

Biological Trait & Biodiversity Difference of Macroinvertebrates Occupying the Intermittency Continuum in California Mediterranean Streams

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ABSTRACT

Ephemeral streams experience flow for a short duration after precipitation events with sporadic discharge patterns. Ephemeral streams are rain-fall dominated with an absence of forest litter. In comparison, perennial streams have continuous surface flow throughout the year with less variable discharge patterns in the absence of extreme prolonged drought. As climate change continues to alter the weather patterns in California, there will be more polarized precipitation events that cause an increased frequency of both floods and droughts. Gradually increasing the proportion of streams that have ephemeral flow regimes in Mediterranean regions. Understanding the ecological difference between ephemeral and perennial streams such as the presence of different biological traits and amount of functional trait space occupied can help us anticipate whether macroinvertebrate communities in different flow regimes are more prone to the ecological impacts of climate change. I hypothesize that the habitat difference in the two distinct flow regimes will affect the trait composition in streams. I looked at the abundance of stream macroinvertebrate from Hunting and Knoxville Creeks in California and linked their abundances to published trait information on 63 modalities of 13 biological traits, potentially reflecting communities' resilience from and resistance to dessication events. Although all but one of the metrics used to quantify taxonomic diversity scored higher in perennial streams than in the ephemeral streams, I did not find a significant difference in the total abundance of macroinvertebrates present at each site. In contrast, functional richness was significantly higher at the ephemeral site. Functional evenness did not differ significantly between ephemeral and perennial streams, but functional divergence was significantly higher in perennial macroinvertebrates communities.

KEYWORDS:

ephemeral, perennial, freshwater ecology, climate change, functional space

INTRODUCTION

Climate change is causing increasingly frequent and prolonged drying events throughout the world. In the past decade, California has experienced severe droughts especially from 2011-2017 which wiped out over 100 million trees in California (Deamer 2016). The increase in temperature intensifies evaporations, making periods with low precipitation even drier than they would be in cooler climates. Climate change is the result of more and more long-wave infrared radiations reflected from the Earth getting trapped in the Earth's atmosphere as a result of our increase in anthropogenic greenhouse gas emissions (Stern and Kaufmann 2014). Particularly, Mediterranean regions such as California are considered to be the most vulnerable to climate change due to our strong seasonality of precipitation, high water demand, and population growth (Chang and Bonnette 2016). By the mid-century, model simulations have predicted the annual number of zero flow day frequencies to increase by 27% (Jaeger et al. 2014). These droughts increase the disturbance frequency to stream networks and make them gradually more fragmented. Hence streams will be increasingly isolated more frequently and for longer periods of time. The disconnectedness creates a similar impact to the "barrier effect" often caused by roads and dams, which can lead to local extinctions of two to six generations caused by the loss of population sources because up/downstream migrations have been interrupted (Letcher et al. 2007). As climate change becomes a prominent issue that the world seems reluctant to engage in head-on, we need to study the various long-term implications that it has on our freshwater ecosystem, with macroinvertebrates being the basis of it.

Aquatic insects play a crucial role as decomposers in the food web, they also assist us in monitoring our streams. Benthic macroinvertebrates(BMIs) grow through processing organic materials like leaf litter in streams (Covich et al. 1999), which has been shown to boost heterotrophic activity and facilitate a more dynamic aquatic ecosystem (O' Brien et al. 2017). Since stream networks mostly flow one way, nutrient recycling is fundamental to a thriving, fast-flowing freshwater habitat. Without macroinvertebrates, the compounds in decaying leaf litter will simply drift downstream and can not be easily recaptured. Additionally, macroinvertebrates are well known as biological indicators of water quality, they are often utilized to inform policy decisions regarding setting pollutant regulations, determining life stressors, and indicating improvements in water quality (Gresens et al. 2009). Thus, despite how miniscule they may look, macroinvertebrates have a crucial role in ecological management and

their importance should not be overlooked. Previous studies have pointed out that aquatic insect community recoveries may be compromised when disturbance frequency increases, especially in fragmented stream networks (Sarremejane et al. 2020). Anthropogenic activities will also severely impact biotic communities and put them at greater risk of biodiversity loss and biotic homogenization (Baker et al. 2021). Understanding how macroinvertebrates' traits allow them to prevail in the desiccation caused by drier terrains as well as human impacts is critical to conserving stream ecosystems in the face of climate change.

Macroinvertebrates have multitudes of traits that allow them to adapt to desiccation and promote their survival in isolated pools and dry bed sediments during drought conditions (Stubbington et al. 2017). These traits are categorized into four groups: life cycle, morphologies, resistance forms and dietary habits. Diversity in ecological traits for macroinvertebrates can improve general aquatic insects communities' resilience to droughts. Some species are even capable of employing multiple drought-resistant mechanisms to ride out drying events (Bogan et al. 2017). The four trait domains for aquatic insects to survive disturbances are dispersal, reproductions, synchronization, and development. For instance, one of the reproductive strategies that allow invertebrates to survive disturbance is polychaete worms that exhibit brooding behavior was shown to be more successful evolutionarily in water bodies that have a lower pH, caused by the increase in the concentration of CO₂ near hydrothermal vents (Lucey et al. 2015). Notably, common dispersal strategies that increase taxa's resilience to droughts include going into dormant stages, swimming, or aerial dispersal of offsprings between perennial refuges (Bogan et al. 2017). Thus, studying macroinvertebrate persistence through the selective pressures of climate-induced drought, and specific drought-survival traits that allow invertebrates to persist, is critical for understanding how macroinvertebrate populations might shift or persist in the future.

Although there has been some research done on trait difference between mediterranean and temperate regions, only a limited amount of studies concerning BMIs biological trait in relation to Mediterranean stream flow regime are done in California. Additionally, the data used in some of the BMIs trait studies have been collected sporadically over several decades, using various sampling techniques by different researchers over a spread out sampling area (Statzner et al. 2004). In particular, my research will look into how macroinvertebrate traits shift along the

intermittency gradient. In this study, I will be testing to see if the predictions made by previous researchers for mediterranean and temperate regions also apply to ephemeral and perennial zones because both variables are characterized by the difference in flow intermittency (Bonada et al. 2007). I will be investigating 1) Does the tested hypotheses proposed in Bonada et al. (2007) for difference of trait categories between mediterranean and temperate regions apply similarly to the difference between ephemeral and perennial streams? 2) Is there a significant difference in habitat types in terms of biodiversity metrics? And 3) How does functional diversity metrics shift along the intermittency continuum? I hypothesize that 1) I will notice a similar difference in abundance weighted trait proportions as referenced in Bonada et al. 2007. 2) Ephemeral sites taxa will have a lower richness, diversity, abundance, but a higher evenness compared to the perennial sites. And 3) perennial sites taxa will have a higher functional richness (FR) and functional evenness (FE), but functional divergence will be higher for taxa at the ephemeral sites. To answer these questions, I will be using a macroinvertebrate trait dataset coded by Dr. Leah Bêche and Dr. Patina Mendez that has fuzzy coded values for various BMIs traits (i.e., body size, voltinism, food type). I will use the trait dataset along with a 20 year long macroinvertebrate abundance dataset at two segments of two mediterranean creeks in California. The abundance data will also include precipitation data, stream orders, catchment size, and location.

METHODS

Study site

Macroinvertebrate samples were collected at 4 sites in Hunting and Knoxville Creeks (Table 1) by Dr. Vincent Resh. The Hunting Creek and Knoxville creek are two low-order streams located ~30 km north of Lake Berryessa. The four sampling sites consist of one 1st Order ephemeral stream at Knoxville Creek (1D), one 1st Order intermittent stream (1P) at Hunting Creek, and two 2nd Order Perennial streams at Hunting Creek with perennial (2D) and ephemeral (2P) flow regimes respectively. The catchment size of these streams ranges from 2.1 km² to 29.3 km². Researchers also collected surber samples (0.093 m², 0.5 mm mesh) five times annually at five randomly selected riffles and he has been sampling the same riffles every year (Resh et al. 2005).

Table 1. Summary of physical characteristics of the study sites (Resh et al. 2005). (Note that the mistake from Resh et al. 2005 which switched up sites 2D and 2P has been rectified in the following table)

	Latitude (N)	Longitude (W)	Elevation (m)	Stream order	Watershed area (km ²)	Flow regime	Total precipitation Mean ± SD (CV)	Range of total precipitation (mm) and years
Hunting/Knoxville Creeks (HKC)								
1D	38°47'56"	122°18'53"	390	1	2.1	ephemeral	605±232	289 -1062
1P	38°51'55"	122°24'54"	634	1	4.4	intermittent	(38.4)	(1990) (1995)
2D	38°49'45"	122°22'45"	402	2	22.1	perennial		
2P	38°48'30"	122°22'36"	348	2	29.3	ephemeral*		

*The channel from which samples are taken is ephemeral but it is adjacent to a perennial channel

Dataset Descriptions

I tested my hypotheses using two large databases on macroinvertebrate communities in freshwater streams in California, North America. The details of the database origin and structure were described in Bêche and Resh (2007). I described only what is essential for understanding this study here.

20 Year-long data (HCKC)

Resh et al. (2005) recorded 206 taxa of macroinvertebrate abundance data at the Hunting Creek and Knoxville Creek (HCKC) mostly to the genus and operational taxonomic units (OTUs). The same person collected, sorted, and identified all the samples continuously over a 20-year period from 1984 to 2003. The data encompassed a six-year period of extended drought from 1987 - 1992, when rainfall was within or close to the driest quartiles based on a 66-year record. Resh chose three sites with different flow regimes at the Hunting Creek and one ephemeral site at the Knoxville Creek to sample. Dr. Vincent Resh noted that there is not a significant difference in habitat between the two streams so I will mainly differentiate the four sites by their flow regimes (V. Resh, pers. comm.). Dr. Resh also recorded other environmental data such as the annual average Flies Wasser Stammtisch-hemisphere values and wet-season precipitation data.

Biological traits data

I used a data set with 13 biological traits with a total of 63 modalities of all traits (Usseglio-Polatera et al., 2000; Tachet et al., 2002; Bêche, 2005; Table 2). These traits are related to life cycle (i.e., Life Cycle Duration, Voltinism, Dispersal), morphologies (i.e., Body Size, Body Armor, Body Shape, Respiration Types, Oviposition, Aquatic Stages), resistance form (i.e., Resistance Method, Locomotion), and diet (i.e., Feeding Habits, Food Types) of 507 taxa. Each taxa is classified in order, family, and genus and most of the trait information was coded at the genus level. Regarding macroinvertebrates' affinity with different traits, the dataset used the fuzzy-coding approach (Chevenet et al., 1994) to indicate if individual taxons have No (0), Weak (1), Moderate (2), or Strong (3) association with various traits. Out of the 507 taxa cataloged, only 114 taxa that were present at the Hunting Creek/ Knoxville Creek thus were selected for this study. Trait information was available for most of the taxa present at HCKC besides body armoring and body shape traits. Additional information related to taxonomic rankings including species, phylum, and class are also available but not needed for the study.

Table 2. Biological traits (13) and modalities (63) are included in the dataset. Most modalities are written as the letter code.

Body Size (mm)	Life Cycle Duration	Locomotion
a1 < 2.5	f1 ≤ 1 year	k1 flight
a2 > 2.5 - 5	f2 ≥ 1 year	k2 surface swimmer
a3 > 5 - 10		k3 swimmer
a4 > 10 - 20	Voltinism	k4 crawler
a5 > 20 - 40	g1 semi, merovoltine	k5 burrower
a6 > 40 - 80	g2 univoltine	k6 endobenthic
a7 > 80	g3 multi-,bivoltine	k7 temporarily attached
		k8 permanently attached
Body Shape	Aquatic Stages	Food Type
b1 flattened	h1 egg	11 FPOM
b2 cylindrical	h2 larva	12 CPOM
b3 spherical	h3 adult	13 periphyton/algae
		14 macrophytes
Body Armoring	Oviposition	15 dead animals > 1mm
c1 none	i1 ovoviviparous	16 microinvertebrate
c2 moderate	i2 fixed, single eggs	17 macroinvertebrates
c3 strong	i3 free, single eggs	18 vertebrates
	i4 fixed, clutches	
Respiration	i5 free, clutches	
d1 cutaneous	i6 endophytic eggs	Feeding Habit
d2 gills	i7 terrestrial eggs	m1 absorber
d3 plastron		m2 gathering - collector
d4 aerial	Resistance	m3 shredder
	j1 Refuge Use	m4 scraper
Dispersal	j2 Diapause	m5 filtering - collector
e1 Aquatic Passive	j3 None	m6 piercer
e2 Aquatic Active		m7 predator
e3 Aerial Passive		m8 parasite
e4 Aerial Active		

Abundance weighted trait values analysis

To perform statistical analysis on the HCKC abundance data, I took the average of the five sampling counts from each site according to the entry numbers to create a composite abundance for each sample. I averaged the species abundance entries differentiated by their taxa, site name, year, and sample number. To maintain consistency, only samples from April were used to perform the analysis so there is no seasonal variability. The fuzzy coded traits values are rescaled as proportions of individuals in a taxon associated with the modality with weak (1) and strong (3) affinity. I then combined the two datasets and created a traits x abundance matrix through matrix algebra. I then used the 20 years of data as replicates since all abundance counts are performed at the same rifles during the sampling period and there wasn't a significant correlation between time and species abundance or richness. I use two-tailed t-tests the significance of the difference in proportions in abundance weighted trait values of the two sites.

