ABSTRACT

JORDT, SAMANTHA E. Accelerating the Recovery of Invertebrate Communities in Restored Streams in North Carolina by Addressing Egg Recruitment Limitation Through Supplementation of Oviposition Habitats. (Under the direction of Dr. Bradley Taylor).

The UN has designated 2021-2030 the decade of restoration, reflecting a global recognition that our planet is changing so rapidly that conservation alone is not enough, and we must restore some of the ecosystems we have lost. Ecosystems, however, are extremely complex and thus far, our attempts to recreate terrestrial, marine, and stream environments have been met with some successes but many failures. Aquatic insects, for instance, rarely reestablish in restored streams even years post restoration. In both terrestrial and marine restored sites, recruitment has been identified as a contributing factor in failed biological recolonization and has been suggested as limiting aquatic insect establishment in stream restoration as well. One way recruitment limitation occurs is when the availability of suitable habitat influences whether new individuals survive long enough to become established and mature to adults (Chesson 1998). In terrestrial restoration, that has manifested in a failure of seed establishment, and in marine restoration, larval mussel establishment. In streams, larval insect habitats are often improved as a result of restoration efforts, yet many taxa have specific habitat requirements for adult female egg laying that may not be available in these streams. An estimated ¾ aquatic insects require emergent instream structures such as rocks, wood, or macrophytes to land on then crawl underwater and attach their eggs onto the side or the underside of those structures, and availability of egg laying habitats limit egg recruitment. We censused 50 m long areas of ten restored streams and three reference streams to compare egg-laying habitat, in the form of emergent rocks, and quantify egg inputs. Both rock and egg mass densities were lower in restored streams than reference streams, though egg mass density on individual rocks was higher in restored streams. This is notable because rocks were also more likely to roll in restored streams, possibly resulting in greater egg mortality due to damage during rock movement or desiccation once the rock settles if eggs are exposed to air. The following year, we experimentally increased oviposition habitat in five out of ten restored streams to test whether egg laying habitat was limited in restored streams and whether, by increasing it, we could increase egg mass density, richness, and eventually larval abundance. Specifically, we tested whether the change between years before and after treatment would differ between restored-treated and restored-untreated streams and between restored-treated and reference streams. Following treatment, egg mass density and richness per stream area increased in restored-treated streams but egg mass density per rock area remained elevated compared to reference streams. Additionally, larvae in the four egg laying taxa did not respond to treatment but was not negatively impacted by rock additions. While treatment reduced the percentage of rocks that rolled or went missing, it continued to occur at a higher rate than in reference streams and may explain, in part, the lack of response from larvae. These results indicate that restored streams were limited by oviposition habitat and possibly stability but even minor increases in egg laying habitat can alleviate one recruitment limitation. However, there may be additional recruitment or post recruitment barriers to aquatic insects in restored streams. This experimental approach to accelerating biological restoration in restored streams is one example of how multidisciplinary collaboration and intersections in science may illuminate missing linkages and improve ecological restoration more broadly.

Accelerating the Recovery of Invertebrate Communities in Restored Streams in North Carolina
by Addressing Egg Recruitment Limitation Through Supplementation
of Oviposition Habitats

by
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BIOGRAPHY

My interests in aquatic ecology and macroinvertebrates started at a very young age. I grew up in Wisconsin, on a long peninsula jutting out into Lake Michigan. I spent many of my days as a child exploring the boreal forests, wetlands, lakes, and streams surrounding my home. I became enchanted by the area's beautiful, colorful, bubbly aquatic worlds and I would often stand motionless in them, watching as they acclimated to my presence and came alive. Though I had no formal training, I learned a tremendous amount about the characteristics of, and contrasts between, the extremely different aquatic environments that make up the peninsula.

I graduated from the University Wisconsin Madison where I continued to develop my knowledge of natural science, focusing on limnology and entomology, earning a BS in Conservation Science. As an undergraduate student, I spent a semester in Australia with the School for Field Studies and a summer in Utah working with the UW Department of Entomology studying Mountain Pine Beetle. Those experiences proved to me that I love working in the field, even in extreme conditions such as being devoured by terrestrial leeches in the Australian rainforest or gathering data under the watchful eye of a black bear in Utah.

After graduating college, I was fortunate enough to get a full-time job with the Wisconsin Department of Natural Resources, working in the Environmental Loan Programs as a project manager. It was a diversion from my desired career path, but the position allowed me to pay off my student loans and develop professional skills. After 5 years, I decided it was time for me to return to my roots and seriously pursue a career that I was passionate about.

I ultimately chose to pursue a master's degree in Brad Taylor's lab in the Department of Applied Ecology because of its focus on aquatic ecosystem ecology and aquatic insect life histories. While at North Carolina State University, I have been able develop my knowledge of stream ecology, focusing on aquatic insect recruitment into restored streams. Studying aquatic insects in restored systems has allowed me to focus on a science that I am fascinated in and believe is incredibly important. I would like my future research to inform conservation practices that improve habitat for aquatic insects and the species that rely on them.

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CHAPTER 1

A rolling stone gathers no eggs: the importance of stream insect egg laying natural history for stream restoration

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Keywords: oviposition, particle size, recruitment, substrate stability, natural channel design

The degradation of waterways has led to the widespread practice of stream restoration aimed at accelerating recovery from damage by land-use change and other disturbances. However, the biological recovery of restored streams often lags behind their physical and chemical recovery (Louhi et al. 2011a). Improving stream restoration is important for many reasons, including mitigating the impacts of development and because annually billions of dollars are invested in stream restoration worldwide. Slow biological recovery is not surprising given the emphasis on restoring physical stream features (e.g., riffle-pool sequence, meanders, bank stability) and the expectation that biological recovery will naturally follow (Palmer et al. 2014). Restoration typically improves physical habitats for fish and larval insects (Bernard et al. 2007); yet, habitats for adult aquatic insects, are overlooked. Here, we report observations of egg laying in restored and reference streams that reveal how natural history science can be used to enhance stream restoration design, particularly for the recovery of stream insects.

