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Spatial and Temporal Heterogeneity of Hyporheic Invertebrates in an Ozark Stream

David Fleshman
Missouri State University, Fleshman33@live.missouristate.edu

SPATIAL AND TEMPORAL HETEROGENEITY OF HYPORHEIC INVERTEBRATES IN AN OZARK STREAM

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David Fleshman

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ABSTRACT

The hyporheic zone (HZ), an ecotone between surface water and groundwater in streams, provides extensive but underappreciated habitat for invertebrates in alluvial systems like the gravel-bed streams common in the Ozark highlands. Relative to its importance as a habitat, little is known about spatial distribution and response to disturbance by invertebrates in the HZ. In riffle-pool systems, surface water typically enters the HZ at the head of riffles (downwelling) and returns to the surface at the tail of riffles (upwelling). Previous research has found differences in invertebrate communities and environmental variables between upwelling and downwelling zones, but results have been inconsistent. I sampled the hyporheic zone at three depths in upwelling and downwelling zones for four months (Oct-Jan) in high flow and three months (June-Aug) in low flow seasons, as well as opportunistically in response to floods. Abundance and richness were significantly greater in downwelling zones than upwelling zones, and greater at shallower depths. Dissolved oxygen and particulate organic matter, two important resources for invertebrates, showed no spatial patterns and no correlation with abundance and richness. Invertebrate communities showed no differences in abundance or richness between pre and post flood samples, suggesting that the HZ was not used as a refuge in response to disturbance. While invertebrates were not observed using the HZ as a refuge, sampling difficulties likely impacted these results and further research is needed on this topic. My research highlights the importance of the HZ as habitat for a high diversity and biomass of invertebrates and emphasizes the need for further research on this understudied part of stream ecosystems.

KEYWORDS: stream ecosystems, invertebrates, connectivity, hyporheic, flow, diversity, resilience, streambed sediments

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Approved:

Debra Finn, Ph.D., Thesis Committee Chair

Chris Barnhart, Ph.D., Committee Member

Sean Maher, Ph.D., Committee Member

Julie Masterson, Ph.D., Dean of the Graduate College

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OVERVIEW

The hyporheic zone, an ecotone between surface water and groundwater habitats in streams, is relatively understudied but is a critical location for multiple physical and ecological processes, including temperature regulation and nutrient cycling. It is also an important habitat for invertebrates, both surface dwelling, and lesser-known groundwater inhabiting species. Invertebrates can occupy the hyporheic zone temporarily in response to disturbances such as drought, as well as obligately for their full life cycles or during specific life stages.

Spatial distribution of invertebrates in the hyporheic zone and the environmental factors that influence them are poorly understood. At the riffle scale, surface water tends to enter the hyporheic zone in areas of downwelling at riffle heads, while hyporheic water returns to the surface at riffle tails in areas of upwelling. Distribution of hyporheic invertebrates between and within zones of upwelling and downwelling have not been extensively studied. Differences in diversity and abundance have been observed between zones, but patterns in flow and chemical/biological factors influencing invertebrate communities can be highly variable, both spatially and seasonally, resulting in inconsistent findings on how communities are distributed. While the hyporheic zone is considered a refuge from disturbance, few studies have focused on hyporheic invertebrate communities' response to flooding, one of the most prevalent forms of disturbance in streams. Due to difficulties associated with sampling during and immediately following floods, it has been difficult to assess immediate behavioral responses by HZ invertebrates to floods.

In my thesis, I addressed several unresolved questions in hyporheic ecology, using a relatively unimpacted gravel-bed stream with extensive hyporheic habitat in Southwest Missouri.

Chapter one focused on comparing hyporheic invertebrate communities between heads and tails of riffles as well as environmental variables that may influence their distribution in space and time. It has been formatted in preparation to submit to the peer-reviewed journal *Freshwater Biology*. I sampled the hyporheic zone at heads and tails of riffles asking if invertebrate communities vary in diversity and abundance between upwelling and downwelling zones. I also sampled invertebrates during high flow and low flow seasons to determine how hyporheic communities change through time. For my second chapter, I sampled hyporheic insect communities pre and post flood, in an attempt to monitor if surface water invertebrates respond to disturbance with vertical shifts into the hyporheic zone. Perhaps unsurprisingly, I was unable to attain a large enough sample size following flood disturbance to come to solid conclusions in Chapter 2, but it is included here to provide a record and data that might be useful for future studies.

SEASONAL VARIATION OF INVERTEBRATE COMMUNITIES BETWEEN UPWELLING AND DOWNWELLING ZONES OF AN OZARK STREAM

Introduction

Lotic ecosystems have four dimensions of connectivity: longitudinal (upstream/downstream), lateral (channel/floodplain), vertical (surface/subsurface), and temporal (through time) (Ward, 1989). Each dimension is important to stream ecosystem function, but research efforts have not focused on them equally. With human impacts such as dams having clear impacts on upstream-downstream connectivity, longitudinal connectivity has historically received more attention (e.g. Vannote et al., 1980; Newbold et al., 1982; Ward & Stanford, 1983). Interaction between the main channel and floodplain/riparian habitats have also been well studied (e.g. Junk et al., 1989; Nakano et al., 1999). Of the three spatial dimensions of connectivity, vertical has historically been the least studied in stream ecology, even though surface/subsurface exchange is critical to stream ecosystem function (Stanford & Ward, 1993).

Vertical connectivity typically refers to exchange between surface waters and the hyporheic zone (HZ), which is commonly defined as an ecotone between groundwater and surface water habitats (Boulton et al., 1998; Williams et al., 2010). High surface area of sediment particles and high microbial activity make the hyporheic zone a crucial component of streams for nutrient and organic matter transformations, as well as an important habitat for invertebrates (Boano et al., 2014). Invertebrate communities within this ecotone include both benthic (henceforth: “epigean”) and groundwater (henceforth: “hypogean”) taxa, in both macroinvertebrate (individuals >1mm) and meiofauna (0.4-1mm) size categories. Epigean invertebrates use the hyporheic zone as a refuge from predation, competition, and adverse

surface conditions, as well as a nursery for early instar insects (Dole-Olivier, 2011; Stubbington et al., 2011), some of which can spend their entire juvenile life stage within the HZ (Stanford and Gaufin 1974). Conversely, hypogean invertebrates live exclusively in groundwater/hyporheic habitats for the duration of their life cycles.

In riffle-pool streams with unobstructed vertical connectivity, along a single riffle, surface water tends to enter the hyporheic zone at riffle heads (downwelling) and return to the surface at riffle tails (upwelling) (Franken et al., 2001). Physical and chemical characteristics typically vary between upwelling and downwelling zones and might drive differences in invertebrate communities (Franken et al., 2001; Stubbington et al., 2011). Variation in two key resources for invertebrates, dissolved oxygen (DO) and particulate organic matter (POM), are potential factors driving these differences (Peralta-Maraver et al., 2018; Stubbington et al., 2011). Increased levels of DO and POM from surface water entering downwelling zones may lead to higher abundance and diversity of hyporheic invertebrates, while conditions of lower oxygen and POM may lead to fewer invertebrates in upwelling zones. In addition to DO and POM, physical factors may impact how hyporheic invertebrate distribution can vary between upwelling and downwelling zones. Clogging of interstitial spaces by fine sediments (colmation) may homogenize communities between upwelling and downwelling zones by preventing the migration of epigean taxa into the HZ (Mathers et al., 2017). The opposite effect may also occur, with differences between zones driven by higher occurrence of colmation in downwelling zones compared to upwelling zones.

Patterns in abundance and diversity within the hyporheic zone are highly variable and results have been inconsistent across studies. There are conflicting reports regarding invertebrate distribution in upwelling and downwelling zones, with some studies finding greater

diversity/abundance in downwelling zones (Hendricks, 1993), and others in upwelling zones (Olsen & Townsend, 2003), with still others reporting no difference between zones (Mathers et al., 2017). Lack of pattern in invertebrate distribution may be influenced by DO and POM, as previous studies have found conflicting results regarding the distribution of these resources and their influence on hyporheic invertebrates (Franken et al., 2001; Hutchins et al., 2020; Strayer et al., 1997; Williams & Hynes, 1974). Lack of spatial pattern might occur when resources such as DO and POM are at levels that are not limiting to invertebrates and are evenly distributed across zones of upwelling and downwelling. Conversely, erratic distribution of DO and POM within upwelling and downwelling zones may also lead to lack of patterns in invertebrate communities. Along with conflicting results among studies, study locations have also been limited. Studies need to be conducted in more ecoregions, and in streams with wide ranges of human impact, from heavily impacted with high levels of fine sediments to minimally impacted systems with coarse substrates and high surface/subsurface exchange.

Along with spatial variation, seasonal variation in hyporheic invertebrate communities is another little-understood topic in hyporheic ecology. Hyporheic communities and the environmental factors influencing them change seasonally, but little research has focused on changes in hyporheic communities through time, with focus on the influence of stream flow. Patterns in subsurface flow can change seasonally, with substantial changes in upwelling/downwelling occurring at the riffle scale driven by variation in overall discharge (Wu et al., 2018). Extremes in surface flow may be major drivers of change in hyporheic invertebrate communities, with both drought and flood driving epigean fauna to migrate vertically into the hyporheic zone (Dole-Olivier, 2011). Extreme low flows resulting in loss of surface flow can force the migration of epigean taxa into the hyporheic zone (Vadher et al., 2017), while

disturbance from high flow has also been demonstrated to cause downward migration of invertebrates seeking refuge from adverse surface conditions (Holomuzki & Biggs, 2000). These behavioral responses to changes in flow likely cause broad-scale changes in hyporheic communities, but prior studies have not incorporated natural seasonality into studies of hyporheic community response.

In this study, I sampled the hyporheic zone of a minimally impacted gravel-bed stream in the Ozark Highlands ecoregion during high-flow and low-flow periods in upwelling and downwelling zones to characterize spatial and temporal patterns of invertebrate distribution. I predicted that overall abundance and diversity of invertebrates in the HZ would be greater in downwelling than upwelling zones, greater during a flood-prone high-flow period than a low-flow period, and greater at shallower depths below the streambed. Furthermore, I predicted that epigean fauna, influenced by DO concentration and POM content, would be the major drivers of these patterns.

Methods

Study site. I conducted this study at Bull Creek, a second order gravel-bed stream in the Ozark highlands ecoregion of southwest Missouri (Figure 1). With a watershed comprised largely of relatively unimpacted National Forest land and karst topography, Bull Creek is designated as an outstanding state resource water (Missouri Department of Natural Resources, 10 CSR, 20-7, 2017). Bull Creek's flow regime is described as groundwater flashy (Leasure et al., 2016), characterized by a spring-fed baseflow and common flash floods, especially during high flow winter/spring seasons. At the study area, reaches alternate between exposed bedrock and beds of coarse gravel and cobble, often exceeding 2m depth (Dorff & Finn, 2020). I chose a

~300m study reach containing three consecutive riffles with deep hyporheic zones. Hyporheic invertebrates are abundant and diverse at this site, making it an ideal location for this study (Dorff, 2019).

Study design. I sampled the hyporheic zone monthly for four months (Oct. 2019-Jan 2020) during a high flow period, and three months (Jun. 2020-Aug. 2020) during a low flow period, using a Bou-Rouch pumping method (Bou & Rouch 1967). I installed PVC wells at depths of 30, 50, and 70 cm (Figure 2) in both upwelling and downwelling zones (Figure 3), replicated across three riffles (Figure 1). Wells were constructed from 1-inch schedule 40 PVC pipe, each with one end plugged and a 15-cm length of a screen comprised of 72 5-mm diameter holes. Sampling depth for each well was measured as the distance from the stream bed to the middle of the screen (Figure 2). I chose these depths because previous studies using a reduced difference in depth between wells (10, 20, 30cm) found no significant differences in invertebrate communities (Stubbington et al., 2011). I also intended to sample deep enough to characterize the hypogean community. This study design resulted in a total of 18 wells pumped to collect invertebrates on each sample date. Due to high flow, only half of the wells were pumped during the late October sampling date. One well was lost, and I pumped the remaining half in early November, resulting in four sample months rather than three for the high-flow period, and 107 total samples collected throughout the study rather than 108 (18 per sampling day x6).

Data collection. To monitor flow for the duration of the study, a Solinst Levellogger was installed at approximately 40 cm depth in the HZ at the beginning of the study period. We measured discharge using a Hach FH950 handheld flowmeter with wading rod at a range of flow stages and made a rating curve to estimate discharge in cubic meters per second. Discharge

measurements were recorded in a bedrock lined reach just upstream of the study reach to avoid underestimation by not accounting for subsurface flow.

On each invertebrate sampling date I measured hydraulic head (difference in water depth between inside and outside of well) in each well to characterize the degree of upwelling or downwelling (Figure 2)(Greenberg et al., 2002). I made these measurements to test the accuracy of predetermined upwelling and downwelling zones at riffle tails and heads, and to assess variability at the finer scale. After measuring hydraulic head, I measured dissolved oxygen at depth in each well with a YSI ProSOLO optical dissolved oxygen meter and reported results as DO percent saturation to facilitate comparisons across dates and locations that varied in temperature.

To collect invertebrates, I pumped 8 liters of water from each well keeping pumping rate consistent between samples to avoid bias in estimates of diversity and abundance (Hunt and Stanley 2000). I poured samples through a 125-µm sieve and preserved them immediately in 95% ethanol. 125-µm is a small enough mesh size to retain all macrofauna and the majority of meiofauna (Hummon, 1981).

In the laboratory, I processed all samples using a Bogorov counting chamber and dissecting microscope at 15-20x. Before processing each sample, I split the entire sample into quarters using a WildCo Folsom plankton splitter. I sorted/counted all macrofauna and counted all meiofauna in the first quarter. I used the first quarter as a subsample for estimating total abundance of copepods (the most abundant taxon), then sorted all individuals of non-copepod taxa in the remaining three quarters of the sample. If there were fewer than 50 copepods in the first quarter, I continued counting them in the remaining three quarters. No copepods were removed from samples. I identified insects to the family level (earliest instars to order) and other

invertebrates to the lowest practical level. These taxonomic resolutions were suitable to quantify the diversity of samples and categorize taxa as either primarily epigean or hypogean. Common hypogean taxa included isopods from the genus *Caecidotea*, amphipods from the family Crangonyctidae, and copepods.

After removing all invertebrates but copepods from 8-L samples, I quantified particulate organic matter (POM) by measuring ash-free dry mass (AFDM) of each sample. Samples were dried at 60ºC for at least 48 hours then ashed for 2 hours at 500ºC in a muffle furnace. To account for the biomass of copepods left in the samples, I used a subset of sorted copepods to estimate mean dry mass of per individual copepod (~0.004mg). I multiplied mean copepod mass by the number of individuals in each sample and subtracted the mass from the total AFDM of the sample to estimate mg of POM/8-L sample (as potential food source for invertebrates).

Analysis. After sorting and counting invertebrates, I calculated insect abundance, taxonomic richness for all invertebrates, and Simpson's diversity index for all invertebrates for each sample. Using program R, I ran three-way ANOVAs to test for differences between sample periods, upwelling/downwelling zones, and depths both for environmental variables (DO and POM) and for invertebrate communities (insect abundance, total taxonomic richness, Simpson's diversity index, copepod abundance, and isopod abundance). I used insect community metrics to represent exclusively epigean communities and used copepod and isopod abundance to represent hypogean taxa, as these two broad taxonomic groups were the most abundant (>85% of all hypogean invertebrates). I also used three-way ANOVAs to test for differences in proportional abundance of epigean vs hypogean taxa among flow periods, upwelling/downwelling zones, and depths. Proportions were arcsin square-root transformed before ANOVAs were conducted.

For comparisons of DO, POM, and invertebrate metrics between upwelling and downwelling zones, I conducted two sets of ANOVAs, the first grouping samples according to pre-determined zones of upwelling and downwelling at the tails and heads of riffles, respectively, and the second grouping samples specifically according to the direct empirical measures of hydraulic head in each well prior to pumping. The purpose of this was to determine if larger-scale areas of upwelling and downwelling (riffle tails and riffle heads) had greater influence on environmental variables and invertebrate communities than finer-scale well-to-well differences. I also used a one-way ANOVA to determine if hydraulic head varied between wells at the heads and tails of riffles (negative HH at riffle heads and positive HH at riffle tails).

I used a Shapiro-Wilkes test for normality before conducting ANOVAs and $\log_{10}$ transformed any non-normal variables before analysis. POM, insect abundance, and Simpson's diversity were not normally distributed, but were normally distributed after $\log_{10}$ transformation. To assess significance, I used a Bonferroni correction that resulted in a conservative alpha value of 0.006. I used Pearson correlations to test for associations between environmental variables (DO and POM) with invertebrate community metrics (abundance, richness, and diversity).

I used the vegan package in R and the metaMDS function with base settings to compare community structure across depths, sample periods, and between upwelling and downwelling zones. For each sample date, I pooled data from the same depth/zone across the three replicate riffles. Before conducting the NMS analysis, I $\log_{10}(n+1)$ transformed the data to decrease the influence of highly abundant taxa and increase the influence of less abundant taxa. I used a stress cutoff of 0.2, and the analysis with the lowest stress value was used after running 20 random starts. I ran NMS with all samples from both flow periods and plotted results on a biplot with 95% confidence ellipses to compare community structure between high- and low-flow periods.

Differences between groups are considered significant if ellipses do not overlap. I also ran NMS separately within each flow period and plotted to compare communities between upwelling and downwelling zones. After each NMS, I used multi response permutation procedure (MRPP) to confirm if groups with non-overlapping confidence ellipses were significantly different. In each flow period separately, as well as combined, I also ran MRPP to test for differences among depths. I used the envfit function to calculate r^2 values between environmental variables (depth, hydraulic head, temp, DO, POM) and invertebrate communities. Any variables with p-values <0.1 were plotted as vectors on biplots, with the length of the vector correlated to the strength of the relationship.

