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Importance of Instream Habitat to Recruitment and Community Structure of Aquatic Invertebrates

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IMPORTANCE OF INSTREAM HABITAT TO RECRUITMENT AND COMMUNITY STRUCTURE OF AQUATIC INVERTEBRATES

by

Catherine R. Billé

A Thesis

Presented to the Faculty of
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Master of Science in Biology

Approved: Adviser: Matth E. McTammany
Matthew McTammany

Department Chairperson: KF
Ken Field

Date

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Table of Contents

List of tables

List of figures

Abstract

This thesis investigates how mechanisms of colonization by aquatic insects, both by ovipositing adults and or larval drift, operate at either micro-, meso-, or macro-scales to influence larval community assemblage in streams. Our study took place in a forested floodplain stream characterized by uniform soft clay and loose detritus substrate. Within this study reach we built three sets of riffles, with each set comprised of three identical riffles built either 15, 10, or 5 m apart. We examined microscale influences on community assemblage by studying recruitment of egg masses to our constructed riffles. We found that riffle habitat additions were used by ovipositing insects and that oviposition behavior and habitat preferences varied across taxa. Mesoscale impacts on community assemblage were addressed by studying how riffle habitat isolation might impact total invertebrate abundance, along with aquatic insect taxa with different oviposition behaviors and larval mobility, within and below isolated riffle habitats. We found that impacts of habitat isolation in our stream were masked at the community level, as total invertebrate abundance did not vary significantly within or below isolated riffles; however, community composition varied by location. Oviposition behavior and larval mobility might be responsible for differences in community structure within and below isolated riffles, but discerning population dynamics requires further investigation. Finally, we focused on the macroscale impacts of habitat diversity on community assemblage by comparing invertebrate communities from mud habitat that was

characteristic to our study reach prior to our experiment with communities from our constructed riffles. We documented a 79% increase in taxa richness at the reach scale after adding riffle habitats to our study reach. We found mud and riffle habitats supported equally abundant and diverse communities of macroinvertebrates but with distinct taxonomic differences based on oviposition behavior and larval habitat preferences.

The results of these three studies suggest that benthic invertebrate communities in streams are influenced by processes operating at multiple life stages. In addition, abundance, distribution, and diversity of instream habitat directly influences abundance and composition of benthic invertebrate communities. Consequently, impairment of habitats preferred by adult or larval invertebrates could present barriers to colonization or population persistence within a stream. Therefore, stream restoration efforts aimed at recruiting and supporting diverse macroinvertebrate communities should include instream habitat diversity, including habitat for oviposition, amongst other primary concerns, such as water quality and best land-use practices. Furthermore, recovery of macroinvertebrate communities following restoration efforts that target improvements in water quality may not be fully observed if instream habitat quality and diversity remain low.

Overview of questions, hypotheses, and predictions

Chapter 1: Riffle habitat additions provide oviposition habitat

Recruitment of egg masses to riffle habitat additions

Question: Do riffle habitat additions facilitate aquatic insect recruitment?

Hypothesis: Riffle habitat additions facilitate aquatic insect recruitment by providing oviposition habitat to taxa preferring inorganic substrates to oviposit.

Prediction: If riffle habitat additions facilitate aquatic insect recruitment by providing a wider variety of oviposition habitat, then if we build riffles in a previously mud-bottomed stream we will find egg masses on substrate in riffles.

Oviposition behaviors and habitat preferences of various taxa

Question: How does oviposition habitat preference in riffles vary among taxa in our stream?

Hypothesis: Habitat preferences of taxa ovipositing in riffles in our stream might vary by rock emergence, rock size, location within the stream channel, and water velocity.

Prediction: If habitat preferences of taxa ovipositing in riffles within our study stream vary, then when we sample riffles for insect eggs and rear them for identification, we will find that egg masses of taxa are associated with unique combinations of the above habitat characteristics.

Chapter 2: Instream habitat isolation may influence spatial patterns of benthic communities

Effects of instream habitat isolation on benthic communities

Question: Does riffle habitat isolation negatively impact macroinvertebrate abundance in isolated riffles and or in habitat downstream of isolated riffles?

Hypothesis: Increased habitat isolation decreases abundance of macroinvertebrates in riffles, potentially as a result of decreased colonization by ovipositing adults or larval movement. Decreased abundance within riffles as a result of habitat isolation also results in decreased abundance of invertebrates in habitats downstream from riffles as a result of decreased export of individuals from riffle habitat.

Prediction: If riffle habitat isolation decreases abundance of macroinvertebrates in isolated riffles, then we will find lower invertebrate densities in the riffle sets with riffles spaced farther apart than in the riffle sets with riffles closer together. These patterns in abundance will also be reflected in habitat downstream from riffles.

Trait-based vulnerability to habitat isolation

Question: How do life history traits affect each taxon's response to habitat isolation?

Hypothesis: The effects of habitat isolation on abundance might be taxon-specific and depend, at least in part, on each taxon's oviposition behavior and larval mobility.

Prediction: We predict that oviposition behavior will determine initial distribution (nonselective leading to even distribution and selective oviposition potentially leading to patchy distribution) and larval mobility will determine whether a taxon can accumulate within and below isolated habitat. These traits are likely to interact, so we predict that distribution and abundance of predetermined model taxa will behave as such:

- Nonselective ovipositing and mobile larvae will be found throughout the study reach and either a) will not significantly differ in abundance below riffle sets or b) accumulate in abundance in downstream habitat as upstream habitat isolation decreases.
- Nonselective ovipositing and immobile taxa will be found throughout the reach but might not accumulate in abundance in downstream habitat as upstream habitat isolation decreases.

- Selectively ovipositing and mobile taxa abundance might be patchy depending on where they are oviposited, but will accumulate in abundance in downstream habitat as upstream habitat isolation decreases.
- Selectively ovipositing and immobile taxa abundance might be patchy depending on where they are oviposited but might not accumulate in abundance in downstream habitat as upstream habitat isolation decreases.

Chapter 3: Reach-scale effects of instream habitat diversity on benthic community structure

Question: Does instream habitat diversity influence macroinvertebrate community composition at the reach scale?

Hypothesis: Increasing instream habitat diversity will increase diversity of macroinvertebrate communities within a stream reach by providing greater variety of oviposition sites and larval habitat, thereby increasing recruitment and retention of macroinvertebrates.

Prediction: If instream habitat diversity increases diversity of macroinvertebrate communities within a stream reach, then when we increase habitat diversity in our study reach by building riffle habitat, we will see increases in richness and diversity metrics and changes in community composition related to oviposition behavior and larval habits.

Chapter 1 Riffle habitat additions provide oviposition habitat

1.1 Introduction

Benthic invertebrates are an incredibly diverse group of organisms that are integral to stream ecosystems and are an established indicator of stream health. Of these invertebrates, aquatic insects are unique in that most have complex life cycles with juveniles living in an aquatic environment before transitioning from the stream to live as terrestrial adults who end their life by laying eggs back into the water. This life cycle thus involves interactions with both aquatic and terrestrial environments, meaning that aquatic insects are subjected to and must survive within markedly different habitats. Both aquatic and terrestrial environments that aquatic insects inhabit present multiple bottleneck opportunities which have the potential to influence population size and distribution at each stage of life.

The focus of most research into stream invertebrates, especially on benthic community assemblage, has been on the biology and ecology of juvenile aquatic insects, as this is most often the longest period in their life cycle. However, much less is known about the ecology and biology of adult aquatic insects. Adult aquatic insects are often logistically complex to study because adults can be short-lived and because periods of emergence depend on many factors and are often site-specific, therefore making them difficult to predict. However, since recruitment of the next generation of aquatic insects relies on successful mating and oviposition, factors influencing population dynamics and behavior during the adult stage have important implications for subsequent generations and benthic community assemblage.

Oviposition (egg-laying) by most aquatic insects occurs by terrestrial adults returning to streams to lay their eggs in an aquatic environment. However, specific oviposition behaviors vary widely among aquatic insects and are often taxon-specific (Merritt et al., 2019). Oviposition behaviors range from “selective” ovipositors, which choose a site on which to carefully attach their eggs, to “non-selective” behaviors like bombing or splashing in which adults release their eggs indiscriminately into the water and eggs attach to substrates or organisms encountered throughout the stream (Encalada & Peckarsky, 2007). Oviposition behaviors are carried out across different stream habitats, and many taxa decide where to oviposit by certain habitat characteristics, such as substrate type or water velocity. Selective ovipositors may have high specificity of preferred oviposition habitat, and there is some evidence that taxa use rock size, rock emergence, location relative to the bank, and water velocity to select suitable oviposition sites (Reich & Downes, 2003; Macqueen & Downes, 2015).

Specificity and variety in selective oviposition behavior and habitat preference make the presence of diverse instream habitat vital for many insects to complete their life cycles. In streams impaired by sedimentation, instream habitat is often altered, simplified, or destroyed, which could potentially create a barrier against colonization by taxa with specific habitat needs for oviposition. Additionally, there are many taxa for which we know very little about their oviposition behaviors and habitat preferences. By understanding how instream habitat is used by a wider variety of selective ovipositors, we can better understand colonization dynamics and potential impact of habitat changes in impaired streams. This information could improve stream restoration projects whose goals may include, along with improving water quality, recruiting and supporting diverse communities of insects. Additionally, information about the influence of instream habitat on insect recruitment could provide insight into the efficacy of implemented restoration efforts, as benthic insects often serve as key indicators of stream health.

This study occurred in a forested floodplain stream characterized by uniform substrata comprised of soft clay and loose detritus. We constructed riffle habitats with the goals of facilitating aquatic insect recruitment by providing coarse rocky substrate as potential oviposition habitat, and adding to our knowledge of oviposition behaviors and habitat preferences. We hypothesized that riffle habitat additions would facilitate aquatic insect recruitment by providing habitat to taxa which oviposit on coarse inorganic substrates and predicted that we would find egg masses attached to various substrates within the riffles. We also hypothesized that oviposition habitat preference would vary among taxa whose eggs we found in riffles. We predicted that oviposition habitat preferences might be highly specific among certain taxa and would relate to factors like rock size, rock emergence, location relative to the bank, and water velocity. To accomplish these goals and investigate our hypotheses, we surveyed the constructed riffles for egg masses and documented characteristics of the locations where we found egg masses. In the lab, we reared the egg masses we found in order to identify which taxa laid the eggs and to describe each taxon's oviposition behavior, possibly for the first time, or for further details about preferred oviposition habitat of known selectively ovipositing taxa.

1.2 Methods

Study area

The study site was an unnamed 1st-order tributary to Chillisquaque Creek, located at Bucknell University Chillisquaque Creek Natural Area in Montour County, Pennsylvania (Figure 1). The tributary originates from a small pond on the property and flows through a floodplain forest where it expands into several large pools before narrowing and increasing in slope and velocity roughly 300 m above its confluence with Chillisquaque Creek.

Our study took place in the last 300 m of the unnamed tributary before it joins Chillisquaque Creek. Due to uniformity in depth and width, the entire reach is essentially "run" habitat, with virtually no areas that could be classified as "pool" or "riffle" habitats (Figure 2). We chose this site for our experiment because it lacked coarse inorganic and emergent substrate, making it an ideal location for us to manipulate the substrata and add riffle habitat.

Prior to our experiments, the invertebrate community in this small tributary consisted mainly of freshwater crustaceans (Amphipoda and Isopoda), worms (Oligochaetes), bivalves, Chironomidae (Diptera), and small populations of

Hydropsyche sp. and *Cheumatopsyche* sp. (Trichoptera) and Baetidae (Ephemeroptera).

Riffle construction

We built nine riffles in the study reach during summer 2019 and surveyed egg masses deposited on the substrate within the riffles. Each riffle was constructed by adding well-rounded sandstones and siltstones (1-3-inch diameter) to a 1-m long section spanning the entire width of the stream (Figure 3). Average water depth in constructed riffles was 3.0 ± 1.0 cm and ranged from 2.0-6.0 cm. We then added 12 large rocks (4-7-inch diameter), equally spaced across the stream and along the riffle, that emerged from the stream surface. Larger rocks introduced to each riffle provided equal total surface area of emergent habitat for oviposition. The submerged rocks and emergent rocks both provided potential habitat for colonization by ovipositing adults.

For the purpose of another study, we constructed riffles in sets of three, each set with three identical riffles. Riffle sets were built with different inter-riffle distances of either 15, 10, or 5 m and a had a 20 m “buffer” devoid of added coarse substrate (i.e. large rocks) separating riffle sets from each other (Figure 4). Insect perception of riffle aggregation might contribute to selection of oviposition sites and will be investigated in our results.

Following construction on August 1, 2019, we left riffles to be colonized by adult insects from the surrounding area for 6-10 weeks, before we surveyed rocks for egg masses in September-October 2019.

Water Quality

Our study occurred over ten weeks, from 1 August to 9 October 2019. Daily measurements of basic water quality indicators were taken at our study site for the duration of our experiment (Figure 5). Measurements of pH, temperature (°C), dissolved oxygen (mg/L), and specific conductance (µs/cm) were taken using a YSI Professional Plus Instrument (Pro Plus) with YSI Pro Series Quatro Field cable. Average pH was 8.06 ± 0.13 and ranged from 7.72-8.47. Temperature ranged from 12.5-22.7°C and averaged 17.76 ± 2.18°C. Dissolved oxygen ranged from 6.75-9.48 mg/L and averaged 8.35 ± 0.63 mg/L. Specific conductivity ranged from 244.6-297.7 µs/cm and averaged 278.85 ± 10.31 µs/cm. None of the water quality parameters changed significantly throughout the duration of our experiment and thus did not appear to influence macroinvertebrate communities.

Egg mass survey

Egg surveys were conducted on three sampling dates. Riffles spaced 5 m apart were sampled on September 10, 2019, riffles spaced 10 m apart were sampled on September 11, 2019, and riffles spaced 15 m apart were sampled on October 9, 2019. Within each of the nine constructed riffles we assigned three sampling points (left, center, right). At each sampling point an emergent rock and a submerged rock were identified and the following parameters were recorded: type and abundance of egg masses attached to rock (if any egg masses were found), rock size (maximum length and maximum width perpendicular to length measured in cm^2), location relative to right and left bank (m), and water velocity (m/s). Twenty-seven emergent rocks and twenty-seven submerged rocks were sampled in total.

For each type of egg mass found on a rock, a subsample was taken back to the lab, where eggs were incubated and reared to mature larvae and adulthood for identification purposes if possible. Each egg mass was put in a labelled rearing cup containing an air stone and bubbler, stream water, detritus from study stream, aquarium gravel, fish food, and fabric mesh extending above the water surface. Stream water was changed weekly at a minimum, and samples were checked daily (as eggs) and weekly (as larvae) for development. Pictures, notes, and samples of larvae and adults, if present, were taken at each check-in.

This egg survey occurred concurrently with studies observing the effects of riffle addition and habitat isolation on benthic macroinvertebrate communities. As a result, care was taken to minimize disturbance of constructed riffles during egg surveys. Researchers avoided stepping into the riffles and sampled egg masses by standing below riffles or on either bank. Removing egg masses from our riffles might have interfered with colonization and dispersal of larvae in our streams. However, we only sampled 25% of emergent rocks added to our study reach, and far less than 25% of the submerged rocks, which comprised the bulk of our riffles.

1.3 Data Analysis

Recruitment of egg masses to riffle habitat additions

We analyzed and visualized abundance of submerged and emergent rocks found with and without egg masses using RStudio and the R stats package (R Core Team, 2020). Average size of both rock types found with and without egg masses was compared with a t-test. Relative abundance of emerged and submerged rocks with egg masses sampled from the left bank, center of stream channel, and right bank was quantified and visually compared. Average water velocity at the location of each rock sampled was compared using a Kruskal-Wallis ANOVA on Ranks.

Oviposition behaviors and habitat preferences of various taxa

Oviposition habitat preference of specific taxa collected from rocks was assessed individually by observing characteristics described above for each egg mass found for which we could successfully rear and identify taxa.

1.4 Results

Recruitment of egg masses to riffle habitat additions

Of the 54 rocks we surveyed, we found 26 egg masses attached to 14 rocks in our constructed riffles (Figure 6). Twenty-three egg masses were found on 11 emergent rocks (three rocks had multiple egg masses attached to them), and the remaining three egg masses were found on three submerged rocks. Within the first set of riffles (riffles spaced 15 m apart), no egg masses were found in the upper or lower riffles, but four egg masses were found across three rocks in the middle riffle. Seven egg masses were found in the second riffle set (riffles spaced 10 m apart), three from two rocks in the middle riffle and four from three rocks in the lower riffle. Fifteen egg masses were found in the final set of riffles (riffles spaced 5 m apart) – ten egg masses across three rocks in the upper riffle, one egg mass in the middle riffle, and four egg masses across two rocks in the lower riffle.

Sizes of the 54 rocks we surveyed for egg masses are summarized in Figure 7. Rock size did not differ significantly between emergent rocks with egg masses (117.6 ± 26.1 cm^2) and emergent rocks without egg masses (130.8 ± 34.0 cm^2) (t-test, p = 0.2891). Similarly, rock size did not differ significantly between submerged rocks with egg masses (17.3 ± 3.1 cm^2) and submerged rocks without egg masses (17.0 ± 5.4 cm^2) (t-test, p = 0.9138).

Locations of rocks with egg masses in relation to either bank are summarized in Figure 8. Of the 11 emergent rocks found with egg masses attached to them, 27.3% were found near the left bank, 36.4% were found in the center of the channel, and 36.4% were found near the right bank. Of the 3 submerged rocks found with egg masses attached to them, one was found near the left bank, none were found in the center of the channel, and two were found near the right bank.

Water velocity did not vary significantly between submerged or emergent rocks with or without egg masses (Kruskal-Wallis ANOVA on Ranks, p = 0.8955) (Figure 9). Water velocity at emergent rocks with egg masses ranged from 0.02-0.41 m/s and averaged 0.15 ± 0.14 m/s. Water velocity at emergent rocks without egg masses ranged from 0.01-0.23 m/s and averaged 0.1 ± 0.07 m/s. Water velocity at submerged rocks with egg masses ranged from 0.03-0.2 m/s and averaged 0.13 ± 0.09 m/s. Water velocity at submerged rocks without egg masses ranged from 0.01-0.41 m/s and averaged 0.13 ± 0.11 m/s.

Oviposition behaviors and habitat preferences of various taxa

Of the 20 representative egg masses brought back to the lab, 11 were able to be reared to at least larvae for identification and 9 egg masses did not hatch. Eight genera from three orders were identified from the 11 egg masses that were able to be reared. Egg mass appearance and rocks used for oviposition by these genera are summarized in Figure 10. Egg mass appearance, characteristics of rocks used for oviposition, and presumed behaviors of these taxa are described below:

One *Baetis* sp. (Ephemeroptera) egg mass was found on an emergent rock in the seventh constructed riffle of our study reach. The rock was 70 cm^2 and found on the right bank of the stream channel. Water velocity at this rock was 0.35 m/s. Eggs of *Baetis* sp. were attached in a 4.5x2.0 mm semioval plate-like structure to the underside of the rock. Eggs were a brown-gold color and oval shaped. Two other egg masses of presumedly different taxa were also found on this rock, but the eggs from these masses did not hatch in the lab.

One *Hydropsyche* sp. (Trichoptera) egg mass was found on a 14 cm^2 submerged rock on the right bank of the sixth riffle in our study reach. Water velocity at the location of this rock was 0.17 m/s. The 5x5 mm egg mass was attached as a plate-like structure on the rock. Eggs were dark golden brown and a squat oval shape.

Three *Helopelopia* sp. (Chironomidae: subfamily Tanypodinae) egg masses were found on rocks in our constructed riffles. Egg masses consisted of small white oval-shaped eggs suspended in a ~4x4 mm sphere of a clear gel-like substance. One egg mass was oviposited on a 110 cm^2 emergent rock in the seventh riffle on the left bank of the stream channel where water velocity was 0.05 m/s. The other two egg masses were oviposited on a 108 cm^2 emergent rock in the sixth riffle in the center of the stream channel where water velocity was 0.02 m/s.

