality, though not significantly different from one another, are still below a score of 7 indicating the presence of pollution or poor and disturbed habitat. Scores scale from 0 to 10 for ASPT<sup>thai</sup> (Metcalfe, 1989), and all sites within UP Diliman display mean scores lower than 5, indicating generally very poor water quality. The values for ATSPT scale from 0 to 100, where lower ATSPT values indicate a higher sensitivity of the present taxa to disturbances and conversely, higher values indicate higher tolerance (Chessman & Giap, 2010). Although low values are found across the sites, this may be a result of the visual assessment of the site disturbance score being lower than the actual state with other factors such as the concentration of nutrients and dissolved oxygen within the water.

#### Analysis of Seasonal and Land Use Effects on Community Structure

The results of the NMDS ordination made use of both seasonality and land use as the groups, which resulted in a moderate 2D stress value of 0.1677, as displayed in Figure 1. The figure shows weak clustering between land uses, but very apparent and distinct groups for seasonality. However, this is overturned by the R of ANOSIM both for between and within groups, where Seasonality + Land Use show a p-value of 0.048 but with a weak R value of 0.0718, Seasonality has a p-value of 0.0001 and a moderately high R value of 0.5869, and Land Use has a p-value of 0.0058 but with a weak R value of 0.1218 (Table 3).



**Figure 1.** NMDS figure showing the relationship between physicochemical parameters with land use and seasonality using available taxa data. Land use is denoted with shapes, while seasonality is denoted with colors.

The SIMPER analysis (Tables 4 & 5) identified several taxa contributing to the dissimilarity between the groups. Both groups share the presence of non-biting midges (Chironomids), specifically the tribes Chironomini, Orthocladiini, and Tanypodini. Additionally, both groups include pond snails, with representation from the sinistral Physidae and dextral Lymnaeidae families. Damselflies and dragonflies (Odonata) are also common to both groups, with individuals of the genera *Brechmorhoga* (Libellulidae) and *Enallagma* (Coenagrionidae). Lastly, both groups contain Oligochaeta 1, further contributing to the observed dissimilarities.

**Table 3.** ANOSIM results. Both and either groups of seasonality and land use are observed to have a significant difference of means between and within (p<0.05).

Seasonality + Lan	d Use	Seasonality		Land Use	
ANOSIM		ANOSIM		ANOSIM	
Permutation N:	9999	Permutation N:	9999	Permutation N:	9999
Mean rank within:	669.5	Mean rank within:	502.1	Mean rank within:	659
Mean rank between:	720.8	Mean rank between:	922	Mean rank between:	746
R:	0.0718	R:	0.5869	R:	0.1218
p (same):	0.048	p (same):	0.0001	p (same):	0.0058

**Table 4.** SIMPER results, showing taxa responsible for dissimilarities between seasonality. Only taxa above 3% contribution are displayed.

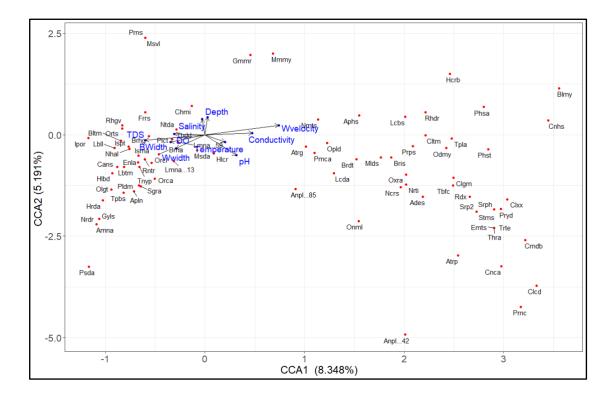
Taxon	Average dissimilarity	Contribution %	Cumulative %	Mean Apr-May	Mean Nov-Dec
t. Chironomini	8.113	9.377	9.377	2.09	3.37
Oligochaeta 1	5.783	6.667	16.044	0	1.81
t. Orthocladiini	4.887	5.634	21.678	0.037	1.56
Brechmorhoga	3.183	3.67	25.348	0.11	1.02
Radix	3.137	3.617	28.965	0.985	0
Physa	3.051	3.518	32.483	0.918	0
Enallagma	2.85	3.286	35.769	0	0.943
t. Tanypodini	2.675	3.084	38.853	0	0.9

**Table 5.** SIMPER results, showing taxa responsible for dissimilarities between land uses. Only taxa above 3% contribution are displayed.

Taxon	Average dissimilarity	Contribution %	Cumulative %	Mean CC	Mean PO	Mean AU
t. Chironomini	7.747	9.81	9.81	2.12	3.2	2.7
Oligochaeta 1	4.601	5.826	15.636	0.978	0.594	1.42
t. Orthocladiini	3.436	4.352	19.988	0.434	1.06	0.823
Physa	2.957	3.744	23.732	0.397	0.293	0.884
Radix	2.746	3.477	27.209	0.851	0.231	0.478
Melanoides	2.738	3.467	30.676	0.343	0.104	1.15
Brechmorhoga	2.497	3.162	33.838	0.278	0.774	0.57

#### **Gradient Length Analysis and Taxa Distribution Patterns**

CCA results as shown in Figure 2 showed weak explanatory power on the pattern of macroinvertebrates in accordance with the physicochemical parameters measured across sampling sites. The first two axes cumulatively explained 13.539% of the variation in the physicochemical and macroinvertebrate data set. The first axis (CCA1) accounted for 8.348% of the variance, while the second axis (CCA2) accounted for 5.191%. However, apparent clustering based on the first axis gradient can still be observed with gastropods and annelids associated with larger river width while dipterans are associated with faster velocity, higher pH, conductivity, and temperature.



**Figure 2.** CCA biplot displaying the relationships between environmental variables and the distribution of sampled taxa. Environmental variables are represented by blue arrows, where the direction and length indicate the gradient and strength of each variable. Taxa abbreviations found in Appendix B.

#### **Difference of Functional Indices Between Sites**

Two-way PERMANOVA of functional indices showed no significant differences between seasonality (p = 0.7553), or land use (p = 0.9834). No significant differences were observed.

**Table 6.** Two-way PERMANOVA results for functional indices according to spatial (land use) groups showing mean values and standard errors in parentheses.