Species diversity analysis

To maintain consistency with the overall analysis, the combined dataset of macroinvertebrate trait and abundance values which has 114 taxa in total was used to calculate the diversity indices. I calculated four total indices including species richness (SRic), species abundance (SAbu), Shannon diversity index (SDI), and species evenness (SEve). Species richness is the total number of species present for a sample, species abundance is the sum count of species present at a site. Shannon diversity index estimates species diversity and it takes into account both richness and abundance, and species evenness is a value that represents evenly distributed are the species in a given community. I then performed a two-sample t-test on R Studio (Version 4.1.2) on all metrics to compare the average values of all indices between ephemeral and perennial sites.

Functional diversity analysis

The type of values represented by functional traits varies from continuous (e.g., body size), ordinal (e.g., voltinism, body armor), binary (e.g., life cycle duration), or nominal (e.g., food type, dispersal). Commonly, the solution would be to estimate the species distance matrix using distances such as the Gower distance which allows a mixed input of both qualitative and quantitative traits through the “gowdis” function in the FD R-package (Podani and Schmera 2006). However, all of the trait categories used in this analysis are fuzzy coded which would not be taken into account in the traditional method (Chevenet et al., 1994). So, I used an extension of the gowdis function, gawdis, which includes an argument that considers fuzzy variables with multiple columns defining a single trait (Bello et al. 2020). Due to a lack of data on some of the taxa, the body armor and body shape physical traits were not included in the calculation for the functional diversity indices. Modalities are grouped together so that traits with a higher number of modalities won't unfairly skew the dissimilarity between species. Moreover, I modified the function so that all traits will have equal weight in affecting the niche of a species and computed a species to species dissimilarity matrix. In addition, I performed a principal coordinate analysis (PCoA) to better visualize species distribution in a multidimensional functional space. The top two principal coordinate axes that explain the most amount of variation in the dissimilarity matrix and thus have the highest coefficient of determination value were used to plot a two-dimensional ordination graph. Next, I used the R Package FD to compute the multidimensional functional diversity index including functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv) (Villéger et al. 2008). Functional richness is the amount of niche space occupied by the BMIs community which is not weighted by abundance. Functional evenness quantifies the regularity of the distribution of species that occupy each functional space. Last but not least, functional divergence means how species diverge in their abundance-weighted distance from the center of gravity in the niche space (Villéger et al. 2008). By entering the Gower dissimilarity matrix into the dbFD function along with the average species abundance, I computed the three functional diversity indices. Once again, I treated the FD values from each year as a replicate and performed a student's test on R Studio (Version 4.1.2) on all functional diversity metrics to compare the means between ephemeral and perennial sites.

Table 3. Proposed hypothesis of the expected differences of trait modalities between Ephemeral and Perennial streams. ‘E’ indicates that the category proportion should be higher in the Ephemeral streams, while ‘P’ implies vice versa. Some of the modalities presented in Table 2 are absent here as none of the taxa are associated with these modalities (E.g. a1, l8, m6, m8... etc.). Others are absent because I did not hypothesize a significant difference. These rationales are partly derived from predictions made by Bonada & Dolédec (2007) and Townsend & Hildrew (1994) on traits that promote resilience or resistance from disturbances like droughts.

Trait	Category	E/P	Rationale
Body Size(mm)	≤ 2.5	E	Smaller sizes require fewer nutrients to sustain and survive low flow disturbances.
	>2.5-5	E	(Same as above)
	>5-10	E	(Same as above)
	>10-20	P	Absence of disturbance allow more time for insects to grow thus permit larger sizes, larger sizes are favored in a competitive environment.
	>20-40	P	(Same as above)
	>40-80	P	(Same as above)
	>80	P	(Same as above)
Life Cycle Duration*	<1	E	Species with a shorter life cycle will be able to reproduce before disturbance occur, hence higher resilience
Aquatic Stages*	Larva	P	Taxa without aerial dispersal is favored in perennial stream
Voltinism (yr ⁻¹)	Multivoltine(>1)	E	More frequent reproduction is favored in streams with higher disturbances
Oviposition	Ovoviviparous	E	Higher resilience capacity from ovoviviparity
	Terrestrial Clutch	E	Terrestrial eggs allow higher drought resistance in dry regions
Dispersal	Aquatic Passive	P	Higher flow rate forces drift along stream
	Aerial Active	E	The ability to fly to wet sites is favored after desiccation
Resistance Forms	Refuge Use	E	Tendency to use refuge to increase resistance to droughts
	Diapause	E	Ability to suspend development during droughts increases resistance
Locomotion	Crawler	P	Permanent flow makes crawling a necessity
	Swimmer	E	No flow condition permits swimming in stagnant water
	Surface Swimmer	E	Same as Above
	Burrower	E	Burrowing increases macroinvertebrate resistance capacity by digging into hyporheic zone
	Endobenthic	E	(Similar to above)
Respiration	Gills	P	Gills are more favorable in permanently wet streams
	Cutaneous	E	Stagnant conditions make oxygen uptake difficult, specialized technique are favored (compared to tegument respiration)
	Plastron	E	(Similar to above)
Body Shape	Aerial	E	Respiration through spiracle increase resistance in high disturbance sites
	Flattened	P	Allow macroinvertebrates to cling on to the substrate at streams with permanent flow
Body Armoring	Cylindrical	E	Cylindrical shape allow taxa to drill into the interstitial layer and increase resistance
	None	E	Traits that promote drought resistance are prioritized in ephemeral streams
	Moderate	P	Higher predation occurrence favor species with body armor
Food Type	Strong	P	(Same as above)
	Periphyton	P	Higher abundance of aquatic plant materials at perennial sites
	Microphyte	P	(Same as above)
Feeding Types	Macrophyte	P	(Same as above)
	Shredder	P	Necessity by higher amount of large litter
	Scraper	P	Higher abundance of algae and biofilms in less disturbed stream

*This trait only had two categories, so we made predictions on only one of the categories here.

Results

Overall, the abundance weighted trait values from ephemeral sites have a higher variability, the average standard error value for trait values from ephemeral sites is 41% higher than the range of values from perennial sites from all of the traits analyzed ($P < 0.01$). There was not a significant shift in macroinvertebrates abundance or any diversity-related indices across the sampling periods ($R^2 \approx 0.196$, $P > 0.05$ for 1D & $R^2 \approx 0.0422$, $P > 0.05$ for 2D, Table 4), which justifies using the 20 years of sampling data as replicates.

Table 4. Power model statistics (adjusted R^2 and P values) of all the diversity indices were analyzed over the 20 years sampling period.

Sites	Species Richness		Species Abundance		Shannon Diversity Index		Species Evenness	
	R^2	P	R^2	P	R^2	P	R^2	P
Ephemeral	-0.01477	0.4062	0.1513	0.05063	0.0258	0.236	0.00068	0.3275
Perennial	-0.04863	0.7342	-0.011	0.3849	-0.04829	0.7281	-0.0518	0.8027

Sites	Functional Richness		Functional Evenness		Functional Divergence	
	R^2	P	R^2	P	R^2	P
Ephemeral	0.0174	0.2629	-0.0315	0.5248	-0.0484	0.73
Perennial	-0.0392	0.6011	0.116	0.07798	0.1312	0.0648

Abundance Weighted Traits Proportions

Life Cycle

For biological traits that are related to macroinvertebrates life cycle development. I found a statistically significant difference for BMIs that have a life cycle duration of greater than one year ($P \approx 0.046$), the proportion of macroinvertebrates that has >1 year life cycle is 120% greater than perennial sites (Figure 1a). However, the difference for taxa with life cycle <1 year is insignificant. Regarding voltinism of macroinvertebrates, a higher proportion of taxa at the ephemeral site are semivoltine ($P \approx 0.019$, Figure 1b) while the reverse is true for univoltine, where taxa in perennial sites are 6% more likely to be univoltine ($P \approx 0.022$), the variance of the abundance weighted trait for multi-voltinism is high ($SE > 3$) for both sites thus the difference is insignificant. Moreover, BMIs that are aerial active for dispersal have a significantly higher proportions in the ephemeral stream than in the perennial stream ($P < 0.012$, Figure 1c), yet the proportion difference for aquatic passive dispersal is non-significant.

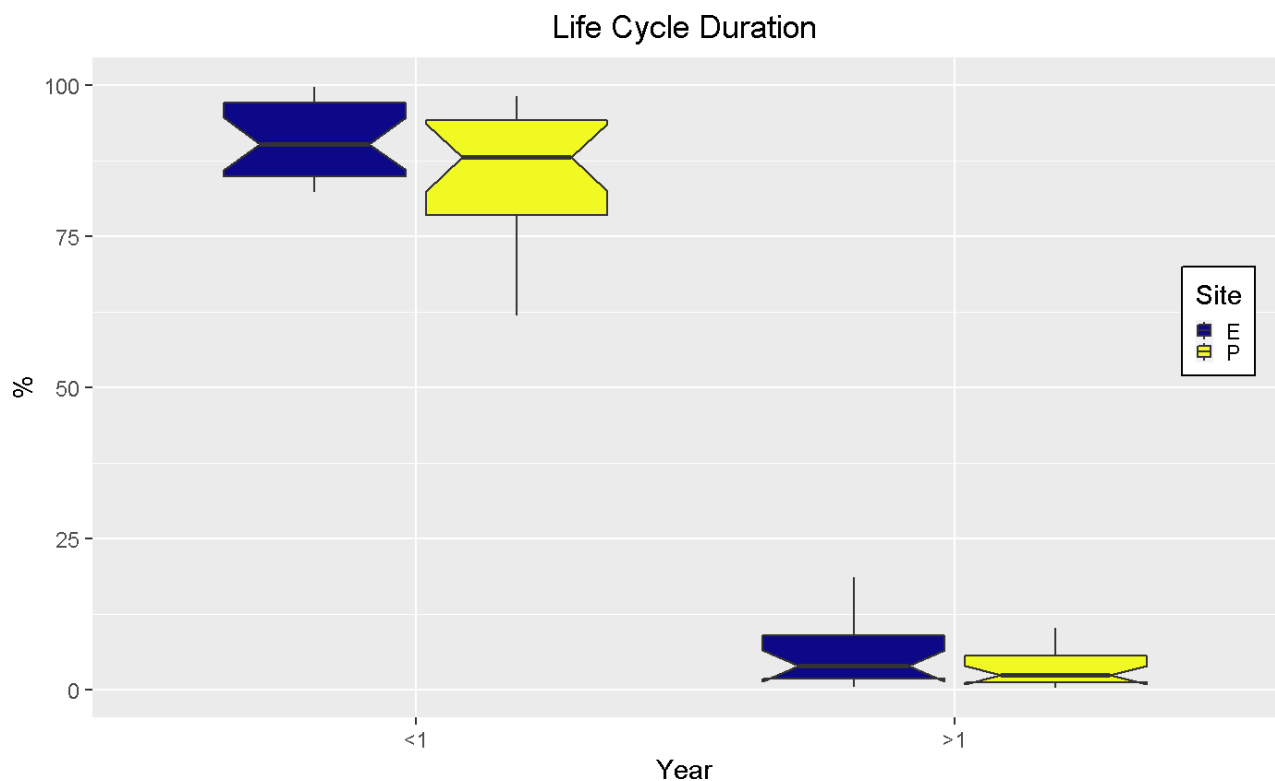


Figure 1a. Difference in proportions of modalities for life cycle duration between ephemeral (E) and perennial (P).