Stream insects have complex life cycles that involve developing in an aquatic habitat, emerging to reproduce terrestrially, then returning to water to lay eggs. Females have various egg-laying strategies; some release their eggs while flying over the water surface, but an estimated 75 % of aquatic insect species attach their eggs to objects within the stream such as rocks (Fig.1.1A-F) or wood (Statzner and Bêche 2010). For example, mayflies from the widespread genus *Baetis* (Ephemeroptera) select rocks partially extending above the water's surface (hereafter emergent rocks, Fig. 1.1A-C) to land on, crawl underneath, and attach tombstone-shaped egg masses (Peckarsky et al. 2000). Caddisflies (Trichoptera) lay eggs in much the same way (Fig. 1.1D) (Smith and Storey 2018). Adult waterpenny beetles (Coleoptera) use emergent rocks as both mating sites and access points for females to crawl underwater and lay their bright yellow eggs (Fig. 1.1E). Gelatinous egg masses of aquatic true flies such as midges (Diptera) are commonly found on emergent rocks (Fig. 1.1F) and are diverse in structure and attachment mechanisms (Reich 2004).

Egg-laying females can be highly selective (Smith and Storey 2018), often preferring emergent rocks that are large, unembedded, and in fast and constant flow. For many species, egg masses containing hundreds to thousands of eggs are highly aggregated, with a few emergent rocks receiving > 90 % of egg masses (Peckarsky et al. 2000). This selective behavior increases egg survival, hatching success (Bovill et al. 2013), and larval recruitment (Encalada and Peckarsky 2012a). Moreover, availability of rocks suitable for egg laying can limit stream insect populations (Alp et al. 2013) and determine stream insect community composition (Kennedy et al. 2016a). Thus, egg laying on emergent rocks represent a linkage between stream physical characteristics and insect natural history that could have applications for stream conservation and restoration to either maintain or accelerate recovery of stream insect diversity, richness, and abundance.

During the summer of 2019, the abundance of rocks suitable for stream insect egg laying and the number of eggs masses they contained was observed throughout the egg-laying period (June to October) in ten restored and three reference streams in western North Carolina (Fig. A1). Streams were restored ≥ 7 years ago, in agriculturally dominated watersheds containing cropland and cattle grazing, and watersheds had < 5 % impervious surface area (Table S1). Restoration involved riverbed and bank reconstruction, riparian buffer planting, and cattle exclusion. Restored streams in North Carolina (Tullos et al. 2009) as elsewhere (Louhi et al. 2011a) have slow biological recovery and low insect diversity and abundance. Reference streams of similar size with predominantly forested watersheds were located nearby (Fig. A1). Suitable egg-laying habitats were identified as emergent rocks with submerged spaces that adult insects could access along the sides and underneath (i.e., unembedded). Emergent rocks were numbered using a nontoxic paint pen and removed from the water to identify and count egg masses, species or morphotype, and sketch mass locations. New masses were identified by comparing sketches to those made previously. Rocks were replaced in their original location and position. We returned every 1-2 weeks to census these and newly emergent rocks for egg masses. Mayfly, caddisfly, and true fly taxa constituted ~ 90 % of the masses in reference and restored streams, with mayflies (45 %) dominant in restored streams and mayflies (39%) and caddisflies (36%) codominant in reference streams.

Densities of emergent rocks (number per m^2 of streambed) were 103 % greater in reference than restored streams, and as water levels declined emergent rocks increased in reference but not restored streams throughout the summer (repeated measures ANOVA, Time × Stream type: $F_{1,24.1}$=6.210, P=0.02; Fig. 1.2A, S2). Emergent rocks were larger in reference than restored streams (repeated measures ANOVA, Stream type: $F_{1,14.1}$=7.907, P=0.01; Fig. 1.2B). As a result of more egg-laying habitat, the number of egg masses standardized for streambed area was 78 % higher in reference than restored streams (repeated measures ANOVA, Stream type: $F_{1,8.4}$=4.343, P=0.04; Fig. 1.2C). However, densities of egg masses on individual rocks (i.e., number masses per m^2 of individual rocks) were 72 % higher in restored than reference streams (repeated measures ANOVA, Stream type: $F_{1,14.6}$=5.867, P=0.04; Fig. 1.2D). The distribution of egg masses among rocks was highly aggregated (observed distribution differed from expected Poisson (random) distribution, reference: P<0.0001 and restored: P<0.0001) and egg masses were more aggregated in restored than reference streams (Kolmogorov-Smirnov test, D=0.1597, P=0.0001), with reference streams containing many rocks with few masses and restored streams containing more rocks with many masses (Fig. 1.2E). These data suggest sufficient numbers of adults were able to disperse to restored streams, but availability of suitable rocks could limit egg laying and thus limit insect recovery. Moreover, greater egg mass densities on emergent rocks in restored streams is notable because the percentage of emergent rocks that rolled or were unrecoverable (rolled out of the study reach or buried in sediment) was 91 % higher in restored than reference streams (generalized linear model, Stream type: χ^2=5.36, df=12, P=0.02; Fig. 1.2F, S2), and differences in emergent rock stability were greater during late summer (generalized linear model, Time × Stream type: χ^2=3.90, P=0.04), when egg laying was high in reference streams. Rolled or missing rocks increased following rainstorms in restored streams, and in one site no submerged or emergent rocks remained after a storm. We could not determine egg survival on rocks that rolled or went missing because some rocks could not be recovered or were recovered more than 2 weeks later and their eggs could have hatched during this time. However, prior to rolling 26 % (16 of 62) of the rolled and recovered rocks had egg masses (n=140 masses), indicating insects lay eggs on unstable substrates. Although survival of eggs on buried or rolled rocks is unknown, mortality due to desiccation can occur in less than 2 hours for some mayflies and caddisflies (Miller et al. 2020) and we observed on multiple occasions that rolling resulted in exposure of the eggs above the water. In reference streams, emergent rocks remained intact, except during heavy rainstorms in early June. These results indicate that in moderate gradient, rocky-bottomed restored streams insect egg laying is limited by the number of emergent rocks. Further, because emergent rocks in restored streams are unstable, egg mortality may be higher due to emergent rocks rolling or being buried in sediment. Thus, even if adult females can disperse to restored streams and all other habitat conditions are suitable, larval insect populations may fail to establish because adult females lack stable egg-laying habitat.

In small rocky-bottomed streams the characteristics of suitable egg-laying habitat (i.e., large, stable, emergent, unlikely to dry rocks in fast-flowing water) for many stream insects are known and could be readily incorporated into restoration projects to increase stream insect recruitment (Storey et al. 2017). However, to maximize cost-effectiveness and achieve biological recovery, restoration should consider site- and taxon-specific natural history and multivariate nature of egg-laying habitat selection. Moreover, restoration projects could be used to test the degree to which insects in restored streams are limited by egg-laying habitat or other factors. For instance, adult dispersal from adjacent reference streams in degraded and disconnected landscapes, or post-recruitment processes such as larval food resources and mortality, factors that may negate the positive effects of restored egg-laying habitat.

