Hydroclimate Impacts on sensitive Benthic Macroinvertebrates in the Drought-stressed Streams of Pinnacles National Park

Anders de Wit

ABSTRACT

Seasonal and interannual changes in hydroclimate regulate stream ecosystems in Mediterranean-climate regions. Drought is a natural part of these regions and can occur seasonally or over multi-year periods. However, climate change will likely increase drought severity and frequency. Studying benthic macroinvertebrates provides an effective means to determine these impacts on streams. In this study, I examined how EPT (mayflies, stoneflies, and caddisflies) in the streams of Pinnacles National Park are affected by seasonal and interannual changes in hydroclimate. I investigated how EPT abundance, richness, and assemblage composition differ seasonally between a dry year during a multi-year drought and a wet year. Furthermore, I studied whether perennial and intermittent sites have differences in EPT. To answer these questions, I used data from two study years in a long-term monitoring project and collected EPT trait information. I found significant seasonal patterns, with perennial sites having greater abundances in the summer and intermittent sites having greater abundance and richness in the winter. The composition of EPT assemblages at the sites was different between winter and summer in both study years. Dry period duration in intermittent sites was associated with lower abundance and richness in the dry year (2016), but not the wet year (2019). These results reinforce how seasonal changes in flow and stream temperature alter habitat for sensitive taxa like EPT, which can be affected differently in perennial versus intermittent reaches. Continued climate change and more frequent multi-year droughts threaten to degrade freshwater ecosystems and remove sensitive taxa.

KEYWORDS

EPT, perennial and intermittent streams, Mediterranean climate, seasonal change, climate change

INTRODUCTION

Climate change poses a significant threat to the global hydrological cycle, with droughts predicted to become more severe, frequent, and long-lasting (Prudhomme et al. 2014). Drought will likely affect Mediterranean-climate regions (MCRs) and their freshwater ecosystems especially harshly. These regions are expected to experience reduced precipitation and increased stream and air temperatures (Filipe et al. 2012). As a result, freshwater biota in MCRs may suffer further declines, as they have been greatly impacted by human activities, such as dam construction and urbanization (Palmer and Ruhi 2019). Freshwater ecosystems in MCRs feature many endemic species and high levels of biodiversity, thus making their conservation even more urgent (Bonada and Resh 2013). Given their ecological importance and vulnerability, it is necessary to further study streams' responses to climate change and to better understand changes to the hydroclimatic variability that characterizes MCRs (Filipe et al. 2012).

Hydroclimate plays a central role in governing the structure and function of stream ecosystems in MCRs (Gasith and Resh 1999). The Mediterranean climate features mild, wet winters and hot, dry summers; stream flows peak in the winter then decrease throughout the summer (Bonada and Resh 2013). In addition to seasonal differences, MCRs often have a high level of interannual variability in precipitation, resulting in alternating periods of wet and dry years (Acuña et al. 2005). These seasonal and interannual changes in hydroclimate have important ecological consequences for streams, which can broadly be classified as perennial (flow continuously), intermittent (run dry periodically) or ephemeral (only flow during high runoff events then quickly dry) (García-Roger et al. 2011). Freshwater biota are adapted to the seasonal cycle of flooding and drying, as well as interannual variability in precipitation. This cycle controls habitat quantity and quality, drives biotic interactions, and determines long-term population and community changes (Gasith and Resh 1999). Although drought is a natural disturbance in stream ecosystems, it can exacerbate seasonal biotic and abiotic changes and exceed the stress tolerances of freshwater biota (Herbst et al. 2019). Therefore, it is critical to understand how specific taxa in streams are affected by drought.

Benthic macroinvertebrates (BMI) are a key part of stream communities and are frequently sampled to help assess changes in stream ecosystems (Lawrence et al. 2010). Drought-induced low flows and associated warmer temperatures can have detrimental effects on

BMI, such as decreased species abundance and richness (Dewson et al. 2007, Herbst et al. 2019). These effects can vary in magnitude when comparing streams with different levels of flow permanence, as distinct differences in BMI community structure have been found between perennial and temporary (intermittent and ephemeral) streams (Bogan et al. 2013, Giam et al. 2017). Temporary streams are very common in MCRs and will likely increase in frequency relative to perennial streams due to climate change (Larned et al. 2010). Within BMI communities, Ephemeroptera, Plecoptera, and Trichoptera taxa (collectively referred to as EPT) are particularly useful for studying environmental stressors like drought, as they are generally rheophilic (Belmar et al. 2013) and temperature-sensitive (Herbst et al. 2019). Although seasonal and multi-year droughts are natural phenomena, severe and extended droughts are becoming more frequent. These long-lasting droughts may have increased impacts on EPT taxa (and likely freshwater ecosystems as a whole) as seasonal flows decrease and stream temperatures potentially rise.

The purpose of this study is to investigate how seasonal and interannual changes in hydroclimate affect EPT taxa in California's Pinnacles National Park. To study these changes, I analyzed a long-term dataset on the BMI community in the streams of Pinnacles National Park, which (like much of California) is a drought-prone area with a Mediterranean climate. I examined how EPT abundance and richness vary between winter and summer in a dry year during an extended drought and during a wet year. Specifically, this comparison involved how flow permanence (perennial versus intermittent study sites) and dry period length impact EPT abundance and richness. Also, EPT taxa exhibit varying responses to flow and temperature changes, with some taxa being more tolerant of harsher conditions (e.g. warm, isolated pools) (Pace et al. 2013). Therefore, I analyzed how these different sensitivities were related to the composition of EPT assemblages in the two study years. I hypothesized that the dry year would have (1) larger seasonal differences in EPT abundance and richness between perennial and intermittent sites due to greater impacts of reduced flows on intermittent sites. (2) The dry year would have stronger relationships between dry period length and EPT abundance and richness due to longer and more intense dry periods at intermittent sites. (3) EPT assemblages would vary more significantly between seasons and between site types in the dry year due to greater changes in habitat conditions.

METHODS

Study area

Pinnacles National Park is a mountainous area located in central California in the Gabilan Range and is a part of the Chalone Creek watershed (Figure 1). The park receives approximately 400 mm of precipitation per year, with most rainfall occurring between January and March due to the region's Mediterranean climate. Temperatures in the winter are generally mild during the day and cold at night (often dropping below freezing), while day-time summer temperatures can reach 40°C (Bogan and Carlson 2018). The area features distinctive formations of volcanic cliffs and spires, as well as a geologic history of extensive fault action and seismicity ("Geology - Pinnacles National Park (U.S. National Park Service)" n.d.). Vegetation in the park is mainly chaparral, but woodlands, grasslands, and riparian vegetation zones are also present (Bogan and Carlson 2018). Chalone Creek is the largest of the several streams flowing through the park and drains roughly 100 km² of land. Surface runoff from rainfall is the most important source of stream discharge (though it can snow in the park at the highest elevations); during the dry summers, groundwater can play an important hydrological role. The stream network in the park is strongly intermittent, meaning few perennial reaches are found, and has a flashy flow regime (Bogan and Carlson 2018). Since the area is protected national park land, the streams in Pinnacles are largely undisturbed. Although there is a reservoir in the park that impounds a portion of Bear Gulch Creek, only one study site in the creek is located downstream of the reservoir.
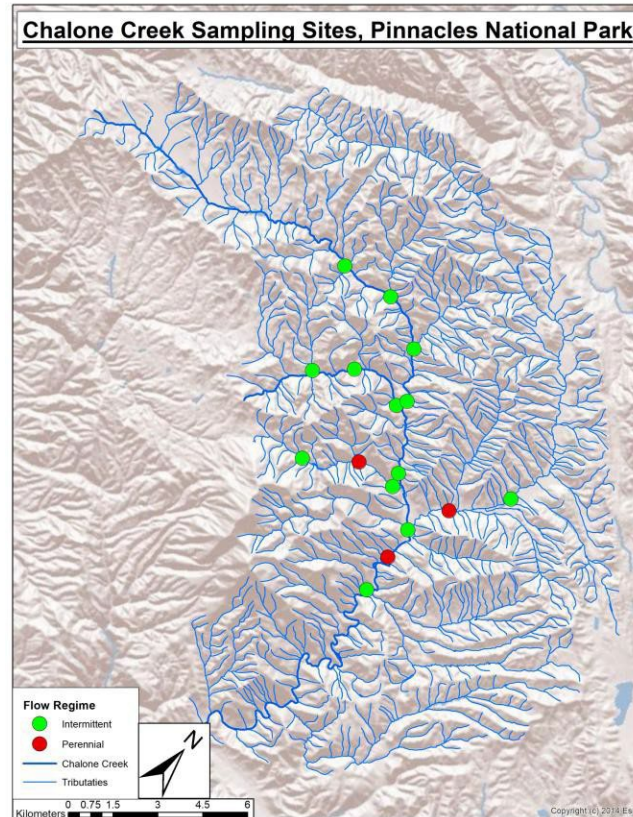
Figure 1. Map of the 16 study sites in the streams of Pinnacles National Park. Intermittent sites are shown in green circles, and perennial sites are shown in red circles. Map courtesy of Travis Apgar.