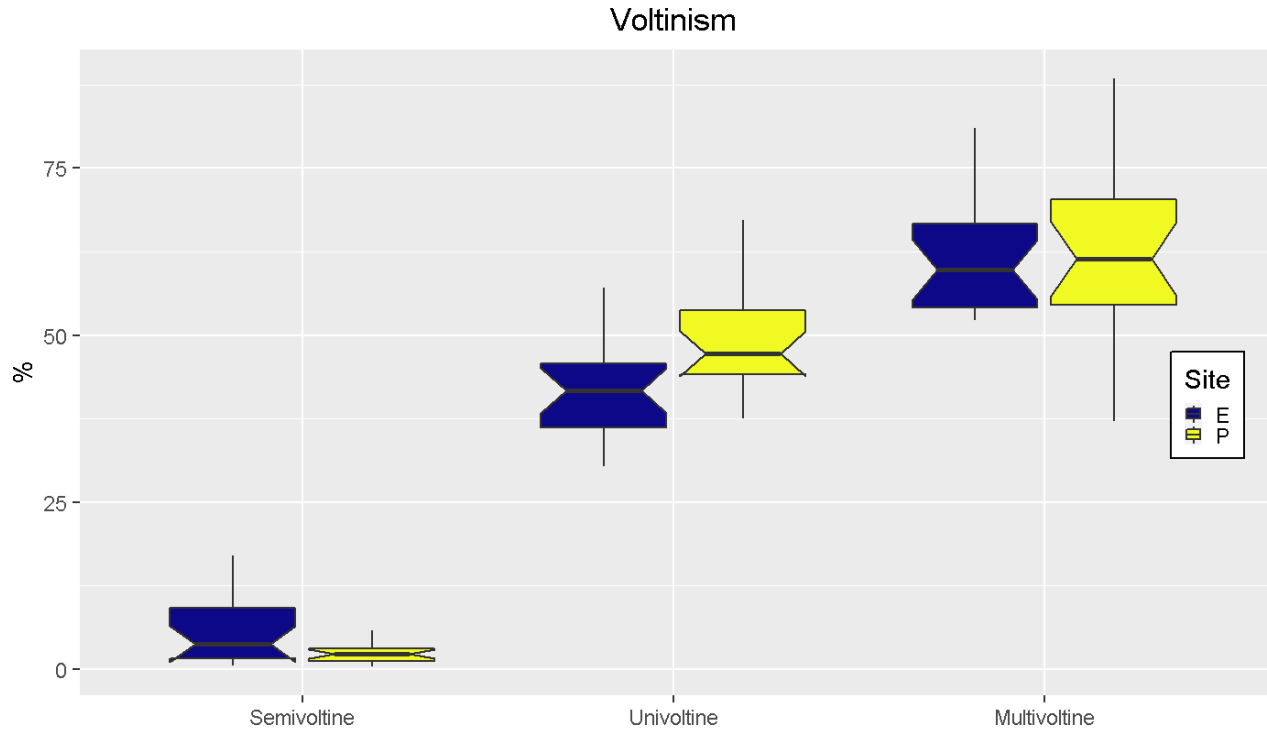


Figure 1b. Difference in proportions of modalities for voltinism between ephemeral (E) and perennial (P).

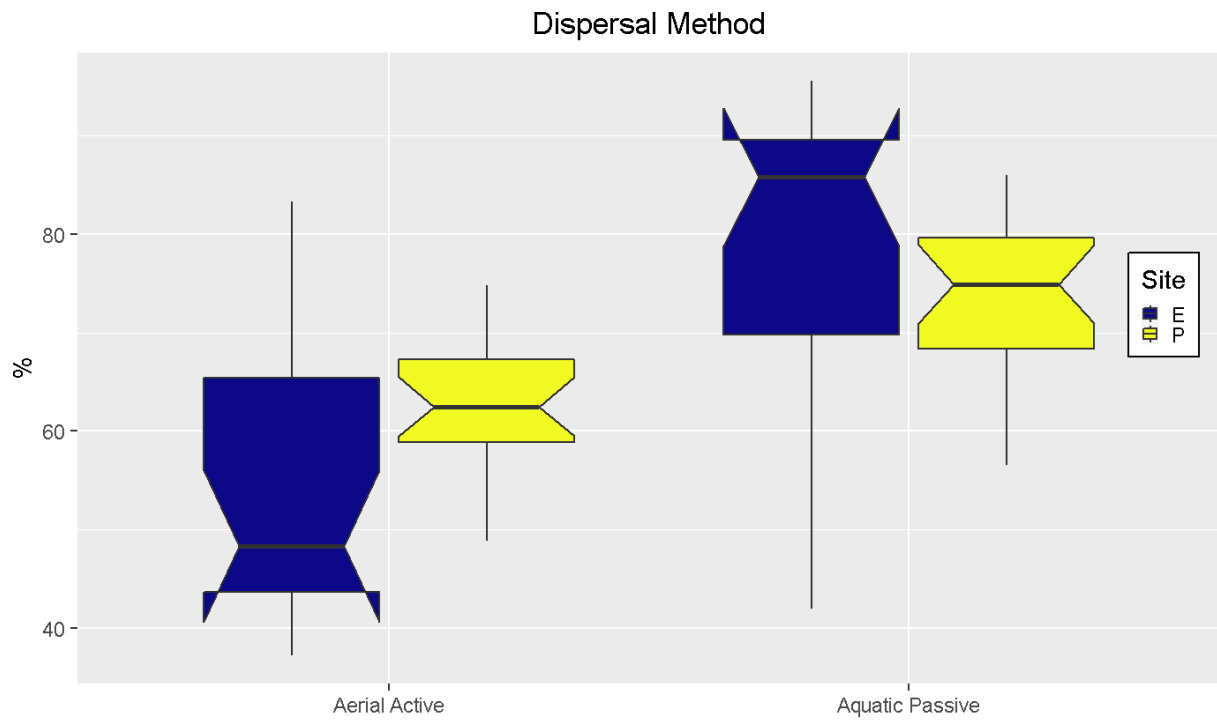


Figure 1c. Difference in proportions of dispersal methods modalities between ephemeral (E) and perennial (P).

Morphologies

When analyzing the difference in proportions that exhibit numerous physical attributes, I found that only two out of six of the body size ranges (>5-10mm & >80mm) have a statistically significant difference in proportions between ephemeral and perennial populations (Figure 2a). More BMIs ranged >5-10mm ($P < 0.005$, Figure 2) and >80mm ($P < 0.05$) are present in ephemeral sites. For the prevalence of the types of respirations used in the two different flow regimes, I found that both cutaneous ($P < 0.03$) and plastron ($P < 0.003$) respirations are more common in perennial sites, but I found no significant difference for aerial and gills respirations (Figure 2b). Regarding egg laying mechanisms of BMIs, I found a higher proportion of taxa that lay single eggs in perennial sites no matter if they are fixed to other objects or free flowing (Figure 2c). On the other hand, a higher proportion of BMIs in ephemeral sites are found to lay terrestrial eggs. Other egg laying modes such as ovoviviparous, free & fixed clutches, and endophytic egg laying showed no significant difference between sites (Figure 2c). Furthermore, the proportion of BMIs who are aquatic living during their larval stages are found in the ephemeral stream ($M \approx 99.96\%$) than in perennial stream ($M \approx 99.69\%$, $P < 0.03$, Figure 2d). Yet I found no significant difference for the taxa that have aquatic adult stages. I also found no significant difference for all the modalities investigated within the body armor and body shape traits (Figure 2e & Figure 2f), which is a result of high variance caused by the limited amount of taxa with such trait data available (~30 OTUs for each trait).

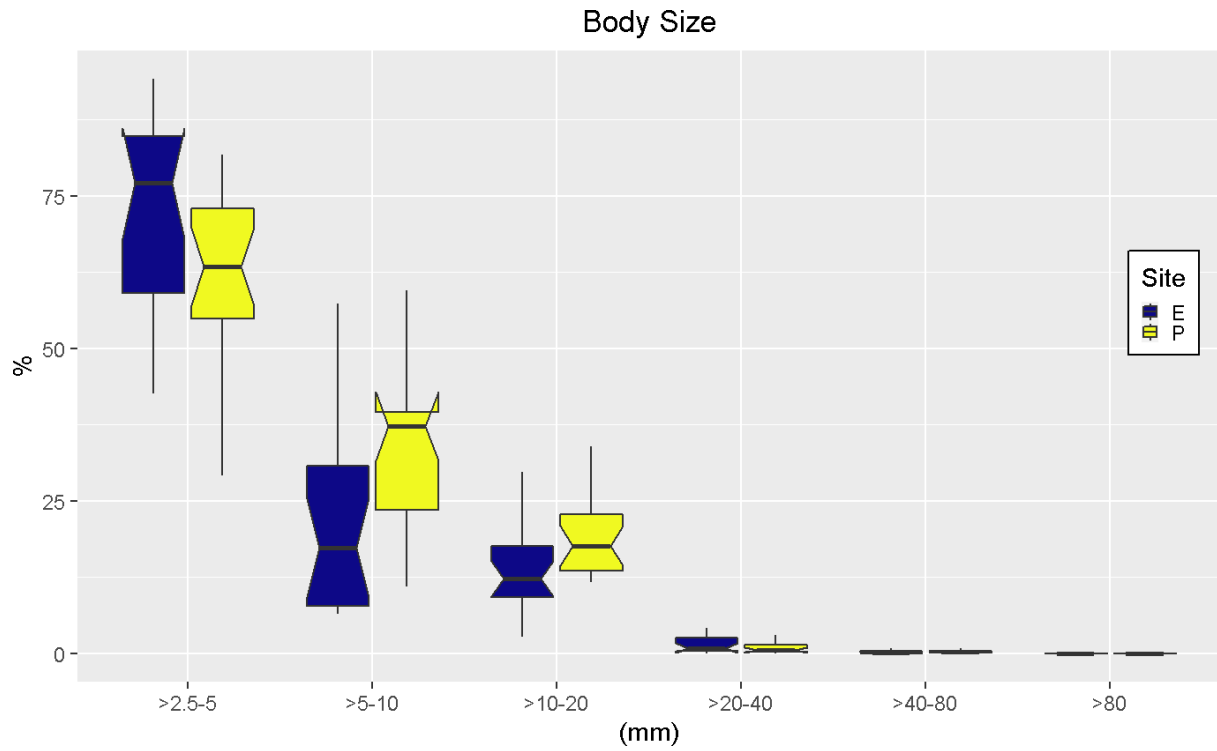


Figure 2a. Difference in proportions for a range of body size between ephemeral (E) and perennial (P).



Figure 2b. Difference in proportions of respiration modalities between ephemeral (E) and perennial (P).

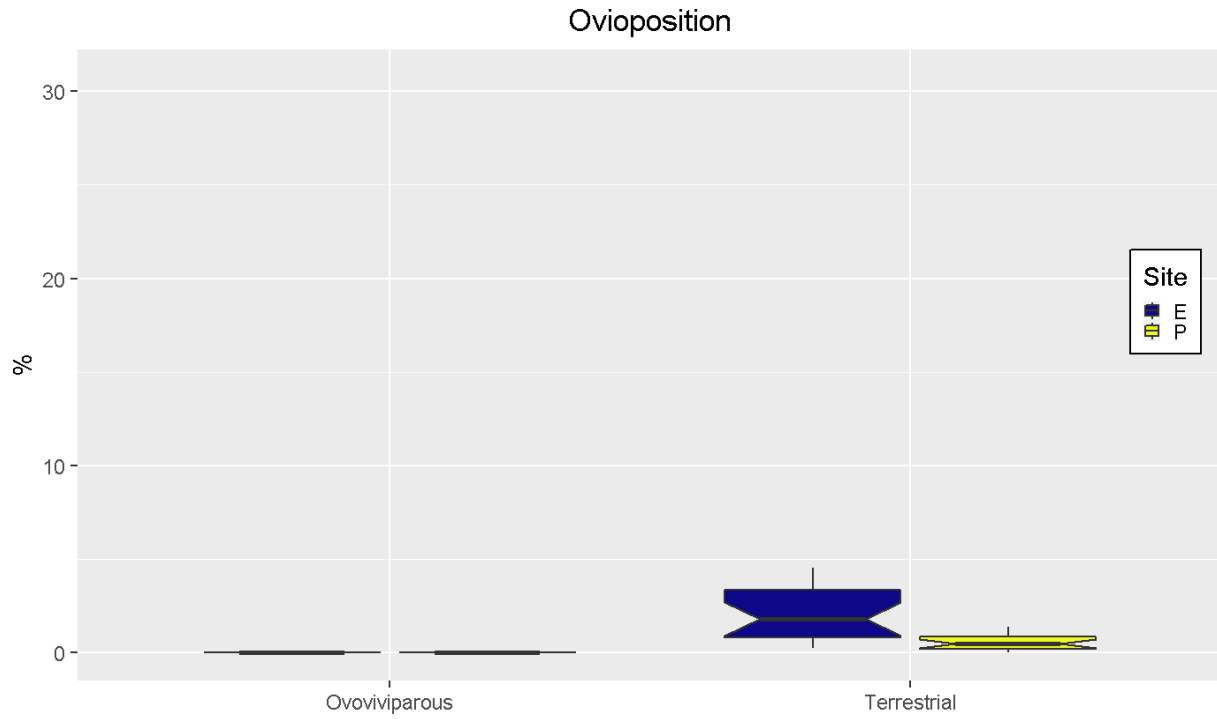


Figure 2c. Difference in proportions of oviposition modalities between ephemeral (E) and perennial (P).