Results

Environmental variables. Mean discharge estimated from rating curves was 2.64 m^3/s during the high-flow sampling period and 0.51 m^3/s during the low-flow sampling period. Short-term flow variation was greater during the high-flow period, with multiple bed moving floods and multiple floods exceeding 20 m^3/s. The highest peak recorded was approximately 94 m^3/s (Figure 4). The final bed moving flood was 63 m^3/s in early June, one week before low-flow sample period collection began. No peaks exceeded 10 m^3/s during the low-flow period.

Mean hydraulic head (HH) during the high-flow period was +26 mm in upwelling zones at riffle tails and -20 mm in downwelling zones at riffle heads (Table 1). During the low-flow period, mean HH was +10 mm in upwelling zones and -0.8 mm in downwelling zones. Among-well variation in HH was common, with negative and positive HH occurring within both zones, but overall HH was significantly negative in riffle heads and positive in riffle tails ($F=23.49$, $P<0.0001$).

Mean DO percent saturation (DO%) was 100.3% in the high-flow period and 96.6% in the low-flow period (F=3.96, *P*=0.50) (Figure 5). Across both flow periods, average DO% was 96.7% in upwelling zones and 100.1% in downwelling zones (F=3.3, *P*=0.072). Interestingly, while DO did not vary significantly between pre-determined zones of upwelling and downwelling, it was significantly higher in individual wells with negative HH measurements compared to wells with negative HH (F=10.9, *P*=0.001)(Table 2). Mean DO% combined across sample periods and between upwelling and downwelling zones was 100.0% at 30cm below the streambed, 98.9% at 50cm, and 96.3% at 70cm (F=1.2, *P*=0.298). There were no interactions among zones, depths, or periods (Table 3).

Mean POM content was 182mg/8-L sample in the high-flow period and 243mg/8-L in the low-flow period (F=3.65, *P*=0.059) (Figure 5). Across both flow periods, mean POM was 197mg/8-L in upwelling zones and 229mg/8-L in downwelling zones (F=0.80, *P*=0.37). POM also did not vary between wells grouped according to positive and negative hydraulic head (F=0.68, *P*=0.41)(Table 2). Mean POM combined across sample periods and between upwelling and downwelling zones was 244mg/8-L at 30cm, 208mg/8-L at 50cm, and 186.4mg/8-L at 70cm (F=0.96, *P*=0.39). There were no interactions among zones, depths, or periods (Table 3).

Invertebrates. Mean insect abundance was 88 individuals/8-L during the high-flow period and 260 individuals/8-L during the low-flow period (F=28.08, *P*<0.0001) (Figure 6). Across both flow periods, mean insect abundance was 90 individuals/8-L in upwelling zones and 258 individuals/8-L in downwelling zones (F=18.7, *P*<0.0001). Abundance did not vary significantly between wells grouped according to positive and negative hydraulic head (F=0.48, *P*=0.49)(Table 2). Mean insect abundance combined across sample periods and between upwelling and downwelling zones was 289 individuals/8-L at 30cm, 148 individuals/8-L at

50cm, and 84 individuals/8-L at 70cm (F=16.28, *P*<0.0001). Tukey's post-hoc test showed that the only significant difference among depths was between 30cm and 70cm (*P*<0.0001). There were no significant interactions among zones, depths, or sample periods for insect abundance (Table 3). There was no correlation between insect abundance and POM (t=1.5, r=0.15, *P*=0.136) and a weak positive correlation between insect abundance and DO (t=2.7, r=0.28, *P*=0.008) (Figure 7).

Mean richness for all invertebrates was 13 taxa/8-L during the high-flow period and 15 taxa/8-L during the low-flow period (F=15.18, *P*=0.0002) (Figure 6). Across both flow periods, mean richness was 12 taxa/8-L in upwelling zones and 16 taxa/8-L in downwelling zones (F=40.87, *P*<0.0001). Richness did not vary significantly between wells grouped according to positive and negative hydraulic head (F=0.48, *P*=0.018)(Table 2). Mean richness combined across sample periods and between upwelling and downwelling zones was 15 taxa/8-L at 30cm, 14 taxa/8-L at 50cm, and 12 taxa/8-L at 70cm (F=14.30, *P*<0.0001). Tukey's post-hoc test showed that the only significant difference among depths was between 30cm and 70cm (*P*=0.0001) There were no significant interactions among zones, depths, or sample periods for richness (Table 3). Richness had weak positive correlations with POM (t=2.4, r=0.23, *P*=0.019) and DO (t=2.0, r=0.21, *P*=0.046) (Figure 7).

Mean Simpson's diversity of all taxa in samples was 2.0 in the high-flow period and 2.5 in the low-flow period (F=9.24, *P*=0.003) (Figure 6). Across both flow periods, mean diversity was 2.1 in upwelling zones and 2.4 in downwelling zones (F=4.43, *P*=0.038). Diversity also did not vary between wells grouped according to positive and negative hydraulic head (F=0.40, *P*=0.53). Mean insect diversity combined across sample periods and between upwelling and downwelling zones was 2.4 at 30cm, 2.4 at 50cm and 2.1 at 70cm (F=2.19, *P*=0.117). There

were no significant interactions among zones, depths, or sample periods for diversity (Table 3). Diversity also had no correlation with POM (t=1.4, r=0.14, *P*=0.158) or DO (t=1.0, r=0.11, *P*=0.30) (Figure 7).

Mean copepod abundance was 388 individuals/8-L during the high-flow period and 659 individuals/8-L during the low-flow period (F=3.56, *P*=0.0004) (Figure 8). Across both flow periods, mean copepod abundance was 391 individuals/8-L in upwelling zones and 656 individuals/8-L in downwelling zones (F=13.05, *P*=0.0005). Abundance did not vary significantly between wells grouped according to positive and negative hydraulic head (F=0.76, *P*=0.385). Mean abundance combined across sample periods and between upwelling and downwelling zones was 573 individuals/8-L at 30cm, 463 individuals/8-L at 50cm, and 535 individuals/8-L at 70cm (F=0.82, *P*=0.444). There were no interactions among zones, depths, or sample periods for copepod abundance (Table 3).

Mean isopod abundance was 29 individuals/8-L during the high-flow period and 68 individuals/8-L during low flow period (F=7.29, *P*=0.008)(Figure 8). Across both flow periods, mean isopod abundance was 28 individuals/8-L in upwelling zones and 68 individuals/8-L in downwelling zones (F=8.21, *P*=0.005). Abundance did not vary significantly between wells grouped according to positive and negative hydraulic head (F=1.53, *P*=0.218). Mean isopod abundance combined across sample periods and between upwelling and downwelling zones was 16 individuals/8-L at 30cm depths, 59 individuals/8-L at 50cm, and 70 individuals/8-L at 70cm (F=5.18, *P*=0.007).

Due to dominance of copepods in nearly all samples, the proportion of epigean individuals only exceeded hypogean individuals in 10 out of 107 total samples. Ratios did not vary between high-flow and low-flow sample periods (F= 2.55, *P*= 0.114) (Figure 9).

Epigean/hypogean ratios did not vary between upwelling and downwelling zones (F= 0.91, *P*=0.343), or between wells with positive and negative hydraulic head measurements. Ratios varied significantly by depth (F=11.60, *P*<0.0001), with Tukey's post-hoc test showing a higher proportion of hypogean fauna in 70cm depths compared to 30cm (*P*<0.0001).

NMS comparing high-flow and low-flow period communities had a stress value of 0.18 in two dimensions, and 95% confidence ellipses for the two flow periods did not overlap on the biplot (Figure 10). MRPP showed significant differences in invertebrate community structure between high-flow and low-flow periods (MRPP, A=0.03, P=0.024). There were no common taxa that were unique to either high flow or low flow period. Some rarer taxa were limited to a single period, including Simuliidae, Ephemeridae, Leptohyphidae, and Dytiscidae that were only collected in low-flow samples. Sialidae was the only taxon unique to high flow samples, and only two individuals were found across all samples.

With the two sample periods separated for further analysis, community structure was different between upwelling and downwelling zones within the high-flow period (MRPP, A=0.09, P=0.0003) and the low-flow period (MRPP, A=0.05, P=0.014). Stress for the high-flow period NMS was 0.14 in two dimensions, while stress for the low-flow period NMS was 0.12. Error ellipses did not overlap between upwelling and downwelling zones for high-flow samples but did overlap between zones for low-flow samples (Figure 11). Several taxa that were common in downwelling zones were rare in upwelling zones in both high flow and low flow periods. Ceratopogonidae were 30x as abundant in downwelling samples compared to upwelling, Caenidae were 22x as abundant, Gomphidae were 13x as abundant, and Heptageniidae were 7x as abundant (Appendix A). Similar to seasonal flow period comparisons, there were no highly abundant taxa that were unique to either upwelling or downwelling zones, but several uncommon

taxa were exclusive to downwelling samples. Ephemeridae, Ephemerellidae, Isonychiidae, and Sialidae were all only found in downwelling samples. Dytiscidae was the only taxon that was found exclusively in upwelling zones across both flow periods, and only one individual was collected. Overall higher abundance and diversity drove the differences in communities collected in downwelling zones compared to upwelling zones.

There were no differences in community structure among depths in either high-flow (MRPP, A=0.04, P=0.08) or low-flow (MRPP, A=0.02, P=0.165) periods separately (Figure 11), but running an analysis with both sample periods combined, there were significant differences among depths (MRPP, A=0.03, P=0.024) (Figure 10). During the high-flow period dissolved oxygen percent saturation (r^2=0.78, *P*=0.003) and temperature (r^2=0.72, *P*=0.004) both had significant correlations with community composition (Table 4). Temperature (r^2=0.72, *P*=0.0001) and dissolved oxygen percent (r^2=0.004 *P*=0.004) also showed significant correlations during the low flow period. Depth, hydraulic head, and POM did not have significant correlations with community composition (Table 4).

Discussion

In support of my hypotheses, hyporheic invertebrate communities showed strong patterns between upwelling and downwelling zones, among depths, and between periods of high flow and low flow in an Ozark gravel-bed stream. Insect abundance was approximately triple in downwelling zones compared to upwelling zones during both high-flow and low-flow periods. Total taxonomic richness was also higher in downwelling zones than upwelling zones regardless of sampling period. Both abundance and richness were also significantly higher in samples collected at 30cm below the streambed compared to 70cm samples. Contrary to my hypothesis,

insect abundance in the hyporheic zone was nearly triple in low flow period samples compared to high flow period samples.

Patterns in hydraulic head measurement in Bull Creek were highly variable within and among zones of primarily upwelling or downwelling, showing high fine-scale patchiness of surface/subsurface exchange. While broad-scale patterns in upwelling and downwelling can be relatively predictable in riffle-pool sequences, as they were in Bull Creek, a variety of physical factors can affect subsurface flow paths at finer scales. Subsurface flow paths are affected by sediment size, bed roughness, depth to bedrock, springs/aquifers, bar locations, and obstacles such as large woody debris (Boulton et al., 1998). While we attempted to select relatively homogenous locations to place wells within either heads or tails of riffles, we had no way of detecting the presence of buried large wood or boulders that could have an impact on subsurface flow. Complexity of subsurface flow paths is likely the biggest factor leading to the highly variable patterns of both environmental variables and invertebrates in the hyporheic zone of Bull Creek.

Insect abundance, richness, copepod abundance, and isopod abundance were all significantly higher in downwelling zones than upwelling zones. Community composition also varied significantly between zones, with varying relative abundances of taxa and multiple taxa that were only found in downwelling zones. These results align with my hypotheses, as well with the results of other hyporheic studies that have found consistent differences between upwelling and downwelling zones (Franken et al., 2001; Hendricks, 1993). While multiple studies have reported differences in communities between zones, lack of difference in invertebrate abundance has also been observed (Bowker et al., 2006) In contrast to my hypothesis, Simpson's diversity showed no significant patterns. Dominance of few taxa led to similar diversity values among all

samples, causing diversity to not vary among upwelling/downwelling zones, depths, or flow periods. This result differs considerably from other studies, with some observing higher diversity in downwelling zones (Fowler & Scarsbrook, 2002) and others observing higher diversity in upwelling zones (Olsen & Townsend, 2003). While abundance of epigean taxa and the most common hypogean taxa (copepods and isopods) each varied significantly between upwelling and downwelling zones, the ratio of epigean to hypogean taxa showed no differences. Hypogean taxa were nearly always dominant over epigean taxa in terms of relative abundance. This was primarily due to high abundance of copepods in nearly all samples, and high abundance of hypogean isopods in many samples. Lack of interaction between upwelling/downwelling and flow period or depth implied that neither of these variables influenced invertebrate distribution between zones.

In support of my hypothesis, insect abundance and richness were both significantly higher in 30cm samples compared to 70cm samples. Patterns in diversity and abundance have been consistent across studies, with both measures decreasing with increased depth below the stream bed (Franken et al., 2001; Peralta-Maraver et al., 2018; Storey & Williams, 2004). Ratio of epigean to hypogean taxa also varied significantly by depth, with 70cm samples having a greater proportion of hypogean taxa compared to 30cm samples. Diversity, copepod abundance and isopod abundance did not vary among depths.

In support of my hypothesis, there were also significant patterns in invertebrate communities between the high-flow and low-flow sample periods, but the pattern of variation was opposite of what I predicted. Both abundance and richness were higher during the low-flow sample period than the high-flow period, and community structure was significantly different between periods. Diversity showed no difference between periods. Lack of interaction between

flow period and upwelling/downwelling and depths implied that spatial distribution of invertebrates was not influenced by time period. Spatial patterns among depths and zones remained constant across both flow periods.

With high-flow samples being collected in winter months and low-flow samples collected in summer months, flow was not the only factor that varied between sample periods. Temperature and total productivity within the stream may have had greater influence on invertebrate communities than the level of flow between the two sample periods. High flows have been demonstrated to cause benthic invertebrates to migrate downward into the hyporheic zone (Dorff, 2019; Palmer et al. 1992), but generally higher invertebrate abundance during warmer months may have outweighed this potential increase in hyporheic zone abundance from downward migrating benthic taxa. Recruitment patterns of more dominant taxa can also have substantial effects on total abundance of taxa. Gibson & Hall (1988) found that the timing of recruitment for dominant insect taxa was directly connected to seasonal patterns of invertebrate abundance in the hyporheic zone.

Dissolved oxygen showed high well-to-well variation and no broad-scale patterns between upwelling and downwelling zones, depths, or flow periods. DO was however, significantly higher in wells with negative hydraulic heads compared to wells with positive hydraulic heads. DO tends to have high spatial variation, influenced by a variety of factors including sediment size composition, flow velocity, organic matter, and microorganism type and abundance (Malard and Hervant, 1999). Most wells tended to have high DO percent saturation regardless of zone. Lack of difference in DO between pre-determined upwelling vs downwelling zones may have been due to the observed high variation in hydraulic exchange, even within zones of primarily upwelling or downwelling (individual wells showing downwelling in

predetermined zones of upwelling and vice versa). Sediment composition could be another cause for lack of difference between zones. Bull Creek's streambed is dominated by course gravels (Dorff & Finn, 2020), which likely leads to a high hydraulic conductivity that can homogenize water chemistry thanks to short residence times of subsurface flow.

This may be why results differed from studies in other locations that have demonstrated significantly higher DO in downwelling zones than upwelling zones (e.g. Dole-Olivier & Marmornier, 1992; Franken et al., 2001). Although DO did not vary between pre-determined upwelling and downwelling zones, it showed patterns on a finer scale well-to-well basis (wells with positive vs negative hydraulic head measurements), demonstrating sensitivity to smaller scale flow pathways. These patterns imply that water chemistry in general may be influenced more by fine-scale patterns of upwelling and downwelling rather than by larger zones at heads and tails of riffles.

Unexpectedly, dissolved oxygen showed only weak correlations with insect abundance and richness, and no correlation with diversity. Conversely, community structure was significantly correlated with DO throughout the whole study, as well as separately during each flow period. This was likely the result of DO concentration differentially affecting specific taxa but not entire communities. Correlation between DO and hyporheic invertebrate communities has been highly inconsistent among studies. Malard & Hervant (1999) found several studies that showed correlation between DO concentration and invertebrate communities, and several studies that found no correlation. Correlations have also been found to vary seasonally, only occurring at certain times of the year (Storey & Williams, 2004). One reason for lack of correlation between whole invertebrate communities and DO at Bull Creek could be the consistently high DO levels, regardless of upwelling/downwelling, depth, or season. While DO had high variation, it never

reached levels low enough to be considered anoxic/hypoxic or detrimental to most taxa. DO concentration less than 2mg/L is considered hypoxic (Coffin et al., 2017), and levels were never close to this concentration in Bull Creek wells. Olsen and Townsend (2003) also recorded consistently high DO levels (average >9mg/L) and found no correlation with communities, while Franken et al. (2001) had high discrepancies in DO among zones (average of 11% saturation in upwelling and 53% saturation in downwelling) and found strong correlation with invertebrate communities. DO likely only influences hyporheic invertebrate communities if it becomes low enough to negatively affect invertebrates. Environmental factors in general may only influence hyporheic invertebrates when their values reach extremes (Richards & Bacon, 1994; Strayer et al., 1997). Lack of significant difference in DO between upwelling and downwelling zones in Bull Creek may have also led to lack of difference in ratios of epigean/hypogean taxa between zones. Lower DO in upwelling zones can make habitat less suitable for epigean taxa in comparison to downwelling zones, leading them to be dominated by hypogean taxa that are more adapted to low oxygen environments (Dole-Olivier & Marmonier, 1992).