Data collection

Dataset description

The dataset used for this study comes from two projects examining changes in the BMI community in the park's streams. One of the projects is a long term-monitoring study, while the other is part of the StreamCLIMES project, which is researching the effects of stream drying across the U.S. The streams have been sampled in the winter (typically February) and summer (typically May or June) from 2014 to the present, with 16 sampling sites (Table 1). Of the 16 sites, three have perennial flow regimes and 13 are intermittent. The reach at each site (30 meters long for the data I analyzed) was sampled using a D-net, and samples were elutriated and then preserved in ethanol. Both riffle and pool microhabitats were sampled along the length of the reach and at alternating spots across the channel width, ultimately yielding one composite sample

per site. HOBO loggers were deployed at each site to record water and air temperatures and to measure electrical conductivity, which is used to determine when sites run dry. Samples were processed in a lab using an established protocol (Herbst et al. 2018), and specimens were identified to the lowest feasible taxonomic level.

Table 1. Site names and locations for all 16 study sites in the streams of Pinnacles National Park. Site type refers to the type of flow at each site.

Stream	Site Name	Site Type	Latitude	Longitude
Bear Gulch	Nature Center	Perennial	36.481647	-121.18071
Bear Gulch	Above Reservoir	Intermittent	36.471242	-121.19437
Bear Gulch	Below Green Gully	Intermittent	36.482809	-121.16825
Chalone Creek	South Wilderness Perennial	Perennial	36.465841	-121.15532
Chalone Creek	Lowermost South Wilderness	Intermittent	36.454224	-121.15365
Chalone Creek	At Highway Bridge	Intermittent	36.486909	-121.16953
Chalone Creek	At first South Wilderness Trail Crossing	Intermittent	36.475996	-121.15626
North Fork Chalone Creek	One mile above Marion Canyon	Intermittent	36.52533	-121.20644
North Fork Chalone Creek	Above Highest Trail Crossing	Intermittent	36.52324	-121.22287
North Fork Chalone Creek	At Marion Canyon	Intermittent	36.51815	-121.19082
North Fork Chalone Creek	Below Willow Spring	Intermittent	36.50494	-121.18197
Sandy Creek	At Campground	Perennial	36.488438	-121.15066
Sandy Creek	Above Campground	Intermittent	36.503186	-121.12913
West Fork Chalone Creek	Below Balconies Cave	Intermittent	36.50183	-121.20023
West Fork Chalone Creek	Above Confluence with North Fork	Intermittent	36.501933	-121.18359
West Fork Chalone Creek	At Chaparral	Intermittent	36.49322	-121.20951

Data sub-set selection

I selected 2016 and 2019 as my study years. California suffered a severe drought from 2012 to 2016, and drought conditions were present in Pinnacles and the surrounding county of San Benito during this period. According to the U.S. Drought Monitor (n.d.), Pinnacles was in

the most severe drought category ("D4 – Exceptional Drought") during 2016, while in 2019 the area was not affected by drought. I tabulated the number of EPT taxa and their abundances for each site in the winter and summer samples for 2016 and 2019. In both study years, some sites were not sampled due to no water or not enough water being present on each sampling date. For these sites, all taxa were assigned an abundance of zero, resulting in a richness value of zero as well. Also, some EPT taxa were not identified fully to species-level. As a result, I used the genus taxonomic rank (and the family rank in some cases) to perform my analyzes. Using hydrological data from the long-term monitoring project. I recorded the number of days with and without water for each site in my study years. The 2016 hydroperiod started from the Summer 2015 sample dates and ended on the Summer 2016 sample dates. The 2019 hydroperiod started from the Summer 2018 sample dates and ended on the Summer 2019 sample dates. Both hydroperiods were 374 days in length.

Trait databases

I used databases from Herbst et al. (2018) and Poff et al. (2006), which contained trait information (e.g. temperature preferences, size, behavioral habit, etc.) for BMI taxa. These trait data are often used in ranking systems to classify how sensitive taxa are to stress. From these databases, I selected two trait categories: temperature preference, where 1 = cold stenotherm, 2 = cool eurytherm, 3 = warm eurytherm, and rheophilic flow/habitat preference, where 1 = erosional, 2 = mixed, 3 = depositional. Erosional habitat refers to riffles (fast flowing), while depositional habitat refers to pools (slow flowing). Mixed habitat means that the taxon prefers both riffles and pools. I cross-referenced the two databases with my Pinnacles dataset to record the values for the two categories for each EPT taxon present at the study sites.

Data analysis

EPT abundance and richness in perennial versus intermittent sites

I compared perennial and intermittent sites to determine if they exhibited seasonal and interannual differences in EPT abundance and richness. To determine which predictor variables and their interactions best explained variation in EPT abundance and richness, I used generalized

linear models, as I did not find that random effects needed to be included. The response variables of abundance and richness were non-Normally distributed, which I established using Shapiro-Wilk tests and data visualization. I did not log-transform the data, since generalized linear models have been shown to perform better than using log-transformation when analyzing ecological count data (O'Hara and Kotze 2010). I used a negative binomial distribution for both my abundance and richness models due to overdispersion when Poisson distributions were fitted.

For my generalized linear models, the three predictor variables were site type (perennial and intermittent), year (2016 and 2019), and season (winter and summer). To decide which combination of predictor variables performed the best, I used changes in corrected Akaikes Information Criterion values (ΔAICc), with the best-fitting model having the lowest ΔAICc value. For the purposes of this study, models with ΔAICc values of two or less are presented. However, models with ΔAICc values between two and seven have been shown to be also worthy of investigation (Burnham et al. 2011). I then conducted post-hoc comparisons using the best-fitting models for each response variable to further analyze my results. I performed these analyses in R, using the "glm2" and "MASS" packages to run the generalized linear models and the "emmeans" package to conduct post-hoc tests (R Core Team 2021).