One *Natarsia* sp. (Chironomidae: subfamily Tanypodinae) egg mass was found attached to an emergent rock on the right bank of the fifth riffle in our study reach. The rock was 133 cm^2 and water velocity at that location was 0.04 m/s. The 7x5 mm egg mass consisted of a clear gel-like substance with what appeared to be newly hatched individuals suspended in the gel. No eggs were seen within the gel mass. One other egg mass of a presumedly different taxa was also found on this rock, but the eggs from this mass did not hatch in the lab.

One *Tanypus* sp. (Chironomidae: subfamily Tanypodinae) egg mass was found on a 117 cm^2 emergent rock on the left bank of the sixth riffle in our study reach. Water velocity at the location of this rock was 0.02 m/s. The 2x2 mm egg mass consisted of pointed oval-shaped beige eggs suspended in a clear globular jelly mass. One other egg mass of a presumedly different taxon was also found on this rock, but the eggs from this mass did not hatch in the lab.

One *Parametriocnemus* sp. (Chironomidae: subfamily Orthocladiinae) egg mass was found on a 99 cm^2 emergent rock in the second riffle of our study reach. The rock was found in the center of the stream channel where water velocity was 0.27 m/s. The egg mass consisted of small white eggs suspended in a globular clear jelly mass.

Two *Paratendipes* sp. (Chironomidae: subfamily Chironominae) egg masses were found on one 104 cm^2 emergent rock on the right bank of the second riffle in our study reach. Water velocity at the location of this rock was 0.03 m/s. The egg mass consisted of small white eggs suspended in a globular clear jelly mass.

One *Polypedilum* sp. (Chironomidae: subfamily Chironominae) egg mass was found on an 18 cm^2 submerged rock on the right bank of the second riffle in our study reach. Water velocity at the location of this rock was 0.03 m/s. The egg mass consisted of small white eggs suspended in a loose clear gel-like mass.

1.5 Discussion

Recruitment of egg masses to riffle habitat additions

As evidenced by the 26 egg masses found attached to substrates in constructed riffles, we can conclude that our riffle habitat additions facilitated recruitment of aquatic insects to our study stream by providing habitat to ovipositing adults. Observations of the type of substrates found with egg masses attached to them show that taxa can have unique microhabitat preferences within the same habitat type and that these preferences can vary even within the same family. In our stream at least, preferred oviposition habitat was predominantly emergent rocks and to a lesser extent, submerged rocks near either bank. Features such as whether a rock is emergent or submerged and the location relative to either stream bank may be predictors of whether microhabitat is used for oviposition, but they do not fully describe if a habitat is "ideal" for oviposition by most taxa as we found many rocks that fit these descriptions (n = 40) without egg masses attached to them.

Despite our attempts to maintain riffle habitat uniformity in our stream, oviposition seemed to be higher in riffles at the downstream end of our study reach, closer to the confluence with Chillisquaque Creek. This distribution of egg masses could be due to differing rates of oviposition between our sampling dates, as riffles closer to the confluence with Chillisquaque Creek were sampled September 10-11 and had higher egg mass abundances than the riffles that were further from the confluence and were sampled on October 9, 2019. Additionally, since our survey occurred over one month and we did not find evidence of any taxa ovipositing in both months, it is likely that patterns in egg mass distribution were the result of taxon-specific behaviors and habitat preferences varying between taxa ovipositing in September and October. Other possible explanations of the observed distribution of egg masses could be that riffle density, which increased closer to the confluence with Chillisquaque Creek, may have attracted more ovipositing adults. Additionally, oviposition might have decreased with distance from Chillisquaque Creek if adults flying upstream into our study stream from Chillisquaque Creek preferred to oviposit in the first riffles they encountered. Adult flight patterns are not well understood, and direction and distance of flight seems to be taxon-specific and dependent on environmental factors like surrounding landscape and weather conditions (Harris & McCafferty, 1977; Jackson & Resh, 1989; Macneale et al., 2004; Smith & Collier, 2006; Vebrova et al., 2018). Environmental factors such as lower water depth and faster flow conditions in riffles closer to the confluence with Chillisquaque Creek also might have made downstream riffles more appealing sites to ovipositing adults than upstream riffles. Water depth (cm) was significantly lower in the most downstream set of riffles (2.0 ± 0.0) compared to the first (3.7 ± 1.5) and middle riffle sets (3.2 ± 0.9) (Kruskal-Wallis ANOVA on Ranks, Dunn's test with Bonferroni correction, adjust p-value < 0.05). Additionally, water velocity (m/s), although not statistically significant was also highest in the final set of riffles (0.16 ± 0.135) compared to the first (0.128 ± 0.086) and middle riffle sets (0.086 ± 0.067). Adult perception of instream habitat, dispersion of flying adults, and environmental factors influencing oviposition behavior are areas needing more research.

Oviposition behaviors and habitat preferences of various taxa

We did not find egg masses in high enough abundance to make conclusions about whether oviposition habitat preferences, such as particular rock size, water velocity, or location relative to the bank, differed significantly in our study reach from previous reports of the taxa we found.

In our study reach, *Baetis* sp. and *Hydropsyche* sp. appeared to selectively oviposit on the underside of rocks in shallow riffles. Our observations of *Baetis* sp. oviposition habitat preference for emergent rocks, as well as our egg mass description agree with previous reports of this well-studied genus (*B. bicaudatus*: Peckarsky et al., 2000; Encalada & Peckarsky, 2006; *B. rhodani*: Lancaster et al., 2010; *B. alpinus*: Knispel et al., 2006). Although we did not directly witness adults ovipositing, the fact that we found the *Baetis* sp. egg mass on an emergent rock supports previous observations that this genus lands on emergent rocks before crawling below the stream surface to inspect the substrate and select a suitable site to oviposit (Peckarsky et al., 2000). Similarly, our observations of *Hydropsyche* sp. oviposition habitat preference for submerged rocks and egg mass appearance agree with previous reports of this genus (Fremling, 1960; Deutsch, 1984; Miller et al., 2020). As stated above, although we did not witness oviposition behavior directly, the fact that we found the *Hydropsyche* sp. egg mass on a submerged rock supports previous reports of this genus diving below the stream surface to oviposit (Deutsch, 1984).

The remaining six genera belonged to the family Chironomidae, which is incredibly diverse. Like most aquatic insects, more is known about the ecology and biology of its larval stages than about oviposition behaviors and egg mass descriptions. While there are some instances of unique oviposition behaviors and egg mass forms from chironomids (Funk et al., 2018), most documentation of Chironomidae egg masses describe them as eggs variably suspended within a jelly-like mass, called spumaline, often with an anchor-thread or extension of the jelly mass to attach eggs to solid structures in streams (Oliver, 1971; Williams, 1982). Egg mass shape and organization of eggs within the jelly mass are unique to different subfamilies, with Orthocladiinae and Diamesinae having linear egg masses with eggs arranged obliquely within the mass and Tanypodinae and Chironominae having round egg masses with eggs arranged peripherally or scattered within the mass (Oliver, 1971). The egg masses we found of Orthocladiinae (*Parametriocnemus* sp.), Chironominae (*Paratendipes* sp. and *Polypedilum* sp.), and Tanypodinae (*Natarsia* sp., *Tanypus* sp., and *Helopelopia* sp.) are consistent with these subfamily egg mass descriptions.

How Chironomidae egg masses end up attached to substrates is somewhat unclear, and likely varies by taxon. Some reports describe female chironomids ovipositing egg masses on rocks themselves (Armitage et al., 2012; Vallenduuk & Moller Pillot, 2013), but there are also reports which state that egg masses are released into the water and later attach to substrate (Williams, 1982; Armitage et al., 2012; Vallenduuk & Moller Pillot, 2013). Regardless of whether adults physically attached egg masses to the substrate or egg masses attached to rocks in our stream after deposition from the drift, all the Chironomidae egg masses in our stream, with one exception, were found on emergent rocks. This may suggest that adult Chironomids in our study reach landed on emergent rocks and crawled underwater to oviposit, much like the oviposition behavior displayed by *Baetis* spp. The one Chironomidae egg mass we found on a submerged rock belonged to *Polypedilum* sp., a genus known to oviposit into the drift with egg masses later attaching to the benthos (Williams, 1982). *Natarsia* sp. are suspected of ovipositing in moist soil (Vallenduuk & Moller Pillot, 2013), but our study suggests that this genus (but possibly a different species) might also oviposit in streams by attaching eggs to instream substrates.

Our study demonstrates that there is still much to learn about oviposition behaviors and habitat preferences of aquatic insects. In our study, every attempt was made to keep constructed riffles uniform. However, site selection by ovipositing adults still seemed to be patchy in our stream. This suggests that habitat characteristics other than the ones we controlled may be perceived by adult insects and contribute to their selection of oviposition sites. Sensory cues used by adults to identify habitat for oviposition vary by taxon and are likely as diverse as habitat preferences and oviposition behaviors (Lancaster & Downes, 2013). Adult aquatic insects may detect instream habitat for the purpose of oviposition from hierarchical spatial scales, such as by stream, habitat unit, and substrate type (Hoffmann & Resh, 2003). Additionally, adults may use visual (Reich & Downes, 2003; Encalada & Peckarsky, 2006), chemical (Trexler et al., 1998; Rejmankova et al., 2000), or mechanical (Reich & Downes, 2003; Encalada & Peckarsky, 2006) sensory cues to discern between habitats and choose an "ideal" site to lay their eggs. Furthermore, the extent to which selectively ovipositing taxa may deviate from their "preferred" oviposition habitat and oviposit on less "ideal" habitat in is unknown.

This study shows that instream habitats, like riffles, add opportunities for insects to lay their eggs, and could possibly lead to recruitment of taxa which previously were unable to oviposit due to lack of preferred oviposition habitat in streams. Any work that adds to our knowledge of oviposition behavior, habitat use for oviposition, and factors influencing availability of preferred oviposition habitat is important; however, we do not know how most insects oviposit or what their preferred oviposition habitat may be. Understanding how colonization of streams by ovipositing adults is facilitated by instream habitat is important, because recruitment of egg masses is likely to impact subsequent generations within a stream and might potentially add to our understanding of community dynamics and ecological processes. For example, recruitment of egg masses of *Baetis* sp., a genus with high oviposition site preference for large rocks in splash zones, could potentially be limited in streams where availability of this microhabitat varies with climate and hydrogeomorphology (Encalada & Peckarsky, 2006). Additionally, connections between *Baetis* sp. egg mass distribution and oviposition habitat availability can also influence distribution and abundance of larvae hatching from those eggs (Lancaster et al., 2011). Studies examining habitat preferences of a wider variety of taxa and studies examining factors that influence microhabitat availability in streams would be helpful in discerning how recruitment of taxa may be limited in streams impaired by land-use practices, such as sedimentation, where instream habitat is often simplified, altered, or destroyed.

Chapter 2 Instream habitat isolation may influence spatial patterns of benthic communities

2.1 Introduction

Aquatic macroinvertebrates are an incredibly diverse group of organisms whose presence and abundance are critical to healthy stream ecosystems. Persistence of macroinvertebrate taxa within a stream reach depends on the presence of a variety of instream habitats, like riffles, which serve as habitats as well as conduits for colonization, either by ovipositing (egg-laying) aquatic insects or drifting juveniles. As riffles often serve as a source of drifting taxa to downstream habitats, impacts to macroinvertebrate communities in upstream habitats are likely to affect downstream communities as well.

Sediment loading caused by upstream erosion and long-standing legacy sediments is one of the leading impairments of water quality in streams and rivers in the United States (United States Environmental Protection Agency, 2017). Legacy sediments are standing deposits of sediment in streams that arrived during historical land-use activities, such as mill-damming and agricultural erosion in the 17th to early 20th century (Walter & Merritts, 2008). Upstream erosion of stream banks and cleared and developed land adds additional sediment loads to streams already impaired by legacy sediments. Continued influx of sediments from eroding banks impairs water quality, disrupts flow regimes, and destroys substrate heterogeneity (Thoms, 1987; Doeg & Koehn, 1994; Walter & Merritts, 2008). Loss of substrate heterogeneity in streams impaired by sedimentation can isolate areas of coarse inorganic substrate, like riffles. Isolation of riffles by distance is likely to be particularly harmful to taxa reliant on coarse substrate for oviposition and larval habitat.

Oviposition behavior by aquatic insects, the manner in which terrestrial adults return to streams to lay their eggs, varies by taxon and ranges in use of and reliance on instream habitat. Non-selective ovipositors are taxa that indiscriminately release their eggs into the water column, while selective ovipositors choose a particular site, such as an emergent rock within a riffle, on which to adhere their eggs (Lancaster & Downes, 2013). Habitat isolation might influence where adults oviposit, if they are able to at all, and result in isolated patches of egg masses. Increasing preferred oviposition habitat increases egg masses and thus early instar juveniles of selectively ovipositing taxa (Lancaster et al., 2010, 2011; Encalada & Peckarsky, 2012). Conversely, impairment of instream habitat due to changes in hydrology in regulated rivers or anthropogenic structures can impact egg mass recruitment (Miller et al., 2020) and adult dispersal (Blakely et al., 2006), both of which are likely to impact larval abundance. Specificity in oviposition habitat might make certain taxa, like those who only oviposit on large emergent rocks, particularly vulnerable to human activities that alter, simplify, or destroy instream habitat, like sedimentation from land disturbance, as patterns in egg masses may persist as larvae grow and develop if populations are isolated from each other.

Additionally, larval mobility could determine population dynamics within and between isolated habitat patches, as some less-mobile taxa may not be able to move successfully from natal habitat to other locations with suitable habitat in the stream. Habits, or modes of life that have specific adaptations and behaviors, make organisms better suited to live in certain environments and indicate mobility and the potential for individuals to colonize downstream habitats by drifting, either actively or passively, to new locations. Habits are taxon-specific, and certain taxa, like baetid mayflies, are considered strong swimmers capable of dispersing easily via drift, though drifting capabilities of aquatic insects in general needs more research (Lancaster et al., 2011). Mobility varies by taxon and determines, in part, whether an individual will be able to successfully travel between desired habitats in a stream. Therefore, organisms moving via the drift depend on presence of downstream habitats suited to their habits, much like adults rely on preferred oviposition sites, to persist within stream reaches.

With this study, we manipulated riffle density by constructing sets of gravel riffles spaced different distances apart in a stream reach previously devoid of rocky habitat to investigate whether riffle habitat isolation negatively impacts total invertebrate abundance and abundance of specific taxa within and below isolated riffles. We hypothesized that isolation of riffle habitat (based on distance between constructed riffles) would also isolate invertebrate communities from each other and potentially disrupt colonization dynamics which might negatively impact total invertebrate abundance within the riffles themselves, as well as in habitats downstream of isolated riffles. We predicted that benthic invertebrate density and diversity would be higher within and below riffles that were less isolated (i.e. spaced more closer together) than within and below riffles that were more isolated (i.e. spaced farther apart).

Additionally, we hypothesized that riffle habitat isolation might affect oviposition distribution or larval movement of certain taxa, which could reduce abundances of specific taxa within the riffles themselves, as well as in habitats downstream of isolated riffles. We predicted that oviposition behavior would determine initial distribution of taxa among riffle habitats (non-selective behaviors resulting in even distribution and selective behaviors potentially leading to patchy distribution) and that larval mobility would determine whether taxa could accumulate within isolated habitat and locations downstream of isolated habitat. Life history traits, like oviposition behavior and larval mobility, are likely to vary in combination among taxa and interact to influence spatial patterns of taxa in habitats with varying degrees of isolation. We predicted that the distribution and abundance of predetermined model taxa would behave such that nonselective ovipositors with mobile larvae would be moderately to highly abundant and evenly distributed throughout the study reach, regardless of level of riffle habitat isolation. We expected similar results for nonselective ovipositors with immobile larvae; however, these taxa might be less abundant than more mobile nonselective ovipositors. We predicted that distribution of selective ovipositors could potentially be patchy throughout our study reach depending on where they oviposit, but that taxa with mobile larvae might accumulate more below areas with low habitat isolation while taxa with immobile larvae might not be able to accumulate due to isolation distance exceeding their drifting capabilities.

2.2 Methods

Study area

Our study was conducted in an unnamed 1st-order tributary to Chillisquaque Creek located at Bucknell University Chillisquaque Creek Natural Area in Montour County, Pennsylvania (Figure 1). The tributary originates from a small pond on the property and flows through a floodplain forest where it expands into several large pools before narrowing and increasing in slope and velocity roughly 300 meters above its confluence with Chillisquaque Creek.

Our experiment took place in the last 300 meters of the unnamed tributary before it joined Chillisquaque Creek. This portion of the stream is 1-2 m wide and characterized by a relatively uniform channel shape with substrata composed of soft clay and loose detritus (Figure 2). Due to uniformity in depth and width, the entire reach is essentially “run” habitat, with virtually no areas that could be classified as “pool” or “riffle” habitats. We chose this site for our experiment because it lacked coarse inorganic and emergent substrate, making it an ideal location for us to manipulate the substrata and add riffle habitat.

Prior to our experiment, the invertebrate community in this small tributary consisted mainly of freshwater crustaceans (Amphipoda and Isopoda), worms

(Oligochaetes), bivalves, Chironomidae (Diptera), and small populations of *Hydropsyche* sp. and *Cheumatopsyche* sp. (Trichoptera), and Baetidae (Ephemeroptera).

Riffle construction

To study how instream habitat influences distribution of larval insects, we built nine riffles in the study reach during summer 2019. Each 1-m long riffle was constructed using 1-3-inch diameter well-rounded sandstones and siltstones, which we added to span the entire width of the stream (Figure 3). Average water depth in constructed riffles was 3.0 ± 1.0 cm and ranged from 2.0-6.0 cm. We then added 12 large rocks (4-7-inch diameter), equally spaced across the stream and along the riffle, that emerged from the stream surface. Larger rocks introduced to each riffle provided equal total surface area of emergent habitat for oviposition. The submerged rocks and emergent rocks both provided potential habitat for colonization by ovipositing adults.

To investigate the impact of habitat isolation on benthic macroinvertebrate community structure, riffles were built in sets of three, with each set comprised of three identical riffles. Riffle sets were built with different inter-riffle distances of either 15, 10, or 5 m and included a 20-m "buffer" devoid of added coarse emergent substrate (i.e. large rocks) to separate sets of riffles from each other (Figure 4). Lengths of inter-riffle distances within riffle sets and of the buffer regions between riffle sets were determined based on previous reports of insect drift distances, which typically ranged from 2-10 m (Naman et al., 2016; Elliot, 2003). Our study design also included an unaltered control reach upstream of the section to which we added riffle habitat. Following construction, riffles were left to be colonized by insects from the surrounding area for two weeks prior to the start of sampling.

Water Quality

Our study occurred over nine weeks, from 1 August to 2 October 2019. Daily measurements of basic water quality indicators were taken at our study site for the duration of our experiment (Figure 5). Measurements of pH, temperature (°C), dissolved oxygen (mg/L), and specific conductance (µs/cm) were taken using a YSI Professional Plus Instrument (Pro Plus) with YSI Pro Series Quatro Field cable. Average pH was 8.062 ± 0.13 and ranged from 7.72-8.47. Temperature ranged from 12.5-22.7°C and averaged 17.76 ± 2.18°C. Dissolved oxygen ranged from 6.75-9.48 mg/L and averaged 8.35 ± 0.63 mg/L. Specific conductivity ranged from 244.6-297.7 µs/cm and averaged 278.85 ± 10.31 µs/cm. None of the water quality parameters changed significantly throughout the duration of our experiment and thus did not appear to influence macroinvertebrate communities.

Survey of benthic communities

In order to avoid disturbing constructed riffles, we used rock baskets (23.5 x 15.0 x 5.0 cm) to sample benthic invertebrates below each riffle, below each set of riffles, and from the upstream control reach (Figure 4). Rock baskets were filled with the same small rocks used to construct the bulk of our riffles (Figure 11). Rock baskets were fully submerged and monitored throughout our experiment to ensure that they did not provide emergent habitat for ovipositing adults. Samples below each riffle were used to study the effects of habitat isolation on macroinvertebrate communities within riffle habitats, with individual riffles serving as replicates for each set of riffles. Samples taken 5 m below each set of riffles and the upstream control were used to study the effects of habitat isolation on communities in habitats downstream of riffle habitat, with multiple samples below each set of riffles serving as replicates. Rock baskets were deployed at the time of riffle construction so that samples would reflect colonization processes (i.e. drift and oviposition) occurring in and below riffle sets throughout the duration of the experiment.