Parameter	Apr-M	Apr-May		Nov-Dec	
Functional Index					
Functional Divergence	0.86	(0.04)	0.83	(0.04)	0.2164
Functional Evenness	0.51	(0.05)	0.53	(0.03)	0.7239
Functional Richness	0.63	(0.04)	0.67	(0.03)	0.3762

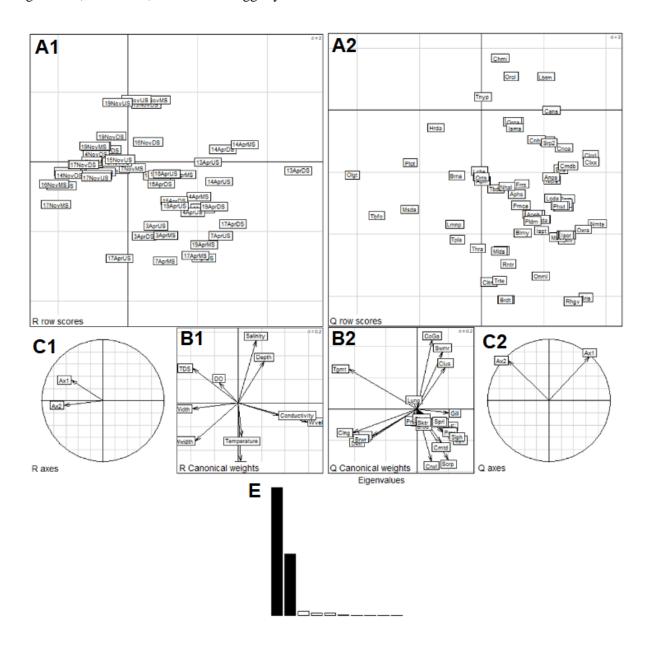
**Table 7.** Two-way PERMANOVA results for functional indices according to temporal (seasonality) groups showing mean values and standard errors in parentheses.

Parameter	cc		PO		AU		P-value
Functional Index							
Functional Divergence	0.86	(0.06)	0.82	(0.04)	0.86	(0.03)	0.7456
Functional Evenness	0.52	(0.05)	0.52	(0.04)	0.52	(0.03)	0.9904
Functional Richness	0.65	(0.05)	0.67	(0.03)	0.63	(0.04)	0.8048

#### Analysis of Macroinvertebrate Distribution and Trait-Environment Relationships

The distribution of macroinvertebrate taxa in the streams was explained by linking the trait characteristics of the species to the environmental conditions. The Monte Carlo test, with 9999 permutations, confirmed that for both models, the observed inertia is significantly greater than what would be expected under the null hypothesis (p=0.035; p=0.049), providing evidence of significant associations in the RLQ analysis, as shown in Figure 3. The first two axes of the RLQ explained 63.643% and 30.666% of the total variance, linking the environmental characteristics in Table R with species traits in Table Q. The first RLQ axis differentiated reaches with higher TDS values, denoting an affinity for harder waters, whereas the second axis differentiated reaches with larger bank-full width and a secondary relationship to the wetted width. For the traits, the first RLQ axis highlighted the

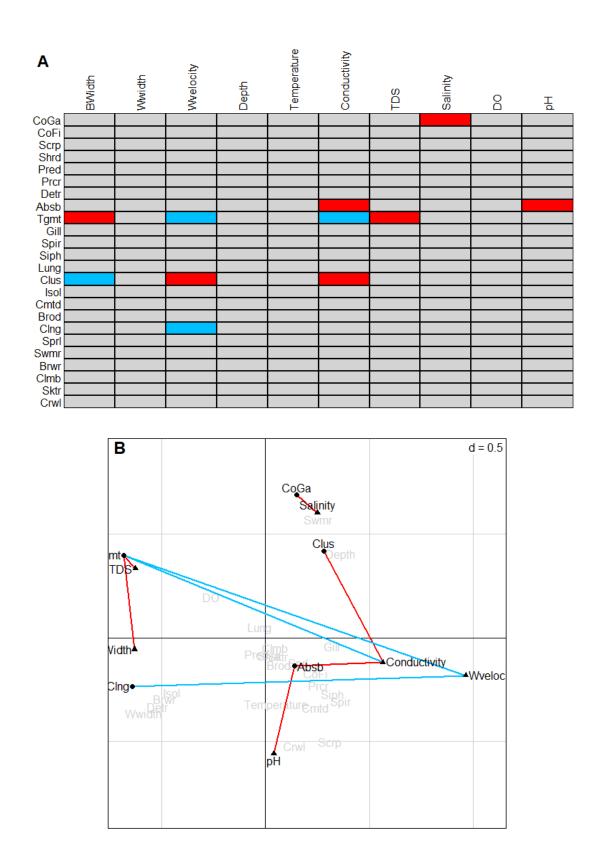
presence of tegument or skin-breathing characteristics, while the second axis differentiated collectorgatherers, swimmers, and clustered egg-layers.



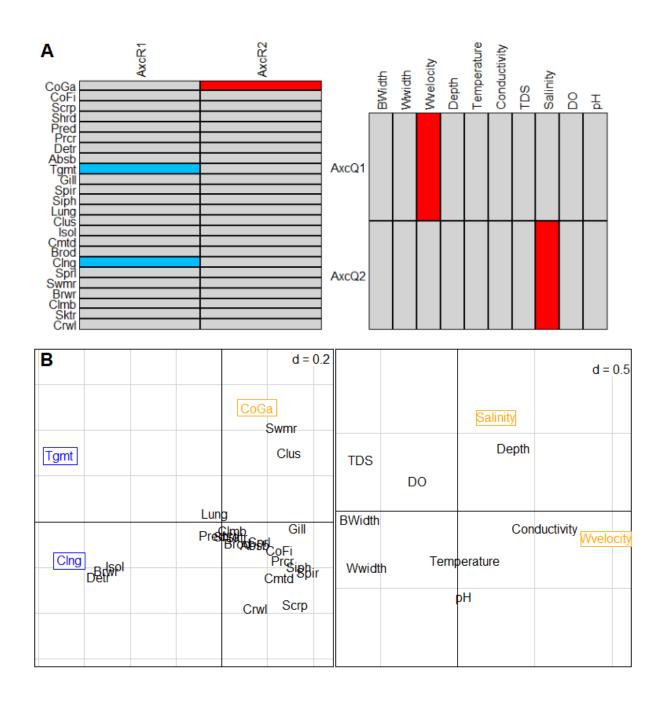
**Figure 3.** RLQ Analysis of macroinvertebrate taxa, environmental parameters, and functional traits.

(A) Distribution of environmental sites and taxa on the first two RLQ axes. (B) Canonical weights, with arrows indicating the correlation strength and direction for environmental parameters and functional traits. (C) R and Q axes for correlation. (D) Bar plot of eigenvalues, indicating the variance explained by each axis, with the first two axes explaining the most variance.