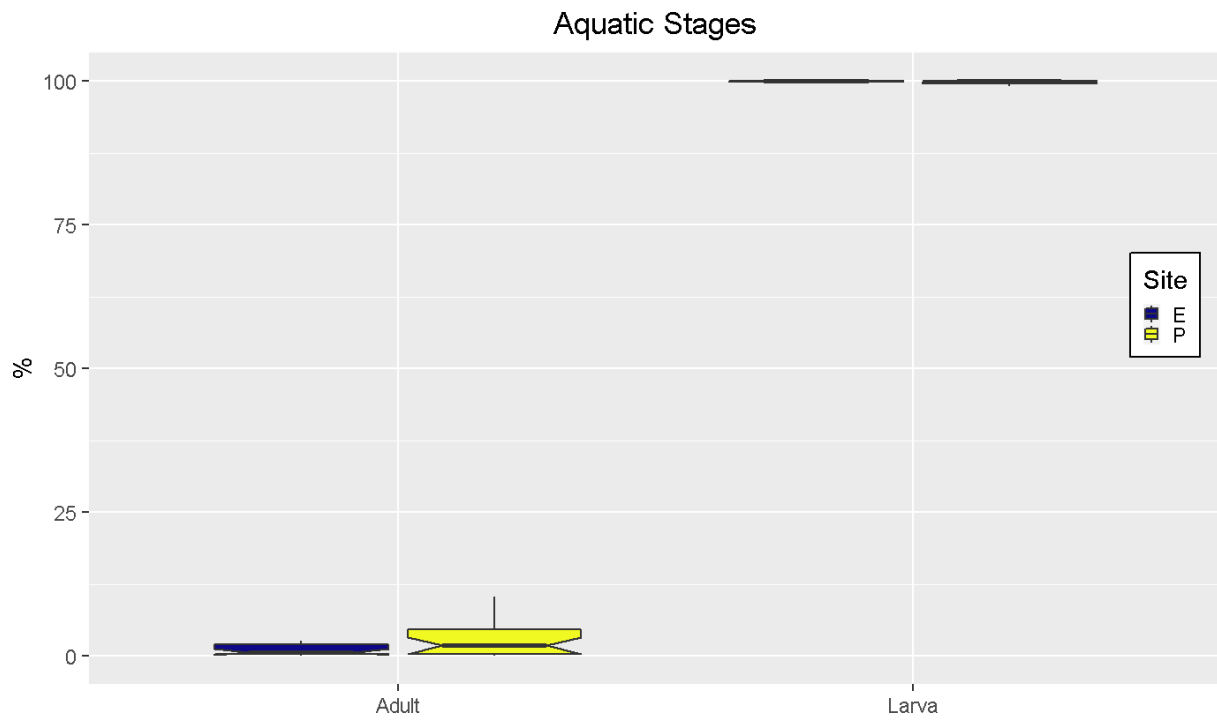


Figure 2d. Difference in proportions for different aquatic stage between ephemeral (E) and perennial (P).

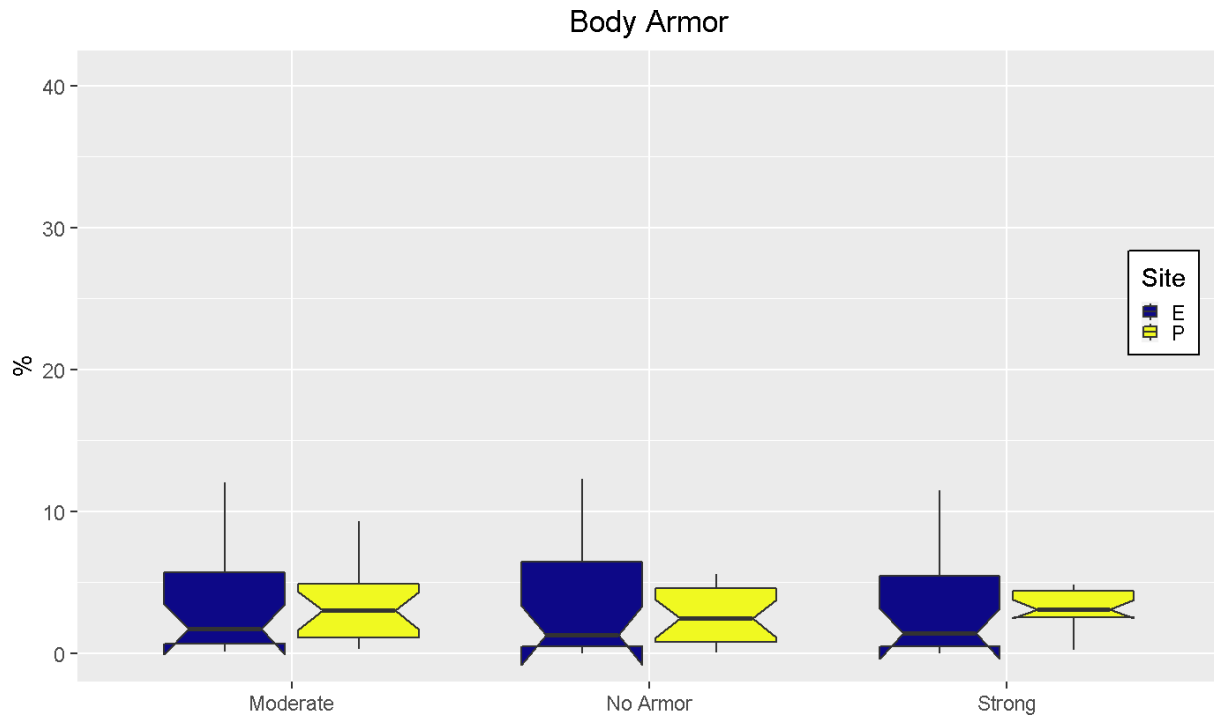


Figure 2e. Difference in proportions for the degree of body armor between ephemeral (E) and perennial (P).

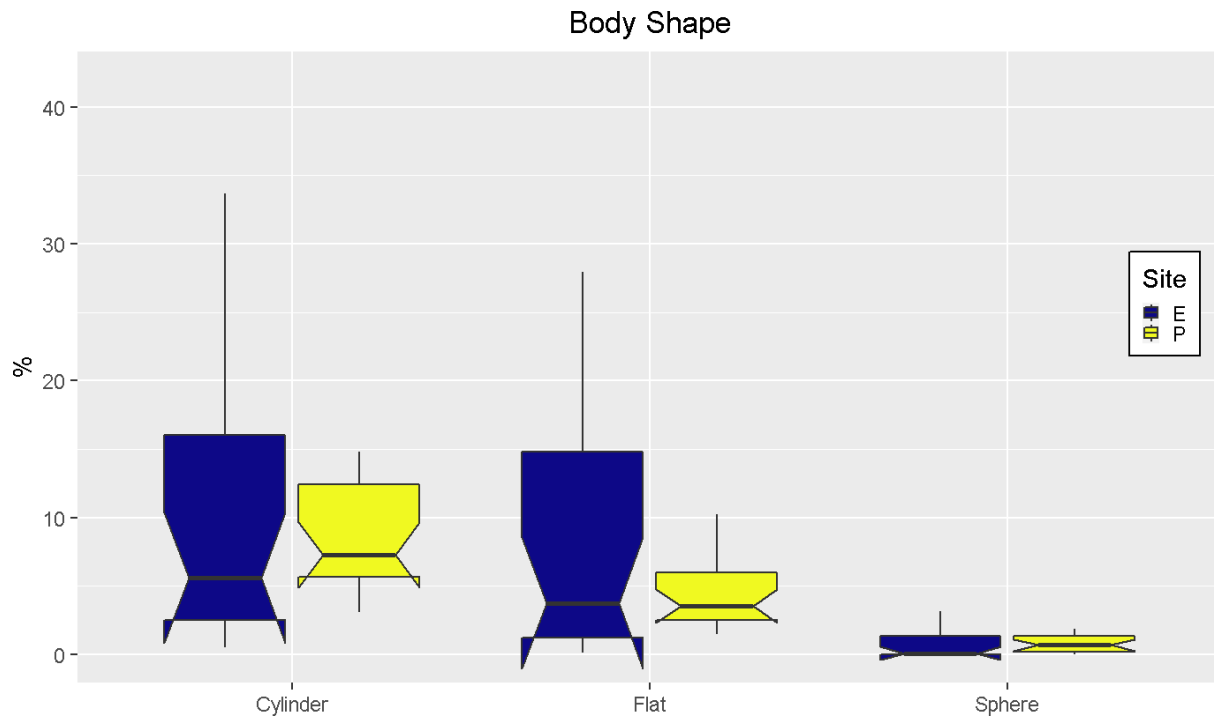


Figure 2f. Difference in proportions for different body shapes between ephemeral (E) and perennial (P).

Resistance Form

For biological traits that increase BMIs resistance to disturbances, I found that a higher proportion of taxa at the ephemeral site make use of refuges ($P < 0.001$, Figure 3a), with refuge using taxa being more than 300% more common at ephemeral sites. Yet I found no significant difference in proportions for taxa that exhibit diapause or have no resistance methods between the two sites. With regards to the locomotion methods of BMIs, I found that macroinvertebrates in ephemeral sites displayed a proportionally higher number of surface swimmers ($P < 0.02$), swimmer ($P < 0.001$), and endobenthic ($P < 0.004$, Figure 3b). Yet, the ability to temporarily attach to other objects is found more prevalent in perennial streams. Other locomotion methods such as flight, crawler and burrower, and permanently attached do not have a significant difference in proportions.

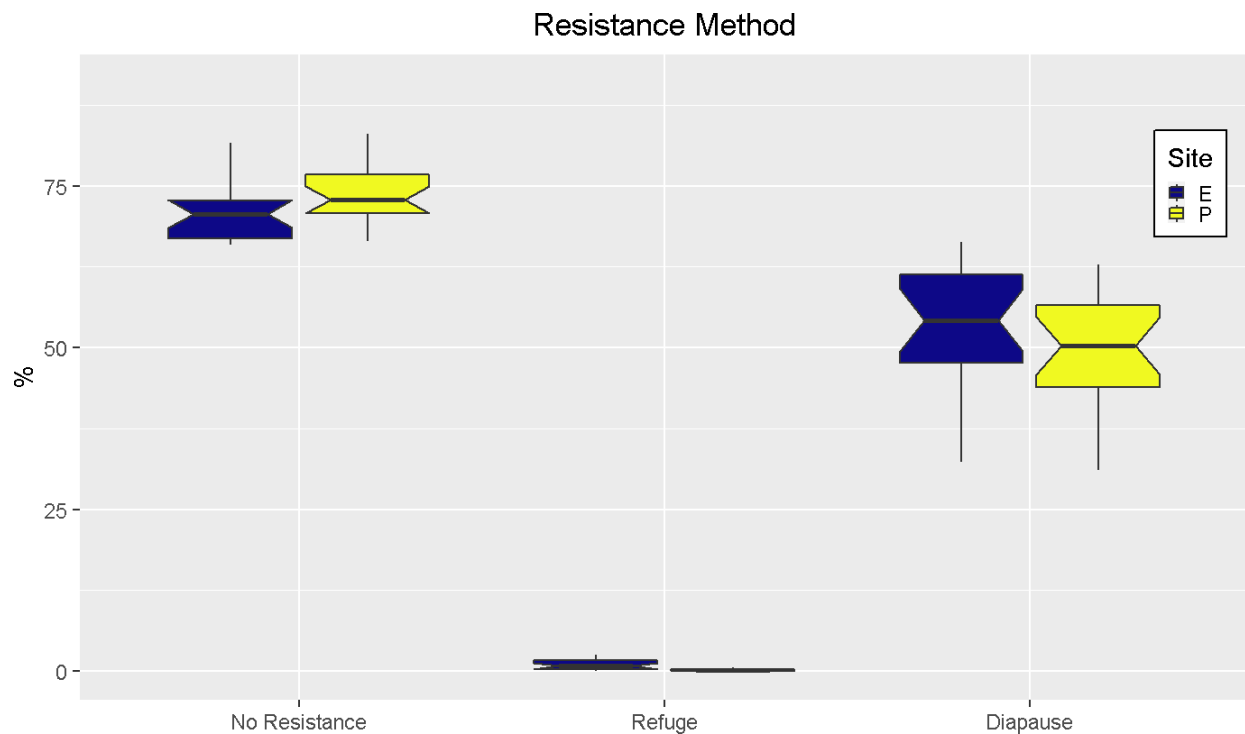


Figure 3a. Difference in proportions for different resistance methods between ephemeral(E) and perennial(P).