Rock baskets were collected every two weeks starting August 20, 2019, approximately two weeks after riffles were constructed and rock baskets were deployed, and continued until October 2, 2019. On each sampling date, samples were taken by removing rock baskets and rinsing material from the rocks into a 250-μm sieve until rocks were free of macroinvertebrates. Macroinvertebrates were transferred from the sieve to plastic containers and preserved in 4% formalin.

To determine larval mobility, we suspended aquarium nets in the water column to collect organisms actively moving via the drift throughout the study reach. We deployed drift nets at the same locations we collected benthic samples (below each riffle, below each set of riffles, and from the upstream control reach) (Figure 12). Drift samples were collected the day prior to rock basket sampling, starting August 19, 2019 and continuing until October 1, 2019. On each sampling date drift nets were deployed for 2-3 hours starting between 12:00-2:30pm. Water velocity and water depth at each drift net was measured. After 3 hours, material was emptied from nets and preserved in 4% formalin.

In the lab, benthic and drift samples were sorted under a dissecting microscope, identified to the lowest practical taxonomic unit (usually genus), counted, and preserved in 80% ethanol. Drift density was calculated at the number of individuals traveling per cubic meter per second.

2.3 Data Analysis

Macroinvertebrate abundance data generated from these samples were analyzed using R, RStudio, and various packages. All figures were made using ggplot2 (Wickham, 2016). Due to time and resource constraints, only samples from the first and last sampling dates were processed and analyzed.

Effects of instream habitat isolation on benthic communities

To test whether increased habitat isolation decreased abundance of macroinvertebrates within riffles, abundance of organisms from samples collected from rock baskets within each set of riffles was compared by conducting a two-way ANOVA with riffle set and sampling date as independent factors using the R stats package (R Core Team, 2020). To test whether habitat isolation decreased abundance of macroinvertebrates in habitats downstream of riffles, abundance of invertebrates collected from rock baskets below sets of riffles and the upstream control were compared on each sampling date by conducting one-way ANOVA and

Kruskal-Wallis ANOVA on Ranks using the R stats package (R Core Team, 2020). Non-metric Multi-dimensional Scaling (NMDS) was used to visualize dissimilarity of communities within and below sets of riffles from both sampling dates and was run using the vegan package (Oksanen et al. 2020). Statistical differences between community composition of samples taken within and below riffle sets on both sampling dates were assessed using an Analysis of Similarities (ANOSIM) (Oksanen et al., 2020). Following the ANOSIM, an Indicator Species Analysis (ISA), from the indicspecies package, was performed to determine which taxa significantly contributed to differences found among benthic invertebrate communities (De Caceres & Legendre, 2009). We calculated richness and diversity indices for rock baskets sampled within riffle sets and for rock baskets below each riffle set from both sampling dates. Richness was calculated as the number of unique taxa per sample. Community diversity was quantified using two indices, Shannon diversity and Simpson's diversity, which describe community diversity by quantifying richness and evenness of taxa. Comparisons of specific taxa found within riffles and/or below riffle sets were made and discussed further.

Trait-based vulnerability to habitat isolation

We hypothesized that taxa would be affected differently by habitat isolation based on oviposition behavior and larval mobility, but this information is not known for most taxa. Because of this, we decided to use distributions of four model taxa found in our study reach with known oviposition behaviors and larval mobilities to examine whether particular oviposition behaviors or degree of larval mobility make certain taxa more vulnerable to habitat isolation.

To choose model taxa, we screened taxa significantly associated with community differences from NMDS and ISA results to find those taxa with known oviposition behaviors. We then used the drift samples to define larval mobility because estimated drift distances are highly uncertain for most taxa. Some studies have even shown that taxa presumed to be highly mobile, like *Baetis rhodani*, do not travel distances as far as previously thought (Lancaster et al., 2011). Taxa found in drift samples collected below sets of riffles were defined as mobile, and taxa not found in those drift samples were defined as immobile. Although our drift samples provided a general idea of which taxa were moving throughout our study reach, our drift samples most likely did not provide a full picture of the drifting community because our samples were taken in late afternoon and not at night when most taxa drift (Waters, 1972).

These efforts to find model taxa resulted in a list of four insect taxa representing four functional groups: taxa with nonselective oviposition behavior and mobile or immobile larvae and taxa with selective oviposition behavior and mobile or immobile larvae. *Baetis* sp., although present in our stream and widely used to study oviposition behavior and interactions with instream habitat, were found in such low abundance that we were unable to use them as a suitable model taxon for this study.

To test whether oviposition behavior and/or larval mobility increased a taxon's vulnerability to habitat isolation, relative abundance of each model taxon from samples below sets of riffles and the upstream control were compared using a one-way ANOVA or Kruskal-Wallis ANOVA on Ranks with the R stats package (R Core Team, 2020). We chose to look at spatial patterns and abundances of taxa in habitats below riffle sets because these samples represented the cumulative impact of habitat isolation in streams. Relative abundance of each model taxon based on riffle set was calculated by dividing abundance of that taxon from a specific sampling location by total abundance of that taxon within the study reach on the date those samples were collected.

2.4 Results

Effects of instream habitat isolation on benthic communities

Invertebrate abundance in benthic samples taken within sets of riffles was not significantly different (Figure 13) (two-way ANOVA, p-value = 0.1378), but invertebrate abundance was significantly higher on the last sampling date in October than on the first sampling date in August (p-value = 0.0432). Although there was no statistically significant difference in average abundance based on riffle set, there was a visual decline in average abundance as inter-riffle distance (i.e. habitat isolation) decreased, which contradicted our original hypothesis.

NMDS (Figure 14) based on benthic invertebrate communities within riffle sets showed separation of samples based on sampling date and, to a lesser extent, riffle set (i.e. degree of habitat isolation). ANOSIM test based on Bray-Curtis dissimilarity determined that community composition differed significantly between sampling dates (p-value = 0.001). ISA determined that Isopoda were significantly more abundant in samples taken on the first sampling date in August (p-value = 0.0075), while Ceratopogonidae, *Lype diversa*, and Chironomidae larvae were more abundant in samples taken on the final sampling date in October (p-values = 0.0004, 0.0311, and 0.0125, respectively). Additionally, community structure differed significantly among riffle sets (i.e. degree of riffle isolation) (ANOSIM test based on Bray-Curtis dissimilarity, p-value = 0.048). Oligochaeta, *Dubiraphia* sp., *Stenelmis* sp., and Elmidae larvae (too small to be identified to genus) abundances were significantly higher in the set of riffles spaced 15 m apart where habitat isolation was the highest (ISA, p-values = 0.0025, 0.0056, 0.0397, and 0.0354 respectively). Additionally, *Stenonema/Stenacron* sp. larvae were significantly more abundant in the set of riffles spaced 5 m apart where habitat isolation was lowest (ISA, p-value = 0.0151).

Invertebrate abundance in rock basket samples taken below each set of riffles and from the upstream control reach (Figure 15) was not significantly different (August: Kruskal-Wallis ANOVA on Ranks, p-value = 0.2815; October: one-way ANOVA, p-value = 0.255). Although there was no statistically significant difference in abundance based on upstream inter-riffle distance on either sampling date, abundance was more than twice as high in habitats downstream from sets of riffles with higher riffle density (i.e. decreased habitat isolation).

Community composition from habitats below each set of riffles differed based on upstream riffle set (i.e. degree of upstream habitat isolation) and sampling date (NMDS, Figure 16). There was a significant difference in community composition based on sampling date (ANOSIM, p-value = 0.043), with Isopoda significantly more abundant in samples in August (ISA, p-value = 0.0113) and Ceratopogonidae, *Tipula* sp., *Pseudolimnophila* sp., *Hydatophylax/Pycnopsyche* sp., and Ancylidae significantly more abundant in October (ISA, p = 0.0003, 0.0141, 0.0068, 0.027, and 0.0061 respectively). There was also a statistical difference between communities based on upstream riffle set (i.e. degree of riffle isolation) (ANOSIM, p = 0.001). Planariidae, *Triaenodes* sp., Nemertea, *Stenelmis* sp., and small Elmidae larvae abundances were significantly higher in the upstream control (ISA, p-values = 0.0033, 0.0076, 0.0405, 0.0067, 0.0188, respectively). *Calopteryx* sp. and *Stenelmis crenata* adults were significantly more abundant in habitat downstream of the riffle set spaced 10 m apart (ISA, p = 0.0003 and 0.0446, respectively). Chironomidae pupae, Hirudinea, Bivalvia, *Clinocera* sp., and *Molanna* sp. were significantly more abundant in habitat downstream of the riffle set spaced 5 m apart (ISA, p = 0.0021, 0.0115, 0.0025, 0.04, and 0.0397, respectively). Amphipoda and Oligochaeta were significantly more abundant in habitats downstream of riffle sets spaced 15 m and 10 m apart (ISA, p = 0.0258 and 0.03, respectively). Isopoda was significantly more abundant in habitat below all three riffle sets than in the upstream control reach (ISA, p-value = 0.0389). Finally, *Cheumatopsyche* sp. were significantly more abundant in samples collected downstream of riffle sets spaced 10 m and 5 m apart (ISA, p = 0.0119).

Richness and diversity metrics of benthic communities within riffles did not differ significantly among the three sets of riffles (Table 1). However, richness in samples collected below the set of riffles spaced 5 m apart (25.67 ± 8.26) was significantly higher than richness downstream from riffles spaced 15 m apart (15.67 ± 3.88) (Table 2, ANOVA with post-hoc Tukey test, p = 0.032). No other significant differences of diversity metrics were detected within or below riffle sets.

Distribution of taxa in samples taken within and below riffle sets was complex. Some taxa were present in every sample we took, while other taxa were found within a riffle set, but not in the habitat below that riffle set and vice versa (See Table 5 in Appendix for raw abundance of taxa with patchy distributions). While this patchiness in distribution may be due to patterns induced by maternal behaviors, larval movement, or habitat filtering, it is hard to make conclusions about these taxa as they occurred in low abundances. Additionally, it is likely that our study captured ongoing colonization processes, and changes in community composition at a given site, especially of rare taxa, are likely to frequently change.

Trait-based vulnerability to habitat isolation

To better understand the effects of oviposition behavior and larval mobility as potential drivers of spatial patterns in macroinvertebrate abundance and distribution, we chose to compare abundances of 4 model taxa among rock basket samples taken below each set of riffles and from the upstream control (Figure 17). Model taxa abundance did not vary by sampling date, so data from the first and final sampling dates were combined for analysis.

Diphetor hageni (Ephemeroptera) served as a model taxon for insects with nonselective oviposition behavior and mobile larvae. Relative abundance of *D. hageni* was significantly higher in habitat below the set of riffles built 10 m apart (18.61 ± 14.37) than in the upstream control (none found) (Kruskal-Wallis ANOVA on Ranks, $p = 0.0079$, Dunn's test with Bonferroni corrections, adjusted $p < 0.05$). Relative abundance of *D. hageni* in habitat below riffles built 15 m apart (6.44 ± 6.15) and in habitat below the set of riffles built 5 m apart (8.29 ± 10.46) did not differ significantly from relative abundance of *D. hageni* in any other habitat.

Stenonema/Stenacron sp. (Ephemeroptera) served as a model taxon for insects with non-selectively ovipositing adults and immobile larvae. Relative abundance of *Stenonema/Stenacron* sp. did not vary significantly in samples taken from rock baskets below the set of riffles built 15 m apart (none), 10 m apart (1.14 ± 1.90), 5 m apart (15.53 ± 19.83), or from the upstream control (16.67 ± 34.31) (Kruskal-Wallis ANOVA on Ranks, $p = 0.2639$).

Cheumatopsyche sp. (Trichoptera) served as a model taxon for insects with selectively ovipositing adults and mobile larvae. Relative abundance of *Cheumatopsyche* sp. was significantly higher in rock baskets below the set of riffles built 10 m apart (19.38 ± 13.1) than in the upstream control (0.11 ± 0.19) and below the set of riffles built 15 m apart (0.61 ± 1.16) (Kruskal-Wallis ANOVA on Ranks, p =

0.0016, Dunn's test with Bonferroni corrections, adjusted $p < 0.05$). Relative abundance of *Cheumatopsyche* sp. did not differ significantly between rock baskets below the set of riffles built 10 m apart (19.38 ± 13.1) or 5 m apart (13.23 ± 14.83).

Calopteryx sp. (Odonata) served as a model taxon for insects with selectively ovipositing adults with immobile larvae. Relative abundance of *Calopteryx* sp. was significantly higher below the set of riffles built 10 m apart (23.27 ± 9.41) than the upstream control (1.55 ± 2.4) and in habitat below the set of riffles built 5 m apart (3.1 ± 3.75) (Kruskal-Wallis ANOVA on Ranks, $p = 0.0011$, Dunn's test with Bonferroni corrections, adjusted $p < 0.05$). Relative abundance of *Calopteryx* sp. below riffles built 15 m apart (5.41 ± 3.39) did not differ significantly from relative abundance of *Calopteryx* sp. in any other habitat.

2.5 Discussion

Effects of instream habitat isolation on benthic communities

Addition of riffle habitat did promote colonization of macroinvertebrates to our study reach over the course of our experiment, and occurred as soon as two weeks after riffle construction. Habitat isolation did not seem to have a significant effect on invertebrate abundance in the constructed riffles, as total abundance did not increase in rock baskets within sets of riffles with decreased levels of riffle isolation. Variability within these samples was quite high, suggesting that colonization dynamics in our study reach were stochastic and likely differed by taxon.

Varying degrees of isolation in upstream riffle habitat also did not significantly impact total abundance of macroinvertebrates in downstream locations. Differences in abundance were not statistically significant, but abundance was approximately 2x higher below riffles spaced 5 m apart than below riffles spaced 15 m apart. Abundance in rock basket samples taken below sets of riffles, particularly on the first sampling date, was highly variable, which may have prevented detection of significant patterns. Larger sample sizes may have helped capture and clarify differences in benthic communities below each set of riffles.

The results of the NMDS plots and subsequent ANOSIM and ISA analyses of invertebrate communities within and below riffle sets showed that the effects of habitat isolation on macroinvertebrate communities are taxon-specific and that abundance and distribution of certain taxa varied within and below riffle sets. Taxon-specific responses to habitat isolation might be obscured by community level response (i.e. total invertebrate abundance). For example, our samples likely captured early stages of emergence and mating of certain taxa, like Ceratopogonidae, Chironomidae, *Tipula* sp., *Pseudolimnophila* sp., *Hydatophylax/Pycnopsyche* sp., *Lype diversa*, and Ancylidae, as their abundance in our samples increased over the course of our sampling efforts. Other taxa, like Isopoda, might already have completed periods of high reproduction by the time we started our sampling, as these taxa decreased in abundance between our sampling dates, which might be attributed to habitat choice, competition, or predation. Other taxa, like *Stenonema/Stenacron* sp., *Cheumatopsyche* sp., *Molanna* sp., Hirudinea, Bivalvia, and *Clinocera* sp., might have been dispersal limited as larvae, as they were only found in high abundance below habitats where riffle isolation was lower. Conversely, Isopoda did not seem to be negatively impacted by upstream levels of habitat isolation, as they were highly abundant below all riffle sets. Other taxa, like Oligochaeta, *Triaenodes* sp., *Dubiraphia* sp., *Stenelmis* sp., small Elmidae larvae, Planariidae, and Nemertea were patchy throughout our stream, and we do not have a clear explanation for this distribution.

Colonization mechanisms, such as oviposition behavior and drift, or invertebrate habitat preferences can influence community structure and composition and may result in patchy distribution of taxa within a stream reach. Mechanisms of colonization are not easy to document, and because multiple mechanisms likely shape community structure and composition, discerning the influence of one mechanism, such as maternal behavior or organism movement, is difficult. This difficulty in attributing colonization mechanisms to organism distributions is why we chose to use model aquatic insect taxa with known life history traits to assess the relative importance of oviposition behavior and larval mobility on spatial patterns of aquatic insect larvae.

Trait-based vulnerability to habitat isolation

Relative abundance of model taxa in habitats below varying degrees of riffle habitat isolation might be determined, at least in part, by maternal behavior and larval mobility. None of our model taxa, except *Stenonema/Stenacron* sp., were significantly more abundant within any of the sets of riffles. Therefore, we used larval mobility and oviposition behaviors to explain patterns in larval abundance in habitats below riffle sets.

Abundance and distribution of our model taxa did not behave exactly as we predicted them to behave based on their maternal behaviors and larval mobilities. For example, relative abundance of most model taxa was lower than expected downstream of the set of riffles with riffles built 5 m apart. To us, this suggests that larval abundance was influenced by factors other than maternal behavior and larval mobility within this habitat. One possibility is that environmental conditions known to influence larval distribution, like flow and water depth, may have differed from habitats further upstream in our study reach and influenced abundances within this riffle set.

While we expected non-selective ovipositors to be found throughout the reach, we expected taxa with mobile larvae to be found in either equally high abundance regardless of upstream riffle habitat isolation or in higher abundance below habitats with low riffle isolation. *D. hageni* was indeed found throughout our study reach, except in the upstream control; however, *D. hageni* was significantly more abundant in habitat below the set of riffles spaced 10 m apart than any other habitat. Although this result was unexpected, abundance of *D. hageni* in habitat below riffle sets did not seem to be linked to abundance of *D. hageni* within riffle sets. We expected *D. hageni* to be mobile enough to accumulate in habitat downstream of riffles built 15 m apart, as this taxon is usually thought of as a strong swimmer. However, despite larvae being abundant within riffles built 15 m apart, larvae were not more abundant in habitat below these riffles. Therefore, abundance of *D. hageni* larvae could be locally variable due to habitat preference and not evenly distributed due to drift. It is also possible that drift distances of *D. hageni* have been overestimated, and therefore, *D. hageni* larvae might not be expected to colonize habitats uniformly across our study reach. In a different study, drift distances of *Baetis rhodani*, another Baetidae, were much shorter than expected for a species typically described as a strong swimmer (Lancaster et al., 2010). These findings support that drift capabilities of some taxa may be lower than previously assumed, even for taxa usually described as strong dispersers. Because capability and propensity for larvae to drift are taxon-specific, the scale at which a taxon is vulnerable to habitat isolation might also be taxon-specific. Further studies that incorporate active and passive drift and investigate drifting distances of taxa would be valuable in discerning population vulnerability to habitat isolation.

Observations of *Stenonema/Stenacron* sp. suggest that non-selective oviposition behavior may also result in patchy initial distribution of larvae. We predicted that *Stenonema/Stenacron* sp. larvae would be found in even distribution regardless of upstream levels of habitat isolation, because adults release their eggs onto the surface of the water (McCafferty & Huff, 1974, 1978). *Stenonema/Stenacron* sp. larvae were found sporadically in our study reach, which suggests that oviposition of this taxon did not occur evenly throughout our study reach, as we assumed would happen for all non-selective ovipositors. Although

Stenonema/Stenacron sp. are thought to dispense their eggs into the water without discretion, there may be habitat preferences for where they release their eggs or where eggs attach once released that could have resulted in uneven distribution of larvae in our study reach. *Stenonema/Stenacron* sp. larvae were not found within or below riffles built 15 m apart or within riffles built 10 m apart; however, they were found below riffles built 10 m apart, suggesting that, like *D. hageni*, presence of *Stenonema/Stenacron* sp. at a given location might not rely on presence in upstream habitats. Although not significant, we did see an increase in abundance of *Stenonema/Stenacron* sp. below riffles built 5 m apart. Larvae were significantly more abundant within these riffles than any other riffle set, so larvae may have been mobile enough to traverse 5 m and colonize our rock baskets downstream. Although abundance and distribution of the model taxa with non-selective oviposition behaviors was patchier than we expected, we did find evidence to support that these taxa are not necessarily impacted by levels of habitat isolation.