Further testing of bivariate relationships between functional traits and environmental parameters was conducted using the fourth-corner test, where 16 functional traits initially showed varying bivariate relationships with all 10 environmental parameters. After adjustment with the Benjamini-Hochberg procedure (FDR) employed to adjust p-values and reduce probable Type I errors in bivariate associations, five functional traits were significantly matched with six environmental parameters. Collector-gatherers exhibited a negative correlation with environments having high salinity. Absorbers showed a negative correlation with environments having high conductivity and pH. Tegument breathers displayed a negative correlation with rivers of high bank-full widths and waters with high TDS, but had a positive correlation with waters of high velocity and conductivity. Clustered egg-layers demonstrated a positive correlation with rivers of high bank-full widths, yet showed a negative correlation with waters of high velocity or conductivity. Clingers showed a positive correlation with waters of high velocity. The fourth-corner table and the overlapping Q and R axes showing their relationships can be seen in Figure 5. The tables and biplots for the Q and R tests were also generated, as seen in Figure 6. For Axis 1, tegument breathers and clingers show significant correlations, with water velocity being the key environmental parameters driving these associations. In contrast, Axis 2 exhibits significant associations for collector-gatherers with salinity being the dominant factor affecting this functional trait.



**Figure 4.** (A) FDR-adjusted fourth-corner tabulation showing negative (red) or positive (blue) correspondences between functional traits and environmental parameters; and (B) corresponding biplot with overlapping Q and R axes.



**Figure 5.** (A) FDR-adjusted fourth-corner table showing negative (red) or positive (blue) correspondences respectively according to the 1st and 2nd axes of both Q and R results. (B) Negatively (orange) and positively (blue) significant factors are shown in their respective canonical weight biplots.

#### **DISCUSSION**

#### **Differences Across Land Use and Seasonality**

Hilsenhoff (1988) discusses in their index findings that the HFBI particularly puts emphasis on mayflies under the Baetidae and Heptageniidae families in indicating clean streams, while there's also an emphasis on blood-red chironomids under the tribe Chironomini in showing dirty streams. In the same vein, BMWP/ASPT<sup>thai</sup> is an index derived from the original UK BMWP scoring system but adapted for tropical streams as with the case in Thailand (Chessman & Giap, 2010). They note that certain taxa indicate specific conditions such as Ephemeroptera as circumneutral indicators, or Syrphidae showing up in organically-enriched areas, or Chironomidae indicating areas with low oxygen concentration or partial pressure. There is an apparent scarcity of mayflies within the streams of this study, whereas the density of chironomids and other pollution-tolerant dipterans are alarmingly prominent among samples. The tribe Chironomini is particularly resistant to low oxygen and high pollutant conditions due to the presence of specialized hemoglobins for oxygen sequestration (Connolly, 2014; Nath, 2018), indicating low oxygen concentration within the campus waterways.

The analyses of Blakely et al. (2014) in their SingScore Index showed that urban streams with poor quality were often located near roads, concrete dikes, or culverts, and were frequently used for recreational activities or army exercises. Similarly, Chessman (2001) found in their study that streams with poor SIGNAL2 scores typically indicate urban, industrial, or agricultural pollution, or downstream effects of dams; in more severe cases, they suggest the presence of toxic pollution or harsh physical conditions. It is highly likely that the poor scores received by the UP Diliman waterways for both indices result from their proximity to areas with frequent construction activity and heavy car traffic, whether for the maintenance of sanctioned staffhouses, the flow of businesses, or the transportation of students and faculty.

Overall, the comparative analysis of the various water quality indices reveals a consistent pattern of poor water quality as a common denominator across all assessments. The HFBI, SingScore, SIGNAL2, and BMWP<sup>thai</sup>/ASPT<sup>thai</sup> indices consistently indicate degraded water quality conditions

within the University of the Philippines Diliman waterways. Despite differences in the specific parameters and assessment methods used by each index, the overarching conclusion remains the same: the presence of high organic and nutrient pollution, low dissolved oxygen levels, and poor stream health.

The findings of a significant difference in groups of seasonality for ATSPT indicate that taxa found in April to May are more tolerant of disturbance compared to their counterparts in November to December. This difference may be corroborated by results in chemical parameters, namely higher temperature, lower TDS, and lower DO differences found between the two sampling periods. Although research shows that an increase in temperature results in waters becoming more favorable for sensitive taxa, this was only true up to a certain point as taxa with cold water tolerance were seen to occur less as well (Khaliq et al., 2024; Li et al., 2013). The hot and dry conditions typical of April and May may have contributed to the decline of sensitive taxa, indicating a shift in their abundance during this season. The study conducted by Olson and Hawkins (2017) also revealed that macroinvertebrates preferring lower Total Dissolved Solids (TDS) levels experience increased osmoregulatory stress and are expected to be more adversely affected by increasing climate and land use changes over time. These observations, consistent with the ATSPT index, are associated with stunted life history traits and higher mortality rates in stream macroinvertebrates. Additionally, Connolly et al. (2004) found that DO concentrations significantly impact the survival and drift of several taxa, with an aversion to waters of 25% concentration and mortality at 10%. Considering these observations, it's likely that only the taxa with higher tolerance to varying environmental conditions would remain in the ecosystem, as they are better equipped to cope with the changing conditions over time.

Another interesting finding that also occurred is the fact that for the biological response metrics, total abundance for April to May was noticeably lower than for November to December but were still significantly higher in Simpson's Diversity Index and Simpson's Evenness Index. This pattern shows that the community was more diverse and evenly distributed during this period, which indicates a more functionally diverse and therefore resilient and balanced ecosystem. The dry season of April to May likely led to a decrease in overall abundance due to environmental stressors such as decreased water availability, as exemplified by the lessened wetted width and increased temperature. However, the

species that persisted during this period may represent a more specialized and resilient community adapted to survive under these conditions, resulting in a more evenly distributed and functionally diverse ecosystem.

April to May had streams that were only 70% as wide but with water flowing more than six times as fast as in November to December. Newbold (n.d.) explains the ecological process of nutrient spiraling in the context of the river continuum, where downstream water flow deposits sediments that provide feeding grounds for macroinvertebrates. This process involves the continuous exchange of inorganic and organic materials within the food web and across the river ecosystem. Covich et al. (1999) supplement this by noting that benthic macroinvertebrates tend to proliferate with the abundance of layered sediments, which accelerates nutrient cycling as a by-product of thriving in rivers with narrow, fast-moving waters. Therefore, the heightened diversity and evenness of the April to May macroinvertebrate communities can be attributed to this phenomenon, despite the narrower aquatic habitats.