Similar to dissolved oxygen, levels of particulate organic matter in samples were highly variable and showed no patterns between upwelling or downwelling zones, depths, or flow periods. These results fall in line with previous studies, as POM is commonly patchily distributed and often lacks spatial patterns within the hyporheic zone (Maridet et al., 1997). Contrary to my hypothesis, POM lacked significant correlations with abundance, richness, diversity, and community structure. Correlation between POM and invertebrate communities has been inconsistent among studies, with some finding correlation between the two (Strayer et al., 1997; Franken et al., 2001; Olsen & Townsend, 2003), but lack of correlation has also been observed (Storey & Williams, 2004). One reason for lack of correlation could be the presence of other

food sources for invertebrates. Brunke and Fischer (1999) observed a positive correlation between invertebrate density and richness, and bacterial density and production. Biofilms on sediment particles within the hyporheic zone are likely an important food source to hyporheic invertebrates (Barlocher & Murdoch, 1989) but were not quantified in this study. Failing to account for all food sources could potentially lead to a lack of significant results when testing for correlation with invertebrate communities.

Environmental factors influencing hyporheic invertebrate communities vary substantially from stream to stream, and there is still much room for new studies on this topic. Sediment characteristics are some of the biggest potential factors influencing communities that were not investigated in this study. This study, as well as the majority of previous studies of hyporheic invertebrates (e.g. Stanford & Gaufin, 1974; Franken et al., 2001; Fowler and Scarsbrook, 2002; Storey & Williams, 2004; Bowker et al., 2006; Dorff & Finn, 2020) have emphasized streams with coarse substrate and extensive interstitial habitat. While these streams generally support high hyporheic diversity, they are not representative of all streams. Studies focused on streams with more anthropogenic impacts such as eutrophication and fine sediment input (e.g. Descloux et al., 2013; Pacioglu & Moldovan, 2015) are severely lacking in comparison to those on more unimpacted systems. More hyporheic studies must be conducted in varying ecoregions, including streams with wide ranges of anthropogenic impacts as well as sediment composition. This was one of very few studies of hyporheic invertebrates in the Ozarks (Hunt & Stanley, 2003; Distefano et al., 2009, Dorff & Finn, 2020), and the first to compare communities between upwelling and downwelling zones in this region. This study demonstrates the value of the hyporheic zone as a habitat for a wide range of invertebrate taxa in the Ozarks and shows the

importance of surface/subsurface exchange in influencing spatial distribution of invertebrates in a gravel-bed stream.

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Table 1. Mean estimated discharge from water level data loggers, hydraulic head, temperature, dissolved oxygen, and POM (ash-free dry-mass of samples) in upwelling and downwelling zones during high-flow (Oct. 2019-Jan. 2020) and low-flow (Jun. 2020-Aug. 2020) periods. ± One standard error.

		Discharge (m^3/s)	HH (mm)	Temp. (ºC)	DO (%)	DO (mg/L)	POM (mg/L)
High-Flow	Upwell	2.46 ± 0.63	26.3 ± 8.6	8.9 ± 0.25	98.4 ± 1.7	11.2 ± 0.2	17.1 ± 3.5
	Downwell		-20.1 ± 4.7	8.8 ± 0.06	102.1 ± 1.9	11.5 ± 0.1	20.5 ± 3
Low-Flow	Upwell	0.51 ± 0.04	10.9 ± 3.8	23.2 ± 0.10	95 ± 1.3	7.9 ± 0.2	28.5 ± 4.9
	Downwell		-0.8 ± 3.7	23.2 ± 0.61	98.1 ± 2.1	8.1 ± 0.2	32.4 ± 5.5

Table 2. ANOVA results for dissolved oxygen, POM, insect abundance, taxa richness, and Simpson's diversity for pre-determined zones of upwelling and downwelling (heads and tails of riffles) and zones of upwelling and downwelling determined by hydraulic head measurement before each sample was collected. Adjusted α=0.007 and significant *P* values bolded.

	DO%	POM	Insect abundance	Taxa richness	diversity	Copepod #	Isopod #
Pre-determined (df=1)	F=3.3 *P*=0.072	F=0.68 *P*=0.412	F=22.94 ***P*<0.0001**	F=40.87 ***P*<0.0001**	F=0.029 *P*=0.866	F=13.05 ***P*=0.0005**	F=8.21 ***P*=0.005**
Actual HH (df=1)	F=10.9 ***P*=0.001**	F= 0.68 *P*= 0.41	F= 0.48 *P*= 0.487	F= 5.78 *P*= 0.018	F=0.40 *P*= 0.530	F= 0.76 *P*= 0.385	F= 1.53 *P*= 0.218

Table 3. Three-way ANOVA results for DO, POM, and invertebrate metrics among upwelling/downwelling zones, depths, and flow periods. Significant *P* values bolded. Adjusted α=0.006.

	DO%	POM	Insect Abund.	Richness	Diversity	Copepod	Isopod	EP/HYP
Up/Down (df=1)	F=3.3 *P*=0.072	F=0.80 *P*=0.372	F=18.70 ***P*<0.0001**	F=40.87 ***P*<0.0001**	F=4.43 *P*=0.038	F=13.05 ***P*=0.0005**	F=8.21 ***P*=0.005**	F= 0.80 *P*=0.374
Depth (df=2)	F=1.2 *P*=0.298	F=0.96 *P*=0.389	F=16.28 ***P*<0.0001**	F=14.30 ***P*<0.0001**	F=2.19 *P*=0.117	F=0.82 *P*=0.444	F=5.18 *P*=0.007	F=12.71 ***P*<0.0001**
Period (df=1)	F=3.96 *P*=0.50	F=3.65 *P*=0.059	F=28.08 ***P*<0.0001**	F=15.18 ***P*=0.0002**	F=9.24 ***P*=0.003**	F=3.56 ***P*=0.0004**	F=7.29 *P*=0.008	F=2.17 *P*=0.144
Up/Down x Depth (df=2)	F=0.46 *P*=0.635	F=0.01 *P*=0.986	F=0.53 *P*=0.591	F=0.42 *P*=0.655	F=2.59 *P*=0.080	F=2.31 *P*=0.105	F=2.57 *P*=0.082	F=3.63 *P*=0.030
Up/Down x Period (df=1)	F=0.02 *P*=0.832	F=0.14 *P*=0.707	F=0.01 *P*=0.920	F=0.40 *P*=0.529	F=0.26 *P*=0.612	F=8.38 *P*=0.005	F=3.97 *P*=0.049	F=0.48 *P*=0.491
Depth x Period (df=2)	F=0.28 *P*=0.754	F=0.75 *P*=0.474	F=0.28 *P*=0.756	F=0.60 *P*=0.550	F=0.45 *P*=0.642	F=0.50 *P*=0.603	F=3.89 *P*=0.024	F=0.84 *P*=0.432
U x D x P (df=2)	F=0.28 *P*=0.759	F=0.26 *P*=0.771	F=0.93 *P*=0.397	F=0.69 *P*=0.50	F=0.69 *P*=0.504	F=0.37 *P*=0.693	F=1.59 *P*=0.209	F=0.98 *P*=0.378

Table 4. Community structure/Environmental variable correlations (envit function in Vegan). Vectors with significance <0.1 plotted on NMS biplots (Figures 10+11). Adjusted α=0.01 and significant *P* values are bolded.

	Depth	HH	Temp	DO	POM
High-Flow	r^2=0.43 *P*=0.087	r^2=0.29 *P*=0.21	r^2=0.72 ***P*=0.004**	r^2=0.78 ***P*=0.002**	r^2=0.04 *P*=0.83
Low-Flow	r^2=0.25 *P*=0.11	r^2=0.12 *P*=0.39	r^2=0.72 ***P*=0.0001**	r^2=0.51 ***P*=0.004**	r^2=0.038 *P*=0.743
Both Periods	r^2=0.26 *P*=0.017	r^2=0.12 *P*=0.18	r^2=0.63 ***P*=0.0001**	r^2=0.31 ***P*=0.007**	r^2=0.09 *P*=0.27

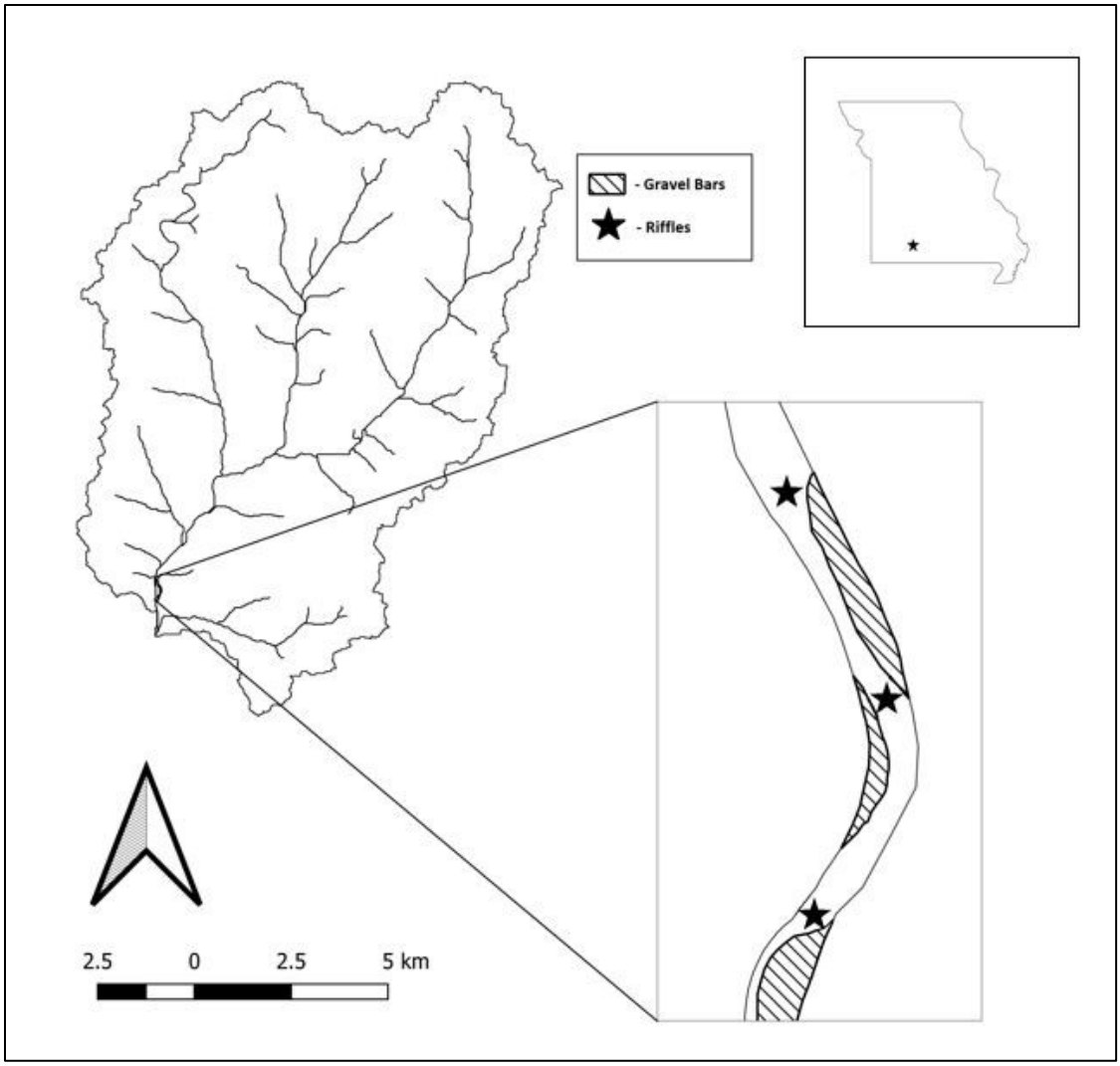

Figure 1. Study reach/riffles and watershed of Bull Creek at the study site in southwest Missouri within the White River basin.

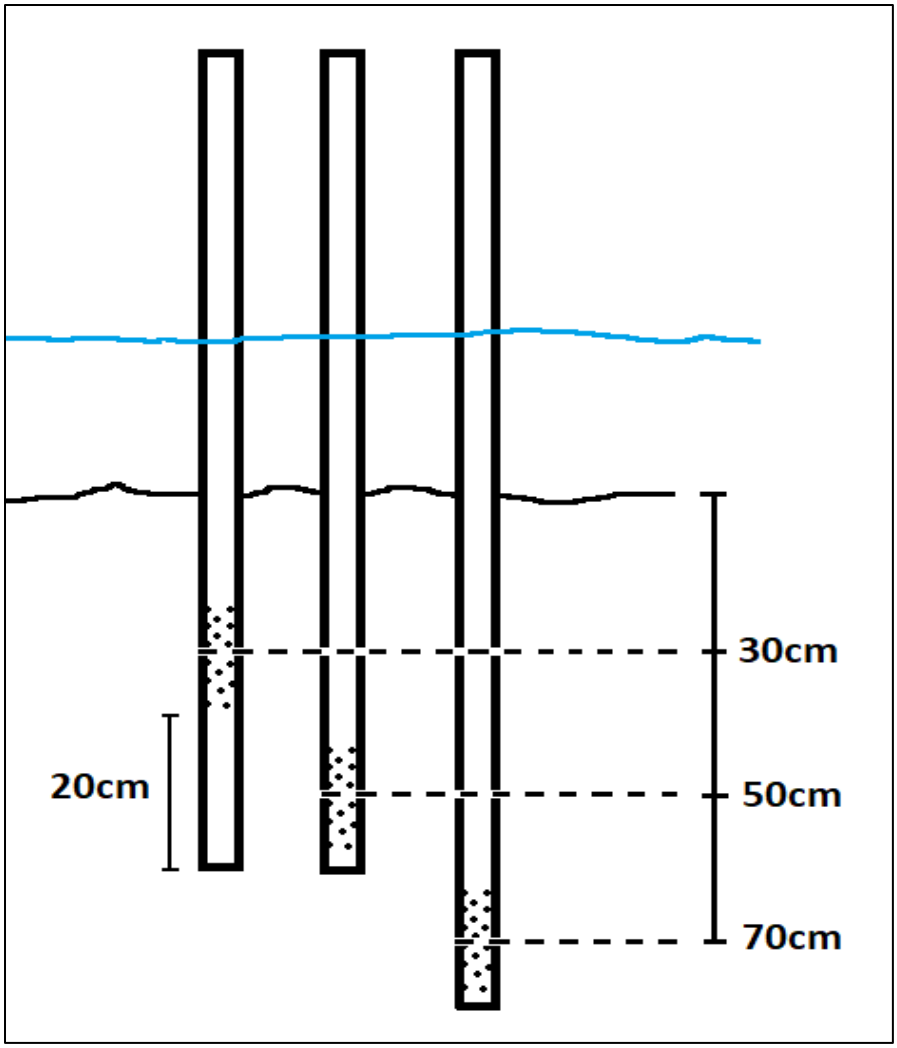

Figure 2. Illustration of three well depths, not drawn to scale. Wells were separated by at least 1.5m. 30-cm wells had a 20cm extension added to the bottom to make them more secure in the stream bed and prevent displacement during floods.

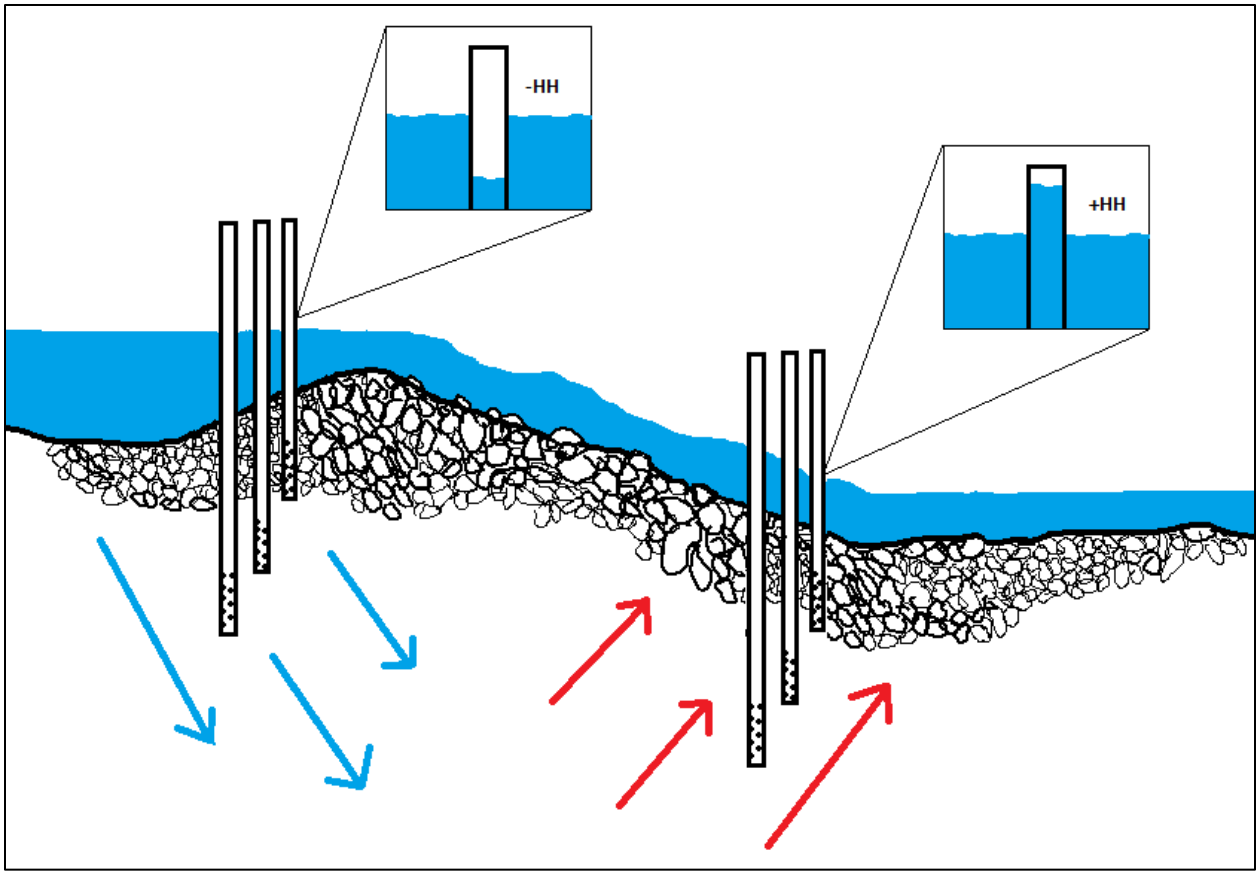

Figure 3. Well configuration for one riffle, illustrating wells at 30, 50, and 70cm depths in both upwelling and downwelling zones. Blue and red arrows represent direction of flow in downwelling and upwelling zones respectively. Zoom panels show negative hydraulic head in downwelling zone and positive hydraulic head in upwelling.