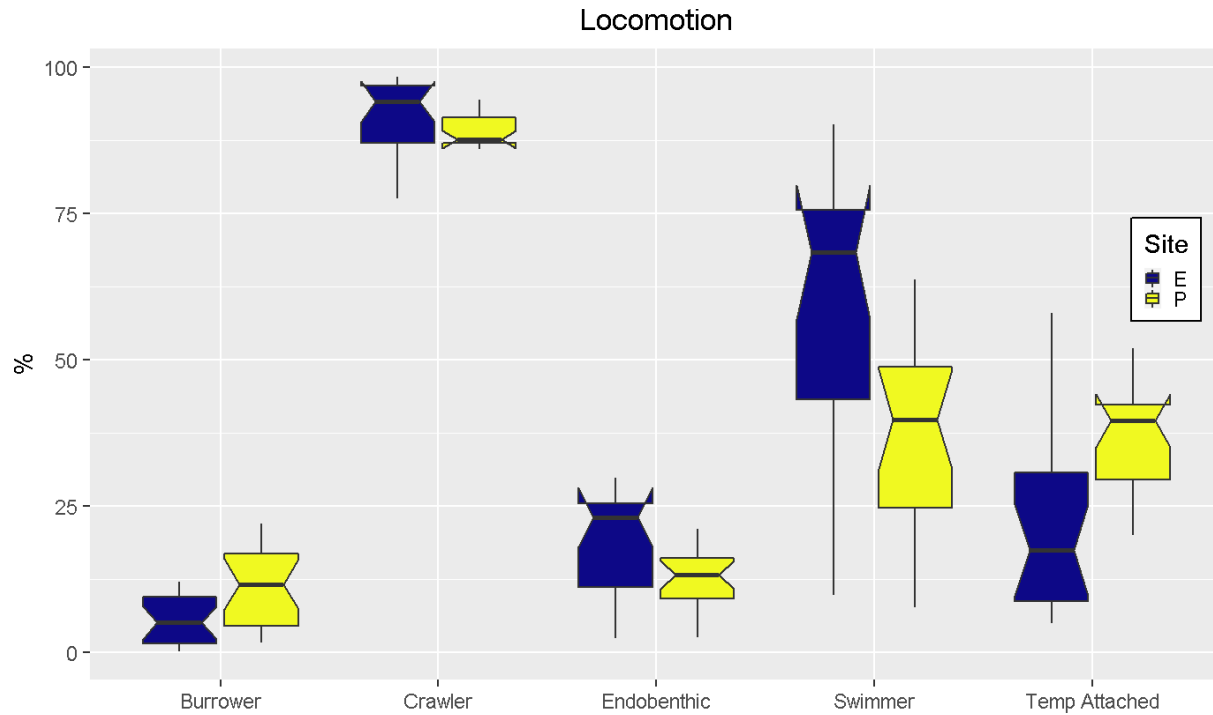


Figure 3b. Difference in proportions for modalities of locomotion between ephemeral(E) and perennial(P).

Dietary Habits

In analyzing BMIs' dietary habits, I found that a high proportion of taxa in ephemeral sites consume CPOM ($P < 0.01$). However, for periphyton and microinvertebrate, taxa in perennial sites tend to consume more of these types of food than the taxa in ephemeral sites ($P < 0.042$, $P < 0.017$, Figure 4a). We didn't find a significant difference for FPOM and macrophyte proportions. Similarly, none of the modalities among feeding habits showed a significant difference besides scraper, which proportionally has a higher presence in perennial sites ($P < 0.034$, Figure 4b).

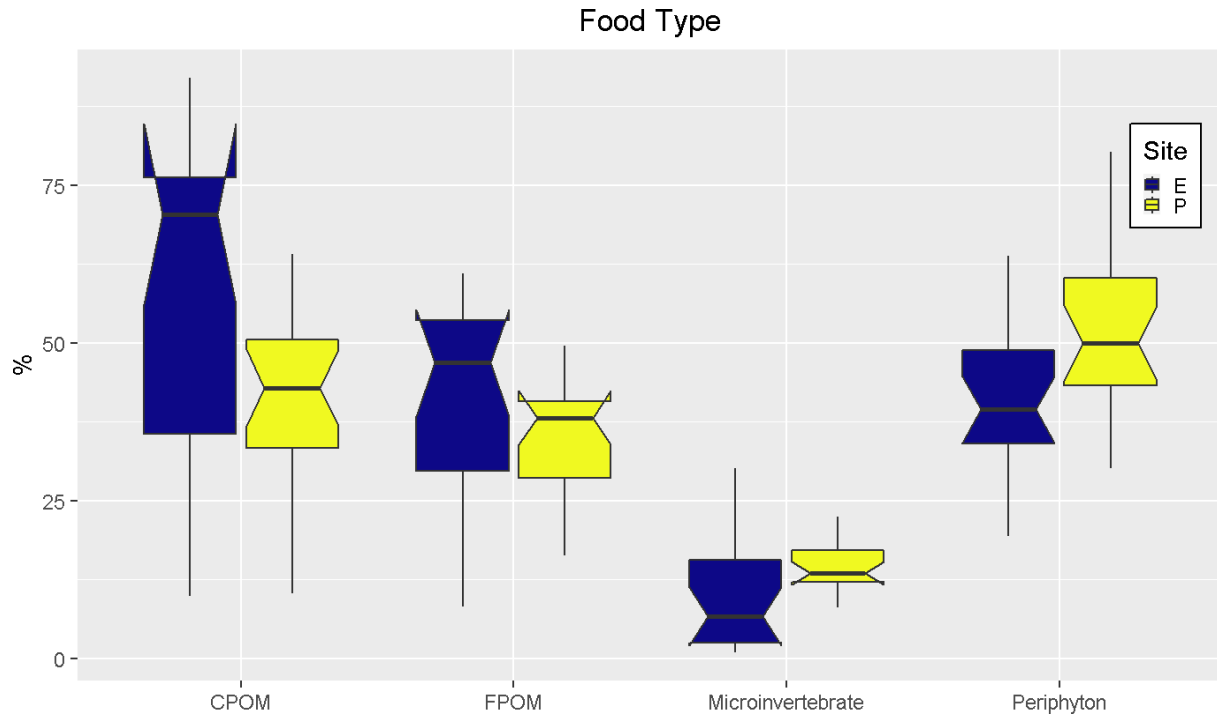


Figure 4a. Difference in proportions for modalities of feeding habit between ephemeral (E) and perennial (P).

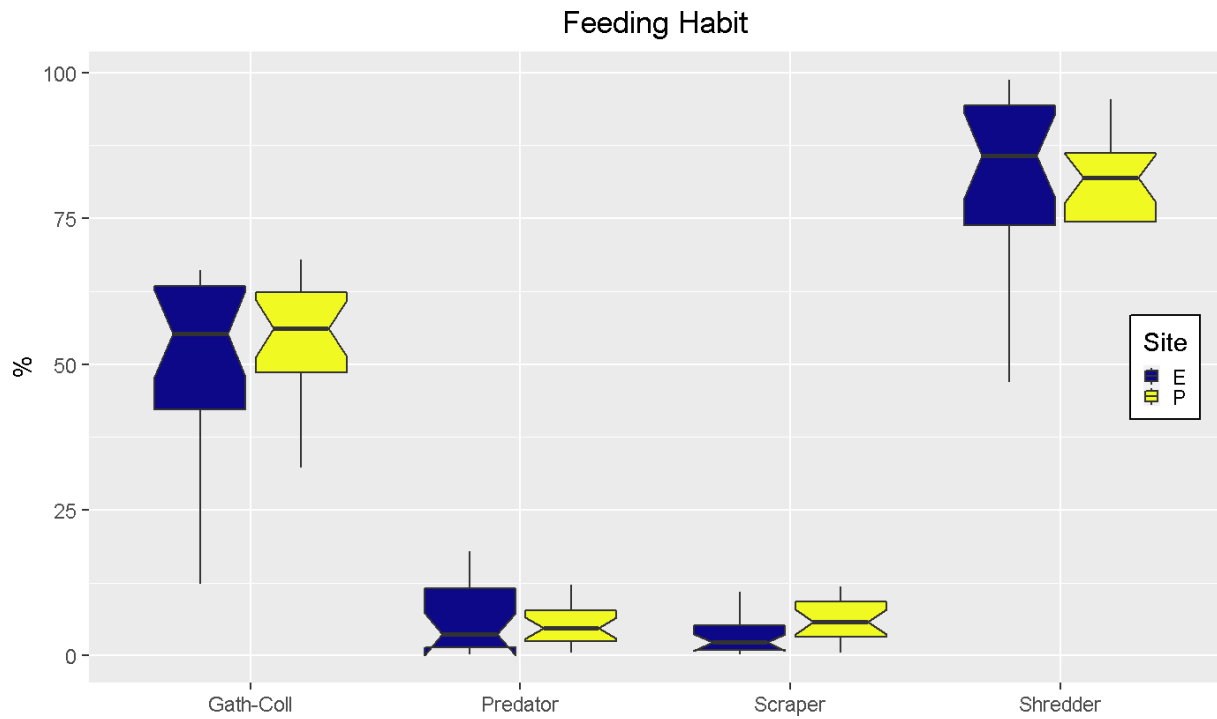


Figure 4b. Difference in proportions for modalities of feeding habit between ephemeral (E) and perennial (P).

Species Diversity Indices

I found that the species richness value of the perennial site ($M \approx 47.15$) and ephemeral site ($M \approx 40.3$) differ significantly ($P < 0.01$, Figure 5a). Despite the fact that the difference in species abundance between the two flow regimes is non-significant, the perennial site does have a higher average abundance ($M \approx 345$, $SE \approx 56$) than the ephemeral site ($M \approx 298$, $SE \approx 29$, Figure 5b). When looking at the Shannon Diversity Index, the estimated diversity value from perennial site ($SE \approx 0.071$) SDI is ~10% higher than that at the ephemeral site ($SE \approx 0.075$, Figure 5c). Lastly, the species evenness at the perennial site ($M \approx 0.65$, $SE \approx 0.018$) is significantly higher than that at the ephemeral site ($M \approx 0.62$, $SE \approx 0.018$, Figure 5d).

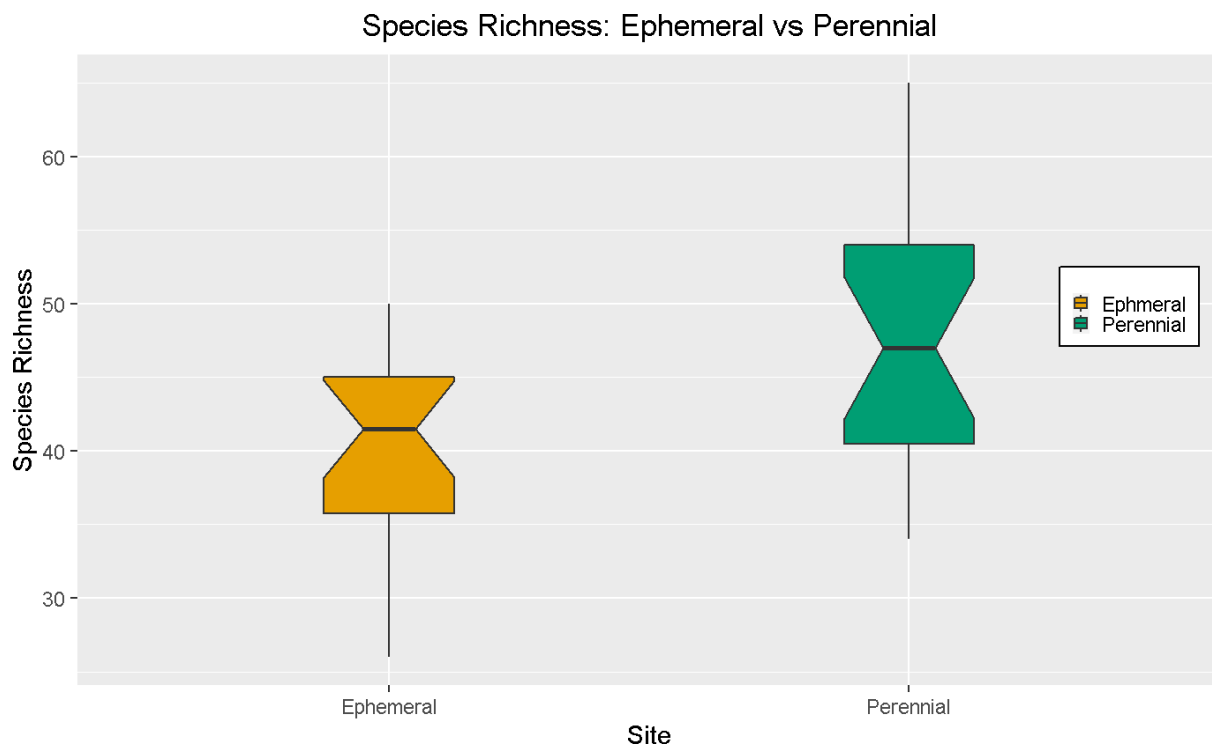


Figure 5a. Difference in species richness between ephemeral and perennial site.