Ancog et al. (2012) found that similar pH levels in rivers were associated with greater biodiversity and richness, likely due to the buffering effects of carbonate-based materials, which stabilize pH and protect ecosystems from acidification. Sanchez et al. (2020) supported this, showing that higher alkalinity can mitigate the effects of acid rain and pollutants. However, despite the significant pH difference between CC and PO, no significant differences in taxa abundance, diversity, or evenness were observed. This may indicate that the biota in both areas are tolerant to the pH range observed, or that the pH variation was not substantial enough to affect community structure within the study period. One possible explanation for the higher pH in the CC area is the constant presence of building projects, which could contribute to alkalinity through limestone runoff. This runoff may be temporarily beneficial by providing a buffer against acidification, but it could also have chronically negative effects if it leads to excessive alkalinity, such as ammonia toxicity and algal blooms which may cause large-scale mortality of present aquatic life (Burdick et al., 2020). Differentially, the constant presence of cars along University Avenue may be causing acidification of PO sites through direct deposition of smoke or via acid rain. As explained by Singh & Agrawal (2006), these mechanisms of

acidification not only harm aquatic ecosystems but also pose health risks for humans, affect soil and crop quality, and compromise building integrity due to the deposition of corrosive chemicals and metallic precipitates from runoff.

#### **Ecological Resilience and Adaptations of Indicator Species**

Chironomids, or non-biting midge larvae, belonging to the family Chironomidae, exhibit specialized adaptations that allow them to thrive in hypoxic conditions. Their elongate shape maximizes surface area, facilitating oxygen diffusion through their cuticle, a process known as cutaneous respiration (Cooper & Uzarski, 2009). Additionally, chironomid midge larvae possess extracellular hemoglobins in monomeric and dimeric forms, unique among invertebrates, which float in their hemolymph. The presence and modulation of hemoglobin concentration in chironomids enables them to tolerate a wide range of environmental stressors, including hypoxia and chemical pollution (Nath, 2018).

The presence of pond snails, specifically belonging to the sinistral Physidae and dextral Lymnaeidae families, in the highly disturbed and polluted waterways of the UP Diliman campus showcases their resilience and adaptability to environmental stressors. These snails are recognized as significant biological indicators due to their ability to accumulate persistent organic pollutants (POPs) and metallic trace elements (Baroudi et al., 2020). According to Voshell and Wright (2002), gilled snails are reliant on high concentrations of dissolved oxygen and are thus sensitive to pollution. In contrast, pond snails, belonging to the pulmonate group, have an internal lung-like structure that allows them to directly absorb oxygen from air or water, making them particularly tolerant to disturbance and pollution because of this adaptation. This suggests that the observed presence of these snails might be an indication of the extent of pollution and the types of contaminants present in the waterways.

The two observed genera of odonates, *Brechmorhoga* (Libellulidae) and *Enallagma* (Coenagrionidae), are specifically cited by Hilsenhoff (1988) to indicate the presence of very prominent stream organic pollution. Hilsenhoff's study delineates a distinction among odonate families in terms of their tolerance to pollution. While families such as Aeshnidae, Calopterygidae, Cordulegastridae,

Corduliidae, and Gomphidae are considered low to moderately tolerant, Coenagrionidae and Libellulidae are identified as highly tolerant to stream organic pollution. The exclusive presence of these two specific tolerant taxa, observed consistently across seasons and different land uses, further strengthens the argument that they are particularly adapted to tolerate the polluted conditions with numerous environmental stressors of the waterways studied.

Oligochaetes, unlike polychaetes, are generally found to be pollution-tolerant and are often associated with dipterans. They exhibit a positive relationship with eutrophication and pollution gradients, as well as turbidity and conductivity gradients (Rashid & Pandit, 2014). Specifically, haplotaxids, including tubificids, are frequently observed in high abundances and densities in waters rich in organic pollutants (Martins et al., 2008).

The constant presence of these taxa across different seasons and land use types suggests a significant influence of anthropogenic pollution and disturbance on the habitat. These taxa are known for their tolerance to various environmental stressors, particularly pollutants and low oxygen levels, indicating a disturbed state of the waterways in the UP Diliman campus. Their resilience and adaptability to such conditions highlight the severity of the environmental degradation and the urgent need for resolution through effective management and restoration efforts.

#### **Functional Stability of Macroinvertebrate Communities**

The consistent functional indices across sites, regardless of land uses or sampling periods, imply that the functional traits of taxa within the study area are relatively stable and consistent over time, suggesting a stable ecological role and function despite variations in environmental conditions. This observation indicates a high degree of functional redundancy within the community, where multiple species can perform similar ecological functions. This redundancy helps maintain ecosystem stability and resilience even under varying environmental conditions. This suggests that the macroinvertebrate community in UP Diliman waterways has likely attained a certain level of resilience and adaptability to the apparent pollution and disturbance. Additionally, it implies that certain species

within the community possess traits that enable them to thrive or persist under such conditions, highlighting the presence of tolerant or resistant species within the ecosystem.

#### Functional Responses of Macroinvertebrate Communities to Environmental Variations

Since collector-gatherers specialize in obtaining nutrition from fine particulate organic matter, high salinity can significantly impact the feeding behavior and nutrient acquisition mechanisms of collector-gatherers. High salinity levels in the water can create an osmotic gradient between the external environment and the internal tissues of macroinvertebrates, necessitating the expenditure of energy to actively regulate the movement of ions and water across their cell membranes. This process can be energetically costly and may divert resources away from other vital functions such as feeding and growth. Furthermore, high salinity conditions can affect the feeding efficiency of macroinvertebrates by altering the availability and quality of their food resources. While it's less likely that high salinity directly reduces the concentration or density of nutrients in fine particulate organic matter, it may impact the overall composition and diversity of the food available. In environments with elevated salinity, certain types of organic matter may become less accessible or less palatable to collector-gatherers. For example, high salinity was found to lead to changes in the composition of aquatic vegetation, detritus, and algae, which are important food sources for many macroinvertebrates (Nielsen, 2003).

Absorbers are organisms that intake dissolved organic matter and nutrients directly from the water column through their thin and permeable body surfaces or specialized structures. These organisms have the capability to passively absorb nutrients and organic compounds dissolved in the surrounding water. Acidic-circumneutral and less conductive environments typically exhibit higher nutrient availability, increased solubility of organic compounds, reduced presence of toxic metals, and decreased osmotic stress (Schneider et al., 2013; Sintorini et al., 2021). These environmental characteristics collectively enhance the absorbers' capacity to assimilate nutrients and organic matter directly from the water column.