The effects of the dry period on intermittent sites

I analyzed the impacts of dry period length on EPT abundance and richness in intermittent sites. To do this, I used the total number of dry days for each of the 13 intermittent sites during the hydroperiods for my two study years. The hydroperiod started from the prior year's summer sample date and ended on the study year's summer sample date. The abundance and richness data were both Normally distributed in each study year, which I established using Shapiro-Wilk tests and data visualization. I then ran linear regression analyses using ordinary least squares on EPT abundance and richness, with the total number of dry days per site as the explanatory variable. I used combined winter and summer data for each study year as the dependent variable in both analyses (abundance and richness). I performed all analyses in R, using the "lm" package to conduct linear regressions (R Core Team 2021).

EPT assemblage composition and flow and temperature preferences

　　To analyze and visualize differences in EPT composition at each site based on my three predictor variables, I used non-metric multi-dimensional scaling (NMDS). I used the Bray-Curtis dissimilarity metric as the basis for my NMDS analysis. Before running my analysis, I applied a square root transformation to the abundance values to decrease the effects of very abundant taxa on the ordination (McCune and Grace 2002). To determine whether EPT composition varied based on my predictors (site type, year, and season), I ran an ANOSIM test for each predictor. To visualize these differences, I generated 95% confidence interval ellipses for the groups in each predictor variable and then plotted these ellipses on the ordinations. Finally, I used indicator species analysis to investigate which taxa were driving the patterns in EPT composition. I fitted significant species vectors to my ordination using the "envfit" function to visualize the influence of key taxa. I performed all analyses in R, using the "vegan" package for the NMDS and ANOSIM analyses and the "indicspecies" package for the indicator species analysis (R Core Team 2021).

　　Using the values from the trait databases, I combined the two categories (temperature preference and flow preference) to make a ranking system on a 0-6 scale (0 = greatest need for cold temperatures and fast-flowing/rheophilic habitat, and 6 = least need). I then assigned each EPT taxon its ranking and grouped taxa into three categories based on their ranking. I put taxa with scores of 2 and 3 into a "Low Tolerance" category, since these taxa need fast-flowing habitat (i.e. riffles) and/or a narrow range of cool water temperatures. I put taxa with a score of 4 into a "Medium Tolerance" category, since these taxa can do well in both riffles and pools and tolerate a broader range of cool temperatures. I put taxa with scores of 5 and 6 into a "High Tolerance" category, since these taxa do well in pools and/or can tolerate warm temperatures.

RESULTS

EPT abundance and richness in perennial versus intermittent sites

　　I found differences in mean EPT abundance and richness based on the three predictor variables of site type, year, and season (Table 2). The dataset for my study years of 2016 (dry

year) and 2019 (wet year) contained 34 total EPT taxa: eight Ephemeroptera taxa, 11 Plecoptera taxa, and 15 Trichoptera taxa. The total abundance was 31,599 specimens.

Table 2. Mean abundance and mean richness of EPT taxa in winter and summer for 2016 and 2019. Thirteen intermittent sites and three perennial sites were sampled in each season and year. The standard deviation (SD) is included for each metric.

Site Type	Year	Season	Mean Abundance	SD	Mean Richness	SD
Intermittent	2016	Winter	161.54	116.73	6.54	3.97
Intermittent	2016	Summer	60.15	130.68	3.77	3.49
Intermittent	2019	Winter	684.93	542.30	6.92	3.38
Intermittent	2019	Summer	135.63	182.10	2.46	2.26
Perennial	2016	Winter	101.33	109.99	8.00	2.00
Perennial	2016	Summer	3717.33	3886.37	8.67	1.16
Perennial	2019	Winter	405.33	213.22	9.00	3.61

Abundance analysis results

The predictor variable combination of site type, year, season, and the interaction of site type and season best explained the variation in EPT abundance, as this model had the lowest ΔAICc value (Table 3). For the 19 possible models, site type, year, season, and the interaction of site type and season were the best predictors, all having variable weights above 0.95 (Table 3). Site type, season, and year were included in the greatest number of models, at 14. Three models had ΔAICc values of two or less, and three models had values between two and seven.

Table 3. The three best-fitting abundance models based on ΔAICc values using generalized linear models. The variable weight is provided for each model term.

Model	df	AICc	ΔAICc	Model Weight	Model Term	Variable Weight Across All Models
Site Type + Year + Season + Site Type:Season	6	821.65	0.00	0.41	Site Type	1
Site Type + Year + Season + Site Type:Season + Season:Year	7	822.67	1.02	0.25	Year	0.9543

Site Type + Year + Season + Site Type:Season + Site Type:Year	7	823.55	1.90	0.16	Season	0.9983
					Site Type:Season	0.9932
					Season:Year	0.3797
					Site Type:Year	0.2935
					Site Type:Season:Year	0.0349

The best-fitting model showed that the difference in abundance across all seasons and sites between 2016 and 2019 was significant (p = 0.004), with 2019 having more EPT than 2016. Through post-hoc tests, I found seasonal differences across both study years based on site type (Figure 2). Intermittent sites had greater abundances in winter (p = 0.004), while perennial sites had greater abundances in summer (p = 0.003). During the winter, abundance differences between perennial and intermittent sites were not significant (p = 0.863). However, perennial sites had higher abundances than intermittent sites in the summer (p = <.001).

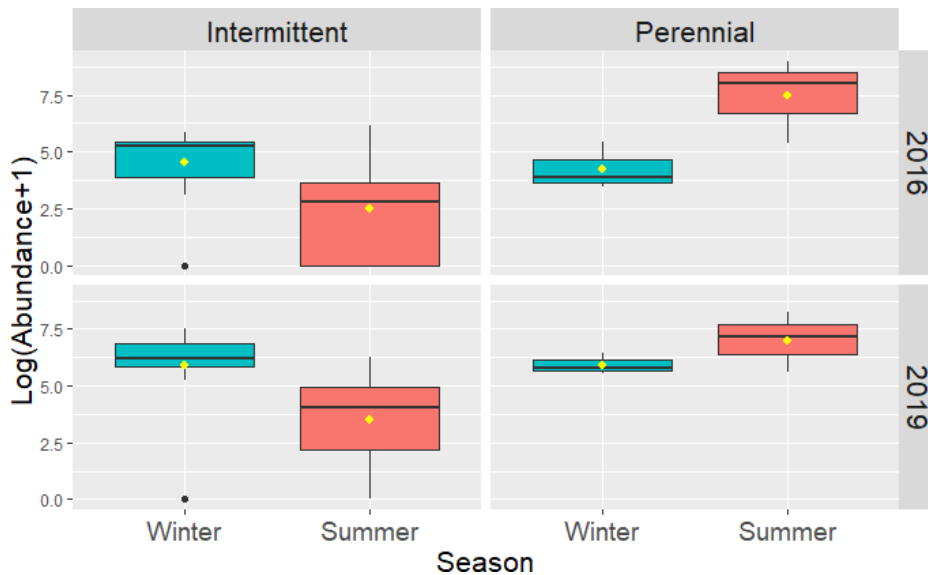**Figure 2. Boxplots for EPT abundances in intermittent and perennial sites for both study years and seasons.** Abundances were log(x + 1) transformed to better visualize the data due to large distribution differences in the data between intermittent and perennial sites. The mean for each boxplot is shown as a yellow dot.

Richness Analysis Results

The predictor variable combination of site type, season, and the interaction of site type and season best explained the variation in EPT richness (Table 4). For the 19 possible models, site type and season were the best predictors, with both having variable weights above 0.98 (Table 4). As with the abundance models, site type, season, and year were the most included predictors, being present in 14 models. However, the variable weight for year was lower across all the richness models than for the abundance models (0.4448 versus 0.9453). Also, the variable weight of the interaction term of site type and season was lower (0.9543 for the abundance models versus 0.6432 for the richness models). Three richness models had ΔAICc values of two or less, and seven models had values between two and seven.

Table 4. The three best-fitting richness models based on ΔAICc values using generalized linear models. The variable weight is provided for each model term.