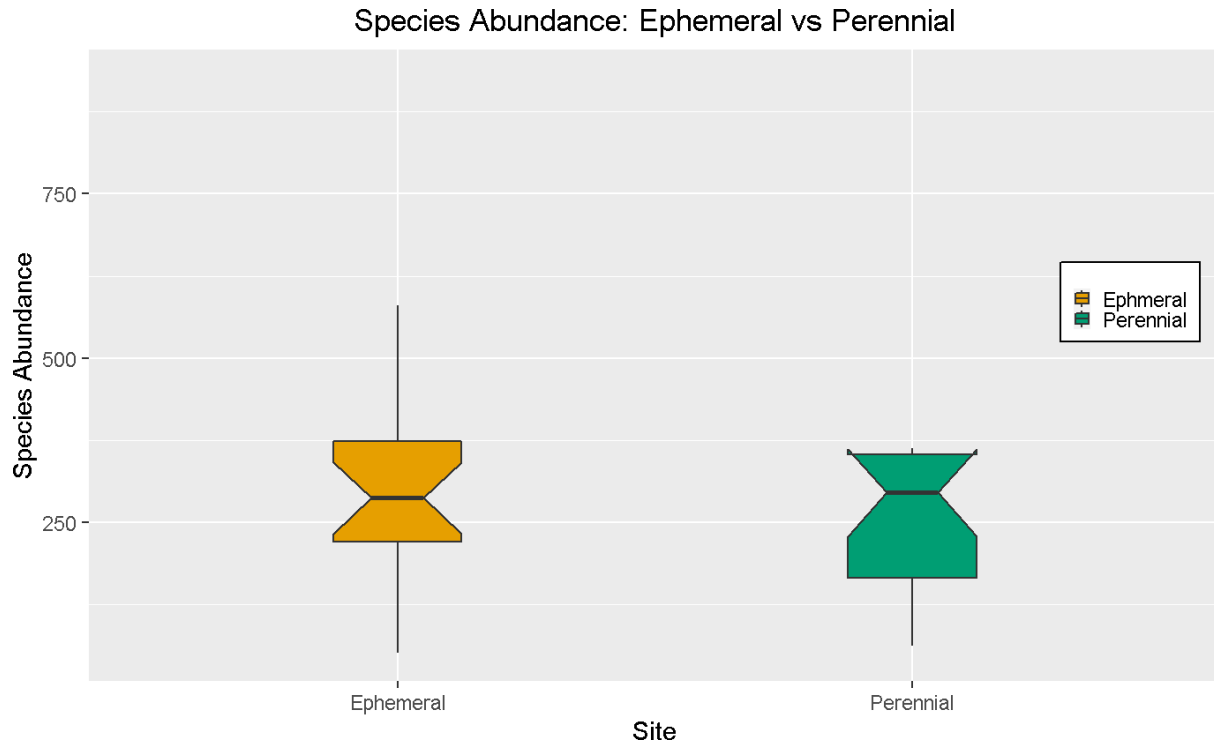


Figure 5b. Difference in species abundance between ephemeral and perennial site.

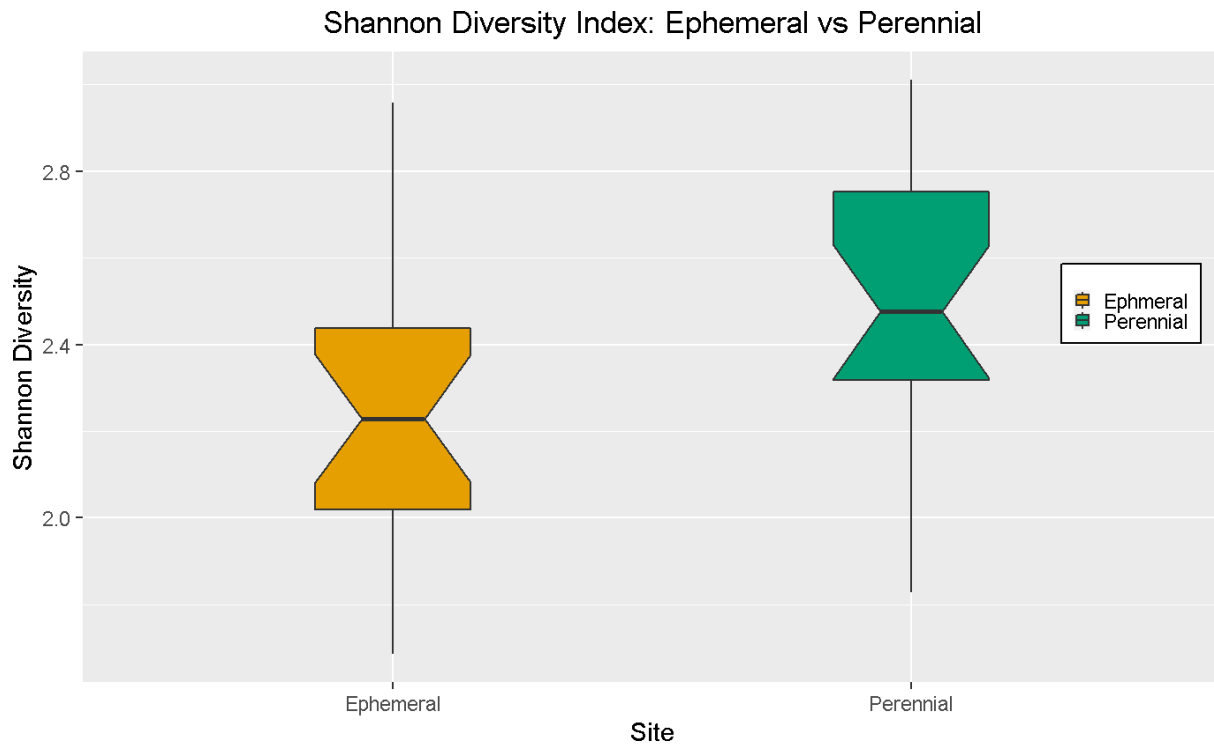


Figure 5c. Difference in Shannon Diversity index between ephemeral and perennial site.

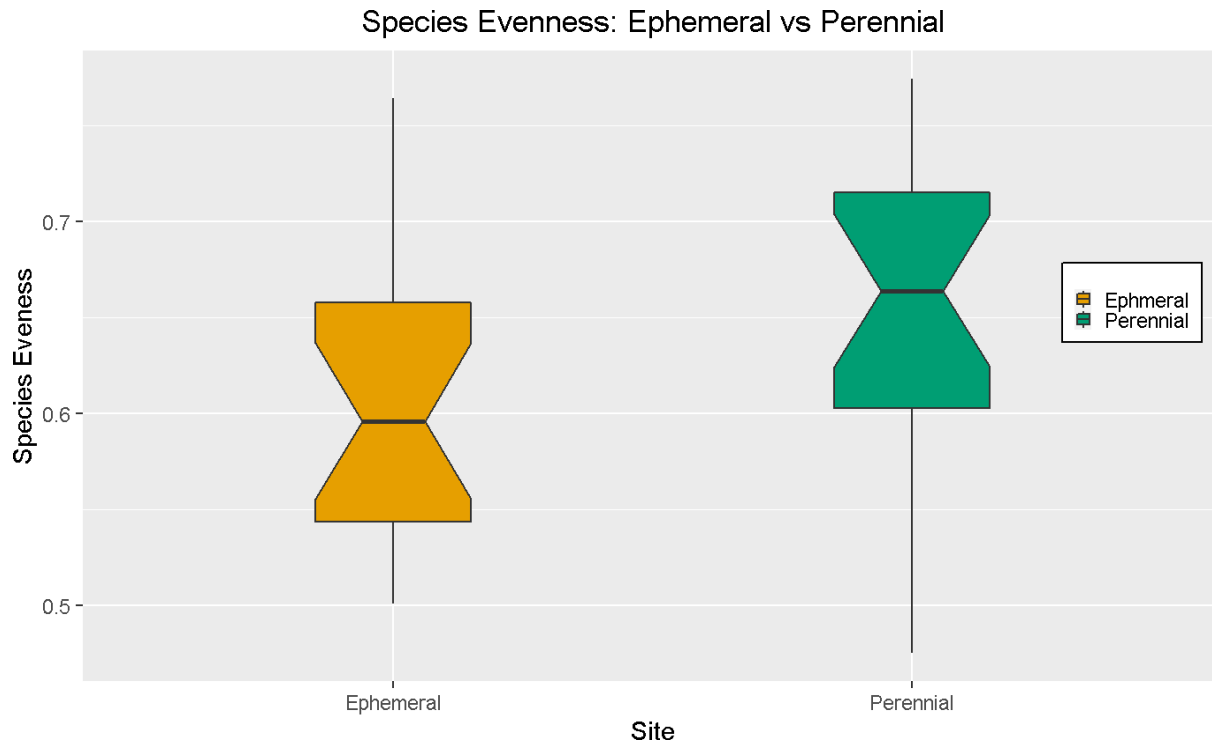


Figure 5d. Difference in species evenness between ephemeral and perennial site.

Functional Diversity Indices

The principal coordinate analysis showed a large overlap in the functional space occupied by the taxa at both sites at least based on the two primary principal coordinate axes (Figure 6). But calculating the functional diversity indices revealed that the ephemeral site actually has a lower functional richness value than the perennial site. According to the FRic value computed based on over 50 modalities, BMIs community at 1D ($M \approx 1342$, $SE \approx 383$, Figure 7a) is over 37 times more functionally diverse than the community at 2D ($M \approx 36$, $SE \approx 16$), and the difference is highly significant ($P < 0.002$). However, there is not a significant difference of functional evenness between ephemeral ($M \approx 0.55$, $SE \approx 0.019$) and perennial ($M \approx 0.54$, $SE \approx 0.016$, Figure 7b) sites. I also found that functional diversity is higher in perennial site ($M \approx 0.76$, $SE \approx 0.014$) than ephemeral site ($M \approx 0.66$, $SE \approx 0.018$) and the result is statistically significant ($P < 0.001$, Figure 7c).

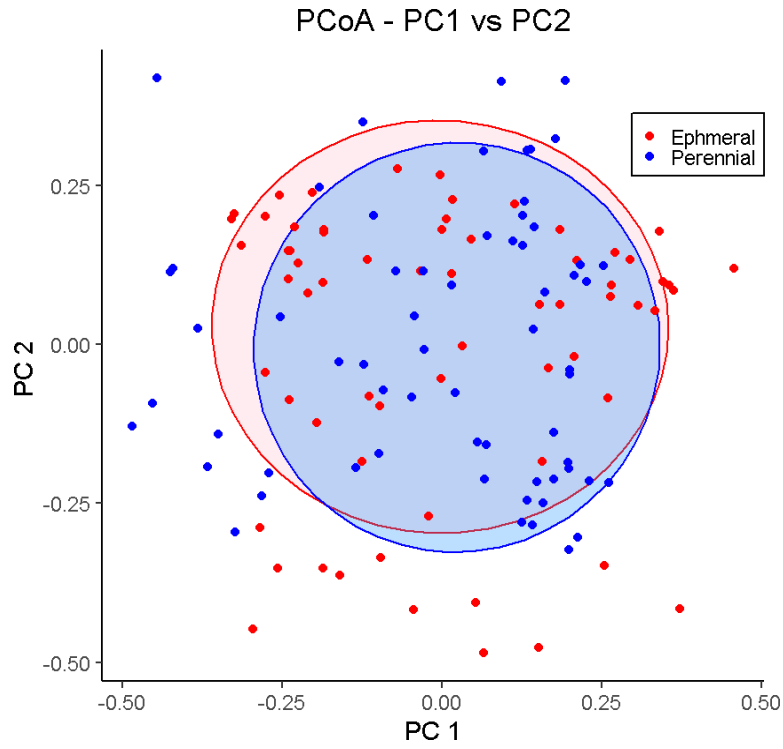


Figure 6. PCoA of top two principal coordinate axes that have the highest R^2 value which explain the variability of taxa dissimilarity present at 1D & 2D. The ellipsoids represent 75% of data points from each site.

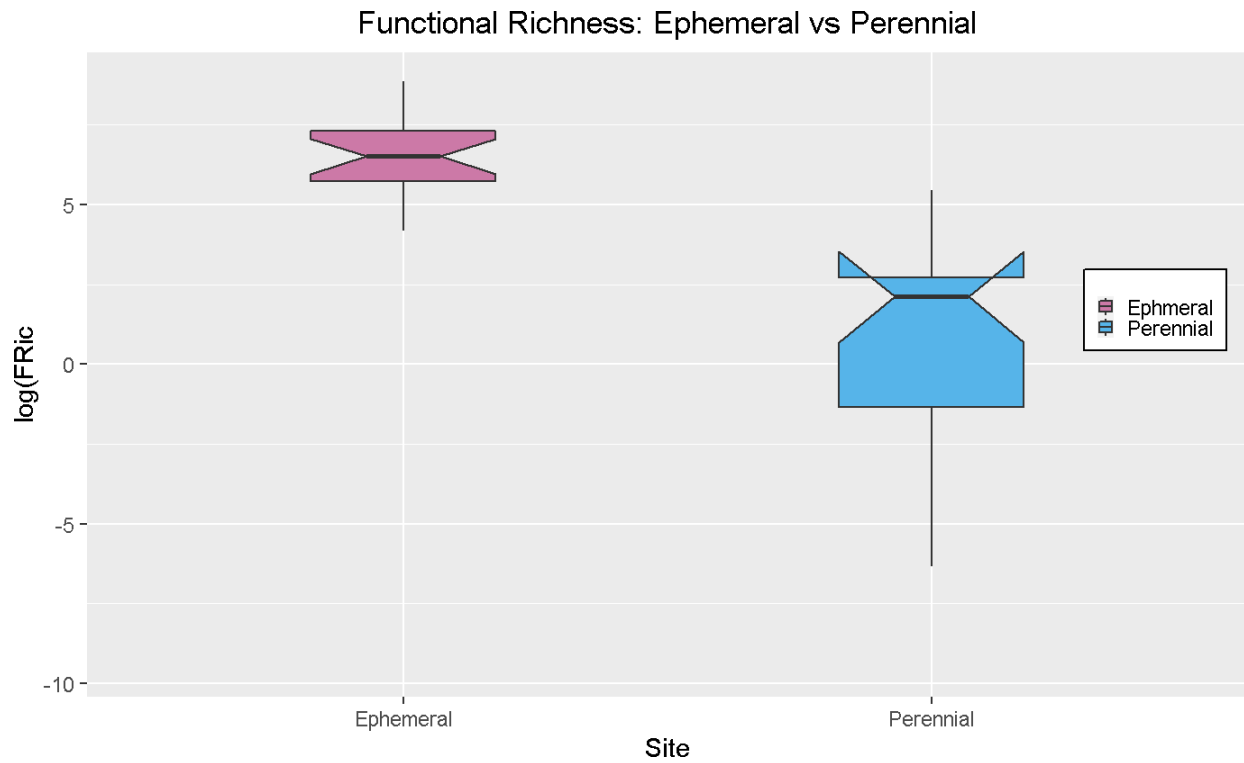


Figure 7a. Difference in functional richness between ephemeral and perennial site.