Tegument breathers, organisms relying on respiratory exchange through their body surface, displayed contrasting correlations within various aquatic environments. In rivers with expansive bank-

full widths, reduced water flow and increased sediment deposition are expected (Rinaldi et al., 2013) and may limit the efficiency of oxygen exchange across the tegument surface, thereby adversely impacting the respiratory processes of tegument breathers. Moreover, high TDS levels often indicate heightened concentrations of dissolved ions and organic matter, further impeding respiratory efficiency. On the other hand, environments with high water velocity typically experience enhanced oxygenation and turbulent flow (Pinto et al., 1982), facilitating more efficient respiratory exchange across the tegument surface. This higher oxygen availability positively influences the abundance of tegument breathers. Additionally, high conductivity levels, indicating the presence of ions, may support the physiological performance of tegument breathers by aiding in maintaining osmotic balance. Therefore, tegument breathers were observed to exhibit preferences for well-oxygenated and ion-rich environments, while showing limitations in habitats characterized by extensive bank-full widths and high TDS levels.

The preference of clustered egg-layers on rivers with high bank-full widths indicates an affinity for habitats with expansive riverbanks. These environments may offer suitable conditions for clustered egg-layers to deposit and incubate their eggs in clusters, providing protection and stability during the incubation period due to reduced water flow and increased sediment deposition (Rinaldi et al., 2013), which may lead to higher hatching success and better initial life history for larvae. This explains the converse, where clustered egg-layers are averse to rivers with high velocity or rapid-flowing waters, which can potentially disrupt egg clusters and hinder their stability. Additionally, elevated conductivity levels may indicate the presence of ions and dissolved substances that could adversely affect egg development or the viability of offspring such delayed pupation and hindered development of larvae (Mamai et al., 2021).

Lastly, though clingers mainly refer to the locomotory habit or mobility, it should be noted that it is possible that the taxa reflected across this functional trait modality has an inconspicuous or unreflected relationship with modalities of other functional traits such as those in functional feeding groups. This could help to better explain or understand the unintuitive relationship of clingers with waters of high velocity. It is possible that members of said functional trait modality are better able to

catch prey, or receive nutrition from a fast-moving flow or water column, as is the case with predators (Hoover & Richardson, 2010) or shredders (Yaagoubi et al., 2023).

## **CONCLUSION**

The findings from various biotic indices highlight severe pollution in the UP Diliman waterways, indicating significant urbanization effects on water quality. The HFBI suggests severe pollution, while the SingScore reflects negative impacts from urbanization. SIGNAL2 points to disturbed habitats characterized by low dissolved oxygen, elevated salinity, turbidity, and disruptive nutrient levels such as nitrogen and phosphorus, with ASPT<sup>thai</sup> confirming generally poor water quality. Significant seasonal variations were observed, with more tolerant taxa predominating during the dry season (April to May) compared to the wet season (November to December), suggesting specialized and resilient communities during drier periods. The dominance of pollution-tolerant taxa such as Chironomids, Physidae and Lymnaeidae snails, specific Odonata genera, and oligochaetes underscores ongoing anthropogenic disturbance. Despite these stresses, functional indices remained stable across seasons and land use changes, indicating the macroinvertebrate community's resilience and adaptability. Notably, significant relationships between functional traits and environmental parameters were identified: collector-gatherers negatively correlated with salinity; absorbers with conductivity and pH; tegument-breathers with river bank-full width and TDS (negatively), and water velocity and conductivity (positively); clustered egg-layers with water velocity and conductivity (negatively), and river bank-full width (positively); and clingers with water velocity (positively). These results demonstrate the variations in macroinvertebrate functional traits and distributions, their relationships with physicochemical parameters, and the ecological impact of urbanization on water quality in the UP Diliman waterways.

## RECOMMENDATIONS

While biotic indices are valuable tools for assessing the health of aquatic ecosystems using family-level taxa, they offer only a limited perspective. These indices may not fully correspond to all the available families in a given area, particularly in regions with unique ecological characteristics such as the Philippines. Thus, there is a need for the development of a biotic index specifically tailored to Philippine streams. Such an index would provide a more accurate and comprehensive assessment of aquatic ecosystem health in the region. It is recommended that future studies focus on the creation and implementation of a biotic index that accounts for the diverse array of families present in Philippine streams. This index could then be utilized in future iterations of research to enhance the precision and relevance of ecological assessments in the region.

From the original 19 sampling sites, it was found that 2 sites did not yield any samples, while 8 sites had incomplete samples. The incomplete sampling could be attributed to the depreciation and degradation of specimens during storage; the samples are now 8 years old. This aging process likely resulted in the degradation of internal structures, leaving behind only exoskeletons, particularly for non-hardy or soft-bodied genera. To reiterate, the protocol includes the removal of exoskeletons and empty shells, as it remains uncertain whether these specimens had contents during the time of sampling. Therefore, it is recommended that future iterations of studies similar to this take into consideration the shelf life of samples and specimens so as not to suffer from severe data loss.

Throughout the duration of this study, only two sampling periods were employed. Given the chronic and evolving nature of climate change, it is recommended that future research consider incorporating additional data points beyond 2016. Increasing the frequency of sampling will provide a more comprehensive understanding of the long-term effects of climate change on the studied ecosystem.

Although no apparent land use changes were observed within the 6-month sampling period of this study, it is crucial to note that significant developments have occurred since the writing of this thesis. These developments include the channelization of waterways along the university belt and the introduction of canals and culverts, among other alterations to the landscape. In future iterations of this study, it is recommended that these changes also be carefully considered in the analyses and discussions.

In future ecological studies, it would also be valuable to investigate the relationships between different functional trait modalities more closely. While many studies have traditionally focused on interpreting functional traits in relation to environmental factors, there is a growing need to explore the potential interactions and correlations among different trait modalities. By shifting the focus towards modality-modality relationships, researchers may gain a deeper understanding of how traits are interconnected within ecological communities. Specifically, investigating the relationships between traits such as clingers in locomotory habit and predators or shredders in functional feeding groups as discussed earlier could provide valuable insights into the relationships of their morphology and how modalities of one functional trait may have dominated in tandem with modalities of other functional traits.