Model	df	AICc	ΔAICc	Model Weight	Model Term	Variable Weight Across All Models
Site Type + Season + Site Type:Season	5	339.92	0	0.35	Site Type	0.9860
Site Type + Season	4	341.17	1.25	0.19	Year	0.4448
Site Type + Season + Year + Site Type:Season	6	341.93	2.00	0.13	Season	0.9970
					Site Type:Season	0.6432
					Season:Year	0.1778
					Site Type:Year	0.1078
					Site Type:Season:Year	0.0071

The best-fitting model showed seasonal richness differences across both study years based on site type (Figure 3). Intermittent sites had higher richness values in the winter than the summer (p < .001), while richness did not vary seasonally for perennial sites (p = 0.999). During the winter, richness differences between intermittent and perennial sites were not significant (p = 0.800). In the summer, however, richness was higher in perennial sites than in intermittent sites (p = 0.002). Although the best-fitting richness model did not include year as a predictor, the

third-best model did include it and had a ΔAICc value of 2.00. This model had the same set of predictors as the best-fitting abundance model. I performed a post-hoc analysis on this richness model and found that, unlike for abundance, the overall richness difference between 2016 and 2019 was not significant (p = 0.509).
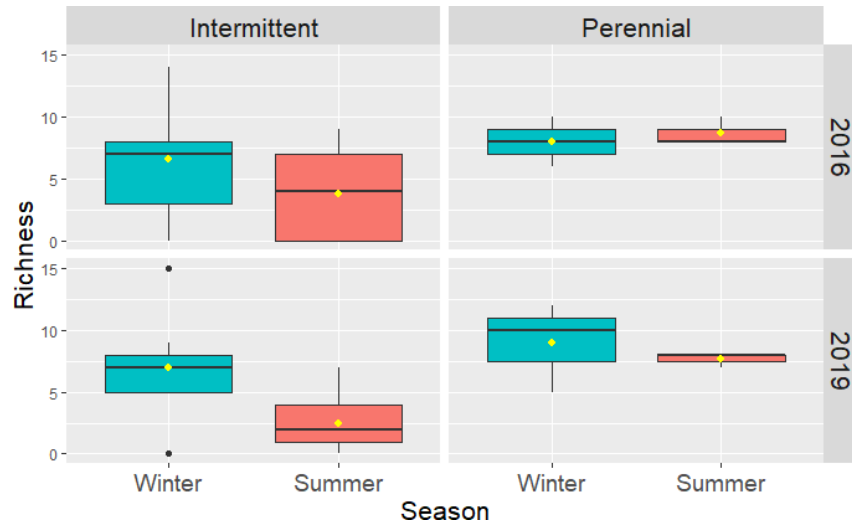

Figure 3. Boxplots for EPT taxa richness in intermittent and perennial sites for both study years and seasons. The mean for each boxplot is shown as a yellow dot.

The effects of the dry period on intermittent sites

The thirteen intermittent study sites showed varying relationships between dry period length and EPT abundance and richness (Figure 4). During the 2016 hydroperiod, the sites had a total of 3310 days without flow, for an average of 255 dry days per site. The 2019 hydroperiod had a total of 2812 days without flow, for an average of 216 dry days per site. For the 2016 linear regression analysis, dry period length explained 31% of the variance in total abundance (R^2 = 0.306, p = 0.05) and 53% of the variance in total richness (R^2 = 0.533, p = 0.005). These results show that the relationships between dry period length and abundance and richness were significant in 2016. For 2019, the linear regression analysis showed that dry period length explained 7% of the variance in total abundance (R^2 = 0.072, p = 0.376) and 7% of the variance in total richness (R^2 = 0.074, p = 0.369). These results show that the relationships between dry period length and abundance and richness were not significant in 2019.
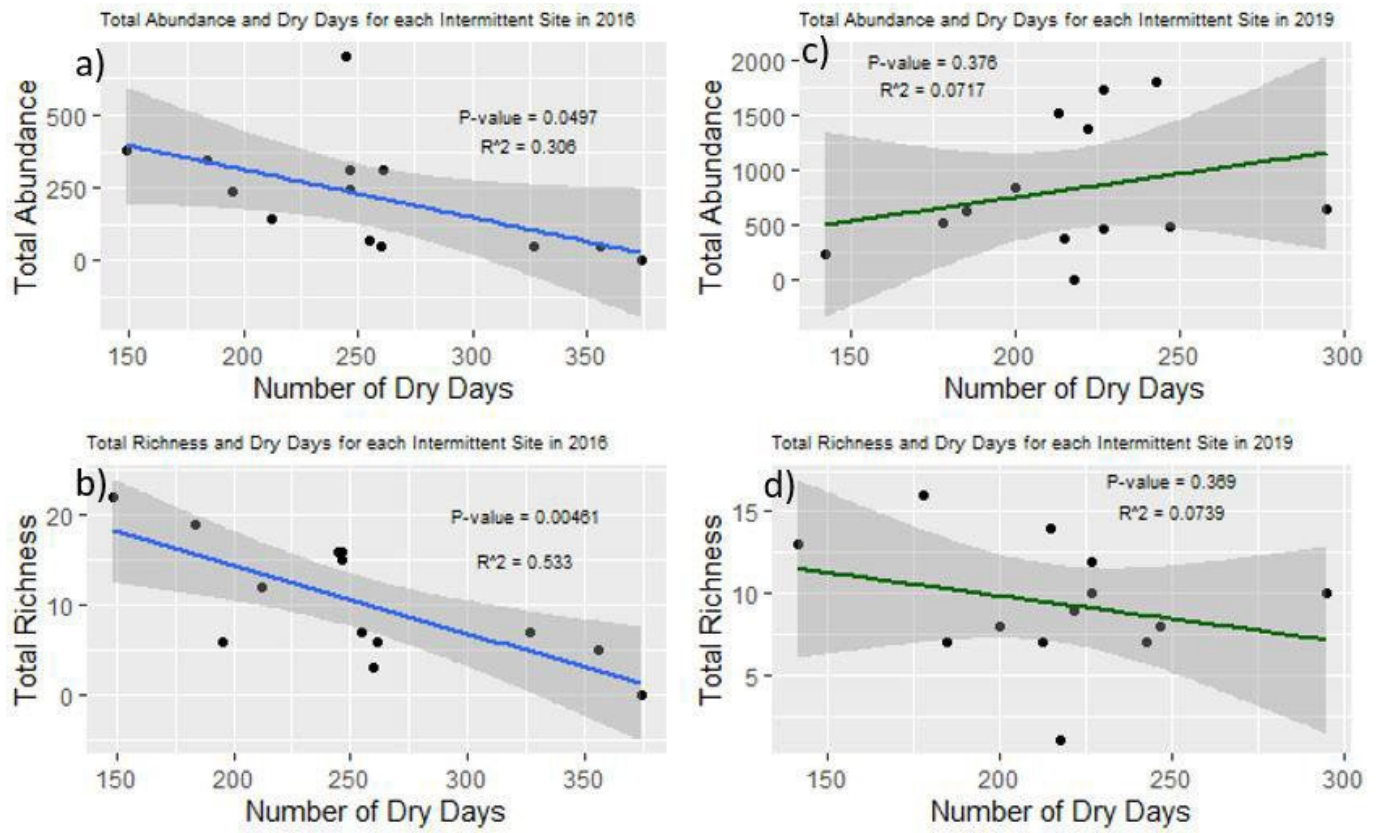
Figure 4. Linear regression graphs for dry period length versus total abundance and total richness for the 13 intermittent sites in 2016 and 2019. Graphs a) and b) are for total abundance and total richness, respectively, in 2016. Graphs c) and d) are for total abundance and richness, respectively, in 2019. The p-value and R^2 value are provided in each graph, along with the 95% confidence interval (shown in dark grey around each regression line).