One of the most vexing problems in stream restoration is that the return of biological conditions lags behind the return of physical habitat and water quality. Our observations suggest that in addition to the number of suitable egg-laying sites, the stability of sites may limit insect recruitment and, hence, recovery of insect diversity and abundance in these and similar restored streams. Indeed, many of the taxa that use the underside of emergent rocks as egg-laying habitat are used as indicators of stream health (Ephemeroptera, Plecoptera, and Trichoptera). Thus, considering the natural history of stream insect egg-laying during the hydrologic and geomorphologic engineering of restored streams is likely to improve stream restoration and accelerate biological recovery, as the importance of natural history has informed other types of restoration projects (Tewksbury et al. 2014).

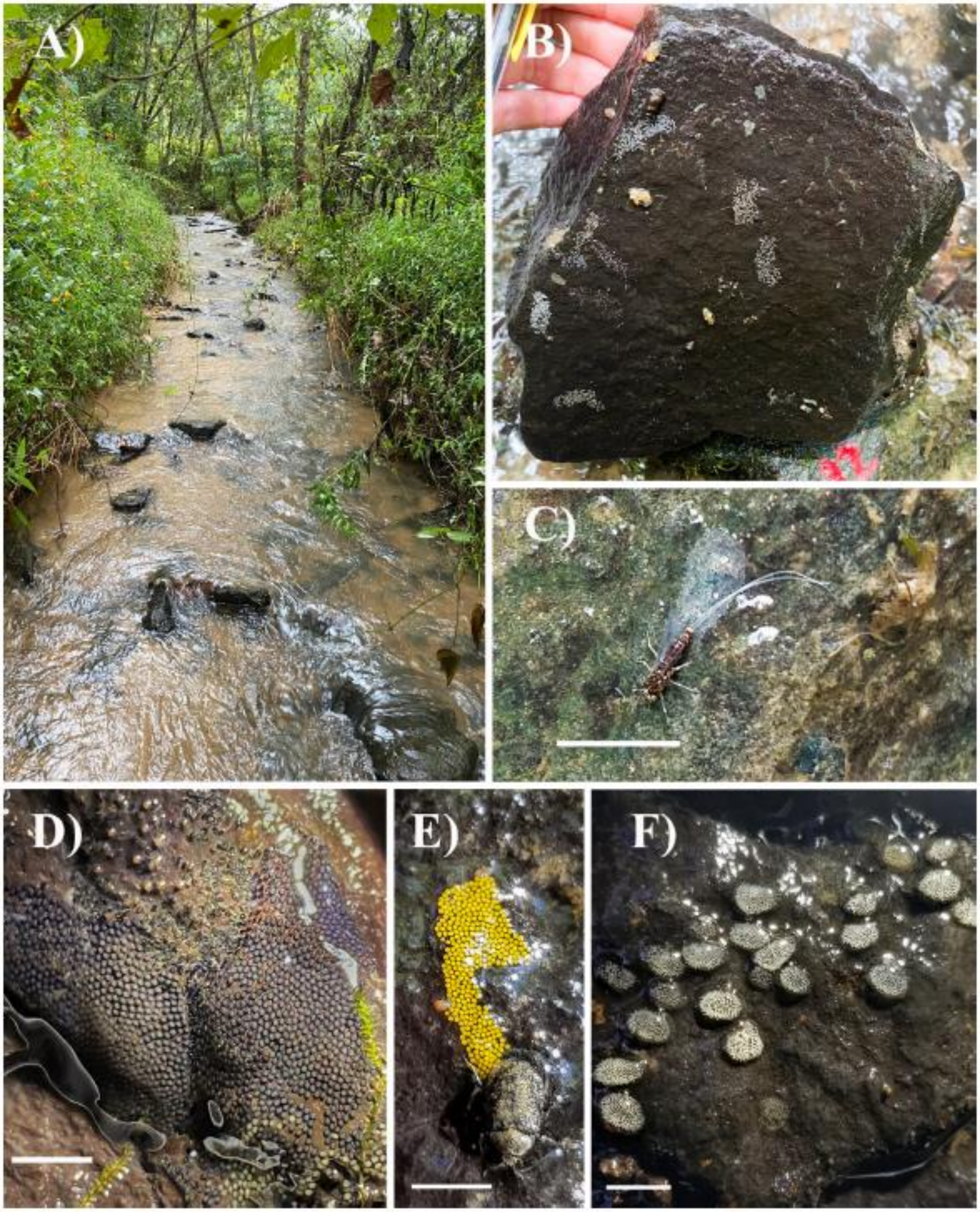

Fig. 1.1. (A) A restored stream in western North Carolina with examples of emergent rocks. (B) Underside of an emergent rock that female mayflies (Ephemeroptera) and caddisflies (Trichoptera) crawled underneath and attached egg masses. (C) Female mayfly (Ephemeroptera: Baetidae) and egg mass. (D) Egg masses of a caddisfly (Trichoptera: Hydropsychidae) taxon. (E) Female waterpenny beetle (Coleoptera: Psephenidae) and egg mass. (F) Egg masses of a midge (Diptera: Chironomidae) taxon. Scale bar in panels C–F is 0.5 cm.