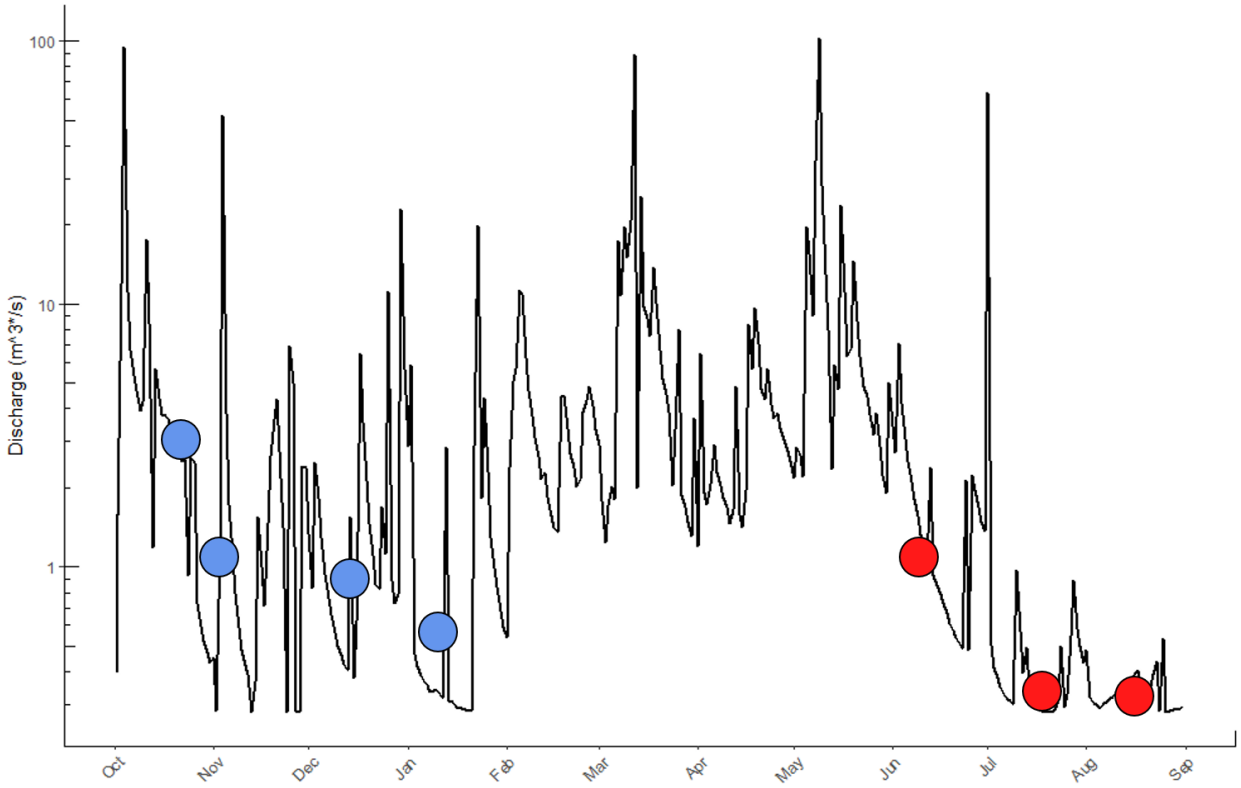

Figure 4. Hydrograph showing discharge in cubic meters per second for the duration of the study (Oct. 2019-Aug. 2020). Dots mark sample collection dates (Blue=High-flow period, Red=Low-flow period).

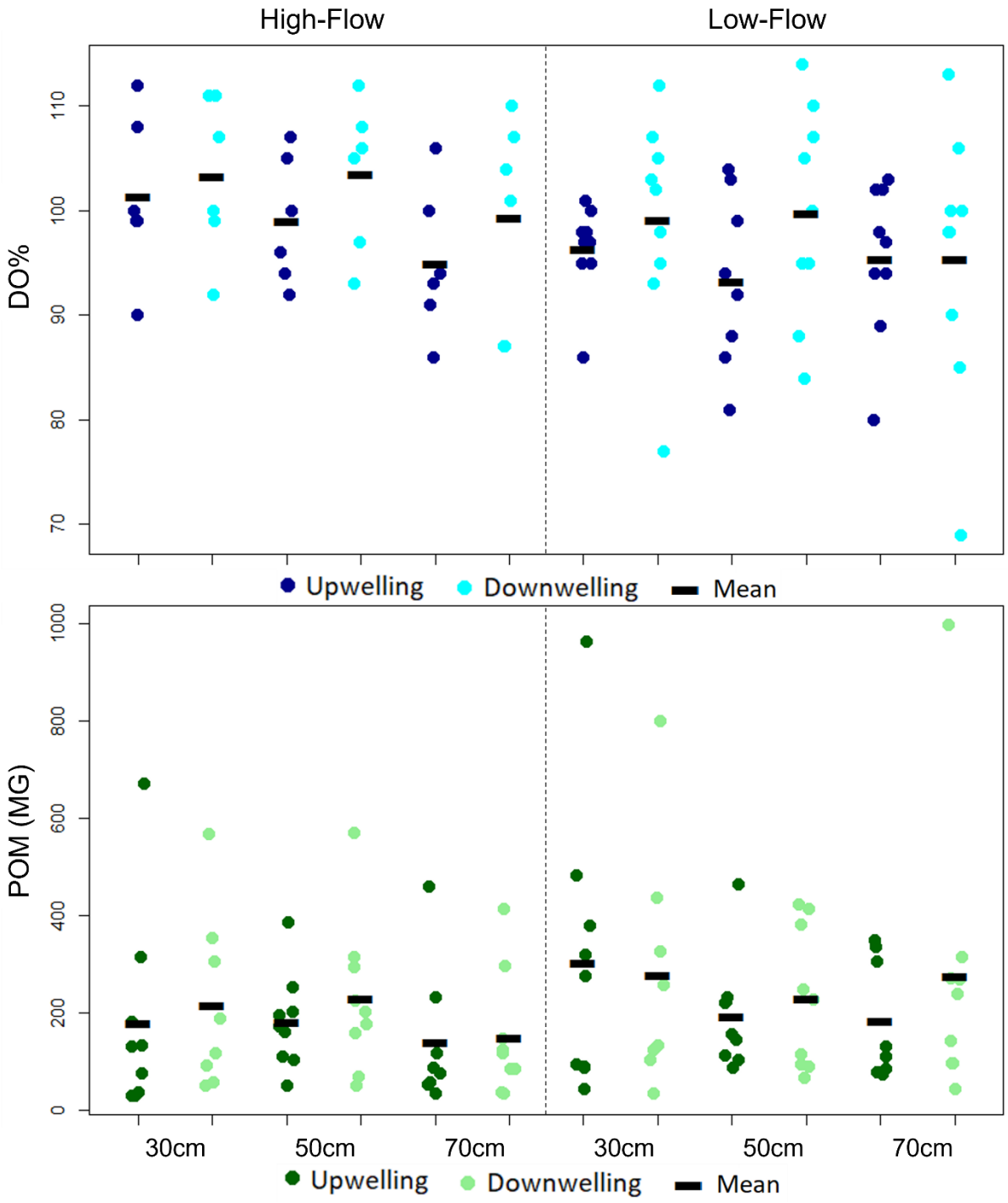

Figure 5. DO% and POM/8L sample at 30, 50, and 70 cm depths in upwelling and downwelling zones during high-flow and low-flow periods. There were no significant patterns, see table 3 for ANOVA results.

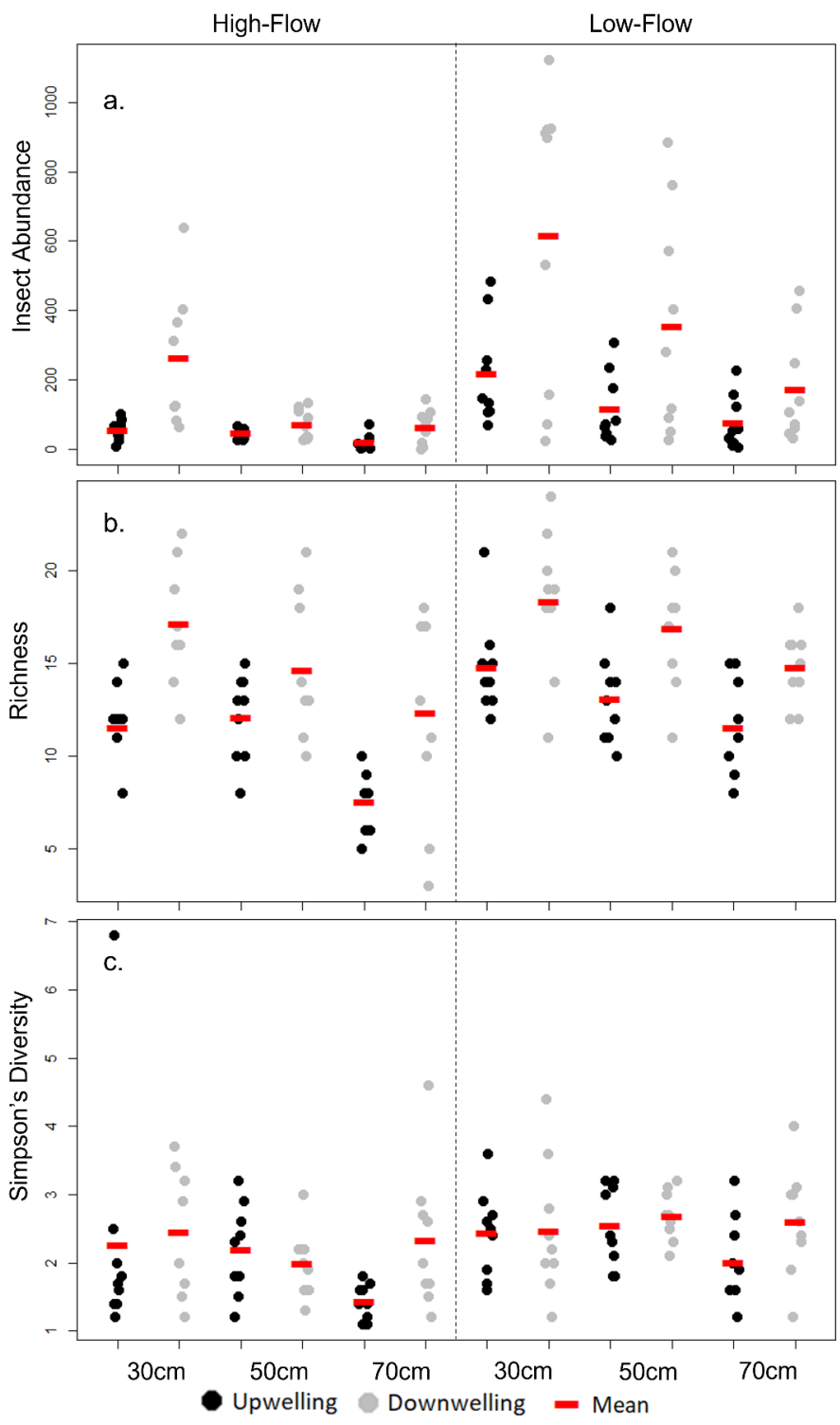

Figure 6. Insect abundance (a), total taxa richness (b), and Simpson's diversity index (.) at three depths in upwelling (black) and downwelling (gray) zones during high-flow (left) and low-flow (right) periods. Circles represent one 8L sample. See table 3 for ANOVA results.
Insect abundance depth Tukey: 30-50 P=0.030, 50-70 P=0.56, 30-70 P<0.0001
Richness depth Tukey: 30-50 P=0.386, 50-70 P=0.012, 30-70 P=0.0002.

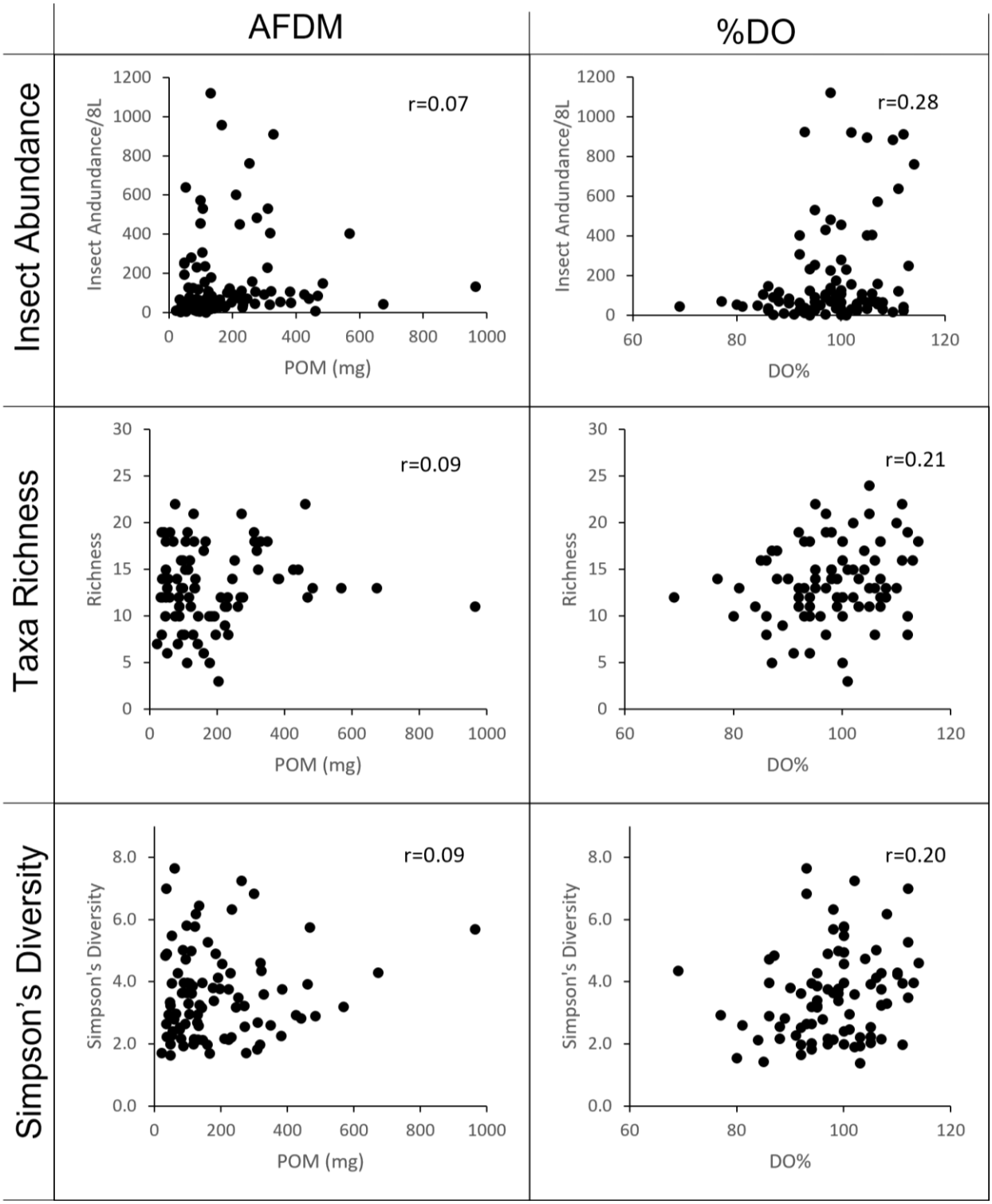

Figure 7. Scatterplots with Pearson's correlation between POM/DO and insect abundance/taxa richness/Simpson's diversity. No correlations were significant.