EPT assemblage composition and flow and temperature preferences

My NMDS analysis found a two-dimensional solution with a stress of 0.16 (Figure 5). Based on my ANOSIM test, I found a significant seasonal difference in EPT composition (R value = 0.598, significance value < 0.001). Although the ANOSIM test found a significant difference in EPT composition between study years (significance value = 0.043), the R value was very low (0.051). There was no significant difference in EPT composition between site types (R value = 0.043, significance value = 0.245). My ordinations fitted with ellipses for the levels of each predictor variable show no overlap between winter and summer and overlap for the other two predictors (Figure 6). This lack of overlap between winter and summer (Figure 6a) indicates

a seasonal difference in EPT composition, while the overlap within year and within site type indicates no relationship with EPT composition (Figures 6b and 6c).
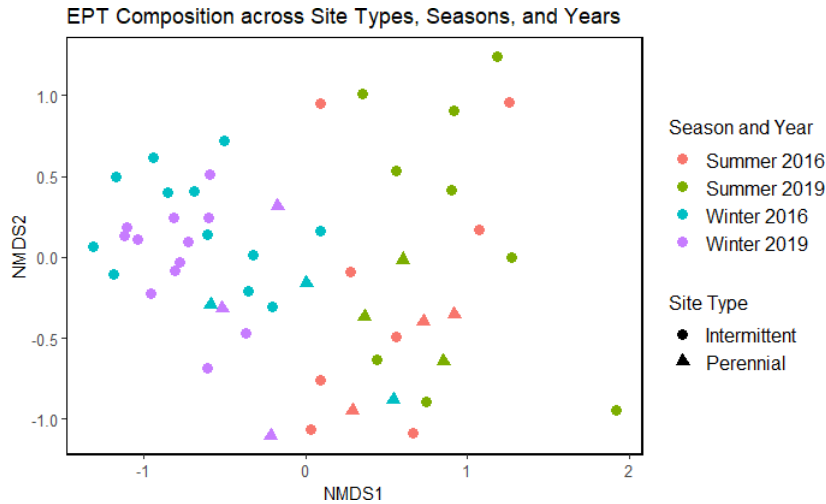

Figure 5. NMDS ordination plot (stress = 0.16) for the EPT abundance data across all site types, seasons, and years.
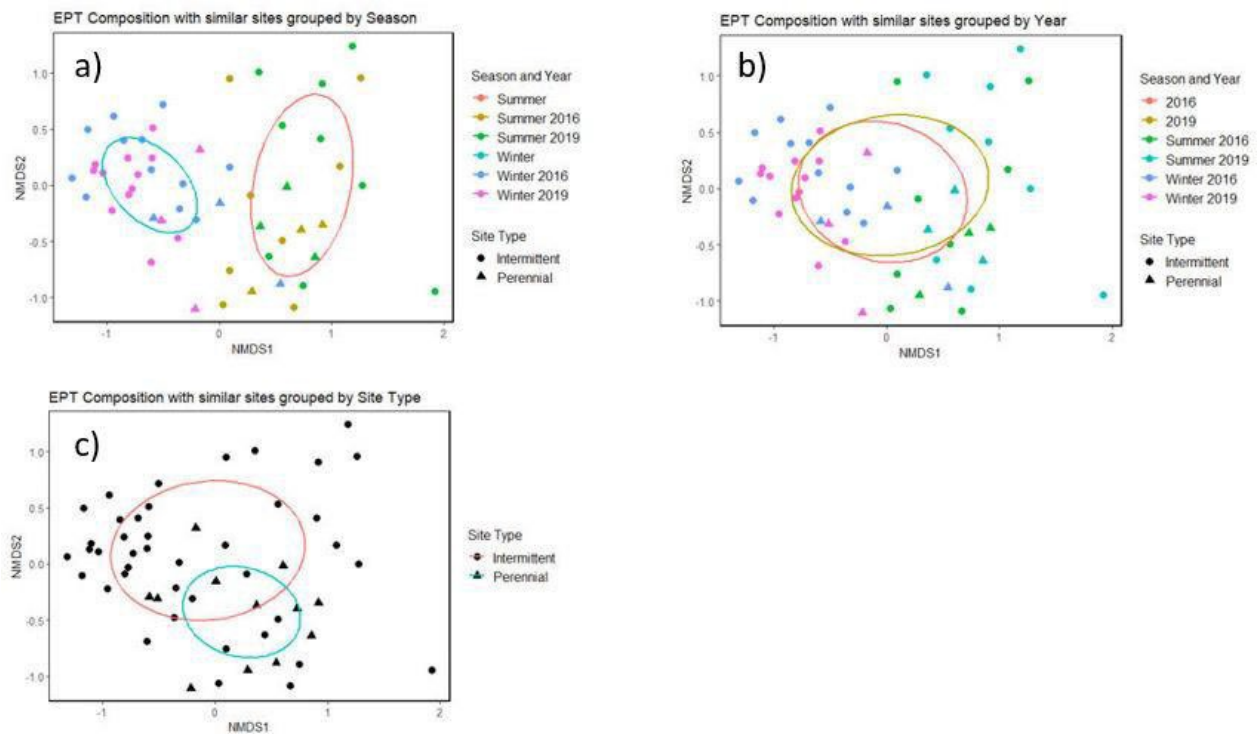

Figure 6. NMDS ordination plots for the EPT abundance data across seasons (graph a), years (graph b), and site types (graph c). Each plot shows the same ordination (stress = 0.16), with 95% confidence interval ellipses plotted for each level of the predictor variable.

The low tolerance category had the highest number of taxa, at 18. The medium tolerance category had 13 taxa, and the high tolerance category had three taxa (Table 5). Sixteen total taxa were significantly associated with season, eight in both winter and summer. The taxa associated with summer were from a mix of the three tolerance categories (four in the low category, three in the medium category, and one in the high category), while significant taxa in the winter were almost all from the low category. Five taxa were significantly associated with the 2019 study year, while no taxa were associated with 2016. Nine taxa were significantly associated with perennial sites and represented a mix of the three tolerance categories. Only two taxa were significantly associated with intermittent sites, both from the low category. Based on my NMDS analysis, I found nine taxa to be drivers of the overall patterns in the data, seven of which were from the low tolerance category (Figure 7). Six of these nine taxa were significantly associated with winter, which can be seen in the direction and length of their species vectors (Figure 7). A longer species vector indicates a stronger association. The one taxon from the high tolerance category was significantly associated with summer, which can be seen in the direction and length of its species vector (Figure 7).