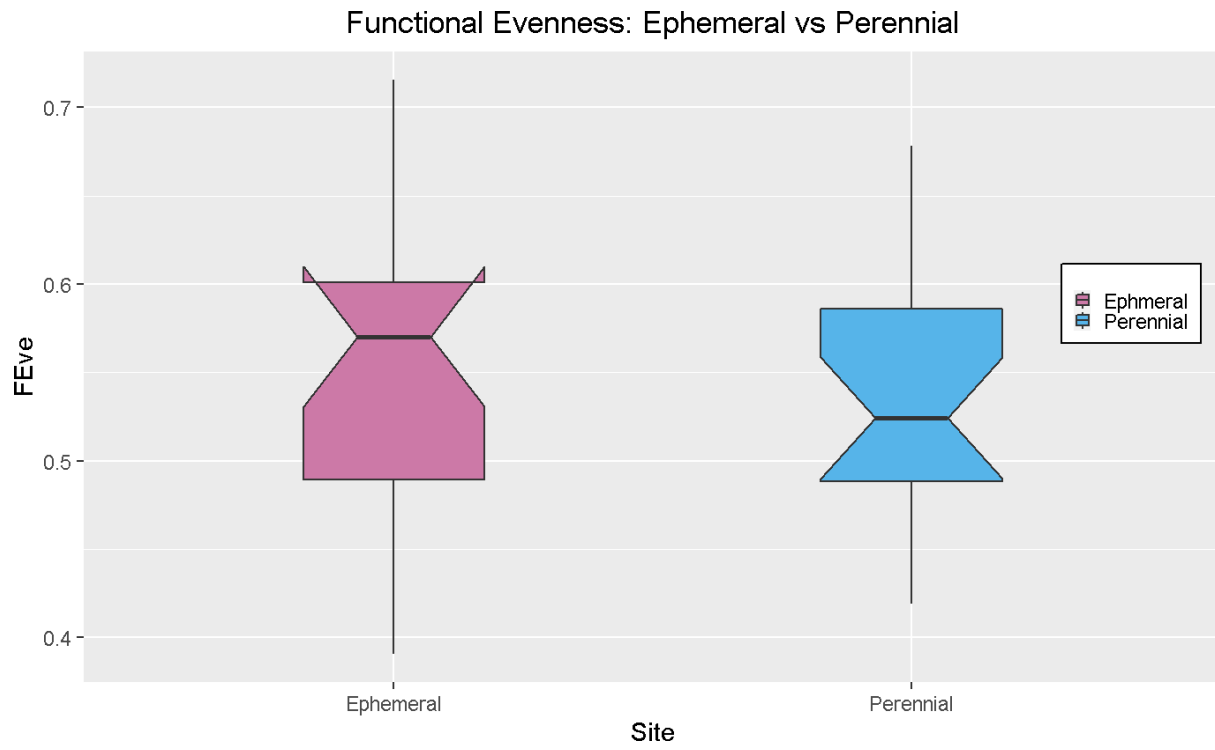


Figure 7b. Difference in functional evenness between ephemeral and perennial site.

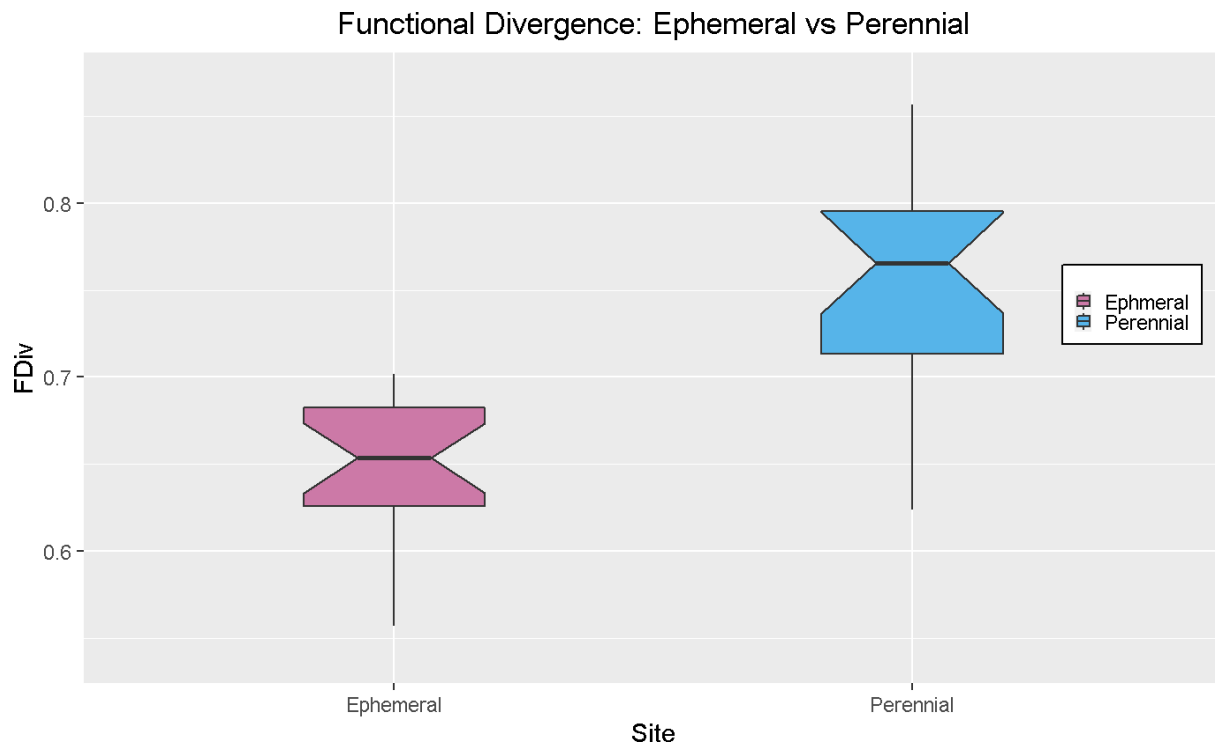


Figure 7c. Difference in functional divergence between ephemeral and perennial site.

DISCUSSION

In this study, I looked at the difference in proportion of 56 modalities that BMIs in 1D and 2D exhibit, 29 of which were examined previously in Bonada et al. (2007). I demonstrated that BMIs assemblage in ephemeral and perennial sites differ substantially; 34% of all modalities investigated showed significantly different proportions when performing two-tailed tests assuming equal variance. This is particularly apparent for modalities related to dietary habits and resistance forms, with 42.9% of categories for life cycle and 45% of categories for resistance forms showing a significant difference in proportions. Out of all the modalities analyzed, a majority of modalities related to dietary habits (80%) and resistance forms (62.5%) match with the difference in proportions found between Mediterranean and Temperate streams (Bonada et al. 2007). In correspondence with past findings, taxonomic richness and shannon-wiener diversity are significantly higher in perennial streams (Santos and Stevenson 2011). But overall species abundance does not differ significantly, suggesting that perennial streams allow for a more diverse BMIs community but the total number of BMIs each flow regime supports remains the similar because nutrients in both flow regimes are approximately the same (Gomes et al. 2020). Principal coordinate analysis (PCoA) displayed somewhat similar distribution BMIs in functional space, where there was a large overlap of trait space occupied by taxa of the two flow regimes. However, functional richness is substantially higher in ephemeral streams, possibly due to the highly variable environmental conditions that ephemeral streams feature, which enhance functional diversity (Stubbington et al. 2017).

Difference in trait categories between flow regimes

As stated, the traits exhibited by BMIs in stream along intermittency gradients are splitted into four categories (Life Cycle, Morphologies, Resistance Forms, Dietary Habits). I expected to see a difference in average proportions of different modalities across the 20 years sampling period because physical harshness of stream habitats often act as a powerful abiotic filter on the biological traits that taxonomic communities exhibit thus resulting in functionally distinct species assemblages in different sites (Poff et al., 1997). Only 48.3% of the proportions difference in 29 modalities matches with the finding in Bonada et al. (2007), 41.3% of the modalities did not match with our hypotheses due to the difference being statistically non-

significant, and 10.4% of the modalities are antithetical to the past findings comparing Mediterranean and Temperate streams (Bonada et al. 2007). Out of all the categories, modalities related to morphologies had the lowest percentage of matching with the previous finding (23.1%), while the traits related to the other three categories had 60% - 80% of modalities that corresponded with the difference described in Bonada et al. (2007).

As hypothesized, aerial active dispersal was more prevalent at the perennial site, with >50% of the communities exhibiting such modality (Figure 1). The high presence of aerial active taxa also corresponds with univoltine BMIs with overwintering adults such as species from the genus *Hydroporus* that are present in the HCKC dataset which colonize new habitats during spring, thus resulting in an overall higher proportion being aerial active in both flow regimes during the sampling month used in the analysis (Miguellez and Valladares 2008). Higher proportion of BMIs consuming periphyton and a significantly higher amount of scraper present at the perennial site (Figure 4) also comply with a study that found plant sapling emergence to be significantly higher in perennial streams than in ephemeral streams (Gomes et al. 2020). In the long term, heterotrophism might become more dominant in ephemeral streams due to its effect on shredders, microbes, and autotrophs. Hence, climate change will decrease the food chain length and trophic diversity in ephemeral streams, leading to a collapse in aquatic food webs (McIntosh et al. 2017). The result of the analyses of biological traits present on each site rejected my hypothesis that trait difference between the Mediterranean and Temperate regions is comparable to trait difference between ephemeral and perennial streams. A possible explanation is a different factor such as macroclimate can make in structuring biological traits in aquatic macroinvertebrate communities, Mediterranean regions can have large diurnal temperature swings during the summer which act as a largescale filter that select BMIs highly resistance to fluctuations in temperature.

Difference in biodiversity metrics

Ephemeral streams were remarkably high in functional richness despite a lower species richness value than the perennial stream (Figure 5), the low species richness supports my hypothesis for species richness but rejects my hypothesis for functional richness. This finding also confirms previous studies that suggest invertebrate richness and density metrics were both

significantly higher in perennials than IRES (Arscott et al., 2010, Santos and Stevenson, 2011). The disparity in the two values indicates that each taxon in ephemeral streams occupies a very unique functional space that might be exclusive to that species with very little functional redundancy. In contrast, during the 20 year sampling period, even when 2D has on average seven more taxa present in each sampling year (Figure 5), the functional richness value is still two orders of magnitude lower than that of ephemeral stream (Figure 6). The radical difference implies a significant overlap in ecological role between species, making perennial streams more prone to changes in environmental conditions. I originally hypothesized that ephemeral streams will have a lower species abundance than perennial stream, despite the general notion being true, the actual difference is not statistically significant therefore my hypothesis is rejected but supported previous findings (Hill et al. 2017). Ephemeral stream with the significantly lower Shannon-Wiener diversity index also rejected my hypothesis and showcased a disparate spread of taxa in the assemblage (Figure 5), but the non-significant difference in functional evenness seems to suggest that the less abundant species in ephemeral stream occupy a relatively larger functional space, thus balancing the spread of functional traits compared to perennial stream.

Limitations & Future Directions

Although my study looked at BMIs abundance data across a 20-year period, it nevertheless only encompassed one ephemeral and one perennial site in the bay area of California, limiting its conclusion to Mediterranean regions only, even if it was part of the study design to only look at Mediterranean region. In addition, my study broadly looked at the traits exhibited by taxa instead of focusing on the distribution of taxa themselves; hence some taxa that possess multiple modalities with a higher abundance could skew the diversity indices without being accounted for. The skewness could create a false perception that some streams are more functionally diverse than they truly are. Another potential impediment is that I generalized the difference between the streams as their difference in flow regimes. However, there might also be ecological interactions unique to Hunting and Knoxville creeks that are not being accounted for.

The analysis of this study also did not examine the effects of Flies Wasser Stammtisch-hemisphere values, wet-season precipitation data on traits composition in BMIs communities and the various diversity indices. For future studies, looking at the impacts of different physical (shear stress, flow rate) and chemical (stream composition) characteristics might be more important to identifying vulnerable streams as they account for more variation in macroinvertebrate community composition than biological or spatial variables (Hill et al. 2017). However, data related to chemical variables for streams are scarce online thus are often difficult to obtain unless a primary research study is conducted, and previous studies suggest that with the exception of electrical conductivity, water quality parameters for the two streams with vastly different intermittency are analogous regardless of sediment to water ratio (Perera and Gomes 2022). Last but not least, identifying ecological relationships is needed in order to more accurately anticipate future fluctuations in flow intermittency to a local scale will be highly beneficial for conservation efforts.