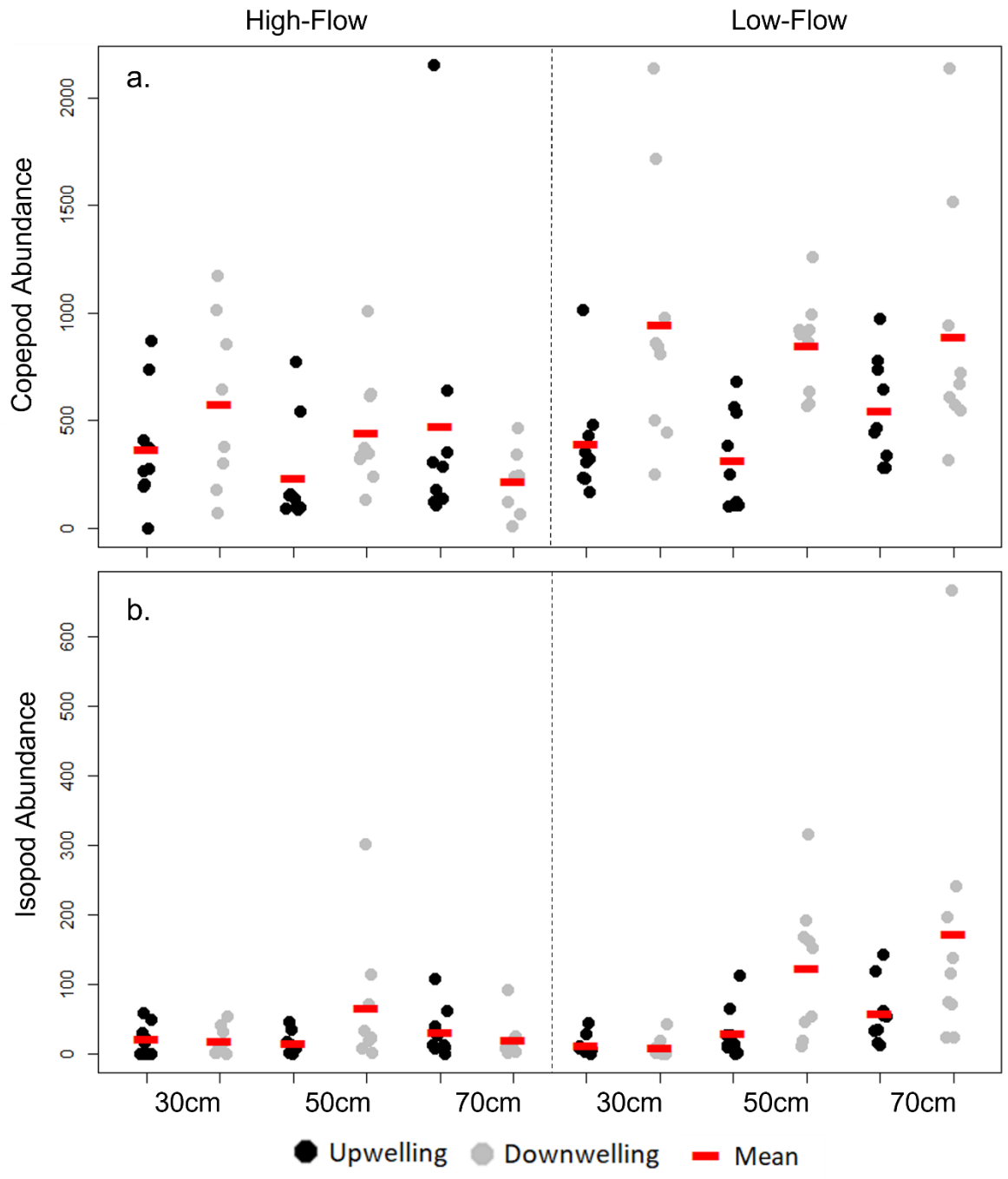

Figure 8. Abundance/8-L sample of two primarily hypogean taxa, Copepods (a) and Isopods (b). Circles represent one sample. See table 3 for ANOVA results.

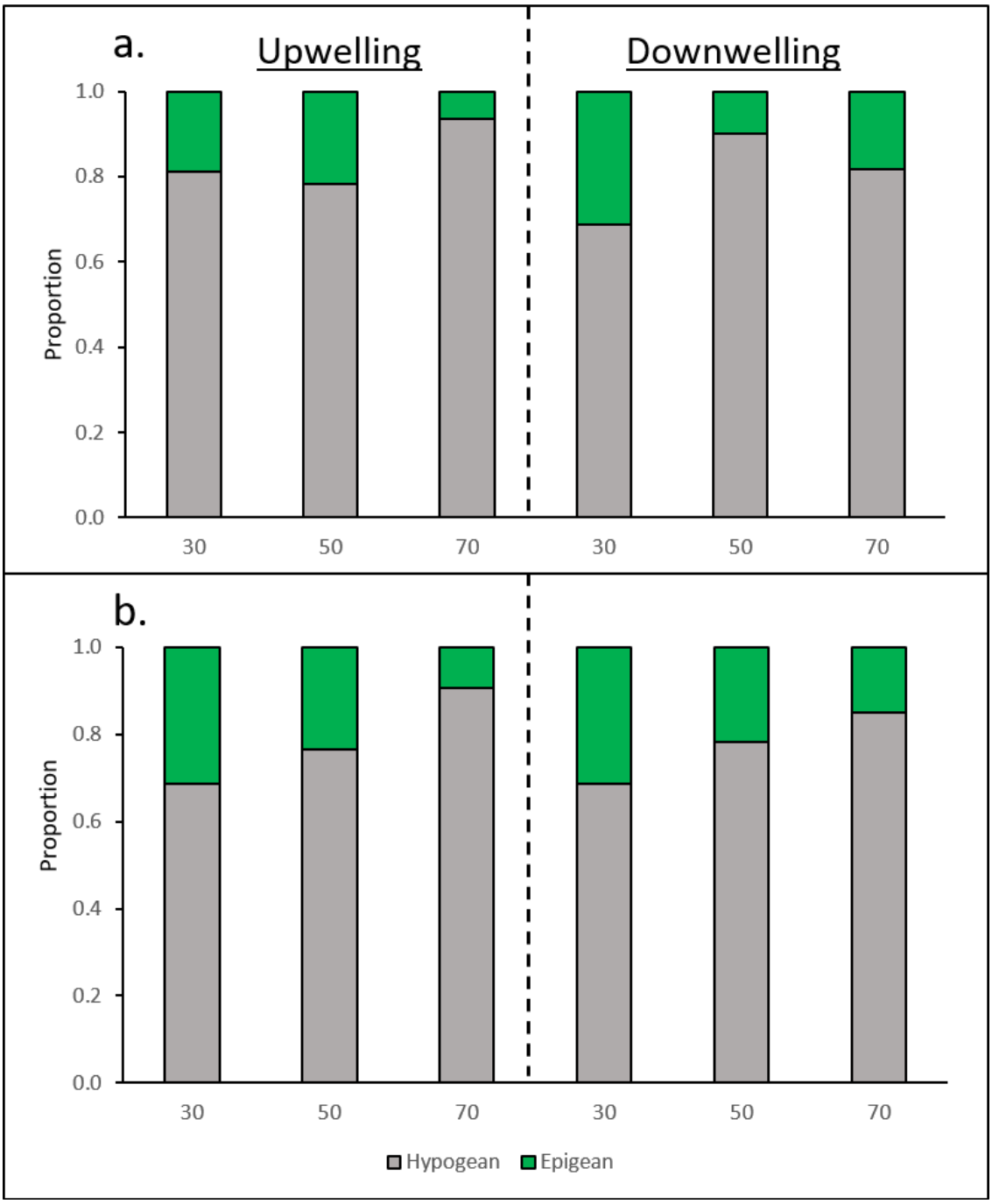

Figure 9. Ratios of epigean/hypogean taxa at depths of 30, 50, and 70cm in upwelling and downwelling zones during high-flow (a) and low flow (b) periods. See table 3 for ANOVA results. Depth Tukey test results: 30-50 P=0.30, 50-70 P=0.055, 30-70 P<0.0001.

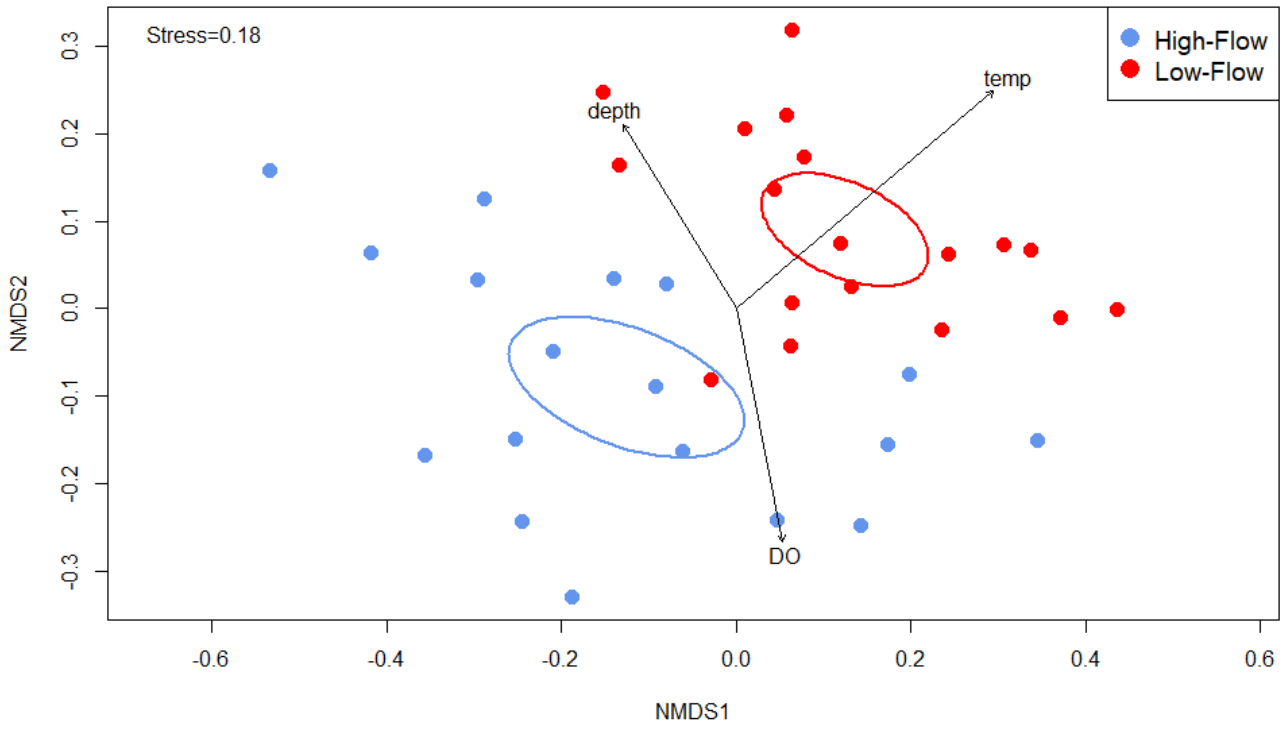

Figure 10. NMS bi-plot of samples collected from high-flow (blue) and low-flow (red) periods with 95% confidence ellipses (categories are not considered significantly different if ellipses are overlapping). Vectors represent significant correlations with environmental variables (dissolved oxygen, depth, and temperature). Periods MRPP (A=0.08, P=0.0004). Depths MRPP (A=0.03, P=0.024).

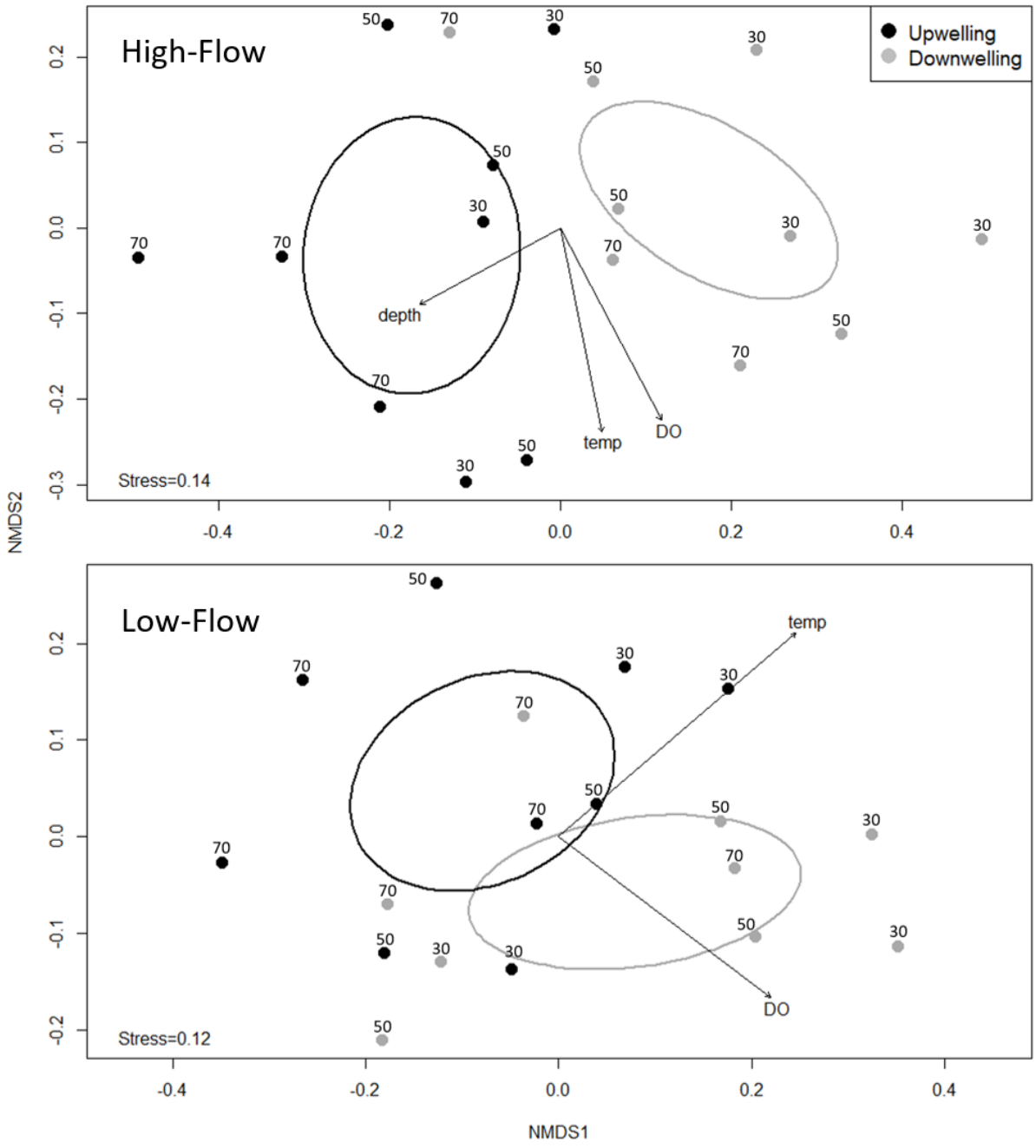

Figure 11. NMS biplots of samples from upwelling (black) and downwelling (gray) zones during high and low-flow periods with 95% confidence ellipses (categories are not considered significantly different if ellipses are overlapping). Numbers above points represent well depths in cm and vectors represent significant correlations with environmental variables (Dissolved oxygen, depth, and temperature). High-Flow upwelling/downwelling MRPP (A=0.09, P=0.0003). Low-Flow upwelling/downwelling MRPP (A=0.05, P=0.014). High-flow depths MRPP (A=0.04, P=0.08), low-flow depths (A=0.02, P=0.165).

HYPORHEIC INSECT COMMUNITY RESPONSE TO FLOODING IN AN OZARK STREAM

Introduction

Streams are dynamic, disturbance-prone systems with floods and droughts naturally and commonly occurring (Poff et al., 1997). Benthic macroinvertebrates have evolved to be resilient to disturbance and are known for their quick recovery after disturbance events (Fritz & Dodds, 2004; Palmer et al., 1992, Vander Vorste et al., 2016). Historically resilience has been attributed to various characteristics of life history and behavior, including aerial colonization (Heino, 2013) and diapause (Stubbington & Datry, 2013). Drift is another important process driving colonization in streams, that has long been credited as the driving force behind invertebrate resilience (Bilton et al., 2001), but evidence has accumulated demonstrating that the hyporheic zone acts as a refugium from flow disturbance events (Vander Vorste et al., 2016). The hyporheic zone has been known for many years to serve as an important habitat for invertebrates (Stanford & Gaufin, 1974) but remains understudied. While some species use the hyporheic zone obligately as part of their life cycle, many may use it facultatively as a refugium from flow disturbance events (Dole-Olivier, 2011; Stubbington et al., 2011).

Migration from the benthos into the HZ has been observed in response to drought (Stubbington et al., 2011; Vander Vorste et al., 2016; Vadher et al., 2017), but fewer studies have tested its use as a refugium in response to floods. Difficulty associated with sampling during high flow is likely one of the main reasons for lack of research on flood response compared to drying response. Sampling the hyporheic zone post-flood, Dorff (2019) found multiple insect taxa that were typically only found in benthic samples. Some taxa were found to use the hyporheic zone

consistently (e.g. Leuctridae), while some were only observed inhabiting it facultatively, presumably in response to increased flows (e.g. Heptageniidae, Perlidae). In laboratory experiments, obligately hyporheic meiofauna have also been observed moving deeper into sediment in response to increased flows (Palmer et al., 1992). The hyporheic refuge hypothesis (HRH, Palmer et al., 1992) in response to high flows is commonly referenced but has had few dedicated studies and results have been inconsistent. More research is needed that directly tests the HRH in response to flooding.

I sampled the hyporheic zone of a gravel bed Ozark stream before and after two major floods in Fall and Winter of a single water year. The main objective was to test the HRH for primarily benthic insect communities, specifically in response to disturbance from high flow. In accordance with the HRH, I hypothesized that insect abundance and taxonomic richness in the hyporheic zone increase, and benthic taxa shift vertically (deeper into hyporheic zone) in response to flood disturbance.

Methods

Study site. Bull Creek is a 2nd order stream in southwest Missouri, USA (Figure 1). Its bed composed of primarily gravel and cobble with minimal fine sediment gives it an extensive hyporheic zone with high levels of interstitial space. Bull Creek is categorized as a groundwater flashy system (Leasure et al., 2016), with fluctuations in flow occurring rapidly and unpredictably. Previous research has found high diversity of benthic insects in its gravel reaches (Dorff, 2019). Bull Creek's combination of an extensive hyporheic zone, flashy flow regime, and high benthic insect diversity make it an ideal site to test the hyporheic refuge hypothesis.