Table 5. Tolerance categories for EPT taxa and results of the indicator species analysis. The three categories represent increasing tolerance of slow-flowing habitat and warm temperatures. The p-value is given for each taxon that was significantly associated with a level for each predictor variable. A blank cell means the taxon was not associated with the corresponding level. Taxa in bold were identified as significant species in the NMDS analysis and were each fitted with a species vector. The genus or family name is provided for each taxon.

| Taxa | Tolerance Category | Predictor Variable | | | | | |
| | | Site Type | | Year | | Season | |
		Perennial	Intermittent	2016	2019	Winter	Summer
Ameletus	Low					0.0037	
Baumannella	Low					0.0187	
Bolshecapnia	Low		0.0401			0.0002	
Capnia	Low				0.0162	0.0001	
Isocapnia	Low						
Isoperla	Low						
Malenka	Low	0.0001					0.0277
Nemoura	Low				0.0246	0.0018	
Perlodidae	Low						
Taenionema	Low		0.0331		0.0011	0.0001	
Gumaga	Low	0.0202					
Hydropsyche	Low	0.0062					0.0024
Lepidostoma	Low	0.0001					0.0275

| Taxa	Category				
Micrasema	Low	0.0015			0.0023
Neophylax	Low				
Polycentropus	Low				
Rhyacophila	Low			0.0005	
Wormaldia	Low				
Baetis	Medium	0.0017			0.0087
Centroptilum	Medium				
Fallceon	Medium				
Paraleptophlebia	Medium	0.0006			
Procloeon	Medium				
Mesocapnia	Medium		0.0017	0.0022	
Sweltsa	Medium				
Hydroptila	Medium				0.0056
Leptoceridae	Medium				
Nectopsyche	Medium				
Ochrotrichia	Medium				
Oxyethira	Medium				0.0261
Oecetis	Medium	0.0453			
Callibaetis	High		0.0139		0.0004
Tricorythodes	High				
Limnephilus	High	0.0453			
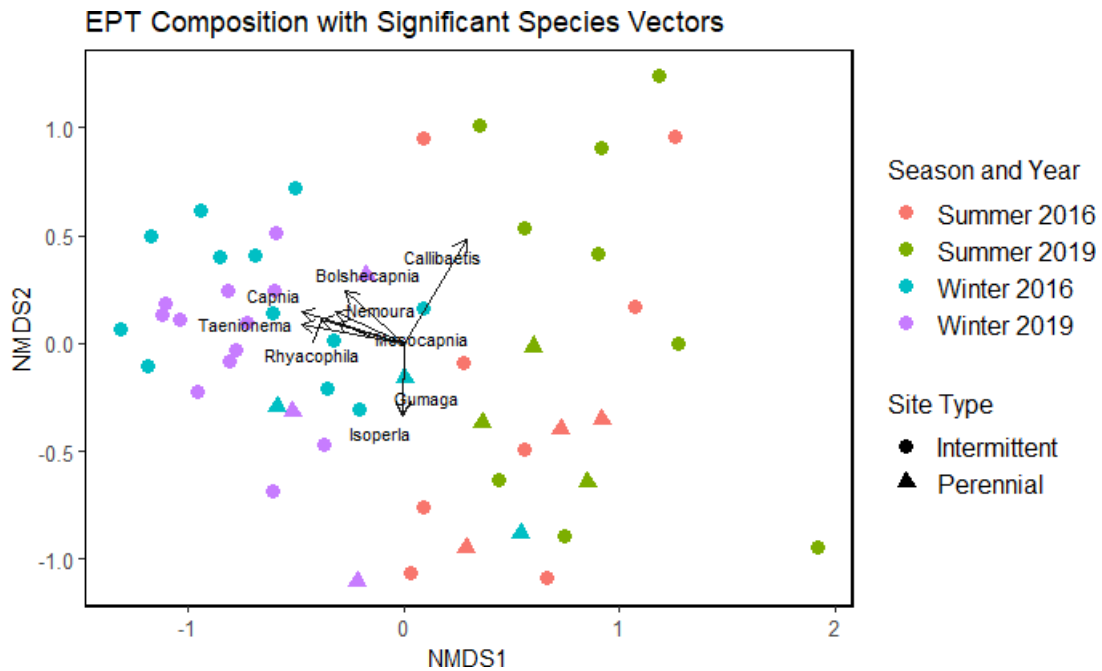
Figure 7. NMDS ordination plot (stress = 0.16) for the EPT abundance data across all site types, seasons, and years. The vectors show the nine taxa identified as significant species and main drivers in the patterns of EPT composition.

DISCUSSION

In this study, I found that seasonal and interannual changes in hydroclimate were related to shifts in EPT abundance, richness, and assemblage composition. Seasonal trends for perennial and intermittent sites and overall EPT composition were more significant than interannual differences. However, intermittent sites had lower abundance and richness in 2016 than 2019, in agreement with one of my main hypotheses. I related these results to key differences between perennial and intermittent streams, such as greater habitat quantity and quality in reaches with perennial flow. Furthermore, I discussed seasonal habitat changes in Mediterranean-climate streams. These changes, such as a decline in riffle-pool sequences in the summer and the onset of drought conditions, have important implications for sensitive taxa like EPT.

EPT abundance and richness in perennial versus intermittent sites

Seasonal and interannual differences between perennial and intermittent sites show the importance of the flow regime in governing EPT abundance and richness. For both study years, perennial sites supported greater overall abundances and species richness than intermittent sites (Figures 2 and 3). Numerous other studies have found this difference between perennial and intermittent streams when analyzing the entire BMI community and EPT specifically (Bogan et al. 2013, Giam et al. 2017, Karaouzas et al. 2019). This difference is likely due to greater habitat connectivity, stability, and diversity in areas with perennial flow (Bonada et al. 2007). However, there was a significant seasonal pattern in both study years, with perennial and intermittent sites having similar abundances and richness in the winter, while perennial sites supported substantially more EPT in the summer. This similarity during the winter has been found in other studies (Bonada et al. 2007). The likely explanation is that intermittent and perennial reaches both feature favorable habitat (i.e. riffles) for EPT in the winter due to greater flow (García-Roger et al. 2011). During the summer, high levels of habitat fragmentation and contraction in intermittent reaches lead to lower abundance and richness compared to perennial reaches (Bêche et al. 2006).

Another aspect of the seasonal pattern was that EPT abundance and richness declined from winter to summer in intermittent sites, while richness did not vary seasonally for perennial

sites (Figures 2 and 3). This greater seasonal variability in intermittent sites is consistent with other studies (Bonada et al. 2006, García-Roger et al. 2011). Perennial reaches have consistent flow and quality habitat, allowing for stable levels of taxa richness throughout the year (Giam et al. 2017). Contrary to my predictions, EPT abundance was highest in summer perennial sites (Figure 2). I had hypothesized that EPT abundance would be highest in winter perennial sites due to more flow and cooler water temperatures. However, perennial reaches act as refuge areas during the dry summer (Bogan et al. 2017a, Larned et al. 2010). My results suggest that as the heavily intermittent stream network in Pinnacles progressively dries, sensitive taxa like EPT move to perennial reaches, resulting in higher abundances in the summer. This "concentration effect" has been observed in other drought-stressed streams (Bêche et al. 2009). Although differences between perennial and intermittent sites are clear, intermittent sites may show varying levels of EPT abundance and richness due to differences in their drying length.

The effects of the dry period on intermittent sites

Based on this study, the magnitude of flow intermittency can negatively impact EPT abundance and richness, though this relationship is not fixed. Total EPT abundance and richness decreased as flow intermittence increased for the 2016 study year (Figures 4a and 4b). Other studies have also observed a significant negative relationship between flow intermittence and EPT abundance and richness (Datry et al. 2014, Feminella 1996). The dry period duration, which can serve as a measure of drying severity, is a key driver of BMI community changes in intermittent streams (Sánchez-Montoya et al. 2018). The dry period creates fragmentation and contraction of habitat both laterally and longitudinally in reaches and across the stream network (Larned et al. 2010). Furthermore, the dry period is associated with harsher environmental conditions, such as lower dissolved oxygen levels and warmer water temperatures (García-Roger et al. 2011). These habitat changes and abiotic conditions in intermittent reaches stress sensitive EPT taxa, which are generally not resistant to desiccation (Datry et al. 2014). The relationship between dry period duration and EPT abundance and richness was not significant in 2019, which was one of the wettest years during the Pinnacles long-term monitoring project. On the other hand, 2016 was during a multi-year drought, which can intensify changes in intermittent streams (Giam et al. 2017). These results show that EPT as a whole are affected by hydroclimate

changes, though individual taxa can exhibit varying responses due to different flow and temperature sensitivities.