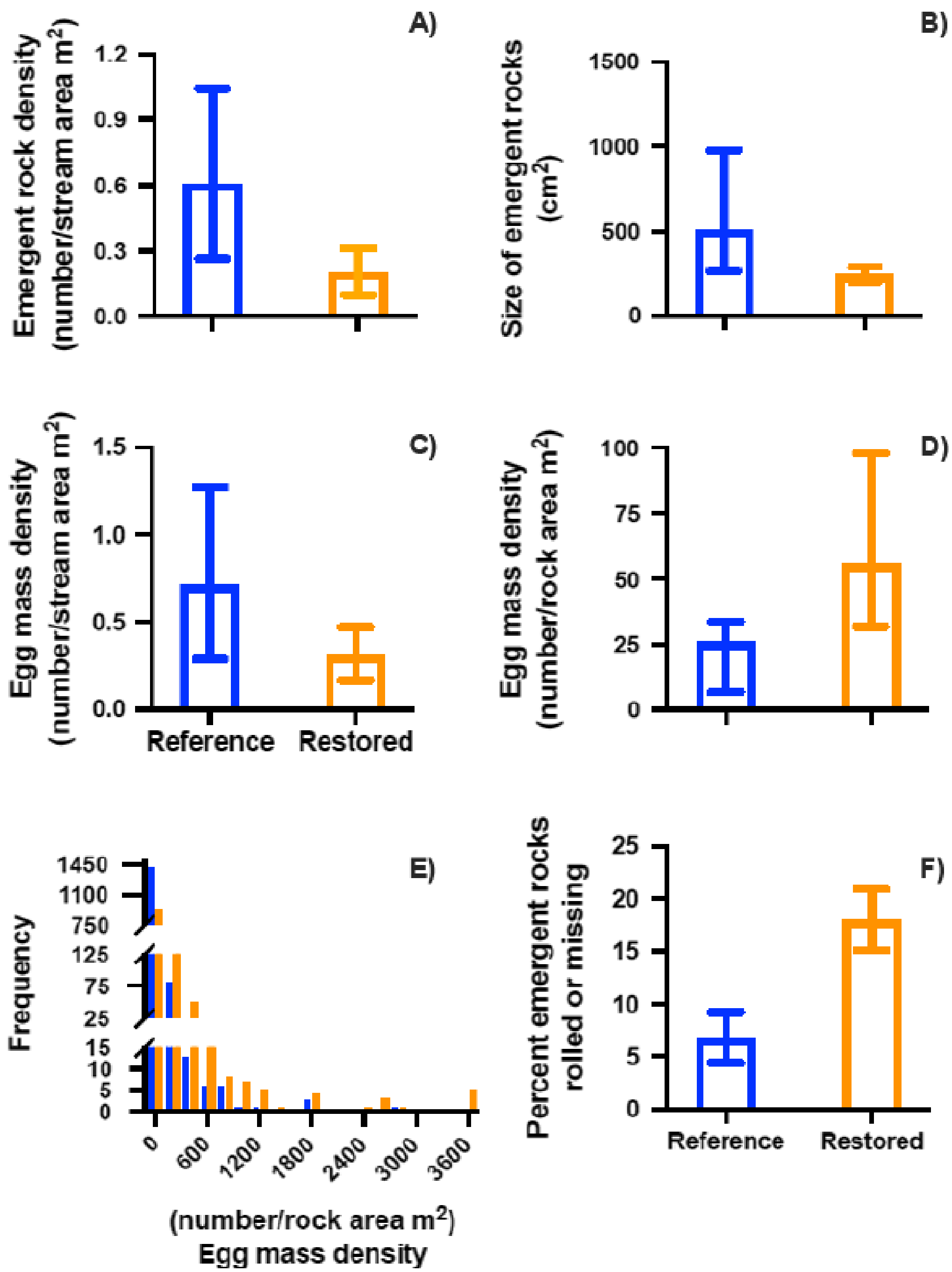

Fig.1.2 Reference (blue bars) and restored (orange bars) streams differed in (A) number of emergent rocks, (B) size of emergent rocks, (C) number of egg masses standardized for wetted stream area, (D) number of egg masses standardized for individual rock area, (E) the distribution of egg mass densities among individual emergent rocks (for display purposes, the restored stream distribution is truncated with bin 3,600 including five rocks: three with 3,600, one with 3,900, and one with 7,200 egg masses per rock area), and (F) percentage of emergent rocks that rolled or were not recovered. Means in panels A–D and F are weighted by the number of weeks sampled for each site from 29 May to 13 October 2019. In panels A–D, means are back transformed and asymmetric error bars are 95% confidence intervals back transformed from the natural-log scale. In panels A, B, and D–F, emergent rocks include only those that were unembedded. The histogram bin width is 300.

CHAPTER 2

Facilitating the recovery of insect communities in restored streams by increasing oviposition habitat

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Abstract

Recruitment limitation is known to influence species abundances and distributions. Recognition of how and why it occurs both in natural and in designed environments could improve restoration success which has often failed at restoring biological communities. Aquatic insects, for instance, rarely reestablish in restored streams even years post restoration. We experimentally increased oviposition habitat in five out of ten restored streams in western North Carolina to test whether egg laying habitat was limiting in restored streams and thus whether increasing oviposition habitat could be used to increase the abundance and richness of aquatic insect eggs and larval insect abundance compared to restored-untreated and reference streams. Following treatment, egg mass abundance and diversity increased by 114% and 67% respectively in restored-treated streams. There were no increases in larval insects based on identification to the taxonomic level of order. The results indicate that these stream insect populations are limited by oviposition habitat and thar adding habitat can alleviate this aspect of recruitment limitation. However, additional recruitment or post recruitment factors may also limit aquatic insects in these restored streams. Nearly all aquatic insect taxa are listed as imperiled due to habitat loss, pollution, and global climate change. Stream restoration is and will

continue to be necessary for rehabilitating and maintaining stream ecosystem structure and function. This study demonstrates the importance of integrating ecological and life history information into restoration practices to improve efficacy for aquatic invertebrates, which are often used to assess stream structure and function.

Keywords: stream restoration, emergent rocks, benthic substrate, oviposition habitat, life history

Introduction

Recruitment limitation occurs when the local abundances of organisms are constrained by the supply rate of new individuals, the availability of suitable habitat, environmental conditions, or species interactions that can individually or collectively influence whether new individuals survive long enough to become established and mature to adults (Chesson 1998). Recruitment limitation can explain variation in the local abundances of many marine organisms (Reed et al. 2004, Doherty and Fowler 1994, Olafsson et al. 1994). In marine barnacles, for example, recruitment limitation has been manifested as an inability to access viable habitat, an inability to settle, or the mortality of settlers (Connell 1985). Beyond marine systems, recruitment limitation has been known to influence terrestrial plants (Connell and Green 2000) and freshwater invertebrates (Downes 1995, Encalada and Peckarsky 2011). Factors such as relative contributions of initial recruitment, size of or distance to source populations, and post-recruitment processes could be targets for management, conservation, and restoration given that recruitment limitation has been known to impede marine restoration efforts (Fariñas-Franco and Roberts 2018, Colsoul et al. 2020) and has been attributed to delayed or failed restoration of forests and prairies (Standish et al. 2007, Acácio et al. 2007, Carrington 2014). Additionally, recruitment limitation has been suggested as a reason for latent recovery of aquatic insects in stream restoration (Heino and Peckarsky 2014, Storey et al. 2017).