We installed a Solinst Levellogger before the first sampling date to record flow stage every hour for the duration of the study. Using a range of discharge measurements taken at various stages, we created a rating curve to estimate discharge for the duration of the study (Figure 4).

I sampled the hyporheic zone of Bull Creek before and after two major floods in October 2019 and January 2020, using a Bou-Rouch pumping method (see chapter 1 for more details on sampling methods). I collected samples from depths of 30, 50, and 70cm in both upwelling and downwelling zones at three replicate riffles (Figure 3). Samples were collected on five total dates, one pre-flood and two post-flood dates in October/November (not all wells were accessible due to high flows on first post-flood trip, half were pumped on 10/28 and the other half were pumped on 11/3), and one pre-flood and one post-flood date in January. Due to lost wells from the flood, samples were only collected from one riffle on the January post-flood collection date.

In the lab, I picked all insects from samples and identified them to the family level, with some early instars only identified to order. I calculated mean abundance among replicate wells for whole insect communities, Ephemeroptera, Plecoptera, and Trichoptera, combined EPT, and Chironomidae, as well as insect taxa richness. I chose EPT taxa due to their usefulness as bioindicator taxa, as well as their common occurrence in hyporheic samples. I evaluated chironomids due to their high abundance in comparison to all other insect taxa in samples. Means among riffles were not calculated for post-flood January samples due to lost wells at 2/3 riffles. I conducted three-way ANOVAs using pre/post flood, upwelling/downwelling, and depth as factors. I conducted separate ANOVAs on mean abundance/richness for October and January floods for each group/taxon.

Results

Discharge was ~0.28 m^3/s on the October pre-flood collection date, peaked at ~139 m^3/s on 10/11/19, and was down to ~5.5 m^3/s and ~1.1 m^3/s for post flood collection dates (10/27 and 11/3, respectively)(Figure 12). Discharge was ~0.44 m^3/s on the January pre-flood date (1/8/20), peaked at ~295 m^3/s on 1/11/20, and was down to ~5.3 m^3/s for the post-flood collection date (1/14/20).

Mean total insect abundance in October was 194 individuals/8-L pre-flood and 94 individuals/8-L post-flood. There was no significant difference between pre- and post-flood insect abundance, due to high variation in abundance among wells (F=2.9, *P*=0.1) and no interaction with upwelling/downwelling or depth (Table 5). Insect abundance also did not differ between pre- and post-flood samples in January, averaging 65 individuals/8-L pre and 90 individuals/8-L post-flood (F=0.5, *P*=0.50)(Table 6). Average EPT abundance per sample in October was 22 individuals/8-L pre- and 19 individuals/8-L post-flood (Figure 13). Abundance did not differ pre- and post-flood for combined EPT or for Ephemeroptera, Plecoptera, and Trichoptera separately. There were no interactions between pre/post flood abundance and depth or upwelling/downwelling. Patterns in pre- and post-flood in January were the same as October. There were no differences in abundance pre- and post-flood for any EPT taxa (Figure 14). Chironomidae abundance was 159 individuals/8-L in pre-flood October samples and 95 individuals/8-L in post flood samples. In January, pre-flood abundance was 30 individuals/8-L and post flood abundance was 48 individuals/8-L (Figure 15). Richness did not vary significantly between pre- and post-flood samples in either October (F=2.0, *P*=0.18), or January (F=1.3, *P*=0.28) (Figure 16).

While there were no significant post-flood increases in abundance or richness, there were multiple taxa found in post-flood samples that were not found in pre-flood samples. Helicopsychidae, Sialidae, and Tipulidae were not present in pre-flood October samples, but were present in post-flood October/November samples (Appendix A). Leptohyphidae, Philopotamidae, Ceratopogonidae, Nemouridae, and Stratiomyiidae were not present in pre-flood January samples, but were present in post-flood January samples. Conversely, in both October and January, Perlidae was the only insect taxon to be found pre-flood but not post-flood.

Discussion

Contrary to my hypothesis, patterns in abundance pre- and post-flood did not show any evidence of benthic taxa migrating into the hyporheic zone in response to floods. There were no increases in abundance in the hyporheic zone for whole insect communities, EPT taxa, or chironomids at any depth after either flood. These results did not support the hyporheic refuge hypothesis, stating that benthic invertebrates use the hyporheic zone as a refugium from floods. However, the difficulties of sampling the hyporheic zone immediately after floods, including both the physical difficulty of accessing the stream and the loss of sample wells reducing sample size for statistical power, prevented me from solidly testing the HRH.

While abundance of insects did not increase between pre- and post-flood samples, several taxa were present in post-flood samples that were absent prior to the floods. These taxa have clear affinities to the benthic zone (Dorff, 2019) and hence might represent some evidence in support of HRH, even in the absence of statistical power to robustly test the hypothesis. While multiple taxa were present in post-flood samples that were absent in pre-flood samples, in most cases there were only between 1-3 individuals of a given taxa. With numbers this low it is

possible that these taxa were only found in post-flood samples simply due to random chance. There was however only a single taxon (Perlidae) that was found in a pre-flood sample that was not found in a post flood sample. This is substantially different from the 8 families that were exclusively found in post-flood samples and supports the possibility that these taxa migrated into the hyporheic zone in response to flooding. Sample timing likely also influenced the outcome of this study. Post-flood samples were collected nearly a week to three weeks after the peak of each flood, likely giving highly mobile invertebrates ample time to migrate back to the benthic zone, returning communities to their pre-flood state. Sampling during or immediately following floods would provide new insight into the topic of the HRH, but there are currently no known methods for sampling invertebrates in these conditions.

It is also possible that invertebrates in Bull Creek simply do not respond to flooding with downward migration. The hyporheic zone at Bull Creek has high invertebrate diversity year-round (see chapter 1) and may act as a source for post-flood recolonization, even if benthic taxa do not use it as a refugium. Sampling the benthic zone and hyporheic zone concurrently pre- and post-flood could potentially track if colonization is occurring from the hyporheic zone. Resilience of invertebrates may also come from other sources. While drift has been demonstrated to play a less significant role in invertebrate resilience than previously thought (Vander Vorste et al., 2016), drift has historically been credited as one of the most important factors supporting resilience (Brittain & Eikland, 1988). Insects may also use other refugia from high flows in Bull Creek, including side channel habitats or floodplains. Snails have been observed colonizing from the edges of Bull Creek following floods when they are typically evenly (and densely) distributed throughout the channel (Personal observation). It is possible that insects exhibit

similar behavior, but they are not as easily observable as grazing snails in the field, without dedicated sampling.

Low sample size and limited sampling methods likely played a role in the lack of difference between pre- and post-flood samples. With many wells being washed away in floods, resulting in few post-flood samples, I likely did not have the statistical power to detect temporal differences, even if a downward migration did occur. There is potential for future studies at Bull Creek with updated sampling methods, and more replicate samples collected. With the shallowest depth sampled being 30cm, it is also possible that even if benthic invertebrates migrated downwards, they didn't travel deep enough to be collected. Future studies could sample at a narrower range of depths immediately following a flood (e.g., 10cm, 20cm, 30cm) to track potentially finer scale movements. Difficulties associated with sampling during floods may have also been a driver of the lack of observed difference between pre- and post-flood samples. If benthic invertebrates migrate down in response to flooding, then return to the benthic zone as flows lower, sampling post-flood may not be adequate for tracking vertical shifts in communities. This may have been especially impactful on the samples associated with the October flood, as post-flood samples were collected 2-3 weeks following the flood peak. Using the Bou-Rouch sampling method, there is no way to feasibly sample the hyporheic zone during a flood. Other forms of sampling would be necessary for collecting samples during flooding that prevent one from safely entering the stream. Future research could involve developing a method to sample the hyporheic zone during high-flow conditions.

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Table 5. Three-way ANOVA results comparing abundance of Ephemeroptera, Plecoptera, Trichoptera, Chironomidae, and all insects, plus total insect taxa richness pre/post October flood, between upwelling and downwelling zones, and among depths. Adjusted α=0.007 and significant *P* values are bolded.

	EPT	E	P	T	Chiron	All Insects	Richness
Up/Down (df=1)	F=23.6 ***P*<0.0001**	F=22.3 ***P*<0.0001**	F=9.6 ***P*=0.005**	F=9.2 ***P*=0.006**	F=14.1 ***P*=0.001**	F=19.8 ***P*<0.0001**	F=45.6 ***P*<0.0001**
Depth (df=2)	F=4.9 *P*=0.02	F=5.3 *P*=0.01	F=1.5 *P*=0.24	F=1.7 *P*=0.20	F=2.7 *P*=0.09	F=3.4 *P*=0.05	F=6.5 ***P*=0.006**
Pre/Post Flood (df=1)	F=0.01 *P*=0.90	F=1.0 *P*=0.33	F=3.4 *P*=0.08	F=4.4 *P*=0.05	F=3.2 *P*=0.09	F=2.9 *P*=0.10	F=2.0 *P*=0.18
Up/Down x Depth (df=2)	F=3.7 *P*=0.04	F=5.2 *P*=0.01	F=2.4 *P*=0.12	F=1.0 *P*=0.40	F=2.2 *P*=0.14	F=2.7 *P*=0.09	F=0.6 *P*=0.54
Up/Down x Pre/Post (df=1)	F=0.05 *P*=0.83	F=0.60 *P*=0.45	F=4.5 *P*=0.04	F=4.3 *P*=0.05	F=2.9 *P*=0.10	F=2.9 *P*=0.10	F=1.6 *P*=0.21
Depth x Pre/Post Flood (df=2)	F=1.0 *P*=0.39	F=2.2 *P*=0.14	F=0.4 *P*=0.65	F=0.8 *P*=0.45	F=0.3 *P*=0.74	F=0.4 *P*=0.68	F=0.3 *P*=0.73
U x D x Pre/Post (df=2)	F=1.7 *P*=0.21	F=3.1 *P*=0.06	F=0.6 *P*=0.58	F=1.0 *P*=0.40	F=0.5 *P*=0.63	F=0.7 *P*=0.49	F=1.5 *P*=0.23

Table 6. Three-way ANOVA results comparing abundance of Ephemeroptera, Plecoptera, Trichoptera, Chironomidae, and all insects, plus total insect taxa richness pre/post January flood, between upwelling and downwelling zones, and among depths. Adjusted α=0.007 and significant *P* values are bolded.

	EPT	E	P	T	Chiron	All Insects	Richness
Up/Down (df=1)	F=5.2 *P*=0.04	F=8.3 *P*=0.01	F=1.9 *P*=0.20	F=0 *P*=1	F=1.7 *P*=0.21	F=3.1 *P*=0.10	F=5.0 *P*=0.05
Depth (df=2)	F=6.3 *P*=0.01	F=7.0 *P*=0.01	F=4.5 *P*=0.03	F=1.5 *P*=0.25	F=1.8 *P*=0.21	F=3.4 *P*=0.07	F=6.6 *P*=0.01
Pre/Post Flood (df=1)	F=0.2 *P*=0.65	F=0 *P*=0.99	F=1.5 *P*=0.25	F=0.6 *P*=0.47	F=0.6 *P*=0.46	F=0.5 *P*=0.50	F=1.3 *P*=0.28
Up/Down x Depth (df=2)	F=0.1 *P*=0.94	F=0.7 *P*=0.51	F=0.5 *P*=0.63	F=0.6 *P*=0.54	F=0.2 *P*=0.79	F=0.2 *P*=0.84	F=0.1 *P*=0.95
Up/Down x Pre/Post (df=1)	F=0.0 *P*=0.96	F=0 *P*=0.97	F=0.1 *P*=0.80	F=0.2 *P*=0.68	F=0.7 *P*=0.41	F=0.3 *P*=0.60	F=0.2 *P*=0.68
Depth x Pre/Post Flood (df=2)	F=1.6 *P*=0.25	F=2.3 *P*=0.14	F=0.6 *P*=0.56	F=0.3 *P*=0.76	F=0.4 *P*=0.67	F=0.8 *P*=0.47	F=0.6 *P*=0.59
U x D x Pre/Post (df=2)	F=3.3 *P*=0.07	F=2.7 *P*=0.10	F=3.1 *P*=0.08	F=2.2 *P*=0.15	F=1.4 *P*=0.28	F=2.0 *P*=0.18	F=0.8 *P*=0.46

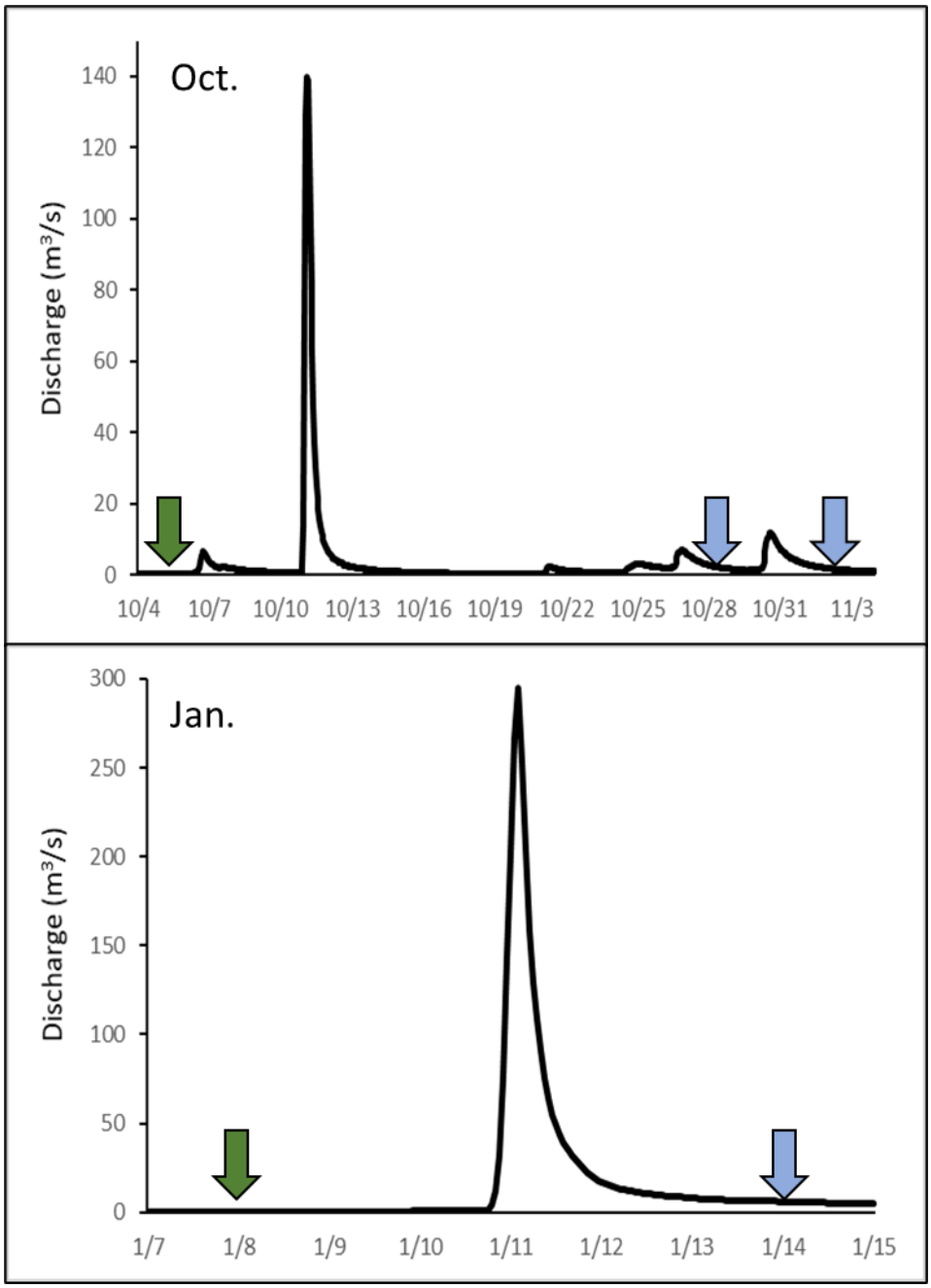

Figure 12. Sample collection dates, pre- (green) and post (blue) flood in October/November.