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# APPENDIX A

Table A1. Affinities of the macroinvertebrate taxa to functional feeding groups based on literature

	References	Yule & Yong (2004)	Young & Fontanilla (2014)	Yule & Yong (2004)	Bruce (1981)	Seymour et al (2015)	Yule & Yong (2004)	Yule & Yong (2004)	Young & Fontanilla (2014)	Govedich et al. (2003)	Qiu et al. (2017)	Yule & Yong (2004)	Yule & Yong (2004)	Yule & Yong (2004)	Lima et al. (2022)	Yule & Yong (2004)	Yule & Yong (2004)	Yule & Yong (2004)	Jia & Fikacek (2014)	Macroinvertebrates.org (n.d.)	Yule & Yong (2004)	Zuzana et al. (2021)	Yule & Yong (2004)	Mahr (n.d.)	Yule & Yong (2004)	Young & Fontanilla (2014)	Yule & Yong (2004)	Young & Fontanilla (2014)	Young & Fontanilla (2014)	Rupert et al. (2004)	Short & Giron (2018)	Rupert et al. (2004)
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Ĭ	Tgmt Gill Spir Siph Lung Clus Isol Cmtd Brod Clng Sprl Swmr Brwr Clmb Sktr Crwl	3		3	3	_	3	2		2				2	3	2	2			2	8	3	2	2			8			2	2	2
	Sprl S											2	2																			
	Clng									-	2	-							2											-		-
uo	Brod										2																					
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	CoG														3																	
	Taxon	Aedes	Amerianna	Anopheles	Anopsilana	Aphelocheirus	Aphelonecta	Austroagrion	Austropeplea	Barbronia	Bellamya	Beris	Brachydentera	Brechmorhoga	Caenis	Canacea	Canthydrus	Clogmia	Coelostoma	Crambidae 1	Culex	Culicoides	Enallagma	Eristalis	Eumetopus	Ferrissia	Gammarus	Gyraulus	Helicorbis	Helobdella	Helochares	Hirudinea 1

Table A1. Affinities of the macroinvertebrate taxa to functional feeding groups based on literature (cont)

			FFG					Respiration	ation		Re	Reproduction	ction			H	Habit			
Taxon Co	CoGa CoFi Scrp Shrd Pred Prcr Detr Absb Tgmt Gill Spir Siph Lung Clus Isol Cmtd Brod Clng Sprl Swmr Brwr Clmb Sktr Crwl	Scrp	Shrd P	red Pr	cr De	tr Absb T	gmt G	ill Sp	ir Siph	Lung	Clus I	sol Cr	ntd Bro	od Clng	y Sprl Sv	/mr Bi	rwr Clı	nb Skt	tr Crwl	References
Indoplanorbis		2						2			2		_						3	Young & Fontanilla (2014)
Isolapotamon			2	3				2			8		2						2	Gherardi & Fiorenza (1989)
Isotoma					3			3 1			2					3				Zettel (1982)
Labiobaetis	3							8			3					9				Wakhid et al. (2021)
Laccobius				3				3 1				2				2				Yule & Yong (2004)
Lethocerus				3				2	2		2		2				2		-	Merritt (2008)
Libellula				3				2 2				3				2				Yule & Yong (2004)
Limnophila	-			2	_			3			_	2				_	2			Yule & Yong (2004)
Lycosidae 1				3				_		3	က		_			_	.,	2	-	Schmitz & Perry (2002)
Lymnaea		2						2			3								3	Young & Fontanilla (2014)
Melanoides		2			2		2	2					3						3	Raw et al. (2016)
Mesovelia				2	_			2			2		_					3		Yule & Yong (2004)
Mimomyia	9								က			3				3				Saari et al. (2019)
Muscidae 1					9			3			3			3						Yule & Yong (2004)
Naucoris				3				2			2		2			3				Yule & Yong (2004)
Nehalennia				3				3 2				2	_			2				Yule & Yong (2004)
Nemotelus				N	2 1			2			3		3			2				Yule & Yong (2004)
Neritina		2						2				2	2						3	Kano & Fukumori (2010)
Neritodryas		2						2				2	2						3	Young & Fontanilla (2014)
Noctuidae 1			3					3			3					2				Seth & Sharma (2002)
Odontomyia				_	1 2			2			8		3			3				Yule & Yong (2004)
Oligochaeta 1					3		3				_	_		2		_	2		-	Martins et al. (2008)
Oncomelania		2						2				2	2						က	Dazo & Moreno (1962)
Oplodontha				,-	1 2			2			က		3			3				Yule & Yong (2004)
Orchestia					3			3			3					3				Mekong River Commission. (2006)
Orthemis				3				2 2				3				2				Yule & Yong (2004)
Oxycera		2						2			3		3			3		_		Yule & Yong (2004)

Table A1. Affinities of the macroinvertebrate taxa to functional feeding groups based on literature (cont)

												Topon or deal						
CoGa	CoFi Scr	p Shro	Pred	Prcr D	etr Abs	b Tgm	t Gill 9	spir Si	ph Lu	ng Clu	s Isol	CoGa CoFi Scrp Shrd Pred Prcr Detr Absb Tgmt Gill Spir Siph Lung Clus Isol Cmtd Brod Clng Sprl Swmr Brwr Clmb Sktr Crwl	Ing Sprl Sv	vmr Brv	ır Clmb	Sktr Crw		References
Paludomus	2						2			3						8	Yule	Yule & Yong (2004)
Paranisops			3				-	2			3			8			Yule	Yule & Yong (2004)
	9								8		3		2	2			Yule	Yule & Yong (2004)
Peripsychoda	2				2			3		3						2	Yule	Yule & Yong (2004)
	2					2	2			2		_				3	Young 8	Young & Fontanilla (2014)
Physastra	2						2			2		-				3	Young 8	Young & Fontanilla (2014)
Polychaeta 1			3			2	_			3				3			Yule	Yule & Yong (2004)
Pomacea	2 1				8		2		3	3		2				8	Yule	Yule & Yong (2004)
Procanace	2						က	_		2				2			Yule	Yule & Yong (2004)
Psychoda	2				2			3		3						2	Yule	Yule & Yong (2004)
Radix	2						2			3						8	Young 8	Young & Fontanilla (2014)
Ranatra			3						3		3					3	Yule	Yule & Yong (2004)
Rhagadotarsus			3					2		3		2				3	Yule	Yule & Yong (2004)
Rhagovelia			2	_	_			3			-	9				3	Yule	Yule & Yong (2004)
			2	_				2		3				_		2	Yule	Yule & Yong (2004)
Stratiomyis				_	2			2		3		3		8			Yule	Yule & Yong (2004)
Syrph 2			3						2	3				2				Mahr (n.d.)
t. Chironomini 3						2	-			2				9			Cooper	Cooper & Uzarski (2009)
t. Orthocladiini 2	_					2	-			2				3			Cooper	Cooper & Uzarski (2009)
t. Tanypodini			2		_	2	-			2				3			Cooper	Cooper & Uzarski (2009)
Tabanidae 1			3						3	3				1 2			Yule	Yule & Yong (2004)
Thiara	2				2		2				3					3	Yule	Yule & Yong (2004)
Tipula		_	2					3		_	2			1 2			Yule	Yule & Yong (2004)
Trepobates			3					2		3		2				3	Yule	Yule & Yong (2004)
		2							3		3			_	2	-	Yule	Yule & Yong (2004)
Tubificidae 1					3	3				2		3		3				Timm (2012)