Nearly all aquatic insect taxa are listed as imperiled due to anthropogenic stressors such as habitat loss, pollution, and global climate change (Sánchez-Bayo and Wyckhuys 2019). Stream restoration, which both provides benefits to aquatic insects and benefits from their presence, has generally been ineffective at restoring biological communities (Tullos et al. 2009, Palmer et al. 2010) even decades post restoration (Louhi et al. 2011b, Leps et al. 2016). Delayed recovery of stream insects is likely due to restoration practices focusing largely on chemical and physical stream components and addressing only the basic needs of larval stream insects, such as stream bed heterogeneity (Wohl et al. 2005, Palmer et al. 2010, Bennett et al. 2013). Yet stream insects have complex life cycles with specific habitat requirements at multiple disparate life stages (i.e., egg, larvae, pupa, and adult) that often extend above and beyond the water to include riparian areas as well as instream structures extending above the water surface, such as rocks, wood, or macrophytes, which are used by adult stream insects. The importance of the aerial adult stage for the recolonization and recruitment dynamics of stream insect populations has long been recognized (Müller 1982), but the importance of restoring oviposition habitat for egg laying adults and thus the initial recruitment of insects to restored streams has been less explored (Lancaster et al. 2010a, Storey et al. 2017). Identifying the factors that influence stream insect recruitment at different stages in their development and in their aquatic and terrestrial environments is a necessary step towards improving the efficacy of restoration. Here, we use an experiment to test whether restoring oviposition habitat affects the recruitment of insects in restored streams which, if successful, can be applied to new and existing restoration projects to accelerate the recovery of stream insect communities.

An estimated three quarters of aquatic insect taxa have an oviposition strategy of landing on rocks partially extending above the water surface, then crawling underwater and attaching their eggs onto the side or the underside of rocks in streams (Statzner and Bêche 2010). This behavior is well documented in Ephemeroptera and Trichoptera and it may occur in some Plecoptera (Smith and Storey 2018), the three aquatic insect orders valued for their use in bioassessment (i.e., EPT richness) because they are known to be sensitive to environmental stressors. Egg laying on emergent rocks is also common in aquatic Diptera (Nolte 1993) and in at least one family of aquatic Coleoptera, Psephenidae (Murvosh 1993) but may occur in Elmidae (Elliott 2008). Following emergence, the adults mate and the females return to the stream to find a rock extending above the water surface on which they can land. Once the rock is selected, they crawl under, and cement hundreds to thousands of eggs, often in a mass, to the side or unembedded underside (Hoffmann and Resh 2003, Encalada and Peckarsky 2007, Lancaster et al. 2010b, Storey et al. 2017, Smith and Storey 2018). This behavior leads to high hatching success (Encalada and Peckarsky 2006; Kennedy et al. 2016). Additionally, many other stream insect taxa use these substrates for egg laying indirectly by releasing globular egg masses into the water, relying on the expanding mass to contact and adhere to emergent or submerged rocks, wood, or vegetation (Williams 1982). Consequently, availability of rocks suitable for egg laying females is known to limit aquatic insect populations (Encalada and Peckarsky 2011, 2012b, Alp et al. 2013, Macqueen and Downes 2015). Thus, even if female insects can disperse to a restored stream, and if all other habitat conditions are met, many insect species may fail to colonize because they lack suitable habitats to lay their eggs. We applied this natural history of stream insects and knowledge from experimental studies in undisturbed streams to investigate how emergent rock abundance differed among restored and reference streams, and whether emergent rock abundance could be manipulated to enhance egg and larval insect recruitment.

Much of stream restoration focuses on the size of benthic substrates, such as rocks, for the establishment of larval stages but does not account for characteristics of benthic substrates that are also used by adults of many species for oviposition and, therefore, insect populations may experience egg recruitment limitation in restored streams. The goal of this study was to test whether enhancing egg-laying habitat (i.e., emergent rock abundance) for adult stream insects could be used as a technique to improve stream restoration by accelerating the recovery of the aquatic insect communities in restored streams. We used an experimental approach in which we surveyed egg laying in restored and reference streams before and after adding emergent rocks to restored streams to test the following hypotheses. 1) Egg recruitment of stream insects (density and morphotype richness) who require emergent rocks for egg laying is limited by the number of suitable oviposition sites (i.e., submerged rocks partially extending above the water surface, hereafter emergent rocks, and rocks with interstitial spaces underneath or along the sides that female insects could access, hereafter unembedded rocks). 2) Larval recruitment of aquatic insects (density) whose adults use emergence rocks for egg laying is limited by the number of oviposition sites. 3) Alternatively, increasing oviposition habitat in restored streams will increase egg inputs (initial recruitment) but will not result in increased larval abundance because post-recruitment processes such as egg hatching success, larval food resources, or priority effects (Barrett et al. 2021) associated with the existing biotic community, will limit larval abundance. 4) Egg and larval recruitment are reduced as emergent rock instability increases, and emergent rocks are less stable and thus more likely to roll or be buried in restored streams. Based on the outcomes of these hypothesis, we discuss the direct application of this research for improving the practice and theory of stream restoration as well as the policy and management implications.

Methods

Site selection

We obtained a list of 378 North Carolina Department of Environmental Quality Mitigation Services projects that included a stream component. To reduce variability among sites, we selected sites from the 378 with similar restoration type, land-use, and stream bed composition. We selected projects that had undergone extensive channel transformations that completely altered the channel pattern, profile, and dimension in accordance with restoration priority type one or two (RP1 or RP2) (Doll et al. 2003). This meant that projects increased channel sinuosity, stream bed heterogeneity, and either elevated channels to reconnect them to floodplains (RP1) or created floodplains at the current stream elevation (RP2). We also selected sites between the ages of 7-15 years because this should allow sufficient time for riparian planting to mature, fine sediments to stabilize, detritus to accumulate, biofilms to establish, and some insects to recolonize, but also minimize individual divergence among sites independent of the restoration, such as colonization and damming by beaver. Further, we selected streams with similar catchment land-use composition (impervious surface less than 5% and developed less than 20%). We identified rocks as the oviposition habitat for manipulation in this study and, for that reason, we selected sites that already contained this type of habitat and excluded projects located in the coastal plain and streams with the median particle size (D50) < 2 mm (i.e., primarily sand-bed streams). Although stream insects use rocks, wood, and live vegetation as well as other material for egg-laying (Smith and Storey 2018), we focused on rocks in this study because they were the most abundant habitat, more permanent than wood, and are usually available or added during stream restoration. Lastly, we selected restored stream projects with multiple tributaries to compare manipulated and unmanipulated sites of similar restoration age (>7 years), insect composition, and other physical, chemical, and biological factors given their close physical proximity.