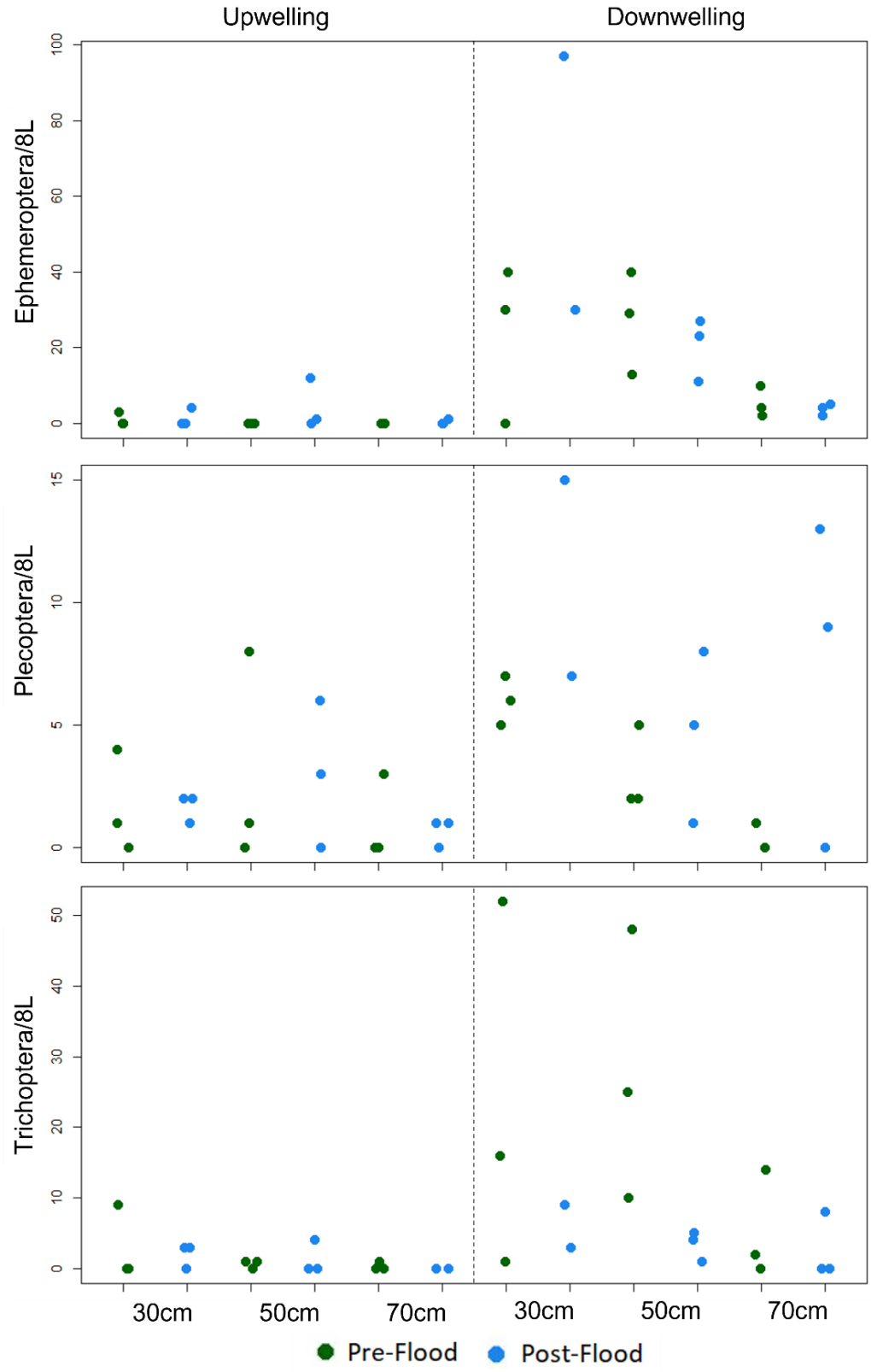

Figure 13. Abundance of Ephemeroptera (E), Plecoptera (P), and Tricoptera (T) per sample. Pre- (green) and post (blue) October flood. Circles represent one 8L sample.

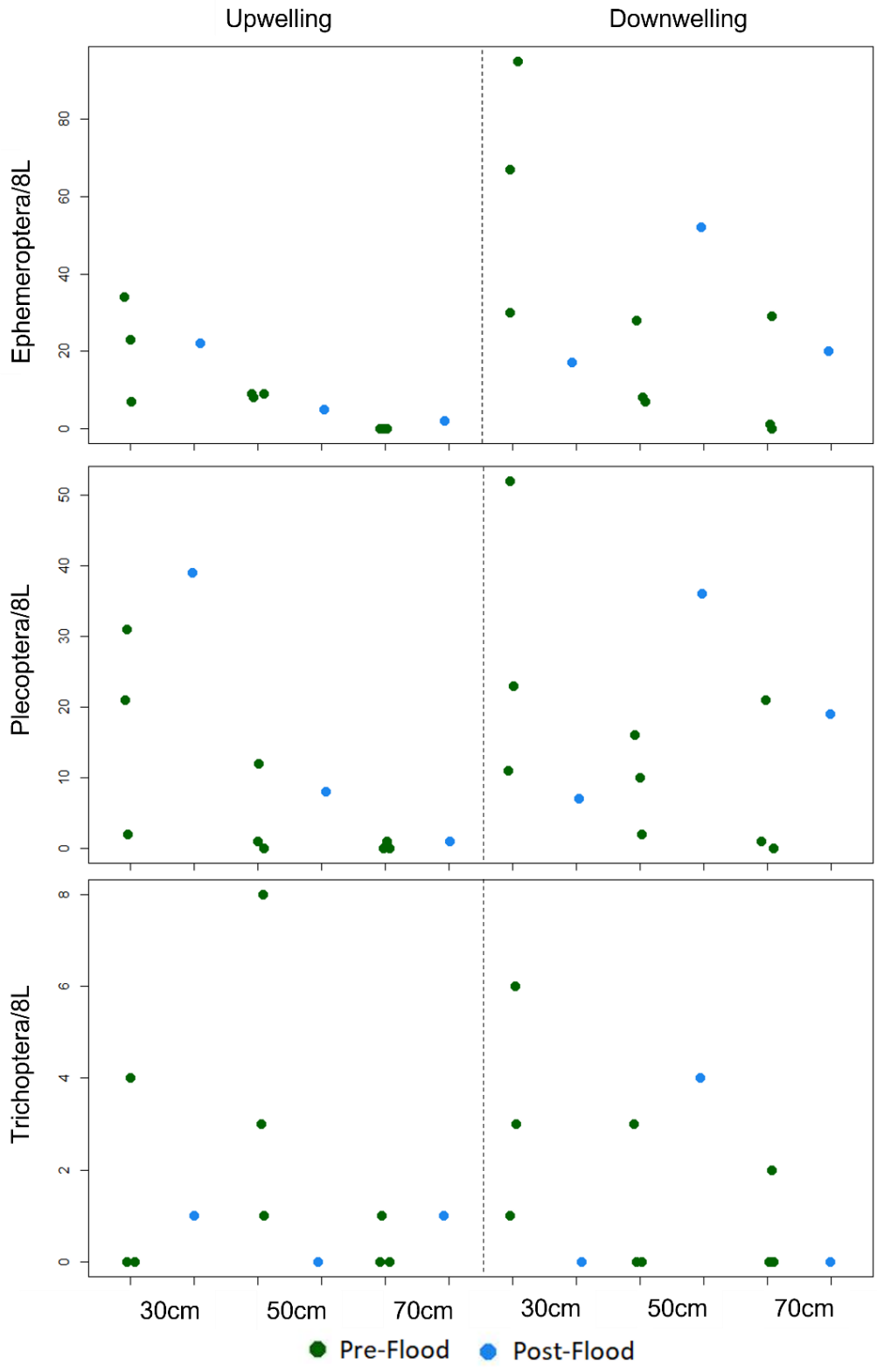

Figure 14. Abundance of Ephemeroptera (E), Plecoptera (P), and Tricoptera (T) per 8L sample. Pre- (green) and post (blue) January flood. Circles represent one 8L sample.

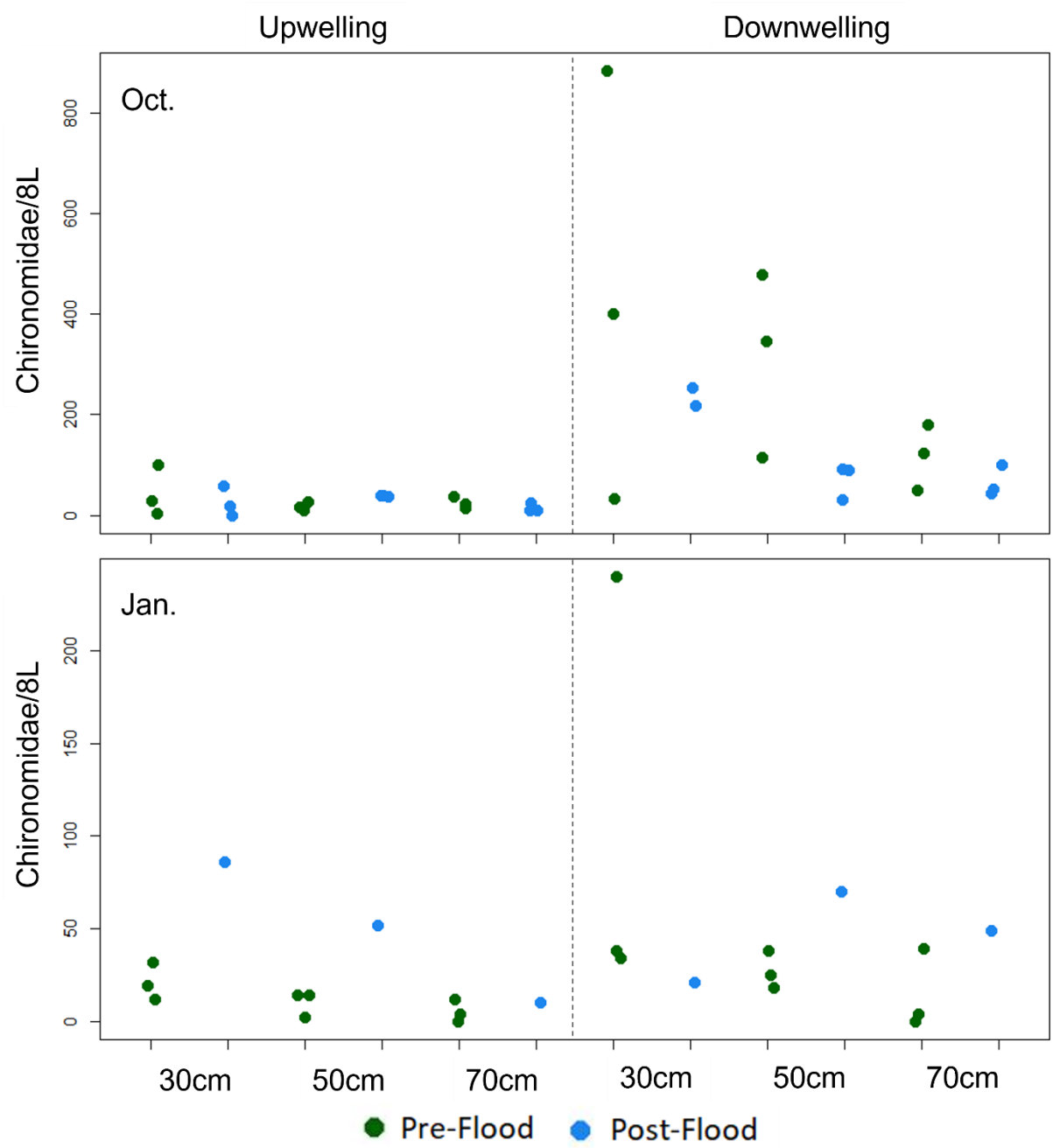

Figure 15. Abundance of Chironomidae per sample. Pre- (green) and post (blue) flood in October and January. Circles represent one 8L sample.

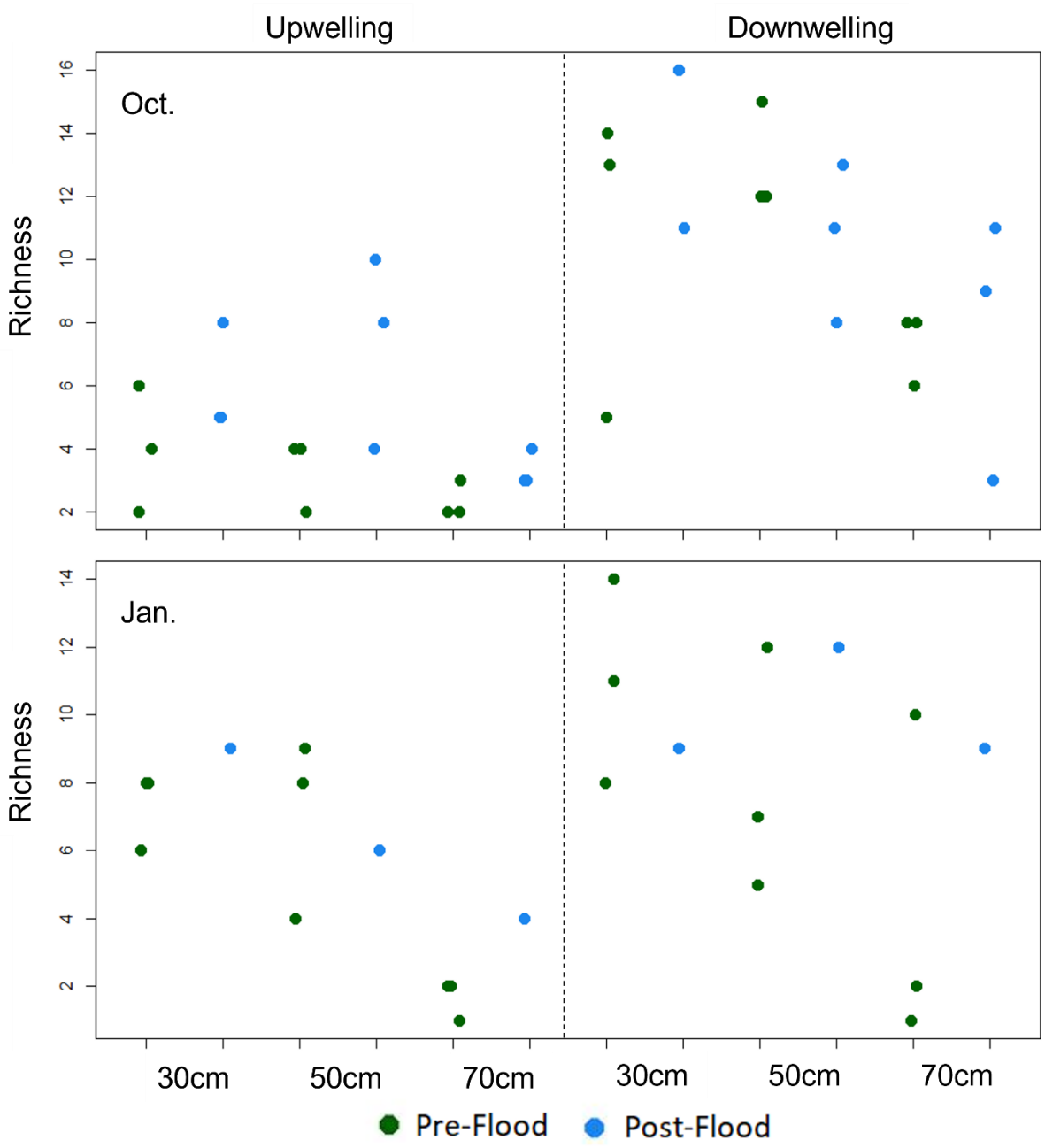

Figure 16. Insect taxa richness per sample. Pre- (green) and post (blue) flood in October and January. Circles represent one 8L sample.

SUMMARY

In chapter 1, I found that hyporheic invertebrate abundance and diversity were significantly higher in downwelling zones than upwelling zones in Bull Creek. Several taxa were also found in downwelling samples that were absent from upwelling samples. While invertebrates showed somewhat predictable patterns in spatial distribution, environmental factors were much more variable, and did not show significant patterns. There were no strong correlations between environmental variables and abundance/diversity, but dissolved oxygen did correlate significantly with community structure.

In chapter 2, there were no statistically significant results showing that insects used the hyporheic zone as a refuge during floods. There were no post-flood increases in abundance or richness in the hyporheic zone in any of the groups that were observed. There were, however, several taxa found in post-flood samples that were absent from pre-flood samples. Lack of significant results may have been due to low sample size from sampling difficulties associated with high flows. While lacking statistical significance, these results are encouraging and highlight the importance of further research in this area. There is much room for development of sampling strategies for monitoring invertebrate response to flooding.

This thesis highlights the importance of the hyporheic zone as a habitat for invertebrates and demonstrates the need for further research of this underappreciated ecosystem. Similar studies have been conducted in other parts of the world, but this research was the first of its kind to be carried out in the Ozarks ecoregion. Understanding how the hyporheic zone functions in a variety of stream types is critical for getting a full understanding of its importance to streams.

APPENDICES

Appendix A. Taxa lists from all samples

Appendix A-1. Taxa list in individuals/8L sample, averaged across three replicate riffles. October-November.