Observations of taxa with selective oviposition behavior suggest that patterns induced by oviposition behavior may persist as larvae hatching from eggs mature, particularly if larval mobility is relatively low. *Cheumatopsyche* sp. distribution was patchy, as we expected it would be, but larvae did not seem mobile enough to colonize habitats below riffles built 15 m apart (despite being found in relatively high abundance within these riffles). *Cheumatopsyche* sp. larvae were mobile enough to colonize habitat below riffles built 10 m apart; however, larvae did not increase in abundance in the same proportion below riffles built 5 m apart, which was unexpected. We also found abundance in habitat below riffles built 10 m and 5 m apart to be unexpectedly high (n = 387 and 237 respectively) compared to abundances within those riffle sets (n = 37 and 7 respectively). This may suggest that *Cheumatopsyche* sp., which oviposit by selectively attaching eggs to rocks in riffles, even diving up to 2-3 m deep to oviposit on submerged rocks (Deutsch, 1984), may have disproportionately used rock baskets over constructed riffles to oviposit, although it is not clear why this preference would exist.

Our observations of *Calopteryx* sp. support that patterns caused by location of oviposition may be more persistent when larvae are not as mobile. *Calopteryx* sp. selectively oviposit in fast flows near riffles, with a preference for emergent vegetation or debris (Johnson, 1962; Siva-Jothy et al., 1995). Based on our observation of *Calopteryx* sp., selective ovipositors with immobile larvae might be the most vulnerable to habitat isolation because maternal behavior results in patchy distribution of larvae that are unable to successfully colonize locations downstream from natal habitat. Similarly, patterns of larval distribution resulting from patchy oviposition habitat can persist in neonate and mid-stage instars of *Baetis rhodani*, despite high levels of drift from riffles at inter-habitat distances of 20-70 m (Lancaster et al., 2010). Our results show that for taxa with low mobility larvae, patterns induced by maternal behavior can persist at distances as small as 5-15 m, but those maternal patterns may be erased at similar distances if larvae are relatively mobile.

In conclusion, drift capabilities and maternal behavior can influence spatial patterns and population dynamics of benthic invertebrates. Furthermore, the scale at which colonization mechanisms, like larval movement and oviposition, operate might be quite small. As a result, some taxa can be isolated from downstream communities at distances as small as 5-15 m. This information is especially relevant in systems where distance between similar habitat units is increased by impairment. In a similar way, the degree to which oviposition behavior or drift capabilities influence larval distribution may change in different sized systems. In large water bodies where distance between similar habitat units is large, oviposition behavior is likely to be a stronger determinant of larval distribution than drift compared to smaller streams, where drift capabilities may erase oviposition patterns and more strongly determine larval distributions because habitat units are closer together. Drift capabilities might also scale to habitat size to some extent in larger systems, or catastrophic drift as a result of flooding may contribute more to colonization dynamics in larger streams. These results are important, as distribution of populations influences community dynamics and ultimately impacts ecological processes across aquatic and terrestrial systems.

Chapter 3 Reach-scale effects of instream habitat diversity on benthic community structure

3.1 Introduction

Aquatic macroinvertebrates are a diverse community of organisms which are integral to freshwater ecosystems. The habits and life histories of these organisms are equally diverse, and they are found in abundance in nearly every freshwater system (Merritt et al., 2019). The composition of macroinvertebrates at a given location is determined by abiotic conditions such as water quality, hydrology, and physical habitat. Each of these environmental features acts as a filter on community composition, determining which taxa can colonize and persist within the stream based on each taxon's tolerance to a suite of conditions (Merritt et al., 2019). Furthermore, macroinvertebrate life cycles are complex, particularly for aquatic insects, which interact with terrestrial and aquatic environments and can experience bottlenecks imposed by both environments at several key life stages, such as recruitment by ovipositing (egg-laying) adults and growth and development as juveniles.

Recruitment of aquatic insect taxa to a stream is, in part, determined by the physical instream habitat, which provides sites for terrestrial adults to oviposit their eggs back into an aquatic environment. Oviposition behavior varies widely among taxa, from non-selective dispersion of eggs into flowing water, to selective behaviors in which adults attach their eggs to specific instream substrate (Lancaster &

Downes, 2013). Oviposition habitat preference is as diverse as oviposition behaviors, and many taxa are highly selective in where they lay their eggs in streams, with some taxa only ovipositing on particular instream habitats, like emergent rocks (Encalada & Peckarsky, 2007). Therefore, diverse physical structures in streams provide potential habitat to a wider variety of ovipositing taxa.

Instream habitat is also important in determining which taxa can persist within a stream as juveniles. Larvae have modes of living, or habits, with associated adaptations that make them especially suited to persist in specific habitat types within aquatic systems (Minshall, 1984; Hynes, 1970a, 1970b). Invertebrates with habits such as crawling, clinging, or swimming are typically found in high flow and turbulent conditions characteristic of riffles, while invertebrates with sprawling and burrowing habits are often found in slow-flow, fine sediment conditions of pool environments (Voshell, 2002). These habits make macroinvertebrate taxa highly associated with specific substrates and, much like instream habitat supporting recruitment of diverse taxa through oviposition, complex habitat structures across stream reaches support diverse larval communities (Brosse et al., 2003; Townsend et al., 2003; Jähnig & Lorenz, 2008).

Environmental factors such as hydrology and geomorphology create unique habitats within streams and rivers at micro and macroscales, and healthy stream systems have a natural variety and complexity of habitat types (Harper & Everard, 1998). Impaired streams, such as those affected by sedimentation that buries instream habitat under fine silt, are often characterized by a loss of instream habitat diversity and complexity (Thoms, 1987). Homogeneous conditions in habitat structure, along with poor water quality and destructive flow conditions, limit the diversity of taxa able to colonize and persist in sediment-impaired streams (Doeg & Koehn, 1994). Restoration approaches that address impaired water quality and habitat conditions are critical to support healthy aquatic communities in streams impaired by human activities. Methods such as planting riparian buffers, stabilizing banks, excluding livestock from streams, and cultivating healthy soils are necessary to combat the effects of poor land-use practices, which result in continued soil erosion and sedimentation in streams. The benefits of these restoration efforts occur slowly, and the benefits to aquatic communities may not be readily apparent and could take several years to appear. Additionally, water quality improvements may not be enough to improve macroinvertebrate community structure if instream habitat diversity is not improved as well. As a result, restoration and management programs aimed at improving benthic community structure may not realize the full ecological benefits of their efforts until instream habitat has been restored or added to streams, even if water quality impairments are remedied.

Our goal with this study was to document the impacts of riffle habitat additions on macroinvertebrate community structure in a hydrologically stable stream with good water quality but minimal instream habitat diversity. Our study reach provided a unique opportunity to assess the impact of riffle habitat additions on benthic communities because the reach had a relatively uniform channel shape with substrata composed of soft clay and loose detritus, most likely as a result of annual flooding and deposition of sediments from a nearby larger stream. Aside from simplified instream habitat, our study reach was relatively unimpaired in terms of water quality. Because most impaired streams suffer from multiple stressors (e.g., water quality and physical habitat degradation), it can be difficult to assess the benefit of different restoration methods in these systems (i.e. restoration of physical habitat or water quality, but not both). Assessing the impact of improving instream habitat complexity in a stream with otherwise good water quality can provide valuable information about using habitat modifications for restoration and help set realistic goals for biological outcomes following restoration projects. We observed how constructing riffle habitat in a homogeneous mud-bottom stream affected benthic community structure and invertebrate abundance at the reach scale. We hypothesized that providing more diverse habitat structure would increase diversity of selectively ovipositing taxa and larval habits across riffle and mud habitats, thereby increasing overall diversity of macroinvertebrates in our stream. We predicted that adding structural diversity to a stream with relatively healthy water quality would increase diversity of macroinvertebrates in the stream by increasing habitats available to support taxa with varying oviposition preferences and larval habits.

3.2 Methods

Study area

Our study was conducted in an unnamed 1st-order tributary to Chillisquaque Creek located at Bucknell University Chillisquaque Creek Natural Area in Montour County, Pennsylvania (Figure 1). The tributary originates from a small pond on the property and flows through a floodplain forest where it expands into several large pools before narrowing and increasing in slope and velocity approximately 300 m above its confluence with Chillisquaque Creek.

Our experiment took place in the last 300 m of the unnamed tributary before it joins Chillisquaque Creek. This portion of the stream is 1-2 m wide and characterized by a relatively uniform channel shape with substrata composed of soft clay and loose detritus (Figure 2). Due to uniformity in depth and width, the entire reach is essentially “run” habitat, with virtually no areas that could be classified as “pool” or “riffle” habitats. We chose this site for our experiment because it lacked coarse inorganic and emergent substrate, making it an ideal location for us to manipulate the substrata and add riffle habitat.

Prior to our experiment, the benthic invertebrate community in this small tributary consisted mainly of freshwater crustaceans (Amphipoda and Isopoda), worms (Oligochaetes), bivalves, Chironomidae (Diptera), and small populations of *Hydropsyche* sp. and *Cheumatopsyche* sp. (Trichoptera), and Baetidae (Ephemeroptera).

Riffle construction

We built nine riffles in the study reach during summer 2019. Riffles were constructed using 1-3-inch diameter well-rounded sandstones and siltstones, which were added to 1-m long sections that spanned the entire width of the stream (Figure

3). Average water depth in constructed riffles was 3.0 ± 1.0 cm and ranged from 2.0-6.0 cm. We then added 12 large rocks (4-7-inch diameter), equally spaced across the stream and along the riffle, that emerged from the stream surface. Larger rocks introduced to each riffle provided equal total surface area of emergent habitat for oviposition. The submerged rocks and emergent rocks both provided potential habitat for colonization by ovipositing adults. Following construction, we allowed riffles to be colonized by invertebrates from the surrounding area for ten weeks prior to sampling.

For the purpose of a study investigating habitat isolation and macroinvertebrate community structure, we constructed riffles in sets of three, with each set comprised of three identical riffles. Riffle sets were built with different inter-riffle distances of either 15, 10, or 5 m and had a 20 m "buffer" devoid of added coarse emergent substrate (i.e. large rocks) to separate each riffle sets from each other (Figure 4). For this study, we were investigating the impacts of instream habitat addition on community composition at the reach scale, so varying degrees of habitat isolation would not likely impact the results.

Water Quality

Our study took place for ten weeks, from 1 August to 11 October 2019. Daily measurements of basic water quality indicators were taken at our study site for the duration of our experiment (Figure 5). Measurements of pH, temperature (°C), dissolved oxygen (mg/L), and specific conductance (μs/cm) were taken using a YSI

Professional Plus Instrument (Pro Plus) with YSI Pro Series Quatro Field cable. Average pH was 8.062 ± 0.13 and ranged from 7.72-8.47. Temperature ranged from 12.5-22.7°C and averaged 17.76 ± 2.18°C. Dissolved oxygen ranged from 6.75-9.48 mg/L and averaged 8.35 ± 0.63 mg/L. Specific conductivity ranged from 244.6-297.7 µs/cm and averaged 278.85 ± 10.31 µs/cm. None of the water quality parameters changed significantly throughout the duration of our experiment thus did not appear to influence macroinvertebrate communities.

Survey of benthic communities in riffle and mud habitats

To test whether invertebrate community structure in our stream was influenced by instream habitat availability at the reach scale, we conducted a benthic survey of our study site on October 11, 2019, which was 10 weeks after the riffles were built and left to be colonized by macroinvertebrates. We used a Surber sampler to collect quantitative benthic invertebrate samples from constructed riffles and habitats that were present in the stream prior to our experiment (i.e. “mud”). We took 9 Surber samples from each habitat type and composited and subsampled them in the field, resulting in three composited samples per habitat type. In order to estimate benthic invertebrate density, samples were split into known subfractions in the lab and sorted under a dissecting microscope using a 200-count minimum. Counts from subsamples were corrected to whole-sample counts, which were then divided by the area of a Surber sample (0.093 m^2) to represent invertebrate density as # of individuals/m^2. Invertebrates were identified to the lowest practical taxonomic unit (typically genus), counted, and preserved in 80% ethanol.

3.3 Data Analysis

Macroinvertebrate abundance data generated from these samples were analyzed and visualized using R, RStudio, and various packages. All tables were made using the kableExtra package (Zhu, 2021), and all figures were made using gglpot2 (Wickham, 2016).

Richness and diversity of macroinvertebrate communities were calculated using the vegan package (Oksanen et al., 2020). Richness was calculated as the number of unique taxa per sample. Community diversity was quantified using two indices, Shannon diversity and Simpson's diversity (Appendix for formulas and explanation). Both indices describe community diversity by quantifying richness and evenness of taxa.

Principal component analysis (PCA) was used to visualize macroinvertebrate community structure among riffle and mud habitats and was carried out using the R stats (R Core Team, 2020) and factoextra (Kassambara & Mundt, 2020) packages. Bubble plots were used to compare relative abundance of different macroinvertebrate taxa and were constructed using ggplot2 (Wickham, 2016) and reshape2 (Wickham, 2007) packages. Statistical differences between riffle and mud communities were determined using base R software (R Core Team, 2020).

3.4 Results

Diversity of macroinvertebrate communities in riffle and mud habitats

The benthic survey of riffle and mud habitat generated 48,688 macroinvertebrates for community analysis. Richness and diversity indices for each composited Surber sample are listed in Table 3. There were no significant differences between density, richness, or either diversity index between riffle and mud habitats (t-test, p-value > 0.05) (Table 4). Density was 8268.82 ± 3139.84 individuals/m^2 in mud habitat and 7960.58 ± 4030.07 individuals/m^2 in riffle habitat. Richness was 15.67 ± 6.03 in riffle habitat and 14.33 ± 2.08 in mud habitat. Shannon diversity index was 1.56 ± 0.2 in mud habitat and 1.38 ± 0.26 in riffle habitat. Simpson's diversity was slightly higher in mud habitat (0.72 ± 0.06) than in riffle habitat (0.59 ± 0.06) but was not statistically significant (p = 0.06). Across both habitats, such low Simpson's diversity values indicate that abundance of macroinvertebrates was dominated by a few taxa compared to a more even distribution of individuals across taxa.

Macroinvertebrate community structure in riffle and mud habitats

Principal component analysis (PCA) revealed patterns of community similarity among samples within each habitat type and differences between habitat types (Figure 18). Principal components one and two captured 93.3% of the variance in our community abundance data (PC1 66.1% and PC2 27.2%). Samples clustered closely by habitat type such that riffle samples were distinctly separated from mud samples, although this separation was not significantly different (PERMANOVA, $p = 0.1$).

Differences in community structure between samples were illustrated by calculating each taxon's contribution to principal components (Figure 19). Both riffle and mud samples consisted of many relatively rare taxa and a few dominant taxa. Dominant taxa contributed greatly to the principal components and thus were responsible for a large proportion of the structural differences in communities between habitat types. Dominant taxa in riffle samples included Chironomidae (Diptera) and *Cheumatopsyche* sp. (Trichoptera), while dominant taxa in mud samples included Oligochaeta and Ceratopogonidae (Diptera).

Macroinvertebrate community composition in riffle and mud habitats

We found 34 taxa in the constructed riffle and original mud habitats within our study reach (Figure 20). Ten taxa were found in both riffle and mud habitats and included various Diptera, Trichoptera, Crustacea, Coleoptera, bivalves, planariids, and oligochaetes. Twenty-five taxa were found in riffle samples, fifteen of which were unique to riffle habitats, including various Diptera, Trichoptera, Ephemeroptera, Zygoptera, and Hemiptera. Nineteen taxa were found in samples from mud habitats, nine of which were found only in mud habitat, including various Diptera, Trichoptera, Ephemeroptera, Coleoptera, Gastropoda, and Megaloptera.

Although community composition varied among samples, no taxa varied significantly between riffle and mud habitats (Wilcoxon Rank Sum test, $p > 0.05$).

This result was surprising and is likely due to taxa shared between habitat types being in relatively equal high abundance and taxa unique to each habitat type occurring in such low abundance that they did not differ significantly from zero, even though present.

In order to test the hypothesis that constructing riffle habitat would increase macroinvertebrate community diversity by providing oviposition habitat to taxa previously unable to colonize our study reach, we examined known oviposition behaviors of taxa found in riffle and mud habitats (Figure 21). A comparison of relative abundance of taxa of four types of oviposition behavior (selective, non-selective, both selective and non-selective, and unknown) revealed differences between community composition in riffle and mud habitat (Figure 22). Relative abundance of macroinvertebrates with non-selective oviposition behavior was significantly higher in mud habitats (44.48 ± 5.28) than in riffle habitats (6.96 ± 4.86) (t-test, 4 df, $p = 0.0008$). Relative abundance of macroinvertebrates with selective oviposition behavior was significantly higher in riffle habitats (26.94 ± 4.49) than in mud habitats (0.89 ± 0.69) (t-test, 4 df, $p = 0.0006$). Relative abundance of macroinvertebrates known to display selective and non-selective oviposition behaviors was significantly higher in riffle habitats (62.4 ± 5.29) than in mud habitats (35.63 ± 6.01) (t-test, 4 df, $p = 0.0044$). Relative abundance of macroinvertebrates with unknown oviposition behavior was not significantly different in mud habitats (19.01 ± 9.81) than in riffle habitats (3.7 ± 1.07) (Wilcoxon Rank Sum test, $W = 9$, $p = 0.0809$).

To determine whether increasing habitat diversity increased macroinvertebrate community diversity by providing habitat that could support a wider variety of larval habits, we examined relative abundance of benthic invertebrate habits from riffle and mud habitats (Figure 23). Invertebrate habits were based on definitions provided by Voshell (2002) and designations provided by Merritt et al. (2019). We then grouped habits based on the habitat they are broadly adapted to live in: “fast flow and firm substrate” (clingers, swimmers, and crawlers), “slow flow and fine sediment” (sprawlers, climbers, and burrowers), “surface skaters” (taxa associated with stream surface), and “mixed habits” (taxa with combinations of habits suited for slow flow and fine substrate as well as fast flow and firm substrate).

All habit groups except skaters were present in both riffle and mud habitats, but relative abundance of habit groups varied between habitat types (Figure 24). Relative abundance of fast flow and firm substrate habits was significantly higher in riffle habitats (29.56 ± 3.39%) than in mud habitats (1.28 ± 1.48%) (t-test, 4 df, $p = 0.0002$). Relative abundance of slow flow and fine substrate habits was significantly higher in mud habitats (53.8 ± 2.65%) than in riffle habitats (4.86 ± 2.64%) (t-test, 4 df, $p = 2.257e\text{-}5$). Relative abundance of mixed habits was significantly higher in riffle habitats (65.15 ± 4.39%) than in mud habitats (44.92 ± 1.64%) (t-test, 4 df, $p = 0.0017$).

3.5 Discussion

From our benthic survey of constructed riffle habitat and mud habitat, which was characteristic of our study reach prior to our experiment, we found that each habitat type supported equally abundant and diverse communities of macroinvertebrates but with distinct taxonomic differences.

Furthermore, community structure of both habitat types was dominated by a small number of taxa. Differences in community structure between riffle and mud habitats apparent on the PCA ordination were driven by Chironomidae and *Cheumatopsyche* sp., which dominated riffle habitats, and Oligochaeta and Ceratopogonidae, which dominated mud habitats. The PCA also showed that each habitat type supported a unique set of many rare taxa found in relatively low abundances. Although the PCA showed that community structure varied among riffle and mud habitats, no taxa varied significantly between riffle and mud habitats, likely due to shared taxa being found in relatively equal abundances and unique taxa occurring in low abundances.

Despite similar abundances of individual taxa across habitat types, our findings support the hypothesis that instream habitat diversity increases macroinvertebrate community diversity at the reach scale. We found 15 unique taxa in the riffle habitats we added and 9 taxa unique to mud habitats. Across both habitat types, our study reach had a richness of 34 taxa after our experiment, which represents a 79% increase in taxa richness at the reach scale when compared to the 19 taxa found in the mud habitat characteristic of this reach prior to our experiment.