Ultimately, 12 projects fit all requirements including access, as the state no longer has the right to access the conservation easement without permission from the property owner after 7 years. Following physical visits, we selected 10 streams. We blocked streams primarily by proximity to one another (Pinch Gut with Candiff Creek and the two Warrior Creek tributaries:

Mountain Creek with the unnamed tributary; Fig. 2.1, Table 2.1) and secondarily by stream characteristics, such as channel size and presence or absence of a culvert above the restored reach (Morgan Creek sites Main Up with Down, UT1 with UT3, and UT2 with UT5; Table 1). We randomly assigned one stream from each of five blocks to receive the treatment (addition of suitable rocks for oviposition) using a coin toss, resulting in five restored-untreated and five restored-treated streams (Table 1). The 10 stream sites were part of four restoration projects located in western North Carolina (Fig. 2.1). Morgan Creek and Warrior Creek had multiple stream sites (at least one treated and untreated) used in the project. There were two sites on different tributaries of Warrior Creek, and Morgan Creek had four sites on different tributaries and two sites in the main channel (the only stream that included two sites in the same channel: an upstream (untreated and downstream treated section of stream). Pinch Gut and Candiff Creek were a block. Three reference streams (Indian Creek, Boone Fork Creek, and Beaverdam Creek) were selected based on their proximity to restored streams, accessibility, similar catchment size, and for having predominantly forested intact watersheds. Two reference streams were included in blocks four and five with restored-untreated and restored treated streams, but the reference stream Beaverdam Creek was included in its own block, six (Table 1). We were limited to three reference streams because of access to private property and physical proximity to the restored streams (Fig. 2.1, Table 1). Indian Creek, near both Pinch Gut and Candiff Creek, was located in Hanging Rock State Park, ~10 meters upstream of a DEQ reference site. Boone Fork Creek was nearest to Warrior Creek but at a higher elevation in the north-eastern portion of Pisgah National Forest, Lenoir, NC. The final reference, Beaverdam Creek, was located east of Morgan Creek on the southern end of the South Mountains State Game Lands.

Experimental Manipulation of Oviposition Habitats

To experimentally test whether increasing the abundance of emergent rocks, or potentially suitable oviposition habitat, increased the abundance and richness of egg masses in restored streams, we used a before and after repeated measures ANOVA experimental design with three treatment types: five replicate restored streams in which we manipulated oviposition habitats by adding suitable rocks (restored-treated), five replicate restored streams that were unmanipulated (restored-untreated), and three reference streams (reference). We intended to treat the five restored streams in March 2020 before insects emerged into adults, and census egg inputs from May to October, similar to 2019. However, due to the COVID-19 pandemic, the treatment was delayed until May 2020 and egg masses censuses started in June 2020. Because of this delay and the seasonality of oviposition that includes a May peak, we omitted 2019 May data from the before-after repeated measures ANOVA and used censuses from June – October for both 2019 (before) and 2020 (after).

In May 2020, we added or repositioned existing rocks within the five restored-treated streams (Table 2) to increase the availability of suitable oviposition habitat. To each 50 m long reach, two people added rocks by hand to all five restored-treated streams in a single visit (Table 2), a reduced effort than originally planned due to delays and restrictions associated with the COVID-19 pandemic. Rocks added were sourced from nearby fields or within the channel itself when rocks were present but submerged. Rocks were then and positioned or repositioned to be unembedded and partially submerged. We adjusted rocks throughout the study period (June-October 2020) to ensure the supplemental habitats remained suitable (i.e., emergent and unembedded) for egg-laying females. We used the mean number of suitable rocks as the response variable for rock abundance, which was calculated as the sum of the number of suitable rocks per m^2 stream area for each stream across all censuses for each year (omitting May 2019 census data), divided by the number of censuses for each year (2019 and 2020).

Census of Suitable Rocks, Insect Egg Mass Abundance, and Egg Morphotype Richness

In 2019, the year prior to treatment (adding rocks to streams), we censused 50 m long reaches of the ten restored and three reference streams for egg masses throughout the egg-laying period (May to October). Suitable egg-laying habitats were identified as emergent rocks with submerged spaces that adult insects could access along the sides and underneath (unembedded). Emergent rocks were numbered using a nontoxic paint pen and removed from the water to identify and count egg masses, family or morphotype, and sketch mass type and locations. Egg masses were identified to either family (e.g. Baetidae, Psephenidae) or order (e.g. Trichoptera, Diptera), using descriptions and photos from literature (Williams 1982, Nolte 1993, Murvosh 1993, Peckarsky et al. 2000, Hoffmann and Resh 2003, Reich 2004, Lancaster et al. 2010a, 2010b, Lancaster and Downes 2014, Smith and Storey 2018, Miller et al. 2020) or by interrupting females actively ovipositing, and then lumped into order for consistency across taxa. New masses were identified by comparing sketches to those made previously and rocks were placed back into the stream in their original location and position. Most masses were easily distinguished from one another due a gelatinous matrix (spumaline) surrounding the eggs each female laid or by the tight fanlike ordering of eggs of other taxa. Some insects, however, lay in loose masses that are difficult to distinguish from one another; quantities of masses were therefore approximated based on the overall pattern of the mass or masses. Lastly, some insects lay individual eggs or small masses in rock crevasses (Smith and Storey 2018), which were not quantified due to the difficulty in reliably locating them. We returned every one-two weeks to census existing and newly emergent rocks for egg masses 10 times in 2019 and 10 times in 2020.

Often, emergent rocks were missing and were recorded as such on the sampling date they could not be found. Missing rocks were then removed from the count of rocks present but were included if they were discovered on subsequent sampling dates. We noted whether rocks rolled (as evidence by the position of the painted number), were under water, out of water, and/or embedded in sediments. All rocks that were emergent and unembedded were censused for egg masses. With the exception of treatment streams (after, 2020), we did not unembed rocks to census them. In treatment streams, embedded rocks were routinely unembedded throughout the 2020 egg laying season to maintain a high level of suitable rock availability but were considered suitable only if they were recovered and unembedded on the following census.