	Oct 5 upwell 30	Oct 5 upwell 50	Oct 5 upwell 70	Oct 5 down 30	Oct 5 down 50	Oct 5 down 70	Oct 27 down 30	Oct 27 down 50	Oct 27 down 70	Nov 3 upwell 30	Nov 3 upwell 50	Nov 3 upwell 70
Coleoptera												
Elmidae	1.00	1.00	1.33	13.67	37.00	9.67	15.00	6.00	9.33	1.67	2.00	1.67
Psephenidae	0.00	0.67	0.00	0.00	0.00	0.00	0.50	0.00	0.67	1.33	3.67	1.00
Dytiscidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diptera												
Early Instar	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chironomidae	44.00	17.67	24.33	439.00	312.67	118.00	235.50	70.67	65.33	25.33	38.67	14.00
Ceratopogonidae	0.00	0.00	0.00	4.33	1.00	0.67	1.50	0.67	4.33	0.33	0.33	0.33
Corethrellidae	0.33	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00
Tipulidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	1.33	0.00	0.00
Simuliidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ephemeroptera												
Early Instar	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.33	0.00	4.00	0.00
Baetidae	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
Caenidae	0.00	0.00	0.00	4.33	6.33	1.67	2.50	3.00	0.33	0.00	0.00	0.00
Ephemeridae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Ephemerellidae	0.00	0.00	0.00	4.33	7.00	0.33	3.50	1.00	1.00	0.00	0.00	0.00
Heptageniidae	1.00	0.00	0.00	14.00	9.67	2.33	52.00	15.33	1.67	0.33	0.33	0.33
Isonychiidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leptohyphidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leptophlebiidae	0.00	0.00	0.00	0.67	3.33	0.67	4.50	1.00	0.33	1.00	0.00	0.00
Megaloptera												
Sialidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
Odonata												
Gomphidae	0.00	0.00	0.00	1.67	0.33	1.00	4.50	1.00	0.00	0.00	0.33	0.00
Coenagrionidae	0.00	0.00	0.00	1.33	2.00	0.33	1.50	0.67	1.00	0.33	0.33	0.00
Plecoptera												
Early Instar	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.67	0.00	0.00	0.00
Chloroperlidae	0.00	0.00	0.00	0.00	0.00	0.00	4.00	3.00	0.00	1.00	1.33	0.33
Leuctridae	0.00	0.00	0.00	1.33	0.67	0.33	3.00	1.00	0.00	0.33	0.33	0.00
Perlidae	1.67	3.00	1.00	4.67	2.33	0.33	4.00	0.67	1.67	0.33	1.33	0.33
Trichoptera												
Early Instar	1.00	0.33	0.00	5.33	4.33	0.00	0.00	0.00	1.67	0.00	0.33	0.00
Hydropsychidae	0.00	0.00	0.00	0.67	2.00	0.00	1.50	0.33	0.00	0.33	0.00	0.00
Leptoceridae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Philopotamidae	1.00	0.33	0.00	16.33	20.33	5.33	4.00	1.67	1.00	0.00	0.33	0.00
Polycentropodidae	1.00	0.00	0.33	0.67	1.00	0.00	0.50	1.33	0.00	0.67	0.67	0.00

Helicopsychidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
Non-Insects												
Chydoridae	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.67	3.67	0.67	0.00	0.00
Copepoda	221.00	74.00	175.00	232.67	178.67	203.00	621.50	569.33	182.67	191.00	111.67	196.33
Crangonyctidae	0.67	0.00	0.67	0.33	0.00	0.67	0.00	1.33	2.00	0.00	0.00	0.67
Hydrachnidia	5.67	1.33	5.00	22.33	4.33	6.33	40.50	21.33	21.33	4.67	9.67	7.33
Ostracoda	22.67	3.67	2.00	77.67	27.67	8.67	64.50	323.00	17.00	2.00	7.33	12.67
Caecidotea	30.00	6.67	23.00	24.67	26.00	42.33	27.50	108.00	39.33	25.00	8.33	17.33
Asellus	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Prosobranchia	0.33	0.33	0.00	6.33	2.67	0.00	7.50	2.33	4.00	1.00	1.00	0.67
Oligochaeta	0.67	0.67	0.33	11.67	4.00	4.00	1.50	6.33	5.00	1.67	1.33	0.67
Turbellaria	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	1.33	3.67	0.00

Appendix A-2. Taxa list in individuals/8L sample, averaged across three replicate riffles. December-January.

	Dec 5 upwell 30	Dec 5 upwell 50	Dec 5 upwell 70	Dec 5 down 30	Dec 5 down 50	Dec 5 down 70	Jan 8 upwell 30	Jan 8 upwell 50	Jan 8 upwell 70	Jan 8 down 30	Jan 8 down 50	Jan 8 down 70
Coleoptera												
Elmidae	0.67	0.67	0.00	2.67	2.67	2.33	1.00	1.00	0.00	0.33	1.33	0.00
Psephenidae	1.33	0.33	0.00	0.67	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00
Dytiscidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diptera												
Early Instar	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chironomidae	38.67	39.33	37.00	192.00	34.00	34.00	21.00	10.00	5.33	104.00	27.00	14.33
Ceratopogonidae	0.00	0.00	0.00	5.33	0.00	1.33	0.00	0.00	0.00	3.00	0.00	0.67
Corethrellidae	0.00	1.33	0.33	0.00	0.00	0.00	0.00	0.33	0.33	0.00	0.00	0.00
Tipulidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00
Simuliidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ephemeroptera												
Early Instar	10.00	3.00	0.33	12.00	9.33	4.00	13.67	5.33	0.00	22.33	4.67	6.33
Baetidae	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	1.33	0.67	0.33
Caenidae	0.00	0.00	0.00	3.67	0.00	1.33	0.00	0.00	0.00	0.33	0.00	0.00
Ephemeridae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ephemerellidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
Heptageniidae	0.67	1.33	0.00	36.33	2.00	1.33	1.67	1.67	0.00	27.00	4.67	1.33
Isonychiidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Leptohyphidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leptophlebiidae	0.00	5.00	0.33	0.00	0.67	2.33	5.33	1.67	0.00	12.67	4.33	2.00
Megaloptera												
Sialidae	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Odonata												
Gomphidae	0.00	0.00	0.00	1.00	1.67	0.00	0.00	0.33	0.00	0.67	1.67	0.00
Coenagrionidae	0.00	1.00	0.00	0.33	0.00	0.67	0.00	0.33	0.00	0.33	0.33	0.00
Plecoptera												
Early Instar	16.33	5.00	0.33	15.00	4.00	16.00	5.33	0.00	0.33	11.00	3.33	1.67
Chloroperlidae	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	2.00	0.33	1.00
Leuctridae	0.00	0.00	0.00	0.00	0.00	0.00	12.00	4.33	0.00	10.33	5.67	4.67
Perlidae	0.00	0.00	0.00	0.33	0.00	0.33	0.33	0.00	0.00	5.33	0.00	0.00
Trichoptera												
Early Instar	0.67	0.00	0.00	1.00	1.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00
Hydropsychidae	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leptoceridae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Philopotamidae	0.00	0.33	0.00	1.00	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.00
Polycentropodidae	2.00	2.00	0.00	1.67	0.00	0.00	1.33	4.00	0.33	3.33	1.00	0.67
Helicopsychidae	0.00	0.00	0.00	1.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
Non-Insects												
Chydoridae	0.67	1.00	1.00	2.00	1.33	2.33	2.00	0.00	0.33	5.67	0.67	0.67

Copepoda	293.67	264.33	362.00	472.67	333.33	235.00	628.00	335.00	873.33	654.67	434.67	237.33
Crangonyctidae	0.33	1.67	1.33	0.00	0.00	2.67	0.00	0.33	1.00	0.00	0.00	2.00
Hydrachnidia	3.33	7.67	6.00	20.00	10.00	10.67	0.67	1.33	2.00	3.67	1.33	1.00
Ostracoda	12.33	9.33	5.00	23.00	69.00	15.00	7.67	8.33	4.33	22.00	57.00	9.00
Caecidotea	12.33	20.33	32.33	18.33	55.00	16.00	29.67	16.33	42.67	14.00	34.00	6.33
Asellus	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00
Prosobranchia	1.67	0.00	0.00	2.00	1.33	0.33	0.33	0.00	0.00	1.67	0.67	0.00
Oligochaeta	1.33	2.33	1.00	5.00	4.67	5.67	1.00	0.33	1.00	1.67	2.33	2.67
Turbellaria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00

Appendix A-3. Taxa list in individuals/8L sample, averaged across three replicate riffles. June-July.

	Jun 15 upwell 30	Jun 15 upwell 50	Jun 15 upwell 70	Jun 15 down 30	Jun 15 down 50	Jun 15 down 70	Jul 15 upwell 30	Jul 15 upwell 50	Jul 15 upwell 70	Jul 15 down 30	Jul 15 down 50	Jul 15 down 70
Coleoptera												
Elmidae	1.67	5.00	0.67	4.33	3.67	3.33	3.67	9.67	0.67	23.67	14.67	4.67
Psephenidae	0.00	0.33	0.00	0.33	0.00	0.00	3.33	0.33	0.00	0.67	0.00	0.00
Dytiscidae	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diptera												
Early Instar	0.67	0.00	0.00	0.33	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00
Chironomidae	73.67	36.00	8.00	31.00	19.00	21.00	184.67	66.00	37.67	770.67	374.33	61.33
Ceratopogonidae	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	1.00	0.33	0.33
Corethrellidae	0.00	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.33	0.00	0.33	0.00
Tipulidae	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Simuliidae	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.67	0.00	0.67	0.00
Ephemeroptera												
Early Instar	4.67	0.00	0.00	0.00	0.00	0.00	5.00	0.00	0.00	23.00	26.00	3.33
Baetidae	1.33	0.00	0.00	0.00	0.33	0.00	1.00	0.67	0.00	5.33	6.67	0.33
Caenidae	0.33	1.00	0.00	0.33	0.00	0.00	0.00	0.67	0.00	6.67	3.67	0.33
Ephemeridae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00
Ephemerellidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
Heptageniidae	0.67	0.00	0.00	3.67	0.00	0.33	3.00	1.67	0.33	12.67	8.00	1.67
Isonychiidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00

Leptohyphidae	0.00	0.00	0.00	0.67	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00
Leptophlebiidae	6.67	7.33	0.33	21.33	6.00	6.67	2.67	0.33	0.00	5.33	3.00	0.00
Megaloptera												
Sialidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Odonata												
Gomphidae	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coenagrionidae	0.00	0.00	0.33	0.67	0.00	0.67	0.00	0.00	0.00	0.67	0.00	0.33
Plecoptera												
Early Instar	0.00	0.00	0.33	0.67	0.00	0.67	0.33	0.33	0.33	1.33	0.00	1.00
Chloroperlidae	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.33	0.00	0.33	0.67	0.00
Leuctridae	25.00	12.00	7.33	20.00	24.67	24.67	3.67	2.00	3.33	23.33	13.33	2.67
Perlidae	0.67	0.33	0.00	0.67	0.00	0.33	0.33	0.00	0.33	3.00	0.33	0.67
Trichoptera												
Early Instar	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hydropsychidae	0.00	0.00	0.00	0.00	0.33	0.00	0.33	0.33	0.00	2.00	0.00	0.00
Leptoceridae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Philopotamidae	0.00	0.00	0.00	0.00	0.00	0.33	3.00	0.33	0.33	26.00	14.67	15.00
Polycentropodidae	0.67	1.67	0.33	0.33	1.00	1.33	3.33	0.00	0.00	7.33	1.67	0.33
Helicopsychidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.33	0.00
Non-Insects												
Chydoridae	20.33	17.00	10.00	17.33	61.00	77.67	3.00	0.33	2.33	11.00	3.00	1.33

Copepoda	407.33	304.33	623.67	839.00	789.33	931.67	273.33	197.67	452.00	982.67	797.33	1000.00
Crangonyctidae	0.00	0.33	0.00	0.33	0.00	0.33	0.33	0.33	0.33	0.00	0.00	0.33
Hydrachnidia	11.33	4.33	2.67	10.33	11.67	18.33	9.00	11.33	3.33	16.00	7.33	8.00
Ostracoda	30.00	44.33	29.67	93.33	347.00	171.67	15.33	16.00	24.00	12.67	107.67	84.33
Caecidotea	5.33	13.67	61.67	20.67	209.33	348.33	12.67	24.67	44.00	3.33	36.33	69.67
Asellus	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00
Prosobranchia	0.67	0.00	0.00	0.00	0.00	0.33	1.00	2.67	3.00	5.67	3.33	1.33
Oligochaeta	6.00	10.33	4.67	6.33	14.33	13.00	16.00	3.67	55.33	21.33	18.33	43.00
Turbellaria	0.00	0.00	0.00	0.67	1.33	0.00	0.00	0.33	0.67	0.33	0.67	0.00

Appendix A-4. Taxa list in individuals/8L sample, averaged across three replicate riffles. August.

	Aug 15 upwell 30	Aug 15 upwell 50	Aug 15 upwell 70	Aug 15 down 30	Aug 15 down 50	Aug 15 down 70
Coleoptera						
Elmidae	9.00	8.33	4.00	46.67	34.00	19.00
Psephenidae	0.33	0.33	0.00	23.67	0.67	0.00
Dytiscidae	0.00	0.00	0.00	0.00	0.00	0.00
Diptera						
Early Diptera	4.67	0.00	0.00	0.00	0.00	0.00
Chironomidae	266.33	165.67	144.67	535.00	373.67	260.00
Ceratopogonidae	0.00	0.00	0.00	5.00	3.67	3.67
Corethrellidae	0.00	0.00	0.00	0.00	0.00	0.00
Tipulidae	0.00	0.00	0.00	0.00	0.00	0.33
Simuliidae	0.00	0.00	0.00	0.00	0.00	0.00
Ephemeroptera						
Early Instar	1.00	0.00	0.33	20.33	0.00	0.00
Baetidae	0.00	0.00	0.00	1.00	0.00	2.67
Caenidae	1.00	0.67	1.00	49.33	28.00	4.67
Ephemeridae	0.00	0.00	0.00	0.00	0.00	0.00
Ephemerellidae	0.00	0.00	0.00	0.00	0.00	0.00
Heptageniidae	18.00	11.33	1.33	94.67	43.00	18.67
Isonychiidae	0.00	0.00	0.00	0.00	0.00	0.00
Leptohyphidae	0.00	0.00	0.00	0.00	0.00	0.33
Leptophlebiidae	0.33	1.67	0.67	9.67	13.33	2.00
Megaloptera						
Sialidae	0.00	0.00	0.00	0.00	0.00	0.00
Odonata						
Gomphidae	0.33	0.00	0.00	7.33	1.33	0.33
Coenagrionidae	0.00	0.00	0.00	2.33	0.00	0.00
Plecoptera						

Early Instar	0.00	0.00	0.00	0.00	0.00	0.00
Chloroperlidae	0.00	0.00	0.00	0.00	0.00	0.00
Leuctridae	2.67	3.67	8.33	29.00	20.00	6.67
Perlidae	5.00	1.00	3.00	4.33	5.33	3.00
Trichoptera						
Early Instar	0.00	1.67	0.00	0.00	0.00	0.33
Hydropsychidae	1.00	0.00	0.00	0.00	0.00	2.00
Leptoceridae	0.00	0.00	0.00	0.00	0.00	0.00
Philopotamidae	8.00	4.33	1.33	20.33	12.33	44.67
Polycentropodidae	5.67	3.67	5.00	6.33	3.00	2.33
Helicopsychidae	0.00	0.00	0.00	0.00	0.00	0.00
Non-Insects						
Chydoridae	1.00	3.33	9.00	10.67	9.00	10.00
Copepoda	502.67	451.00	573.33	1026.67	962.67	746.67
Crangonyctidae	0.00	0.00	0.67	0.00	0.00	0.67
Hydrachnidia	29.67	13.33	14.33	19.67	10.00	12.67
Ostracoda	43.00	66.33	337.00	37.33	119.00	134.67
Caecidotea	20.33	50.33	69.67	3.67	126.00	98.00
Asellus	0.00	0.00	0.00	0.33	0.33	0.00
Prosobranchia	0.00	0.67	1.67	2.67	6.33	0.67
Oligochaeta	5.67	5.67	9.00	15.67	34.33	54.67
Turbellaria	0.00	0.00	0.00	0.00	0.00	0.00

Appendix B. Environmental data from all samples

Environmental variables averaged across three replicate riffles. Data not collected marked with "--".

date	up/downwell	Depth (cm)	HH (mm)	Temp (C)	DO (%)	DO MG/L	AFDM (MG)
Oct 5	Upwell	30	53.5	--	--	--	69.6
Oct 5	Upwell	50	26.3	--	--	--	81.9
Oct 5	Upwell	70	34.0	--	--	--	99.3
Oct 5	Downwell	30	-42.7	--	--	--	236.6
Oct 5	Downwell	50	-13.7	--	--	--	187.8
Oct 5	Downwell	70	1.3	--	--	--	55.4
Nov 3	Upwell	30	35.0	--	--	--	127.3
Nov 3	Upwell	50	18.3	--	--	--	279.8
Nov 3	Upwell	70	51.7	--	--	--	131.6
Oct 27	Downwell	30	-3.0	--	--	--	329.2
Oct 27	Downwell	50	-3.3	--	--	--	311.1
Oct 27	Downwell	70	-20.0	--	--	--	214.4
Dec 5	Upwell	30	35.0	9.9	106.3	11.7	329.4
Dec 5	Upwell	50	-43.3	9.8	102.7	11.3	151.9
Dec 5	Upwell	70	28.3	9.9	94.3	10.3	211.0
Dec 5	Downwell	30	-15.0	10.2	109.7	11.9	110.3
Dec 5	Downwell	50	-28.3	10.2	108.7	11.8	145.1
Dec 5	Downwell	70	-36.7	10.2	107	11.6	80.7
Jan 8	Upwell	30	43.3	7.9	96.3	11.4	78.3
Jan 8	Upwell	50	38.3	7.8	95.3	11.2	111.2
Jan 8	Upwell	70	30.0	7.9	95.7	11.2	44.1
Jan 8	Downwell	30	-8.7	7.4	97	11.2	247.6
Jan 8	Downwell	50	-9.0	7.4	98.3	11.4	231.1
Jan 8	Downwell	70	-28.3	7.5	91.7	10.7	149.8

Jun 15	Upwell	30	-12.3	20.4	98.3	8.7	554.0
Jun 15	Upwell	50	-7.0	19.8	98.3	8.8	306.3
Jun 15	Upwell	70	4.7	19.4	96	8.6	81.0
Jun 15	Downwell	30	19.0	20.1	95.7	8.5	242.8
Jun 15	Downwell	50	8.3	18.8	91.3	8.3	343.9
Jun 15	Downwell	70	10.3	18.7	85.7	7.8	216.6
Jul 15	Upwell	30	5.0	23.6	92.7	7.6	221.7
Jul 15	Upwell	50	16.7	23.5	88.7	7.3	173.5
Jul 15	Upwell	70	15.0	23.7	92.3	7.6	271.2
Jul 15	Downwell	30	4.7	24.2	100	8.1	466.4
Jul 15	Downwell	50	-8.3	24.1	101	8.2	205.6
Jul 15	Downwell	70	-19.3	23.7	94.3	7.7	455.2
Aug 15	Upwell	30	20.0	26.1	98	7.7	135.2
Aug 15	Upwell	50	32.3	26	92.7	7.3	100.3
Aug 15	Upwell	70	24.0	26	98	7.7	163.0
Aug 15	Downwell	30	-1.3	26.4	101.7	7.9	184.3
Aug 15	Downwell	50	-9.3	26.3	107	8.3	136.4
Aug 15	Downwell	70	-11.0	26.4	106.3	8.3	152.1