Community assemblage of benthic macroinvertebrates could be influenced by availability of instream habitat used as sites for oviposition. Taxa requiring a hard substrate to attach their eggs, either exclusively or in addition to other oviposition behaviors, were significantly more abundant in riffle habitat compared to mud habitat. Since the study reach did not contain any riffles with coarse inorganic substrates or emerging rocks prior to our experiment, taxa with selective oviposition behaviors found in the study reach at the end of our experiment potentially colonized the reach via oviposition by terrestrial adults. Previous studies have also documented higher recruitment of selectively ovipositing taxa when preferred oviposition habitat is increased experimentally (Encalada & Peckarsky, 2012) or in streams with more naturally occurring preferred oviposition habitat (Encalada & Peckarsky, 2006; Lancaster et al., 2010). Conversely, taxa with non-selective oviposition behaviors were significantly more abundant in mud habitat. Stream habitats with fine sediment substrate are typically described as lotic-depositional and likely have comparatively slower flows than lotic-erosional habitat like riffles. Higher abundance of aquatic insect taxa with non-selective oviposition behavior in mud habitats in our study reach may be due to the depositional nature of these habitats which may allow eggs that are released freely into the water to fall out of the water column and settle on the stream bottom where the larvae remain once eggs hatch.

We also found that selective ovipositors were not only more abundant in riffles, but not surprisingly, the majority of taxa with this oviposition behavior were found only in riffle habitat, whereas the majority of non-selective ovipositors were found in both riffle and mud habitats, despite taxa with this behavior being more abundant in mud habitat. Selective ovipositors found only in riffles show that overlap in preferred oviposition habitat and ideal larval habitat might mean that taxa with selective maternal behaviors account for larval habitat requirements when deciding where to oviposit. Selection of oviposition sites by female adult aquatic insects may also provide higher certainty that eggs remain in an ideal habitat until they hatch (Thompson & Pellymyr, 1991) and that larvae hatching into ideal natal habitat will have higher survival (Encalada & Peckarsky, 2007). Spatial patterns in egg abundance and distribution created by maternal behavior may also persist over time as larvae mature and may influence larval distribution (Macqueen & Downes, 2015; Lancaster & Downes, 2014; Encalada & Peckarsky, 2012; Lancaster et al., 2011).

Additionally, adding riffles to our study reach introduced novel habitat that potentially supported a wider variety of larval habits. Larvae with habits specialized for withstanding or navigating turbulent flow conditions (clingers, swimmers, and crawlers) were found in higher abundance in our constructed riffles. Additionally, larvae with habits specialized for soft sediment habitats (sprawlers, climbers, and burrowers) were more abundant in mud habitats of our study reach. Our results are in line with other studies that show increasing habitat diversity at multiple scales can introduce habitat niches and support a more ecologically diverse community of macroinvertebrates (Beisel et al., 1998, 2000).

The addition of novel riffle habitat, which could support colonization by selectively ovipositing taxa and persistence of larvae with diverse habits, suggests that instream habitat diversity influences stream biodiversity via multiple stages throughout an insect's lifecycle. Consequently, a lack of preferred habitat at the adult or larval stage could present a barrier to colonization or persistence within a stream. Therefore, stream restoration efforts aimed at recruiting and supporting diverse macroinvertebrate communities should include instream habitat diversity amongst other primary concerns, such as water quality and best land-use practices. Furthermore, recovery of macroinvertebrate communities to restoration efforts that target improvements in water quality may not be fully realized if instream habitat quality and diversity remains low.

Table 1: Richness and diversity metrics of invertebrate communities within riffle sets

Distance between riffles in set	Richness	Shannon Diversity Index	Simpson Diversity Index
15m	22.17±5.49	1.57±0.12	0.66±0.08
10m	17.67±5.09	1.41±0.17	0.62±0.09
5m	17.83±2.86	1.4±0.23	0.64±0.07

Metrics calculated from rock basket samples taken within sets of constructed riffles.

[1] Average richness represents the average number of unique taxa found in riffle set.

[2] Average Simpson Diversity Index represents average 1 - Simpson Diversity Index.

[3] One-way ANOVA revealed no significant difference in richness or diversity metrics based on riffle set.

Table 2: Richness and diversity metrics of invertebrate communities below riffle sets

Location	Richness	Shannon Diversity Index	Simpson Diversity Index
Upstream Control	18.83±2.71	1.42±0.22	0.59±0.11
Below 15m	15.67±3.88	1.36±0.19	0.63±0.09
Below 10m	23.83±6.43	1.6±0.13	0.66±0.03
Below 5m	25.67±8.26	1.4±0.36	0.56±0.11

Metrics calculated from rock basket samples taken below each set of constructed riffles.

[1] Average richness represents the average number of unique taxa found in habitat.

[2] Average Simpson Diversity Index represents average 1 - Simpson Diversity Index.

[3] One-way ANOVA with a post-hoc Tukey multiple comparisons test revealed a significant difference in average richness between the habitat below the riffle set with riffles built 15 meters apart and below the riffle set with riffles built 5 meters apart (p-value = 0.032). No other comparisons were significantly different.

Table 3: Richness and diversity metrics of invertebrate communities in study reach

Habitat	Sample	Density (#/m2)	Richness	Shannon Diversity Index	Simpson Diversity Index
Riffle	r1	6505.38	10	1.18	0.56
	r2	4860.22	15	1.28	0.54
	r3	12516.13	22	1.68	0.66
Mud	m1	4677.42	15	1.58	0.71
	m2	9634.41	12	1.35	0.66
	m3	10494.62	16	1.75	0.78

Metrics calculated from surber samples taken from riffle and mud habitats in study reach.

[1] Richness represents the number of unique taxa found in each sample.

[2] Simpson Diversity Index represented as 1 - Simpson Diversity Index.

Table 4: Summary of richness and diversity metrics of invertebrate communities in study reach

	Riffle	Mud	p-value
Density (#/m2)	7960.58±4030.07	8268.82±3139.84	0.92
Richness	15.67±6.03	14.33±2.08	0.74
Shannon Diversity Index	1.38±0.26	1.56±0.2	0.40
Simpson Diversity Index	0.59±0.06	0.72±0.06	0.06

Summary of metrics calculated from surber samples taken from riffle and mud habitats in study reach.

[1] Average richness represents the average number of unique taxa found in habitat.

[2] Average Simpson Diversity Index represents average 1 - Simpson Diversity Index.

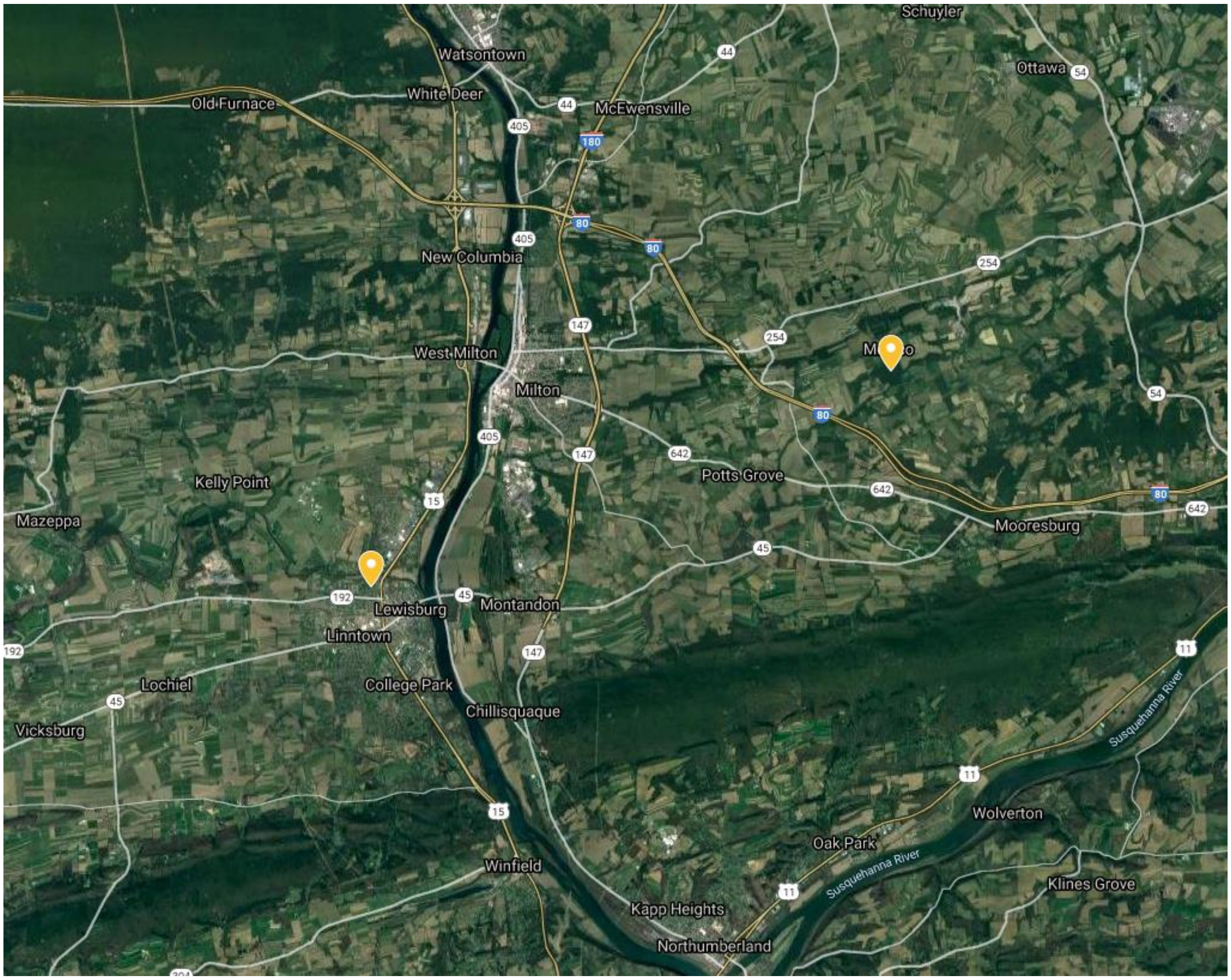


Figure 1 Map of The Bucknell University Chillisquaque Creek Natural Area (upper right) in relation to Lewisburg, Pennsylvania (lower left).

Figure 2 Study reach prior to riffle construction. Substrate was fine silt that we characterized as mud habitat. There was little to no inorganic substrate present in the reach prior to our experiment.

Figure 3 Example of one of the nine riffles built in our study reach. Riffles were identically constructed out of small submerged and large emergent rocks which provided habitat for oviposition and for larval colonization.

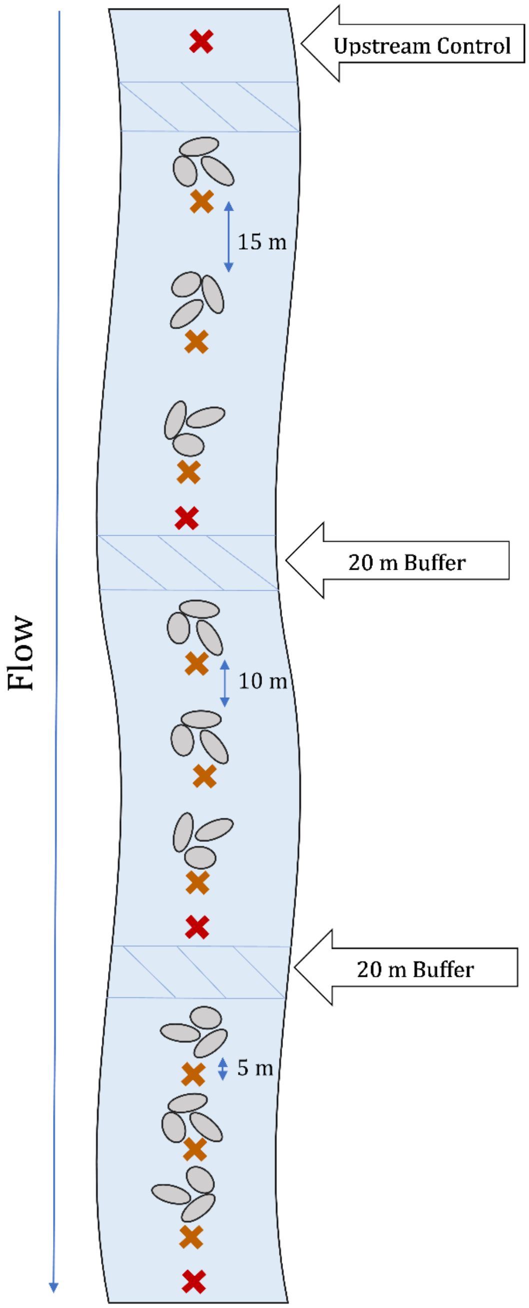


Figure 4 Map of study site with ovals representing nine constructed riffles and hashed areas representing "buffer" regions between sets of riffles. The first three riffles were built 15 meters apart, the second three riffles were built 10 meters apart, and the last three riffles were built 5 meters apart. Rock basket and drift sampling points are marked for samples taken within riffle sets (brown X's) and for samples taken below riffle sets and from the upstream control (red X's).

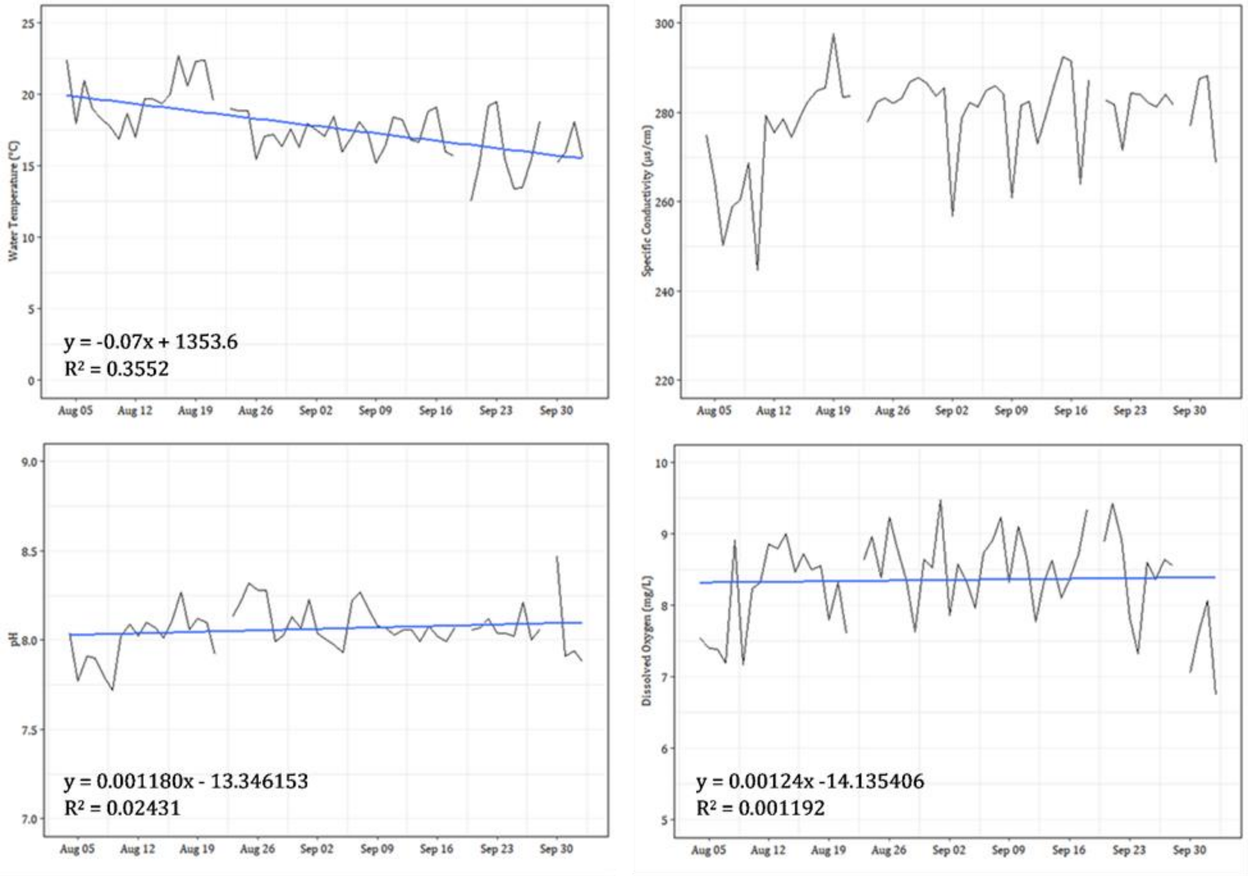


Figure 5 Daily measurements of basic water quality indicators taken at our study site for the duration of our experiment (August 4 – October 2 2019) apart from three days denoted as blank spaces in the line graph. Regression lines are shown in blue with line equations and R^2 values shown on each graph.

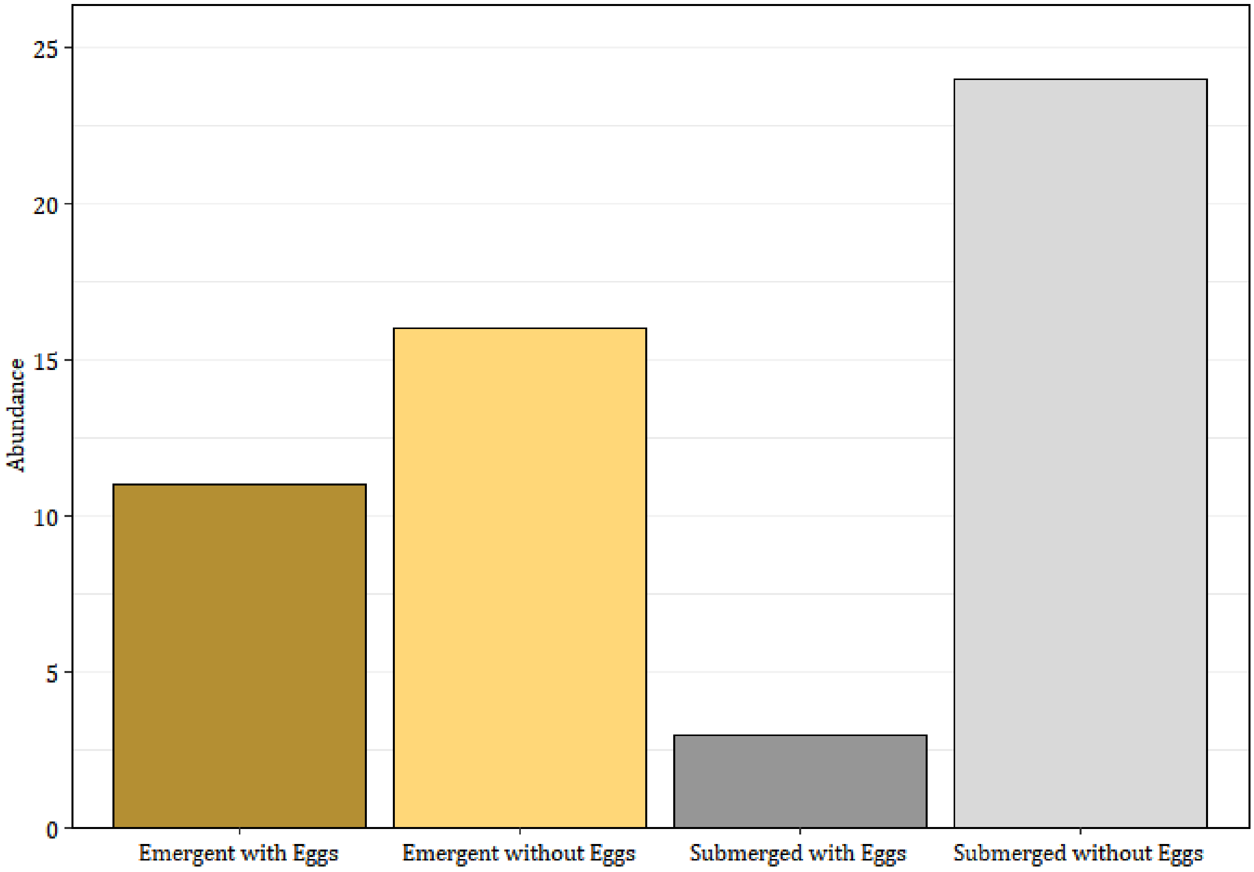


Figure 6 Histogram showing the 54 rocks surveyed for egg masses in the riffles constructed in the study reach. Bars show the abundance of emergent and submerged rocks that were either found with egg masses attached to them or found without egg masses.