As the response variable for egg inputs, we used the mean number of egg masses (either total masses or by morphotype) per stream area, which was calculated as the sum of the number of egg masses divided by the stream area censused for each stream, divided by the number of censuses for each year (2019 and 2020). Egg masses per stream area censused was used rather than total stream area of each 50 m study section because on multiple occasions (8 of 114 site visits) the entire 50 m study section could not be censused either due to lightning storms or time constraints. We also calculated the mean number of egg masses per individual rock area, as previous research has shown when rocks are scarce, the density of egg masses on individual rocks is high (Jordt and Taylor 2021); therefore, we tested whether the density of egg masses on individual rocks in the restored-treated streams changed from before to after treatment. Mean egg masses per individual rock area was calculated as the mean of the sum of masses on an individual rock divided by the underside two-dimensional area of the rock for each stream per census date per year.

Larval Insect Abundance

We sampled the abundance of larval invertebrate on the stream bottom during spring 2020 (before rock addition) and spring 2021 (after rock addition) using a 0.09 m2 Surber sampler with 363 µm mesh size net. Invertebrates were preserved in ethanol, picked under 10x magnification, stained with Rose Bengal, and counted and identified to order.

Statistical Analysis

We used a mixed model repeated measures ANOVA to test whether adding rocks increased 1) the abundance of suitable oviposition habitat, 2) the abundance and richness of egg masses, 3) the abundance of larval insects, and 4) the stability (i.e., percent of rocks rolled, buried, or missing) of suitable oviposition habitat. We used a priori contrasts because we were interested in testing specific hypotheses associated with the stream type x time interaction, namely before-after differences between restored-treated and restored-untreated streams and restored-treated and reference streams, and not before-after differences between restored-untreated and reference streams. Stream type (i.e., restored-untreated, restored-treated, and reference), time (2019 and 2020), and their interaction were fixed factors. Block and block x stream type were random factors. The design was a randomized incomplete block design. We transformed response variables to meet assumptions of normality and homoscedasticity, including a log transformation of Ephemeroptera, Trichoptera, Diptera, and Coleoptera mean egg mass densities per stream area and a logit transformation of the mean percentage of oviposition habitats that rolled or were missing. We used a linear regression to test whether the annual egg mass density of all taxa per stream area decreased as the percentage of suitable rocks rolled or missing increased in each stream. We also used linear regression to predict the effects of the change in emergent rock density on changes in egg mass density and egg mass morphotype richness from 2019 to 2020. For graphical display of transformed variables, we report the back transformed means and 95% confidence intervals calculated in the transformed scale.

Results

Experimental Manipulation of Oviposition Habitats

Prior to the experimental addition of suitable rocks to half of the restored streams, both restored-treated and restored-untreated streams had similar numbers of rocks suitable for oviposition (t_{10} = 1.80, P=0.10; Fig. 2.2a), whereas reference streams had 257 % more suitable rocks than restored streams (t_{10} = 3.07, P=0.012; Fig. 2.2a). Treatment increased the mean number of suitable rocks per m^2 in restored-treated streams compared to restored-untreated streams by an average of 239% (repeated measures ANOVA, Type x Time: $F_{2,10}$=14.71, P=0.001; *a priori* contrast: restored-treated vs restored-untreated, t_{10}=5.41, P=0.02; Fig. 2.2a). Before-after changes in the abundance of suitable rocks were similar in restored-treated streams compared to reference streams (*a priori* contrast: restored-treated vs reference, t_{10}=1.95, P=0.08, Fig. 2.2a). After the experimental addition, abundance of suitable rocks was similar between restored-treated and reference streams (t_{10}=0.44, P=0.6, Fig. 2.2a). A total of 283 rocks were added to five treatment streams, which increased emergent rock availability from 2.35 to 7.82 rocks per 10 m^2 (t_{10}=3.26, P=0.008). There was no change in the availability of emergent rocks in restored-untreated streams (0.89 to 1.42 rocks per 10 m^2; t_{10}=0.56, P=0.6) or in reference streams (6.04 to 9.94 rocks per 10 m^2; t_{10}=0.22, P=0.8).

The addition of oviposition habitats also increased the mean size of emergent rocks in restored-treated streams compared to restored-untreated streams by an average of 40% (repeated measures ANOVA, Type x Time: $F_{2,10}$=3.91, P=0.0558; *a priori* contrast: restored-treated vs restored-untreated, t_{10}=2.70, P=0.02; Fig. 2.2b). Following treatment, emergent rocks in restored-treated streams (mean ± 1 SE, 346.80 ± 26.21) remained smaller, but not significantly so, than those in reference streams (mean ± 1 SE, 526.58 ± 135.87; t_{10} = 1.63, P=0.13).

Insect Egg Mass Abundance and Richness

Before treatment, both restored-treated and restored-untreated streams were similar in the mean number of egg masses per stream area (t_{10} = 0.33, P=0.7; Fig. 2.2c), whereas reference streams had 240 % more egg masses than restored-untreated (t_{10} = 3.08, P=0.01) and 276% more than restored-treated streams (t_{10} = 3.36, P=0.007; Fig. 2.2c). Treatment increased the mean number of egg masses per stream area in restored-treated streams compared to the restored-untreated streams by an average of 186% (repeated measures ANOVA, Type x Time: $F_{2,10}$=5.62, P=0.02). The mean number of egg masses increased significantly in restored-treated streams compared to both restored-untreated (*a priori* contrast: restored-treated vs restored-untreated, t_{10}=2.86, P=0.01; Fig. 2.2c) and reference streams (*a priori* contrast: restored-treated vs reference, t_{10}=2.81, P=0.02, Fig. 2.2c). After treatment, egg mass densities in restored-treated streams were similar to reference streams (t_{10}=0.04, P=0.9, Fig. 2.2c). Change in mean egg mass density per individual rock area from 2019 to 2020 did not differ between restored-treated streams and restored-untreated streams (repeated measures ANOVA, Time × Type: $F_{2,10}$=0.12, P=0.88; Fig. 2.2d). However, there were differences among stream types in the egg mass density per individual rock area (repeated measures ANOVA, Type: $F_{2,5}$=10.44, P=0.02, Fig. 2.2d), with higher densities in restored-untreated compared to both the restored-treated and reference streams ($P<0.05$).