Implications for climate change in Mediterranean streams

It is well known that IRES macroinvertebrate communities are affected by human activity including anthropogenic emissions, Bae and Park (2019) also found that heavy rain and dry events were the primary factors for determining the spatial and temporal extent of habitat diversity and stability in streams under the least disturbance conditions. Climate change is a response of human activity and it is increasing both the intensity and frequency of floods and droughts (Trenberth 2008). The stream networks where flow regimes have been altered due to factors such as climate change, water management policies, and change in land use were the most heavily impacted (Stubbington et al. 2017). Our findings suggest that ephemeral stream taxa have higher resistance to disturbance events due to a higher proportion of BMIs exhibiting resistant related modalities such as refuge use, diapause, and crawling in Mediterranean climate. As high amount of precipitation and high humidity level has been associated with a reduction in aquatic macroinvertebrates dispersal (Migueluez and Valladares 2008), more proportions of streams exhibit ephemeral characteristics in Mediterranean regions might encourage higher

dispersal activities thus higher resilience to sporadic disturbances. However, the knowledge regarding the precipitation threshold required to maintain BMIs dispersal activities to local refuges is limited (Robson et al. 2011). Despite the advantage of having a taxa assemblage with higher resistance/resilience, taxa richness has been associated with flow permanence and flow duration, where it is predicted that 10% decrease in flow permanence can lead to 1.9taxa/m^2 removed, and a reduction of 10 days in flow duration will also remove 0.5taxa/m^2 (Arscott et al. 2010). Because my finding suggests that ephemeral streams' taxonomic richness is lower than perennial streams, flow permanence and flow durations diminishing due to the effect of climate change will have a cascading effect on functional richness in ephemeral streams and weaken its resilience to disturbance. As Céréghino et al. (2018) had suggested, given the potential functional space invertebrates can occupy, they have only utilized a limited number of successful ecological strategies within such space. As climate change continues to alter our stream systems and apply selective pressures on our BMIs communities, there is hope that more of the available space will be occupied by BMIs adapting to traits modalities best suitable for the increasing intermittent conditions in the Mediterranean climate.

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REFERENCES

- Arscott, D. B., S. Larned, M. R. Scarsbrook, and P. Lambert. 2010. Aquatic invertebrate community structure along an intermittence gradient: Selwyn River, New Zealand. *Journal of the North American Benthological Society* 29:530–545.

- Bae, M.-J., and Y.-S. Park. 2019. Evaluation of precipitation impacts on benthic macroinvertebrate communities at three different stream types. *Ecological Indicators* 102:446–456.
- Baker, N. J., F. Pilotto, P. J. Haubrock, B. Beudert, and P. Haase. 2021. Multidecadal changes in functional diversity lag behind the recovery of taxonomic diversity. *Ecology and Evolution* 11:17471–17484.
- Bêche, L. A., and V. H. Resh. 2007. Biological traits of benthic macroinvertebrates in California mediterranean-climate streams: long-term annual variability and trait diversity patterns. *Fundamental and Applied Limnology* 169:1–23.
- Bêche, L., E. McElravy, and V. Resh. 2005. Long-Term Seasonal Variation in the Biological Traits of Benthic-Macroinvertebrates in Two Mediterranean-Climate Streams in California, USA. *Freshwater Biology* 51:56–75.
- Bello, F., Z. Botta-Dukat, J. Leps, and P. Fibich. 2020. Towards a more balanced combination of multiple traits when computing functional differences between species. *Methods in Ecology and Evolution*.
- Bogan, M. T., E. T. Chester, T. Datry, A. L. Murphy, B. J. Robson, A. Ruhi, R. Stubbington, and J. E. Whitney. 2017. Resistance, Resilience, and Community Recovery in Intermittent Rivers and Ephemeral Streams. Pages 349–376 *Intermittent Rivers and Ephemeral Streams*. Elsevier.
- Bonada, N., S. Dolédec, and B. Statzner. 2007. Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. *Global Change Biology* 13:1658–1671.
- Céréghino, R., V. D. Pillar, D. S. Srivastava, P. M. Omena, A. A. M. MacDonald, I. M. Barberis, B. Corbara, L. M. Guzman, C. Leroy, F. Ospina Bautista, G. Q. Romero, M. K. Trzcinski, P. Kratina, V. J. Debastiani, A. Z. Gonçalves, N. A. C. Marino, V. F. Farjalla, B. A. Richardson, M. J. Richardson, O. Dézerald, B. Gilbert, J. Petermann, S. Talaga, G. C. O. Piccoli, M. Jocqué, and G. Montero. 2018. Constraints on the functional trait space of aquatic invertebrates in bromeliads. *Functional Ecology* 32:2435–2447.
- Chang, H., and M. R. Bonnette. 2016. Climate change and water-related ecosystem services: impacts of drought in california, usa. *Ecosystem Health and Sustainability* 2:e01254.
- Chevenet, Fran., S. Doleadec, and D. Chessel. 1994. A fuzzy coding approach for the analysis of long-term ecological data. *Freshwater Biology* 31:295–309.
- Covich, A. P., M. A. Palmer, and T. A. Crowl. 1999. The Role of Benthic Invertebrate Species in Freshwater Ecosystems. *BioScience* 49:119.

- Deamer, K. 2016, December 7. California's Long Drought Has Killed 100 Million Trees | Live Science. <https://www.livescience.com/57124-california-drought-killed-100-million-trees.html>.
- Gomes, P., O. Wai, and G. Dehini. 2020. Vegetation dynamics of ephemeral and perennial streams in mountainous headwater catchments. *Journal of Mountain Science* 17.
- Gresens, S., R. Smith, A. Sutton-Grier, and M. Kenney. 2009. Benthic macroinvertebrates as indicators of water quality: The intersection of science and policy. *Terrestrial Arthropod Reviews* 2:99–128.
- Herbst, D. B. 2018. A comparison of the taxonomic and trait structure of macroinvertebrate communities between the riffles and pools of montane headwater streams:21.
- Hill, M. J., R. G. Death, K. L. Mathers, D. B. Ryves, J. C. White, and P. J. Wood. 2017. Macroinvertebrate community composition and diversity in ephemeral and perennial ponds on unregulated floodplain meadows in the UK. *Hydrobiologia* 793:95–108.
- Jaeger, K. L., J. D. Olden, and N. A. Pelland. 2014. Climate change poised to threaten hydrologic connectivity and endemic fishes in dryland streams. *Proceedings of the National Academy of Sciences* 111:13894–13899.
- Khudhair, N., C. Yan, M. Liu, and H. Yu. 2019. Effects of Habitat Types on Macroinvertebrates Assemblages Structure: Case Study of Sun Island Bund Wetland. *BioMed Research International* 2019:1–13.
- Letcher, B. H., K. H. Nislow, J. A. Coombs, M. J. O'Donnell, and T. L. Dubreuil. 2007. Population Response to Habitat Fragmentation in a Stream-Dwelling Brook Trout Population. *PLoS ONE* 2:e1139.
- Lucey, N. M., C. Lombardi, L. DeMarchi, A. Schulze, M. C. Gambi, and P. Calosi. 2015. To brood or not to brood: Are marine invertebrates that protect their offspring more resilient to ocean acidification? *Scientific Reports* 5:12009.
- McIntosh, A. R., C. Leigh, K. S. Boersma, P. A. McHugh, C. Febria, and E. García-Berthou. 2017. Chapter 4.7 - Food Webs and Trophic Interactions in Intermittent Rivers and Ephemeral Streams. Pages 323–347 *in* T. Datry, N. Bonada, and A. Boulton, editors. *Intermittent Rivers and Ephemeral Streams*. Academic Press.
- Miguélez, D., and L. Valladares. 2008. Seasonal dispersal of water beetles (Coleoptera) in an agricultural landscape: A study using Moericke traps in northwest Spain. *Annales de la Société Entomologique de France* 44.
- O'Brien, J. M., H. J. Warburton, S. E. Graham, H. M. Franklin, C. M. Febria, K. L. Hogsden, J. S. Harding, and A. R. McIntosh. 2017. Leaf litter additions enhance stream metabolism,

- denitrification, and restoration prospects for agricultural catchments. *Ecosphere* 8:e02018.
- Perera, M. D. D., and P. I. A. Gomes. 2022. Do sediments of ephemeral and perennial streams show different impacts on water quality when subjected to the same drying conditions? *Ecohydrology* 15:e2385.
- Poff, N. L. 1997. Landscape Filters and Species Traits: Towards Mechanistic Understanding and Prediction in Stream Ecology. *Journal of the North American Benthological Society* 16:391–409.
- Resh, V. H., L. A. Bêche, and E. P. McElravy. 2005. How common are rare taxa in long-term benthic macroinvertebrate surveys? *Journal of the North American Benthological Society* 24:976–989.
- Robson, B. J., E. T. Chester, and C. M. Austin. 2011. Why life history information matters: drought refuges and macroinvertebrate persistence in non-perennial streams subject to a drier climate. *Marine and Freshwater Research* 62:801–810.
- Santos, A. N., and R. D. Stevenson. 2011. Comparison of Macroinvertebrate Diversity and Community Structure among Perennial and Non-Perennial Headwater Streams. *Northeastern Naturalist* 18:7–26.
- Sarremejane, R., N. Cid, R. Stubbington, T. Datry, M. Alp, M. Cañedo-Argüelles, A. Cordero-Rivera, Z. Csabai, C. Gutiérrez-Cánovas, J. Heino, M. Forcellini, A. Millán, A. Paillex, P. Pařil, M. Polářek, J. M. Tierno de Figueroa, P. Usseglio-Polatera, C. Zamora-Muñoz, and N. Bonada. 2020a. DISPERSE, a trait database to assess the dispersal potential of European aquatic macroinvertebrates. *Scientific Data* 7:386.
- Sarremejane, R., J. England, C. E. M. Sefton, S. Parry, M. Eastman, and R. Stubbington. 2020b. Local and regional drivers influence how aquatic community diversity, resistance and resilience vary in response to drying. *Oikos* 129:1877–1890.
- Sarremejane, R., R. Stubbington, J. England, C. E. M. Sefton, M. Eastman, S. Parry, and A. Ruhi. 2021. Drought effects on invertebrate metapopulation dynamics and quasi-extinction risk in an intermittent river network. *Global Change Biology* 27:4024–4039.
- Schmera, D., and Podani, J. 2006. On dendrogram-based measures of functional diversity. *Oikos* 115:179–185.
- Statzner, B., N. BONADA, and S. Dolédec. 2007. Conservation of taxonomic and biological trait diversity of European stream macroinvertebrates communities : a case for a collective public database.

- Stern, D. I., and R. K. Kaufmann. 2014. Anthropogenic and natural causes of climate change. *Climatic Change* 122:257–269.
- Stubbington, R., R. Sarremejane, A. Laini, N. Cid, Z. Csabai, J. England, A. Munné, T. Aspin, N. Bonada, D. Bruno, S. Cauvy-Fraunie, R. Chadd, C. Dienstl, P. Fortuño Estrada, W. Graf, C. Gutiérrez-Cánovas, A. House, I. Karaouzas, E. Kazila, A. Millán, M. Morais, P. Pařil, A. Pickwell, M. Polášek, D. Sánchez-Fernández, I. Tziortzis, G. Várбірó, C. Voreadou, E. Walker-Holden, J. White, and T. Datry. 2022. Disentangling responses to natural stressor and human impact gradients in river ecosystems across Europe. *Journal of Applied Ecology* 59:537–548.
- Stubbington, R., M. T. Bogan, N. Bonada, A. J. Boulton, T. Datry, C. Leigh, and R. Vander Vorste. 2017. Chapter 4.3 - The Biota of Intermittent Rivers and Ephemeral Streams: Aquatic Invertebrates. Pages 217–243 in T. Datry, N. Bonada, and A. Boulton, editors. *Intermittent Rivers and Ephemeral Streams*. Academic Press.
- Tachet, H., Richoux, P., Bournaud, M. and Usseglio-Polatera, P., 2000. *Invertebres D'eau Douce. Systematique, Biologie, Ecologie*. CNRS Editions: Paris, France
- Townsend, C. R., and A. G. Hildrew. 1994. Species traits in relation to a habitat templet for river systems. *Freshwater Biology* 31:265–275.
- Trenberth, K. E. 2008. The Impact of Climate Change and Variability on Heavy Precipitation, Floods, and Droughts. Page hsa211 in M. G. Anderson and J. J. McDonnell, editors. *Encyclopedia of Hydrological Sciences*. John Wiley & Sons, Ltd, Chichester, UK.
- Usseglio-Polatera, P., M. Bournaud, P. Richoux, and H. Tachet. 2000. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. *Freshwater Biology* 43:175–205.
- Villéger, S., N. W. H. Mason, and D. Mouillot. 2008. New Multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* 89:2290–2301.