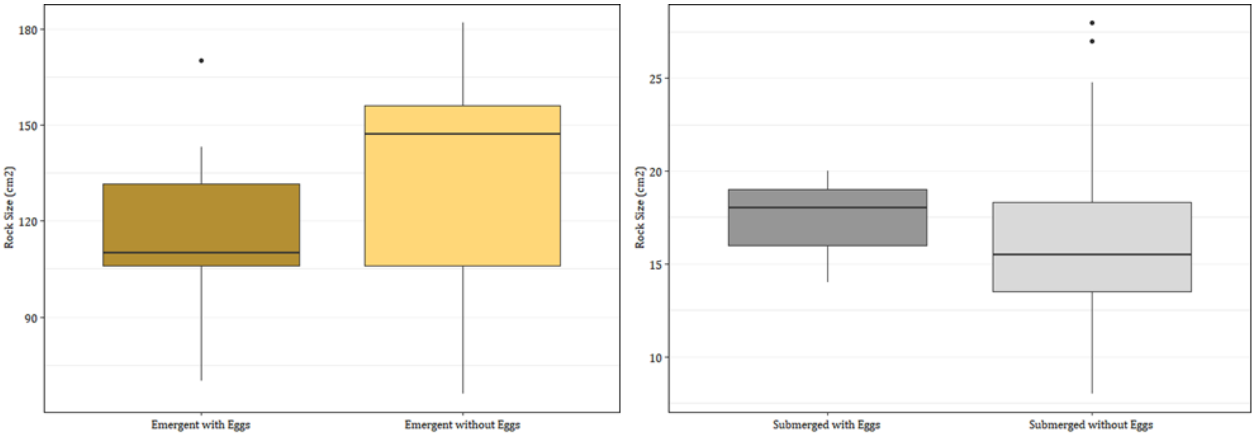


Figure 7 Rock size of the 54 rocks surveyed for egg masses from the constructed riffles in the study reach. Rock size did not differ significantly between emergent rocks with egg masses and emergent rocks without egg masses (t-test, p = 0.2891). Rock size did not differ significantly between submerged rocks with egg masses and submerged rocks without egg masses (t-test, p = 0.9138).

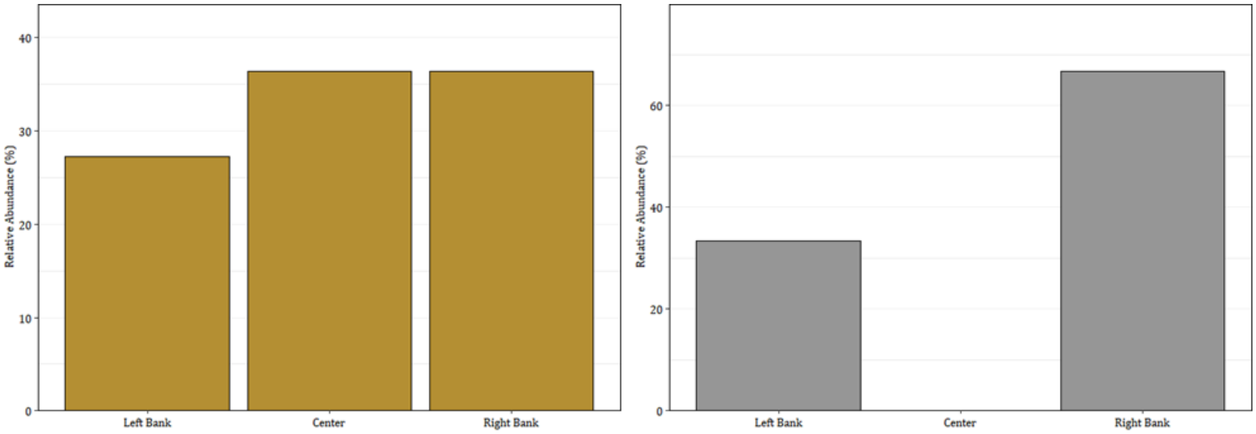


Figure 8 Location within the stream channel of emergent (left) and submerged rocks (right) found with egg masses attached to them. Bars represent the relative abundance of rocks found with egg masses at each location within the stream channel.

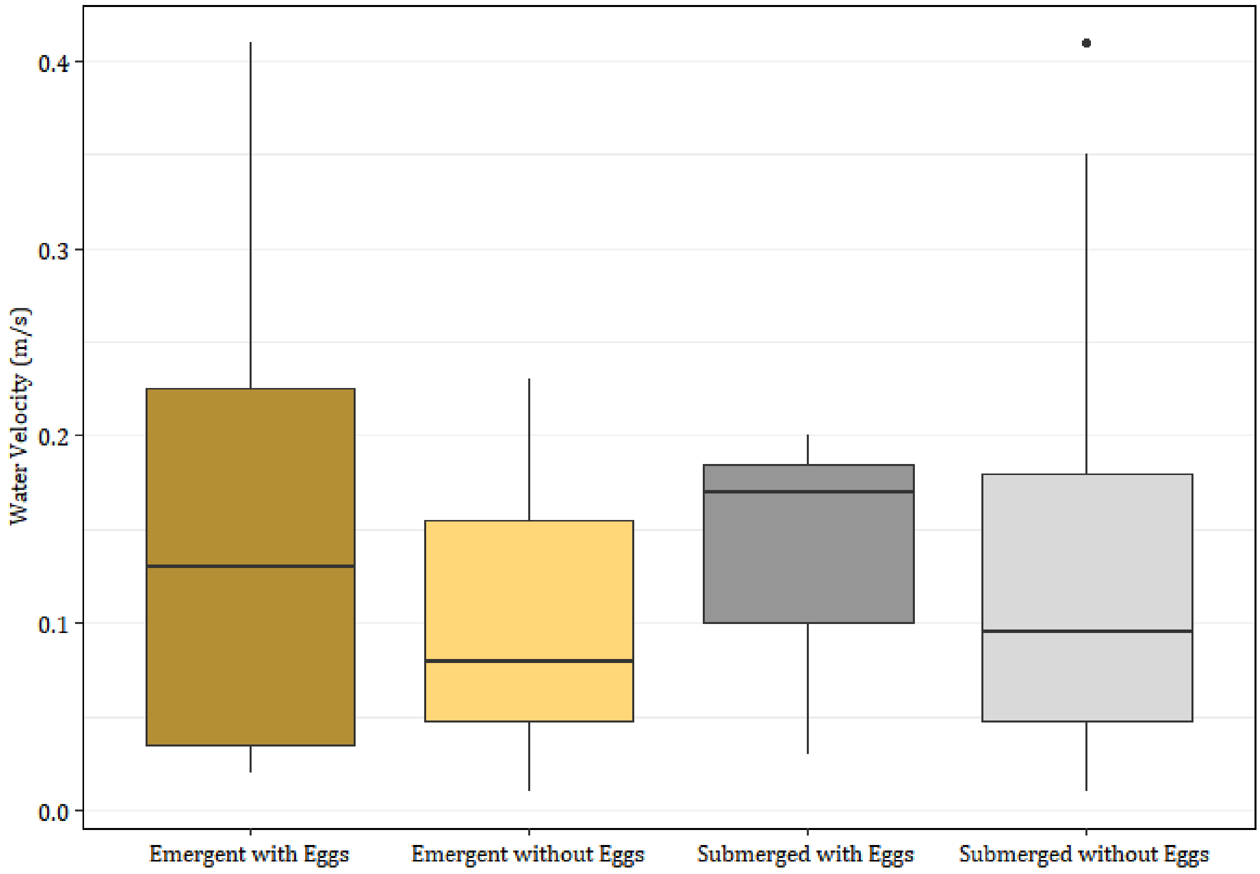
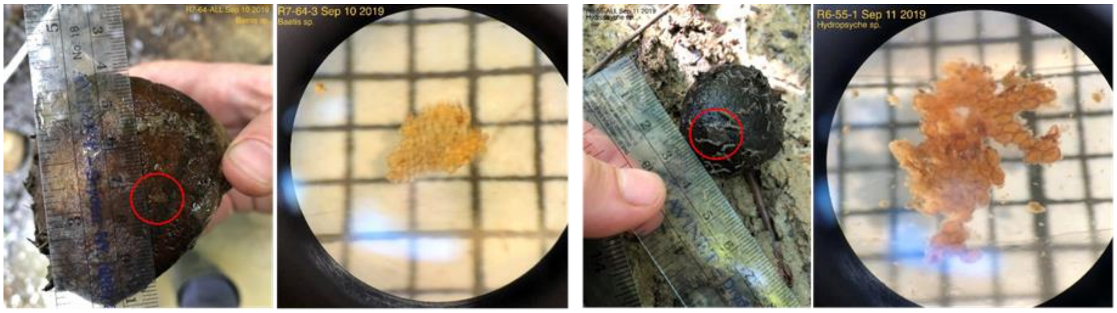
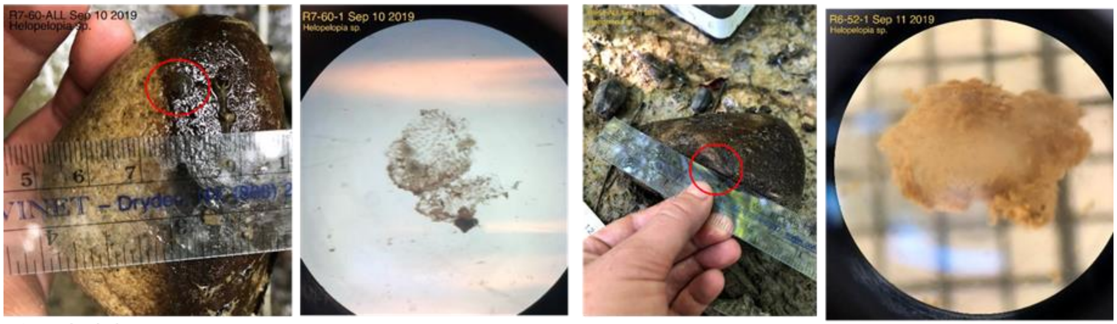


Figure 9 Water velocity at the rocks surveyed for egg masses from the constructed riffles in the study reach. Water velocity did not vary significantly between submerged or emergent rocks with or without egg masses (Kruskal-Wallis ANOVA on Ranks, p = 0.8955).

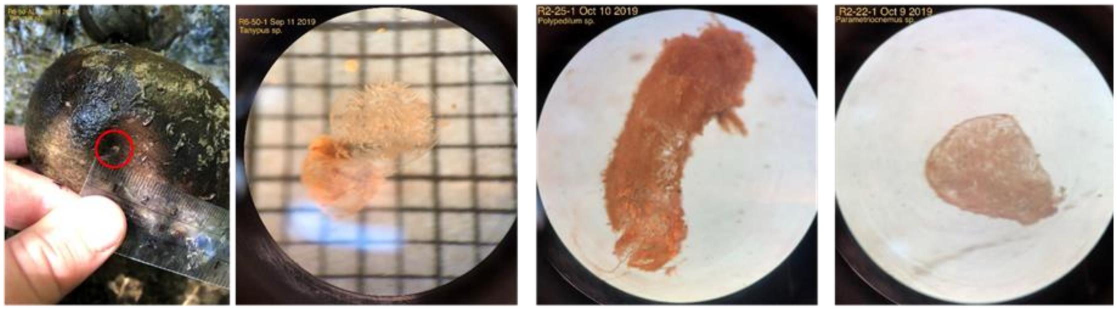


A. ***Baetis* sp.**

B. ***Hydropsyche* sp.**



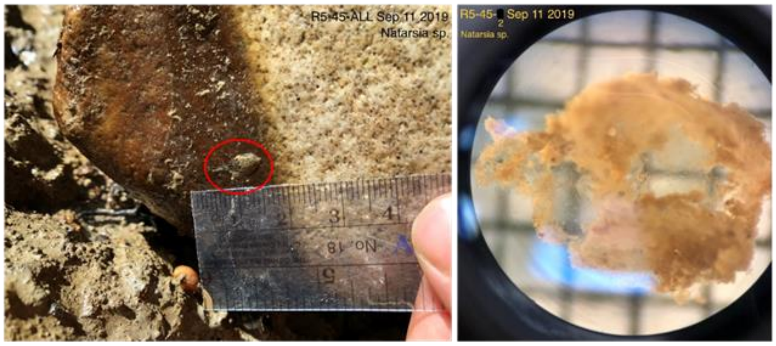
C. ***Helopelopia* sp.**



D. ***Tanypus* sp.**

E. ***Polypedilum* sp.**

F. ***Parametriocnemus* sp.**



G. ***Natarsia* sp.**

Figure 10 Photos of egg masses of the eight genera surveyed from constructed riffles in our study reach. Egg masses, as they were found on the rocks, are circled in red.

Figure 11 Rock baskets used to sample benthic insects below individual riffles (left) and below sets of riffles (right).

Figure 12 Drift nets used to sample larval insects moving between constructed riffles.

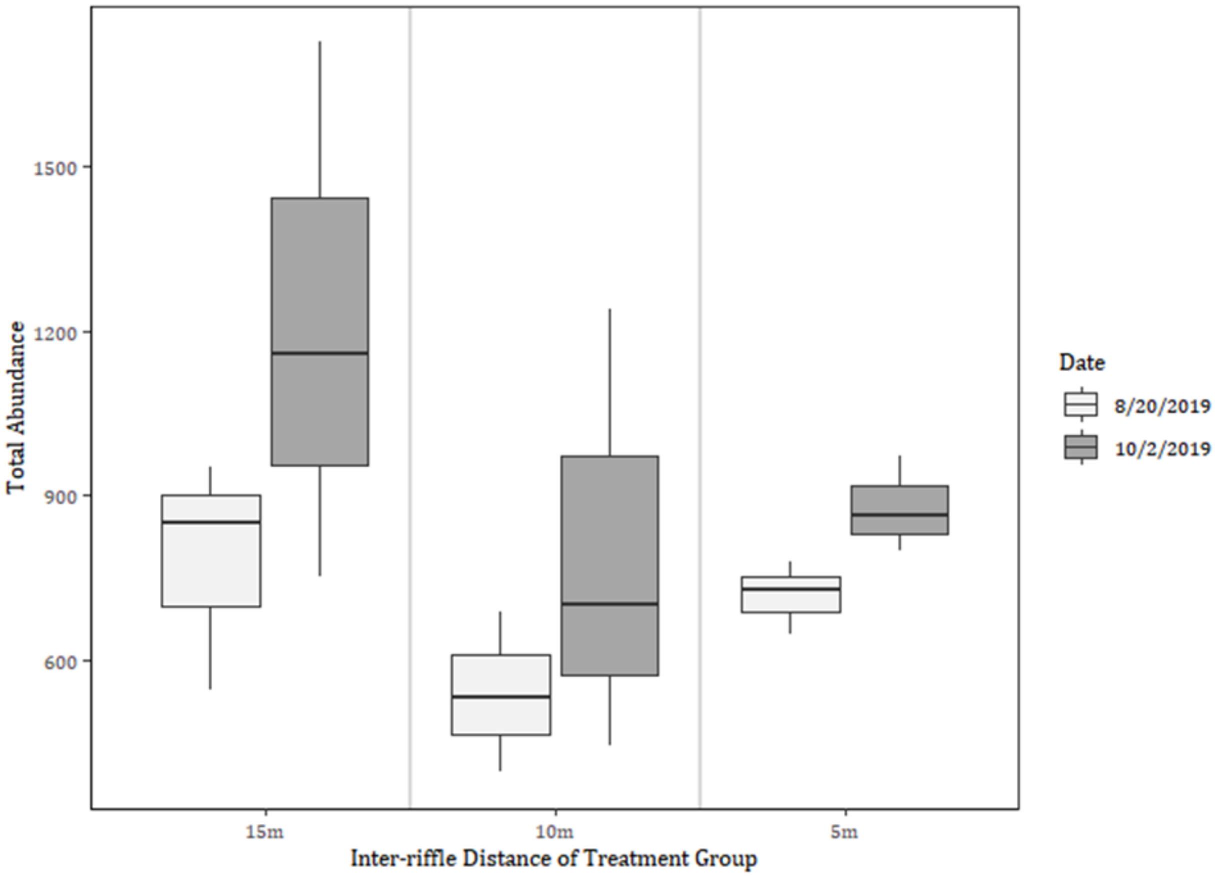


Figure 13 Boxplot showing total abundance of benthic invertebrates within riffle sets. There was no significant difference in abundance based on set of riffles (two-way ANOVA, p-value = 0.1378) but total abundance was significantly higher on the final sampling date on October 2, 2019 (two-way ANOVA, p-value = 0.0432).

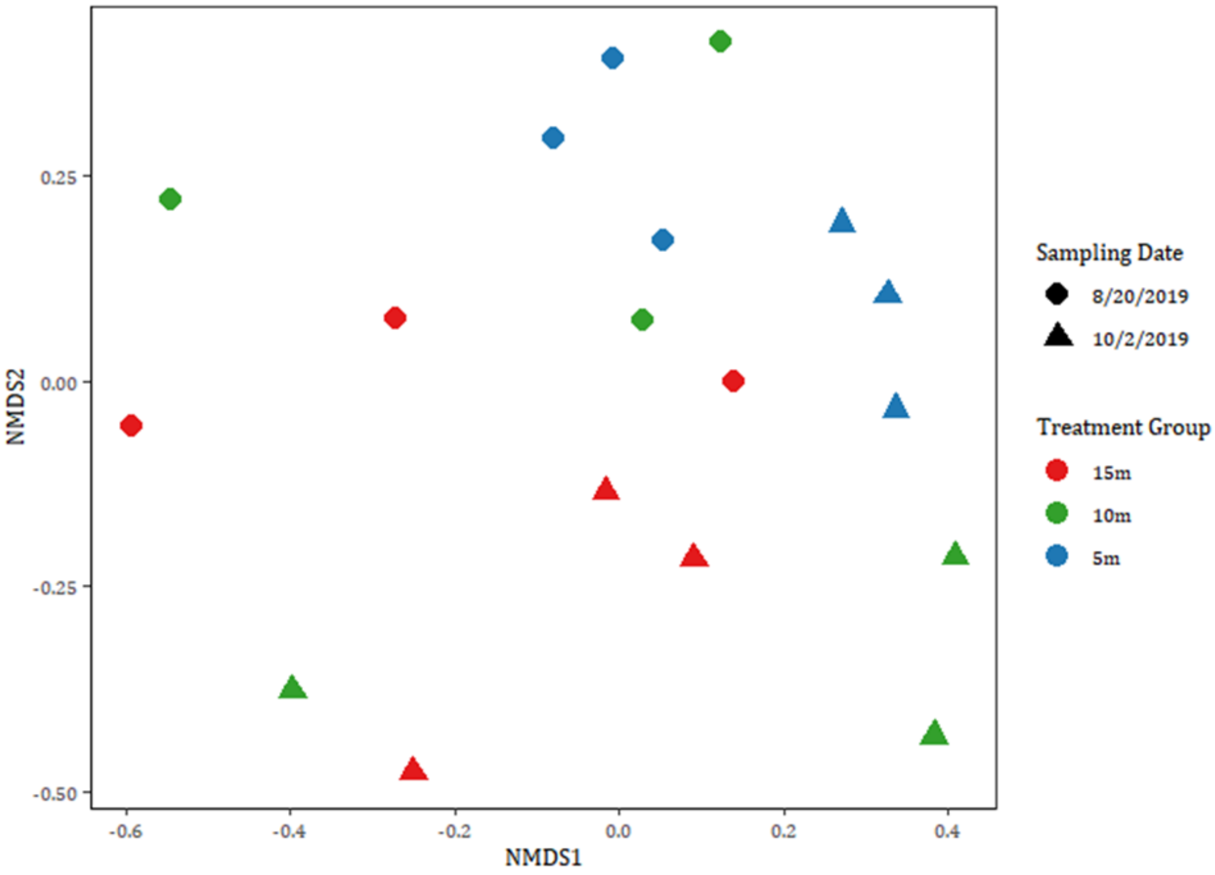


Figure 14 NMDS plot showing community composition of rock basket samples taken from within each set of riffles on both sampling dates.