## APPENDIX B

Table B1. Taxa Names and Corresponding Codes for Data Visualization

Table DT. Taxa	Ivanics	and Correspond	uning Cot	les for Data Visuali	Zation
Taxa	Code	Taxa	Code	Taxa	Code
Helobdella	Hlbd	Canthydrus	Cnhs	Labiobaetis	Lbtm
Barbronia	Brna	Procanace	Prnc	Caenis	Cans
Hirudinea 1	Hrda	Canacea	Cnca	Aphelocheirus	Aphs
Tubificidae 1	Tbfc	Culicoides	Clcd	Belostomatidae 1	Bltm
Oligochaeta 1	Olgt	t. Chironomini	Chmi	Sigara	Sgra
Polychaeta 1	Plct	t. Orthocladiini	Orcl	Rhagadotarsus	Rhdr
Pomacea	Pmca	t. Tanypodini	Tnyp	Trepobates	Tpbs
Ferrissia	Frrs	Culex	Clxx	Mesovelia	Msvl
Austropeplea	Atrp	Aedes	Ades	Naucoris	Ncrs
Lymnaea	Lmna	Mimomyia	Mmmy	Ranatra	Rntr
Radix	Rdx	Anopheles	Anpl	Paranisops	Prns
Neritina	Nrti	Parydra	Pryd	Aphelonecta	Apln
Neritodryas	Nrdr	Brachydentera	Brdt	Rhagovelia	Rhgv
Paludomus	Pldm	Muscidae 1	Msda	Austroagrion	Atrg
Physa	Phsa	Clogmia	Clgm	Enallagma	Enla
Amerianna	Amna	Peripsychoda	Prps	Nehalennia	Nhal
Gyraulus	Gyls	Psychoda	Psda	Brechmorhoga	Brhg
Helicorbis	Hcrb	Beris	Bris	Libellula	LbII
Indoplanorbis	lpor	Nemotelus	Nmts	Orthemis	Orts
Physastra	Phst	Oxycera	Oxra	Crambidae 1	Cmdb
Melanoides	Mlds	Oplodontha	Opld	Noctuidae 1	Ntda
Thiara	Thra	Odontomyia	Odmy	Lycosidae 1	Lcda
Oncomelania	Onml	Stratiomyis	Stms	Trite	Trte
Bellamya	Blmy	Syrph 1	Srph	Gammarus	Gmmr
Eumetopus	Emts	Syrph 2	Srp2	Orchestia	Orca
Coelostoma	Cltm	Tabanidae 1	Tbdd	Anopsilana	Anpl
Laccobius	Lcbs	Tipula	Tpla	Isotoma	Isma
Helochares	Hlcr	Limnophila	Lmna	Isolapotamon	Ispt