EPT assemblage composition and flow and temperature preferences

My NMDS analysis showed a significant seasonal pattern in EPT composition (Figure 6a), with related differences in flow and temperature preferences. Seven out of the eight taxa significantly associated with winter were from the low tolerance category, meaning that these taxa strongly favor riffles and cool temperatures (Table 5). Higher flows in winter compared to summer create an abundance of fast-flowing, well-oxygenated riffle habitat, which is beneficial for EPT (Herbst et al. 2019). As flows decrease during the spring and into summer, riffles decline, and disconnected pools can become the dominant habitat (Cid et al. 2017). This seasonal decrease in favorable habitat may explain the observed shift in EPT composition to less sensitive taxa, a shift that frequently occurs across the BMI community (Bonada et al. 2007). In my study, EPT taxa significantly associated with summer came from a mix of tolerance categories, with an increase in representation from the medium and high tolerance categories compared to winter. For example, the mayfly *Callibaetis* was strongly associated with summer and was the most abundant taxon in the high tolerance category. This mayfly is pool-favoring, can tolerate warm temperatures, and was one of the main drivers of the patterns in EPT composition (Figure 7). Other studies have found declines in sensitive EPT taxa and increases in pool-favoring EPT taxa between wet and dry periods (Bogan et al. 2017b, Pace et al. 2013). The pattern in EPT composition that I found reinforces the importance of seasonal changes in Mediterranean-climate streams.

Seasonal and interannual changes in perennial and intermittent streams

Seasonal and interannual changes in flow and temperature regulate stream ecosystems through shifts in biotic and abiotic factors (Gasith and Resh 1999). I found more significant seasonal patterns than patterns between my two study years. BMI community composition can vary more significantly between perennial and temporary (intermittent and ephemeral) streams in droughts years than wet years (Bêche et al. 2009). Although I did not study the entire BMI

community, EPT abundances were higher overall in the wet year of 2019, and abundance and richness were negatively impacted by dry period length in the drought year of 2016. These results support the roles drought and interannual variability play in structuring BMI assemblages (Resh et al. 2013). At the same time, the seasonal patterns in EPT abundance, richness, and assemblage composition provide evidence that the shift from winter to summer is key for these taxa. As flows decrease, riffle-pool sequences with cool water temperatures begin to fragment and dry up, resulting in disconnected habitat patches and increases in isolated, warm pools (Bonada et al. 2006). My results reinforce the seasonal shift in community composition to lentic taxa and seasonal declines in sensitive taxa, two key patterns that characterize Mediterranean-climate streams (Cid et al. 2017). Finally, the large increases from winter to summer in perennial sites support the concept in stream ecology that perennial reaches serve as refuge and recolonization areas in intermittent stream networks (Bogan et al. 2017a). My study demonstrates the importance of perennial flow regimes, which are vital to EPT and the entire BMI community.

Limitations and future directions

This study's focus on EPT precludes conclusions regarding how the entire BMI community in Pinnacles is responding to seasonal and interannual changes in hydroclimate. Although EPT are useful as bioindicators, knowledge of changes in other benthic macroinvertebrates and other taxa in the food web would give greater insight into how streams are affected by drought and climate change. Another limitation in this study is that analyzing two years of data only provides a snapshot of change, as drought operates on both long and short timescales. Despite the significance of riffles, pools, and water temperature to changes in EPT, I did not quantify these variables in my study. Other studies have investigated differences in BMI community composition between riffles and pools (see Herbst et al. 2018). Moreover, I only studied the flow and temperature sensitivities of EPT taxa. These organisms and other BMI taxa have numerous life-history strategies and traits that are important to consider (Bogan et al. 2017a). This study's limitations provide opportunities for further research into how EPT and other benthic macroinvertebrates are affected by changes in hydroclimate.

My study's results should be analyzed within the context of how the entire BMI community in Pinnacles' streams is changing over time. Studies of specific habitat types (sampling riffles and pools separately) could help determine how EPT are responding to drought in Pinnacles. For example, such studies could quantify the shift from riffle-pool sequences in the winter to greater pool dominance in the summer. Also, seasonal water temperature differences, particularly between riffles and isolated pools, could be studied. Another valuable research area would be investigating spatial relationships between perennial and intermittent sites in the Pinnacles stream network. For example, other studies have related differences in BMI community composition to distances between and among perennial and intermittent reaches (see Bogan et al. 2013, Sánchez-Montoya et al. 2018). These potential future studies in Pinnacles would help determine how Mediterranean-climate areas are responding to drought and climate change.

Broader implications

Studies of freshwater ecosystems continue to show that drought and climate change will have increasing impacts on biota, especially in the western United States (Pyne and Poff 2017). A critical aspect of climate change in this geographic area is that low flows and higher stream and air temperatures are predicted to occur together more frequently (Arismendi et al. 2013). This synchronization could cause disproportionate impacts on sensitive freshwater biota, such as EPT. Although I observed stronger seasonal patterns, I did find evidence of negative effects on EPT from a dry year during a multi-year drought. Importantly, multi-year droughts can have larger impacts on freshwater ecosystems than short droughts (Bêche et al. 2009) and are expected to increase in frequency (Cid et al. 2017). These longer droughts may stress and alter BMI communities, with communities in perennial streams being at high risk due to having fewer drought-adapted taxa compared to intermittent streams (Saffarinia et al. 2022). The significant differences in EPT abundance and richness between the perennial and intermittent sites in my study are indicative of these broader dissimilarities between these flow regimes. Despite the importance of perennial flow regimes to sensitive taxa, streams in Mediterranean-climate areas may shift to greater intermittency due to climate change and human development, such as surface water diversion and groundwater pumping (Larned et al. 2010). Studies like mine

demonstrate the need for the continued protection of freshwater ecosystems and better ways to address the challenges of climate change moving forward.

ACKNOWLEDGEMENTS

REFERENCES

Acuña, V., I. Muñoz, A. Giorgi, M. Omella, F. Sabater, and S. Sabater. 2005. Drought and postdrought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. Journal of the North American Benthological Society 24:919–933.

Arismendi, I., M. Safeeq, S. L. Johnson, J. B. Dunham, and R. Haggerty. 2013. Increasing synchrony of high temperature and low flow in western North American streams: double trouble for coldwater biota? Hydrobiologia 712:61–70.

Bêche, L. A., P. G. Connors, V. H. Resh, and A. M. Merenlender. 2009. Resilience of fishes and invertebrates to prolonged drought in two California streams. Ecography 32:778–788.

Bêche, L. A., E. P. Mcelravy, and V. H. Resh. 2006. Long-term seasonal variation in the biological traits of benthic-macroinvertebrates in two Mediterranean-climate streams in California, U.S.A. Freshwater Biology 51:56–75.

Belmar, O., J. Velasco, C. Gutiérrez-Cánovas, A. Mellado-Díaz, A. Millán, and P. J. Wood. 2013. The influence of natural flow regimes on macroinvertebrate assemblages in a semiarid Mediterranean basin: Influence of natural flow regimes on macroinvertebrates in a Mediterranean basin. Ecohydrology 6:363–379.

Bogan, M. T., K. S. Boersma, and D. A. Lytle. 2013. Flow intermittency alters longitudinal patterns of invertebrate diversity and assemblage composition in an arid-land stream network: *Intermittent flow alters longitudinal patterns*. Freshwater Biology 58:1016–1028.

Bogan, M. T., and S. M. Carlson. 2018. Diversity and phenology of Stoneflies (Plecoptera) from intermittent and perennial streams in Pinnacles National Park, California, u.s.a.:11.