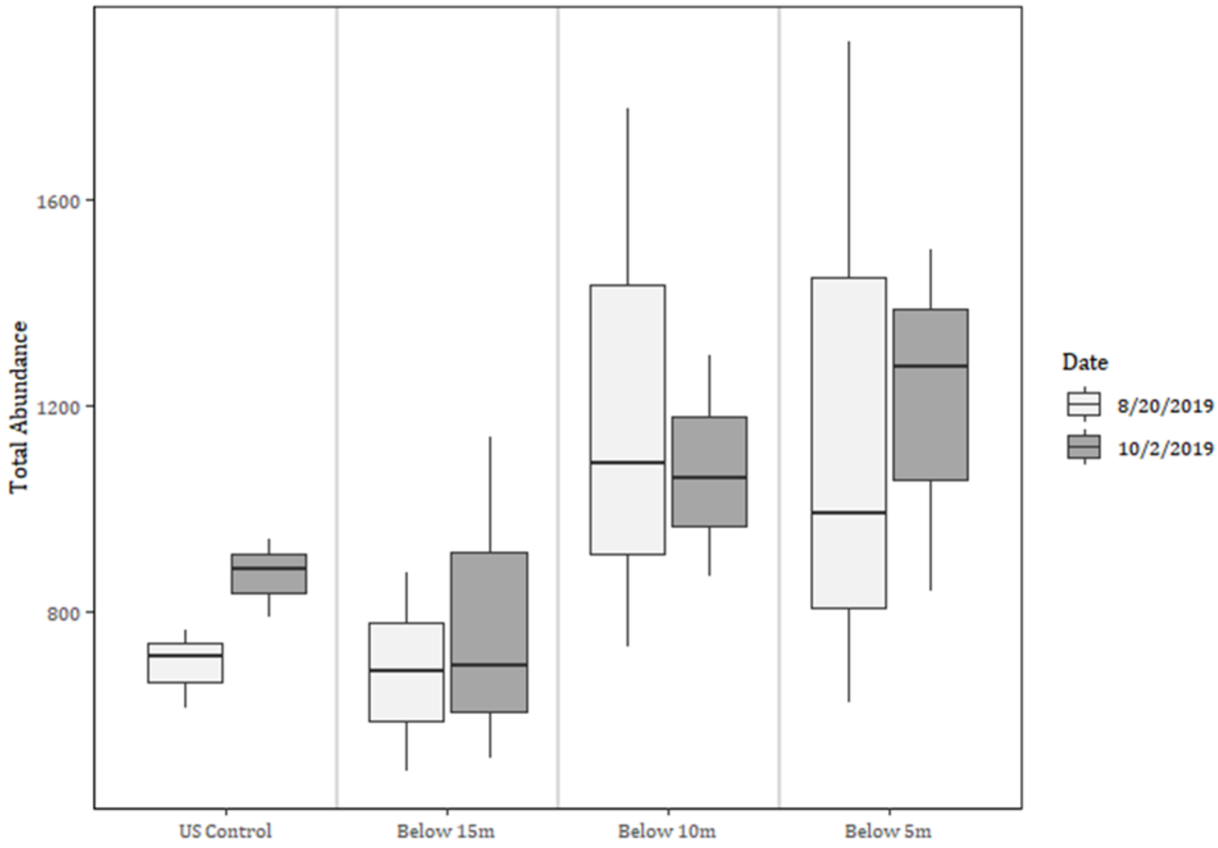


Figure 15 Boxplot showing total abundance of benthic invertebrates in habitats downstream of riffle sets. There was no significant difference in total abundance based on upstream set of riffles (Kruskal-Wallis ANOVA on Ranks, p-value = 0.2815). There was also no significant difference in total abundance based on upstream set of riffles (one-way ANOVA, p-value = 0.255).

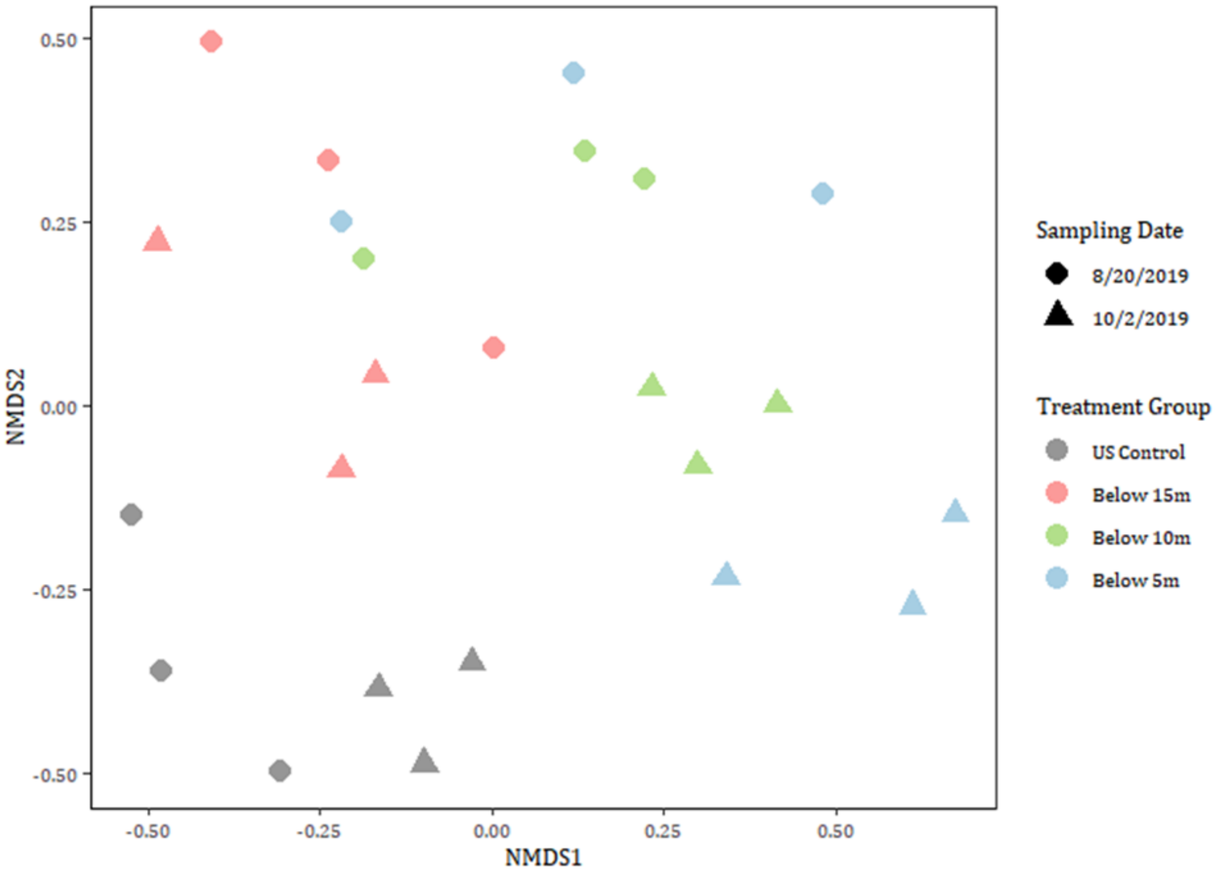


Figure 16 NMDS plot showing community composition of rock basket samples taken from habitats below each set of riffles and upstream control reach on both sampling dates.

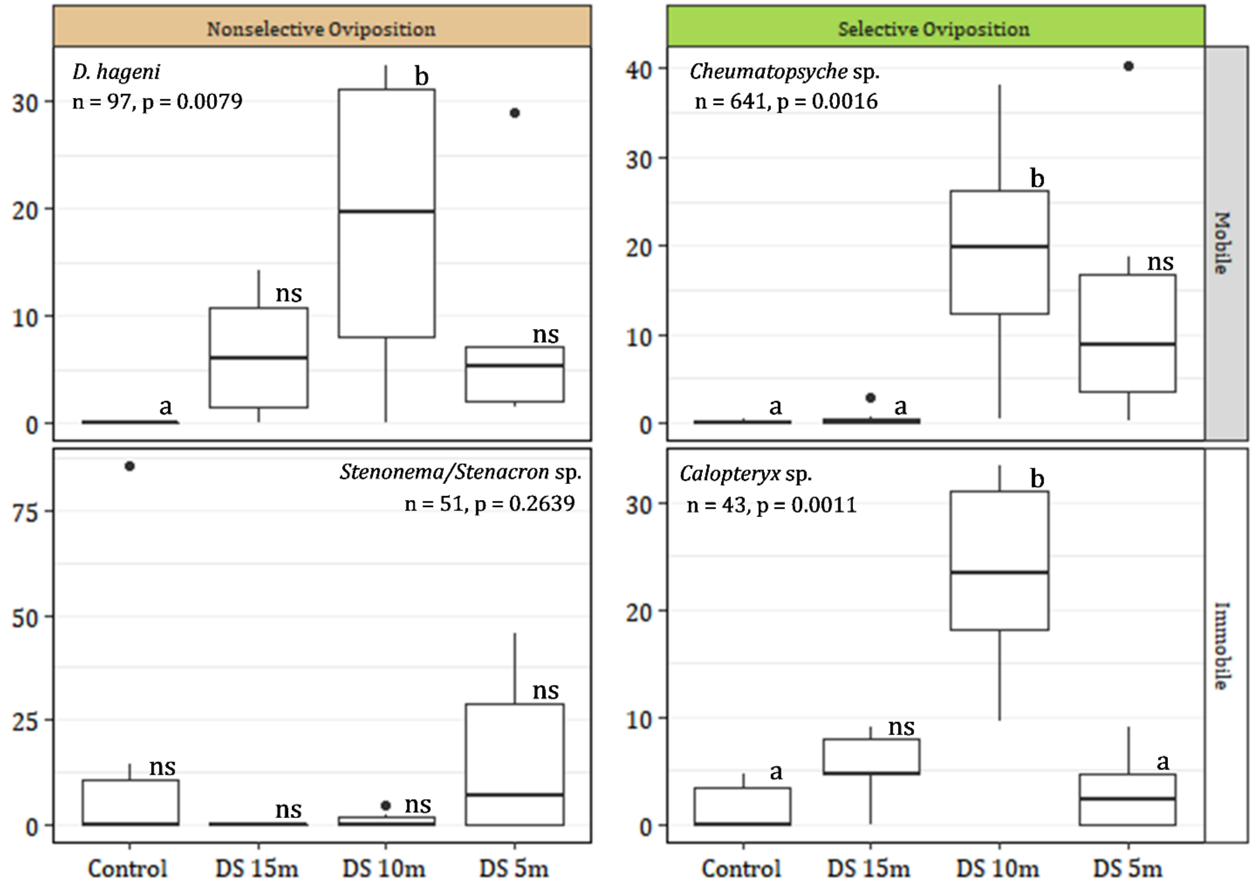


Figure 17 Boxplots of relative abundance of model taxa in locations below each riffle set and upstream control. Boxplots on the left show relative abundance of insects with nonselective oviposition behavior and mobile or immobile and larval behavior. Boxplots on the right show relative abundance of insects with selective oviposition behavior and mobile or immobile larval behavior. N is the total number of individuals found in rock baskets below riffle sets. Letters next to boxplots indicate significant differences among samples (ns = not significant).

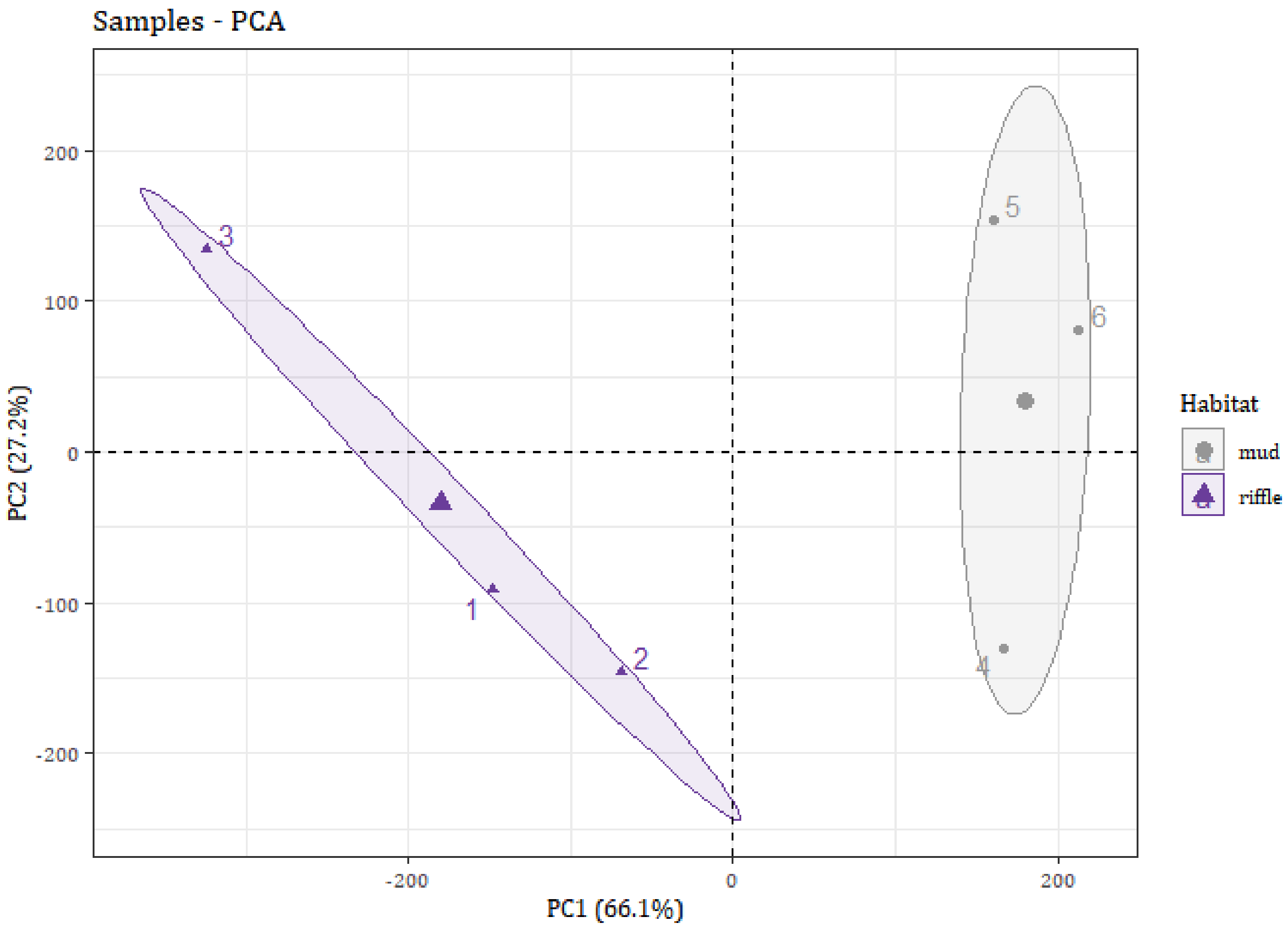


Figure 18 Principal component analysis (PCA) of benthic macroinvertebrate communities in riffle and mud habitats. Samples clustered closely by habitat type such that macroinvertebrate communities in each habitat are distinctly different from each other. Riffle samples are shown in purple triangles and mud samples are shown in grey circles with 95% confidence ellipses delineating the estimated true population mean.

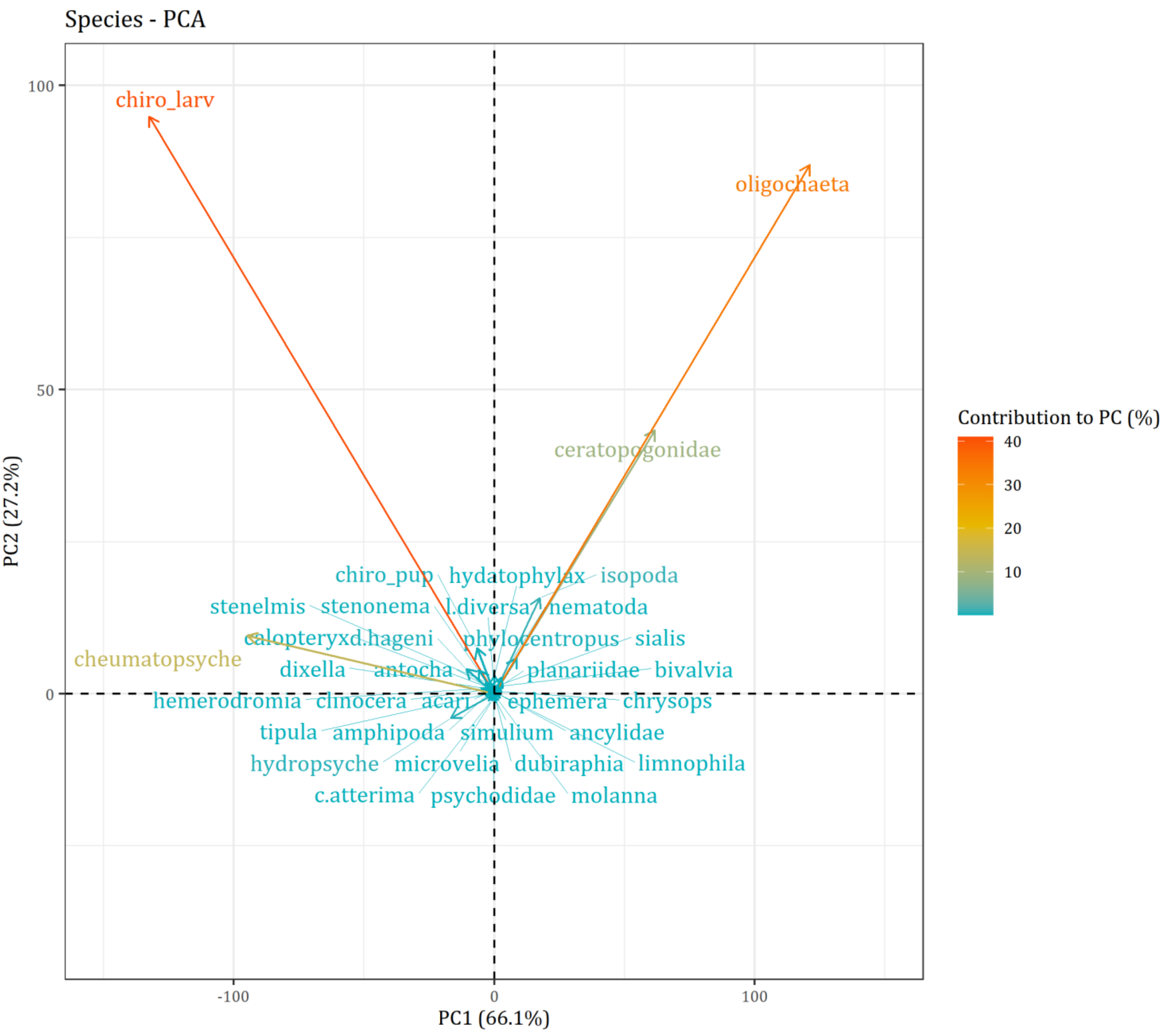


Figure 19 Principal components analysis (PCA) detailing the compositional differences in invertebrates among riffle and mud habitats. Each taxon's contribution to the principal components is indicated by color. Taxa in warmer shades (red) contributed more significantly to separating samples than taxa in cooler shades (blue).

Figure 20 Bubble plot showing relative abundance of taxa found in riffle and mud habitats of our study reach. Samples represent composited Surber samples taken in October 2019. Wilcoxon rank sum tests revealed that no taxa varied significantly between riffle and mud habitats ($p > 0.05$).