## APPENDIX C

```
library(vegan)
library(tidyverse)
library(car)
set.seed(100)
nmds<-read.csv(file.choose())</pre>
nmds data<-nmds[,2:85]</pre>
nmds result<-metaMDS (nmds data, distance = "bray")</pre>
plot (nmds result)
stressplot(nmds result)
nmds result$stress
nmdsscores <- data.frame( scores(nmds result, display="sites"))</pre>
ggplot(nmdsscores, aes(NMDS1, NMDS2)) +
 geom point()+ geom text(label=rownames(nmds data), nudge x =
0.015, nudge y = 0.015, + theme bw()
#-----
-#
#test for modal distrib
env <- nmds[,4:87]</pre>
env.dca <- decorana(env,ira=1)</pre>
env.t1 <- decostand(env, "total")</pre>
env.t2 <- decostand(env.t1, "max")</pre>
#dca after transformation
env.t2.dca <-decorana(env.t2)</pre>
env.t2.dca.DW <-decorana(env.t2,iweigh=1)</pre>
env.t2.dca.DW
                        DCA1 DCA2 DCA3 DCA4
                      0.7879 0.4552 0.4593 0.2882
#Eigenvalues
#Additive Eigenvalues 0.7879 0.4541 0.4540 0.2892
#Decorana values 0.8007 0.5424 0.4861 0.2916
#Axis lengths
                     7.5254 5.2169 5.8409 2.7056
#DCA 7.5 > 3.0 = unimodal; perform CCA
```

```
-#
#cca
library(vegan)
library(ggplot2)
library(ggrepel)
data("varechem")
data("varespec")
species data <- varespec[, 4:87]</pre>
species data
env data <- varechem[, 4:13]</pre>
env data
cca result <- cca(species data, env data)</pre>
summary(cca result)
plot(cca result)
cca.biplot<-plot(cca result, scaling=1, display=c("wa", "bp"))</pre>
cca.biplot2<-plot(cca result, scaling=2, display=c("sp", "bp"))</pre>
veg 1 = as.data.frame(cca result$CCA$biplot)
veg 1["env"] = row.names(veg 1)
veg 2 = as.data.frame(cca result$CCA$v)
veg 2["genus"] = row.names(veg 2)
plot = ggplot() +
  geom point(data = veg 2, aes(x = CCA1, y = CCA2), color = "red") +
  geom point(data =
                veg 1, aes(x = CCA1, y = CCA2), color = "blue")
plot
plot +
  geom text repel(data = veg 2,
                   aes(x = CCA1, y = CCA2, label = veg 2$genus),
                   nudge y = -0.05) +
  theme bw() +
  geom segment(
    data = veg 1,
    aes(
      x = 0,
      y = 0,
      xend = CCA1,
      yend = CCA2
```

```
),
    arrow = arrow(length = unit(0.25, "cm"))
  geom text repel(
    data = veg 1,
    aes(x = CCA1, y = CCA2, label = veg 1$env),
    nudge y = -0.05,
    color = "blue",
    size = 5
  ) +
  theme(axis.text = element text(size = 16),
        axis.title = element text(size = 18))
#-----
-#
#rlq
library(ade4)
library(tidyverse)
data(aravo)
ffbert <- list(data.frame(spe), data.frame(traits),data.frame(env))</pre>
names(ffbert) <- c("spe", "traits", "env")</pre>
dim(ffbert$spe)
dim(ffbert$traits)
dim(ffbert$env)
w1 <- data.frame(ffbert$traits)</pre>
w2 <- prep.fuzzy.var(w1, c(8,5,4,7))
row.names(w2) <- names$Gen</pre>
row.names(ffbert$spe) <- sites$Site</pre>
row.names(ffbert$env) <- sites$Site</pre>
row.names(ffbert$traits) <- names$Gen</pre>
afcL.aravo <- dudi.coa(ffbert$spe, scannf = FALSE)</pre>
acpR.aravo <- dudi.hillsmith(ffbert$env, row.w = afcL.aravo$lw,</pre>
                             scannf = FALSE)
#acpQ.aravo <- dudi.pca(ffbert$traits, row.w = afcL.aravo$cw,</pre>
                        scannf = FALSE)
acpQ.aravo <- dudi.pca(w2, row.w = afcL.aravo$cw,</pre>
                        scannf = FALSE)
rlq.aravo <- rlq(acpR.aravo, afcL.aravo, acpQ.aravo,</pre>
                 scannf = FALSE)
```

```
plot(rlq.aravo)
par(mfrow=c(1,3))
s.arrow(rlq.aravo$11)
s.arrow(rlq.aravo$c1)
s.label(rlq.aravo$lQ,boxes=FALSE)
summary(rlq.aravo)
nrepet <- 4999
four.comb.aravo <- fourthcorner(ffbert$env, ffbert$spe,</pre>
                                 ffbert$traits, modeltype = 6,
p.adjust.method.G = "none",
                                 p.adjust.method.D = "none", nrepet =
nrepet)
plot(four.comb.aravo, alpha = 0.05, stat = "D2")
four.comb.aravo.adj <- p.adjust.4thcorner(four.comb.aravo,</pre>
                                           p.adjust.method.G = "fdr",
p.adjust.method.D = "fdr")
fourthcorner(ffbert$env, ffbert$spe, ffbert$traits, modeltype = 6,
             p.adjust.method.G = "fdr", p.adjust.method.D = "fdr",
             nrepet = nrepet)
plot(four.comb.aravo.adj, alpha = 0.05, stat = "D2")
testrlq.aravo <- randtest(rlq.aravo, modeltype = 6, nrepet = nrepet)</pre>
testrlq.aravo
plot(testrlq.aravo)
Srlq <- fourthcorner2(ffbert$env, ffbert$spe, ffbert$traits,</pre>
                       modeltype = 6, p.adjust.method.G = "fdr",
nrepet = nrepet)
Srlq$trRLQ
plot(four.comb.aravo.adj, x.rlq = rlq.aravo, alpha = 0.05,
     stat = "D2", type = "biplot")
testQaxes.comb.aravo <- fourthcorner.rlq(rlq.aravo, modeltype = 6,</pre>
                                          typetest = "Q.axes", nrepet
= nrepet, p.adjust.method.G = "fdr",
                                          p.adjust.method.D = "fdr")
testRaxes.comb.aravo <- fourthcorner.rlq(rlq.aravo, modeltype = 6,
                                          typetest = "R.axes", nrepet
= nrepet, p.adjust.method.G = "fdr",
```

```
p.adjust.method.D = "fdr")
print(testQaxes.comb.aravo, stat = "D")
print(testRaxes.comb.aravo, stat = "D")
par(mfrow=c(1,2))
plot(testQaxes.comb.aravo,alpha=0.05,type="table",
     stat="D2")
plot(testRaxes.comb.aravo,alpha=0.05,type="table",
     stat="D2")
par(mfrow=c(1,2))
plot(testQaxes.comb.aravo,alpha=0.05,type="biplot",
     stat="D2",col=c("black","blue","orange","green"))
plot(testRaxes.comb.aravo,alpha=0.05,type="biplot",
     stat="D2",col=c("black","blue","orange","green"))
#findices
install.packages("ade4")
install.packages("geometry")
install.packages("fundiversity")
library(tidyverse)
library(FD)
library(fundiversity)
#load abundance and matrix here
ffbert$spe
ffbert$traits #rawmatrix
w2 #standardized
data(traits birds)
fd fric(traits birds)
fd fric(ffbert$traits,ffbert$spe)
fd fdiv(ffbert$traits,ffbert$spe)
fd fdis(ffbert$traits,ffbert$spe)
fd feve(ffbert$traits,ffbert$spe, dist matrix = NULL)
_____
#here be dragons, YOU'VE BEEN WARNED (scratch paper lang)
weights = c(1, 2, 3)
dbFD(ffbert$traits, ffbert$spe, w.abun = TRUE, stand.x = TRUE,
     ord = c("podani", "metric"), asym.bin = NULL,
     corr = c("cailliez"),
     calc.FRic = TRUE, m = "max", stand.FRic = FALSE,
```

```
scale.RaoQ = FALSE, calc.FGR = FALSE, clust.type = "ward",
     km.inf.gr = 2, km.sup.gr = nrow(x) - 1, km.iter = 100,
     km.crit = c("calinski", "ssi"), calc.CWM = TRUE,
     CWM.type = c("dom", "all"), calc.FDiv = TRUE, dist.bin = 2,
     print.pco = FALSE, messages = TRUE)
rich1 <- data.frame(modrMatrix$s, modrMatrix$m, modrMatrix$1,
modrMatrix$X..100, modrMatrix$X.100.1000,
                    modrMatrix$X.1000.3000, modrMatrix$X.3000,
modrMatrix$semi, modrMatrix$uni,
                    modrMatrix$pluri, modrMatrix$X..1,
modrMatrix$X.1.10, modrMatrix$X.10.30,
                    modrMatrix$X.30.365, modrMatrix$X.365)
rich1 <-
data.frame(ffbert$traits$CoGa,ffbert$traits$CoFi,ffbert$traits$Scrp,
ffbert$traits$Shrd,
ffbert$traits$Pred,ffbert$traits$Prcr,ffbert$traits$Detr,ffbert$trai
ts$Absb,
ffbert$traits$Tgmt,ffbert$traits$Gill,ffbert$traits$Spir,ffbert$trai
ts$Siph,
                    ffbert$traits$Lung)
col.names(rich1) <- col.names(ffbert$traits)</pre>
row.names(rich1) <- row.names(ffbert$traits)</pre>
w3 = select(w2, -17:-24)
fd fric(w3, ffbert$spe, stand=FALSE)
sp dist fruits <- mFD::funct.dist(</pre>
  sp tr
               = ffbert$traits,
 metric
            = "gower",
  scale euclid = "scale center",
  ordinal var = "classic",
  weight type = "equal",
  stop if NA = TRUE)
data("fruits traits", package = "mFD")
fruit traits cat
#little john
#galvanized square steel
#eco friendly wood veneers
#screws borrowed from your aunt
```