Bogan, M. T., E. T. Chester, T. Datry, A. L. Murphy, B. J. Robson, A. Ruhi, R. Stubbington, and J. E. Whitney. 2017a. Resistance, Resilience, and Community Recovery in Intermittent Rivers and Ephemeral Streams. Pages 349–376 Intermittent Rivers and Ephemeral Streams. Elsevier.

Bogan, M. T., J. L. Hwan, K. Cervantes-Yoshida, J. Ponce, and S. M. Carlson. 2017b. Aquatic invertebrate communities exhibit both resistance and resilience to seasonal drying in an intermittent coastal stream. Hydrobiologia 799:123–133.

Bonada, N., and V. H. Resh. 2013. Mediterranean-climate streams and rivers: geographically separated but ecologically comparable freshwater systems. Hydrobiologia 719:1–29.

Bonada, N., M. Rieradevall, and N. Prat. 2007. Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. Hydrobiologia 589:91–106.

Bonada, N., M. Rieradevall, N. Prat, and V. H. Resh. 2006. Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California. Journal of the North American Benthological Society 25:32–43.

Burnham, K. P., D. R. Anderson, and K. P. Huyvaert. 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65:23–35.

Cid, N., N. Bonada, S. Carlson, T. Grantham, A. Gasith, and V. Resh. 2017. High Variability Is a Defining Component of Mediterranean-Climate Rivers and Their Biota. Water 9:52.

Datry, T., S. T. Larned, K. M. Fritz, M. T. Bogan, P. J. Wood, E. I. Meyer, and A. N. Santos. 2014. Broad-scale patterns of invertebrate richness and community composition in temporary rivers: effects of flow intermittence. Ecography 37:94–104.

Dewson, Z. S., A. B. W. James, and R. G. Death. 2007. A review of the consequences of decreased flow for instream habitat and macroinvertebrates. Journal of the North American Benthological Society 26:401–415.

Feminella, J. W. 1996. Comparison of Benthic Macroinvertebrate Assemblages in Small Streams along a Gradient of Flow Permanence. Journal of the North American Benthological Society 15:651–669.

Filipe, A. F., J. E. Lawrence, and N. Bonada. 2012. Vulnerability of stream biota to climate change in mediterranean climate regions: a synthesis of ecological responses and conservation challenges. Hydrobiologia.

García-Roger, E. M., M. del Mar Sánchez-Montoya, R. Gómez, M. L. Suárez, M. R. Vidal-Abarca, J. Latron, M. Rieradevall, and N. Prat. 2011. Do seasonal changes in habitat features influence aquatic macroinvertebrate assemblages in perennial versus temporary Mediterranean streams? Aquatic Sciences 73:567–579.

Gasith, A., and V. H. Resh. 1999. Streams in Mediterranean Climate Regions: Abiotic Influences and Biotic Responses to Predictable Seasonal Events. Annual Review of Ecology and Systematics 30:51–81.

Geology - Pinnacles National Park (U.S. National Park Service). (n.d.). https://www.nps.gov/pinn/learn/nature/geology.htm.

Giam, X., W. Chen, T. A. Schriever, R. Van Driesche, R. Muneepeerakul, D. A. Lytle, and J. D. Olden. 2017. Hydrology drives seasonal variation in dryland stream macroinvertebrate communities. Aquatic Sciences 79:705–717.

Herbst, D. B., S. D. Cooper, R. B. Medhurst, S. W. Wiseman, and C. T. Hunsaker. 2018. A comparison of the taxonomic and trait structure of macroinvertebrate communities between the riffles and pools of montane headwater streams. Hydrobiologia 820:115–133.

Herbst, D. B., S. D. Cooper, R. B. Medhurst, S. W. Wiseman, and C. T. Hunsaker. 2019. Drought ecohydrology alters the structure and function of benthic invertebrate communities in mountain streams. Freshwater Biology 64:886–902.

Karaouzas, I., C. Theodoropoulos, A. Vourka, K. Gritzalis, and N. Th. Skoulikidis. 2019. Stream invertebrate communities are primarily shaped by hydrological factors and ultimately fine-tuned by local habitat conditions. Science of The Total Environment 665:290–299.

Larned, S. T., T. Datry, D. B. Arscott, and K. Tockner. 2010. Emerging concepts in temporary-river ecology. Freshwater Biology 55:717–738.

Lawrence, J. E., K. B. Lunde, R. D. Mazor, L. A. Bêche, E. P. McElravy, and V. H. Resh. 2010. Long-term macroinvertebrate responses to climate change: implications for biological

       assessment in mediterranean-climate streams. Journal of the North American
       Benthological Society 29:1424–1440.

McCune, B. P., and J. Grace. 2002. Analysis of Ecological Communities. Page Journal of
       Experimental Marine Biology and Ecology – J EXP MAR BIOL ECOL.

O'Hara, R. B., and D. J. Kotze. 2010. Do not log-transform count data. Methods in Ecology
       and Evolution 1:118–122.

Pace, G., N. Bonada, and N. Prat. 2013. Long-term effects of climatic-hydrological drivers on
       macroinvertebrate richness and composition in two Mediterranean streams. Freshwater
       Biology 58:1313–1328.

Palmer, M., and A. Ruhi. 2019. Linkages between flow regime, biota, and ecosystem
       processes: Implications for river restoration. Science 365:eaaw2087.

Poff, N. L., J. D. Olden, N. K. M. Vieira, D. S. Finn, M. P. Simmons, and B. C. Kondratieff.
       2006. Functional trait niches of North American lotic insects: traits-based ecological
       applications in light of phylogenetic relationships. Journal of the North American
       Benthological Society 25:730–755.

Prudhomme, C., I. Giuntoli, E. L. Robinson, D. B. Clark, N. W. Arnell, R. Dankers, B. M.
       Fekete, W. Franssen, D. Gerten, S. N. Gosling, S. Hagemann, D. M. Hannah, H. Kim,
       Y. Masaki, Y. Satoh,
T. Stacke, Y. Wada, and D. Wisser. 2014. Hydrological droughts in the 21st century,
       hotspots and uncertainties from a global multimodel ensemble experiment.
       Proceedings of the National Academy of Sciences 111:3262–3267.

Pyne, M. I., and N. L. Poff. 2017. Vulnerability of stream community composition and
       function to projected thermal warming and hydrologic change across ecoregions in
       the western United States. Global Change Biology 23:77–93.

Resh, V. H., L. A. Bêche, J. E. Lawrence, R. D. Mazor, E. P. McElravy, A. P. O'Dowd, D.
       Rudnick, and S. M. Carlson. 2013. Long-term population and community patterns of
       benthic macroinvertebrates and fishes in Northern California Mediterranean-climate
       streams.   Hydrobiologia 719:93–118.

R Core Team (2021). R: A language and environment for statistical computing. R
       Foundation for Statistical Computing, Vienna, Austria. https://www.R-
       project.org/

Sánchez-Montoya, M. M., D. von Schiller, G. G. Barberá, A. M. Díaz, M. I. Arce, R. del
       Campo, and K. Tockner. 2018. Understanding the effects of predictability, duration,
       and spatial pattern of drying on benthic invertebrate assemblages in two contrasting
       intermittent streams. PLOS ONE 13:e0193933.

Saffarinia, P., K. E. Anderson, and D. B. Herbst. 2022. Effects of experimental multi-season

drought on abundance, richness, and beta diversity patterns in perennially flowing stream insect communities. Hydrobiologia 849:879–897.

U.S. Drought Monitor (USDM). (n.d.). https://www.drought.gov/data-maps-tools/us-drought-monitor.