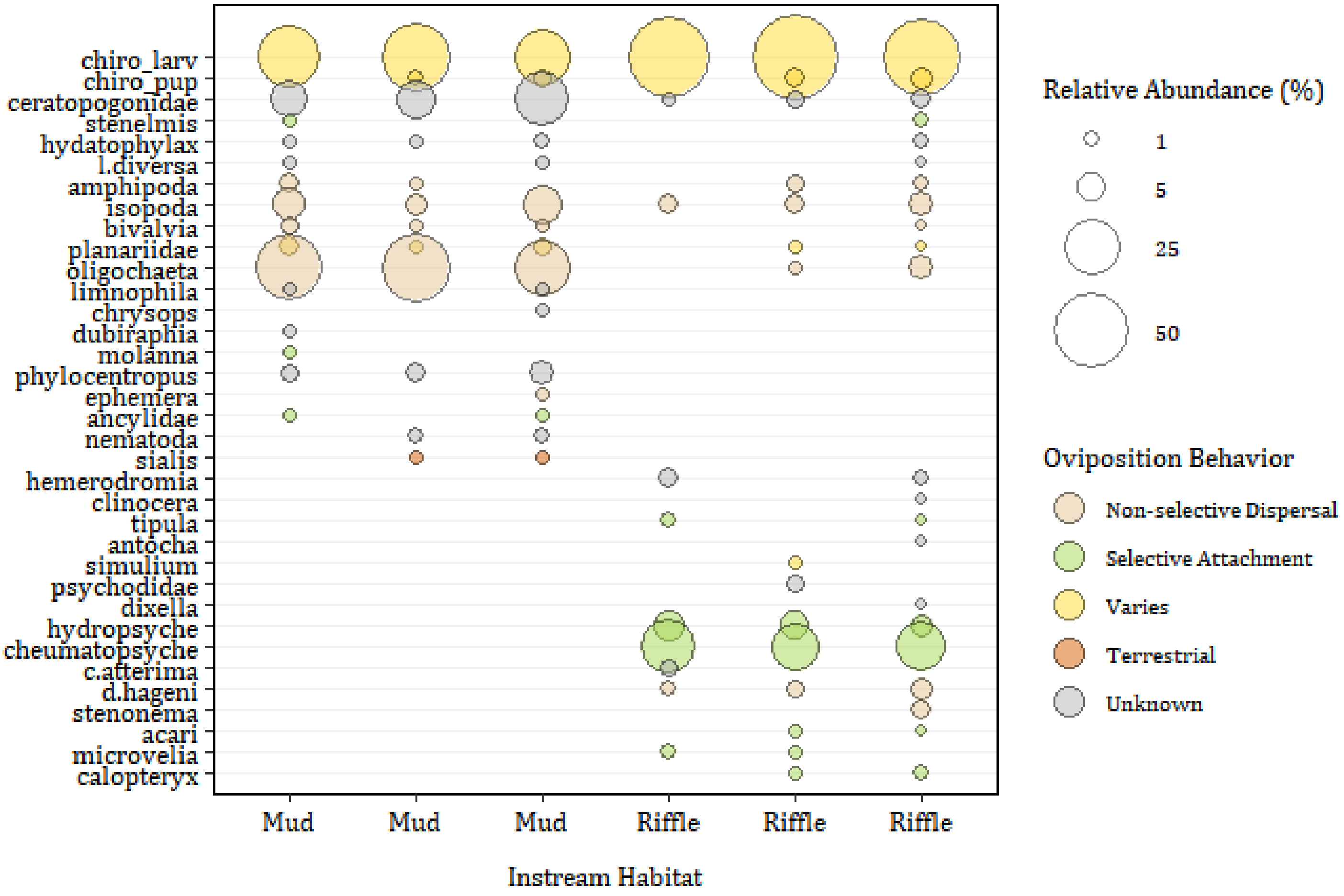


Figure 21 Bubble plot showing oviposition behaviors of taxa found in riffle and mud habitats in our study reach. Samples represent composited Surber samples taken in October 2019. Non-selective, selective attachment, varied, and unknown oviposition behaviors are found in both riffle and mud habitats.

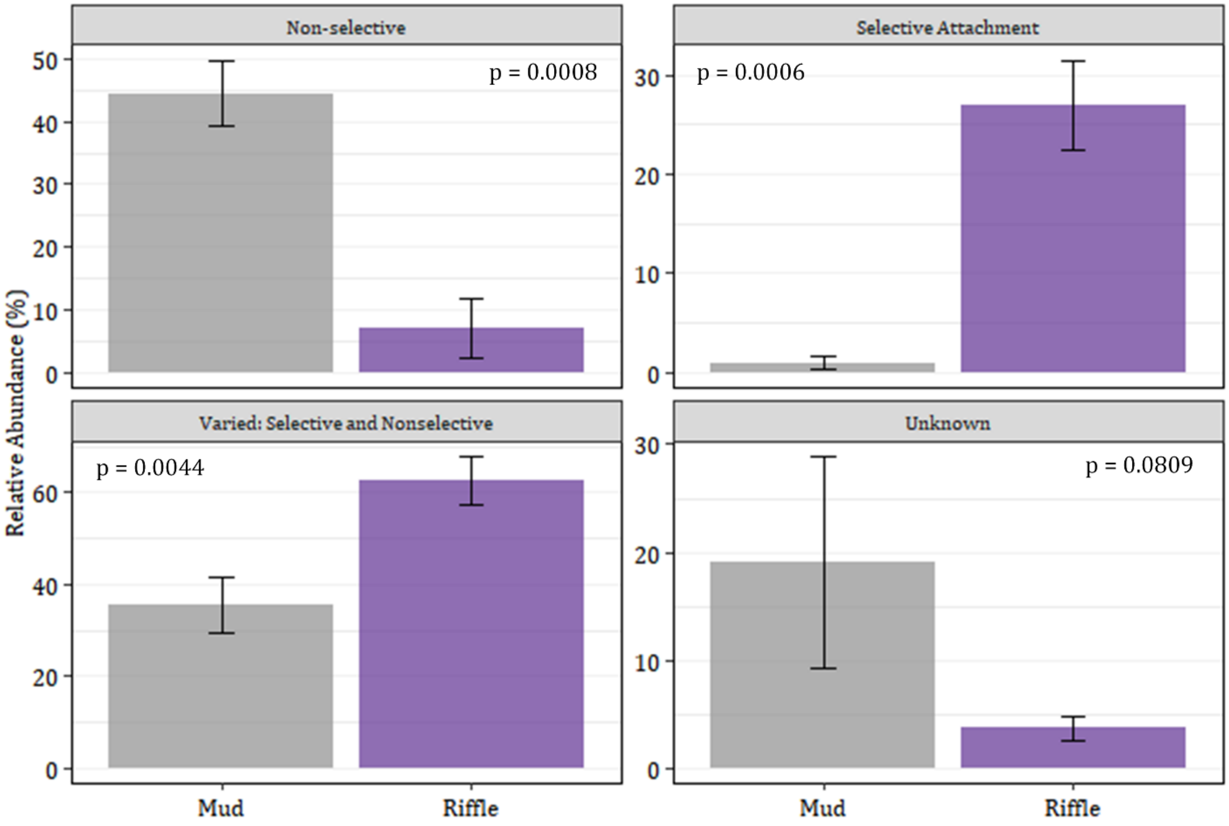


Figure 22 Relative abundance of oviposition behaviors found in riffle and mud habitats in our study reach. Abundance data are from the benthic survey in October 2019. P-values of t-tests comparing relative abundance oviposition behavior in riffle and mud habitats are shown on each plot.

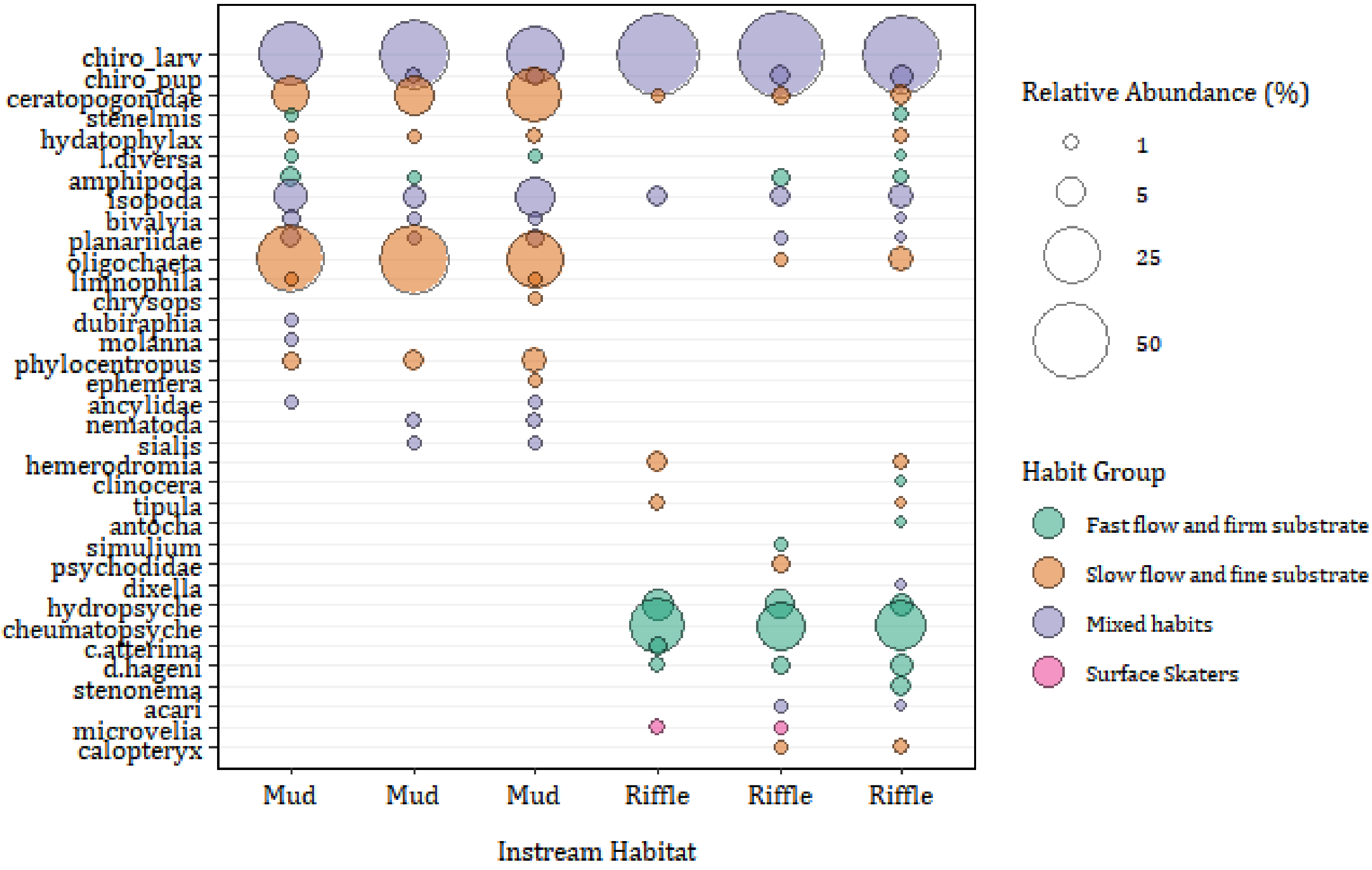


Figure 23 Bubble plot showing habit groups of taxa found in riffle and mud habitats in the study reach. Samples represent composited Surber samples taken in October 2019. All habit groups were found in riffle and mud habitats, although composition of habits varied by habitat type.

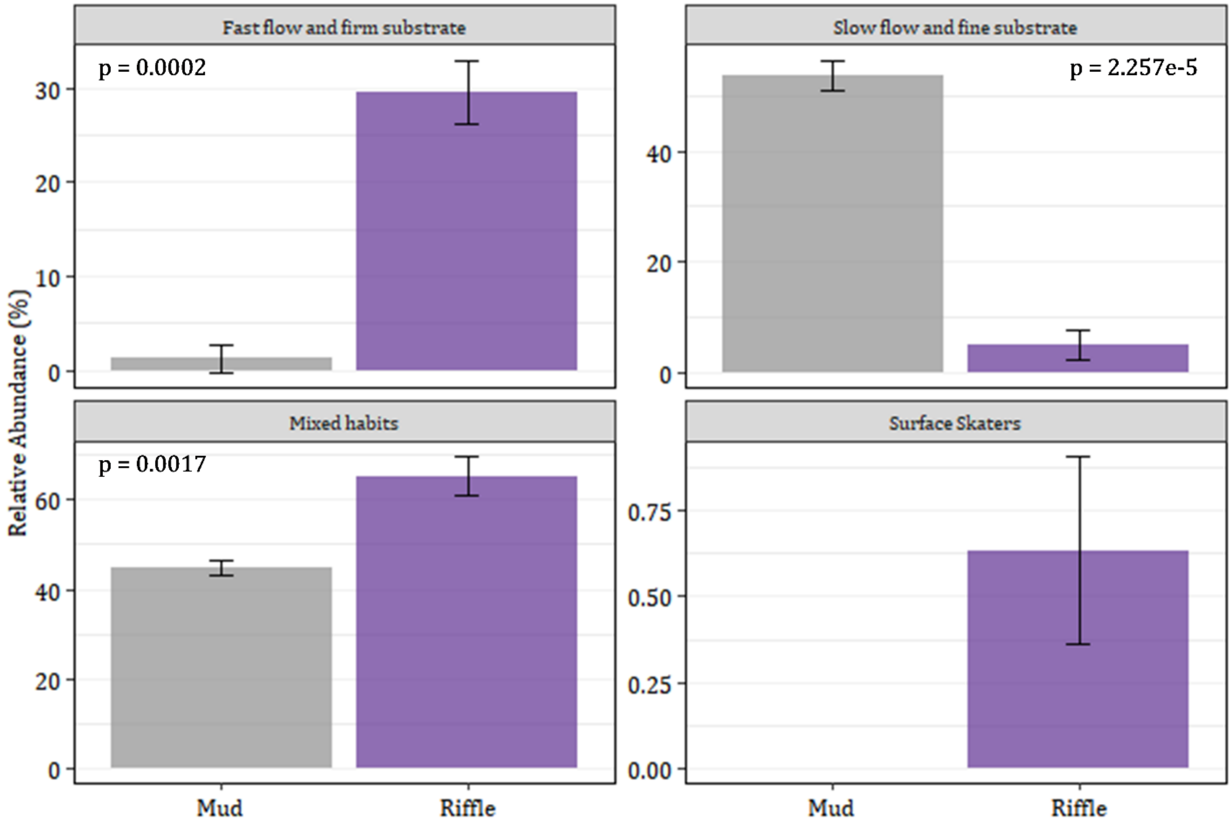


Figure 24 Relative abundance of macroinvertebrate habit groups found in riffle and mud habitats in our study reach. Abundance data are from the benthic survey in October 2019. from the benthic survey of the study reach in August 2019. P-values of t-tests comparing relative abundance of habits in riffle and mud habitats are shown on each plot.

Appendix

Shannon diversity Index:

$$H' = \sum_{i\,=\,1}^{S} (p_i \ln p_i)$$

Where S is the sample richness and p_i is the relative abundance of taxon i

Values typically are 1.5-3.5 with high values occurring when richness is high and most taxa are equally abundant in the sample (high evenness).

Simpson's diversity index:

$$\lambda = \sum p_i^2$$

Where p_i is the relative abundance of taxon i

Simpson's index is often used as a "concentration of dominance" index as it represents the probability that any two individuals chosen at random from a sample will belong to the same taxon. It essentially measures the extent to which individuals in a sample are concentrated into a few taxa.

$$D = 1 - \lambda$$

There are several ways to represent Simpson's index, however by subtracting Simpson's index (λ) from 1, values of D will range from 0-1, with values closer to one representing more diverse communities. D now represents the probability that any two individuals chosen at random from a sample will belong to different taxa.

Table 5 Abundance data for taxa with patchy distribution in rock basket samples from study reach

Taxon	Date	Control	Within riffle set (15m)	Below riffle set (15 m)	Within riffle set (10m)	Below riffle set (10 m)	Within riffle set (5m)	Below riffle set (5 m)
Coleoptera								
Elmidae								
Optioservus sp.	Aug							
	Oct		1					
Optioservus ovalis	Aug	7						
	Oct							1
Oulimnius sp.	Aug							
	Oct	1			1			
Stenelmis crenata	Aug	1	2	1		4	1	
	Oct		4	1	2	3		
Diptera								
Ceratopogonidae pupae	Aug					1		
	Oct							
Culicidae	Aug		1					
	Oct							
Empididae								
Empididae pupae	Aug							
	Oct							2
Clinocera sp.	Aug	2						1
	Oct	1	7	1	5	1		13
Psychodidae	Aug							
	Oct	1			4		1	
Psychomyiidae	Aug							
	Oct				1			
Ptychopteridae								
Ptychoptera sp.	Aug							

Taxon	Date	Control	Within riffle set (15m)	Below riffle set (15 m)	Within riffle set (10m)	Below riffle set (10 m)	Within riffle set (5m)	Below riffle set (5 m)
	Oct					1		
Simuliidae								
Prosimulium sp.	Aug					1		
	Oct							
Simuliidae pupae	Aug							
	Oct		1					1
Simulium sp.	Aug					2		5
	Oct							
Stratiomyidae								
Odontomyia sp.	Aug							
	Oct					1		
Tabanidae								
Chrysops sp.	Aug	1	4		2		2	2
	Oct	2	4		2	1	5	3
Tipulidae	Aug							
	Oct					1		1
Tipulidae pupae	Aug							
	Oct					1		
Antocha sp.	Aug							
	Oct					1		3
Hexatoma sp.	Aug			1				
	Oct					1		
Molophilus sp.	Aug							
	Oct		1					3
Pseudolimnophila sp.	Aug							
	Oct	1	7		3	34		15
Tipula sp.	Aug							
	Oct				5	13		8

Taxon	Date	Control	Within riffle set (15m)	Below riffle set (15 m)	Within riffle set (10m)	Below riffle set (10 m)	Within riffle set (5m)	Below riffle set (5 m)
Ephemeroptera								
Baetidae	Aug		4		8			
	Oct	8						
Baetis sp.	Aug							1
	Oct				1			1
Caenidae								
Caenis sp.	Aug							
	Oct							1
Ephemerellidae	Aug							
	Oct		1					
Ephemeridae								
Ephemera sp.	Aug		4	1		1		
	Oct	3	1			3	2	1
Heptageniidae								
Stenonema/Stenacron sp.	Aug	7					4	
	Oct					3	30	41
Odonata								
Sialis sp.	Aug	1		1	2			
	Oct	4	3	2	5			1
Trichoptera								
Trichoptera	Aug	1					4	
	Oct	2				6		1
Hydropsychidae								
Hydropsyche sp.	Aug					4		4
	Oct		1		1	1		2
Leptoceridae								
Oecetis sp.	Aug							
	Oct		2				1	

Taxon	Date	Control	Within riffle set (15m)	Below riffle set (15 m)	Within riffle set (10m)	Below riffle set (10 m)	Within riffle set (5m)	Below riffle set (5 m)
Triaenodes sp.	Aug	11					6	
	Oct	4	4	2		1	1	1
Limnophilidae								
Hydatophylax/Pycnopsyche sp.	Aug		1		2	2	1	1
	Oct	5	3		2	6	2	15
Molannidae								
Molanna sp.	Aug							1
	Oct							5
Philopotamidae								
Chimarra aterrima	Aug					1		
	Oct		1		4			2
Phryganeidae	Aug							
	Oct				1			1
Psychomyiidae								
Lype diversa	Aug							1
	Oct		2		6	9	2	24
Non-insect invertebrates								
Acari (water mites)	Aug	11		1			4	1
	Oct	5	4			3	5	4
Gastropoda	Aug							
	Oct							1
Hirudinea	Aug		1			1	3	2
	Oct				2	5	4	15
Nemertea	Aug	2						
	Oct	1			2			

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