Increases in the egg mass densities were driven by the combined contributions from taxa in the orders Ephemeroptera, Trichoptera, Diptera, and Coleoptera (the four insect orders that contain egg cementing taxa using emergent rocks that we could readily identify) and not an increase by one particular taxon (Fig. 2.3a-d) (Ephemeroptera: repeated measures ANOVA, Type x Time: $F_{2,10}$=1.79, P=0.19; Trichoptera: repeated measures ANOVA, Type x Time: $F_{2,9}$=2.86, P=0.10; Diptera: repeated measures ANOVA, Type x Time: $F_{2,10}$=1.49, P=0.27; Coleoptera: repeated measures ANOVA, Type x Time: $F_{2,10}$=3.18, P=0.09).

A total of 24 egg mass morphotypes were found during the 2019 and 2020 censuses and were identified as belonging to the following orders: Ephemeroptera (one), Trichoptera (twelve), Diptera (seven), Coleoptera (one), Nematomorpha (one), and Other (two). Only three masses could not be identified to order in 2019 and 2020 combined. The addition of oviposition habitats increased the mean number of egg morphotypes (i.e., richness) in restored-treated streams (repeated measures ANOVA, Type x Time: $F_{2,10}$=6.45, P=0.02; Fig. 2.4) compared to restored-untreated (*a priori* contrast: restored-treated vs restored-untreated, t_{10}=3.58, P=0.01; Fig. 2.4). Before-after changes in the egg mass morphotype richness were similar in restored-treated streams compared to reference streams (*a priori* contrast: restored-treated vs reference, t_{10}=1.32, P=0.22, Fig. 2.4).

Larval insect abundance

These results are preliminary because only three of the five samples from each stream were processed and individuals identified to order. There was no detectable response in total invertebrate abundances in restored-treated streams (repeated measures ANOVA, Type x Time: $F_{2,10}$=0.14, P=0.86. Fig. 2.5a) compared to restored-untreated (*a priori* contrast: restored-treated vs restored-untreated, t_{10}=0.53, P=0.60) nor reference streams (*a priori* contrast: restored-treated vs reference, t_{10}=0.29, P=0.78). Larval insects in orders with egg cementing taxa (Ephemeroptera, Trichoptera, Diptera, Coleoptera) responded differently to treatment.

Ephemeropteran larval abundance remained consistent across restored stream types (repeated measures ANOVA, Type x Time: $F_{2,10}$=0.01, P=0.98; *a priori* contrast: restored-treated vs restored-untreated, t_{10}=0.08, P=0.94; Fig. 2.5b) and increased in reference streams, though the change was not significant (*a priori* contrast: restored-treated vs reference, t_{10}=0.16, P=0.93). On average, there were fewer Trichopteran larvae in 2020 samples than 2019 samples across stream types, but the decrease was not consistent across stream types (repeated measures ANOVA, Type x Time: $F_{2,10}$=6.83, P=0.01; Fig. 2.5c). Trichopteran larval abundance decreased by 17.65% in restored-treated streams and 97.57% in restored-untreated (*a priori* contrast: restored-treated vs restored-untreated, t_{10}=3.60, P=0.005), and by 36.81% reference streams (*a priori* contrast: restored-treated vs reference, t_{10}=2.32, P=0.04). Dipteran larval abundance was unchanged across years for all treatment types (repeated measures ANOVA, Type x Time: $F_{2,10}$=0.01; P=0.98; Fig. 2.5d). Lastly, the change in coleopteran egg mass density was consistent across restored stream types (repeated measures ANOVA, Type x Time: $F_{2,10}$=5.91; P=0.02; *a priori* contrast: restored-treated vs restored-untreated, t_{10}=0.14, P=0.90; Fig. 2.5e), but decreased in reference streams compared to restored-treated streams (*a priori* contrast: restored-treated vs reference, t_{10}=3.04, P=0.01).

Oviposition Habitat Stability

Prior to treatment (2019), a greater percentage of rocks rolled in restored streams than in reference streams (Fig. 2.6). After treatment the mean percentage of rocks that either rolled or were missing decreased in restored-treated streams compared to the restored-untreated streams by 363 % (repeated measures ANOVA, Type x Time: $F_{2,10}$=4.76, P=0.04). The mean percentage of rocks rolled or missing decreased significantly from before to after treatment in restored-treated streams compared to restored-untreated (*a priori* contrast: restored-treated vs restored-untreated, t_{10}=2.89, P=0.04; Fig. 2.6). The before-after change in mean percent of rocks rolled or missing was not significantly different between restored-treated and reference streams (*a priori* contrast: restored-treated vs reference, t_{10}=1.55, P=0.15, Fig. 2.6). The percentage of rocks rolled or missing in restored-treated streams was similar to reference streams after treatment (t_{10}=1.27, P=0.23, Fig. 2.6).

The annual mean number of egg masses per stream (log transformed) decreased as the mean percentage of rocks rolled or missing (logit transformed) increased across all stream types combined (linear regression: r^2 = 0.17, t_{24}=2.19, P=0.04; Fig. 2.7). Furthermore, at least one rock with a disproportionately high number of egg masses ("a motherload rock") rolled in one of the restored-treated streams (Morgan Creek MUT1). Prior to rolling, this rock (120 cm^2) had 44 baetid mayfly egg masses, which constituted 75 % of the egg masses in the stream on that sampling date and 21 % of the egg masses in the survey area during 2019. We recovered this rock the following week and no egg masses remained; however, six new masses had been laid.

Discussion

At the outset, rock availability in restored streams was much lower than reference streams, though there were exceptions (WUT & UT1_R4). Prior to treatment, the two restored stream types (treated, untreated) were indistinguishable in their rock and egg mass densities, but the addition of emergent, unembedded rocks increased eggs laid in treated streams. In fact, this augmentation brought treated streams into comparable range with reference streams' total egg mass density and egg morphotype richness. Treatment, however, did not decrease egg mass density on an individual rock basis and larval abundance did not show a response to treatment. Lack of a larval response could be due to post-recruitment limitations such as substrate stability.

Rocks used for oviposition were more likely to roll or be buried in sediment in restored streams (Jordt and Taylor 2021) which could result in greater egg mass or larval mortality. Notably, adding emergent rocks to treated streams increased the size of rocks and decreased the proportion of rocks that rolled or were buried. These results suggest that suitable rock availability, as well as potentially size and stability, are limiting insect recruitment in restored streams, and supplementing oviposition habitats by adding and positioning rocks readily found in the area could provide a cost-effective and value-added addition to the practice of stream restoration.

Insect egg mass abundance and richness

On average, there were 32 more masses per 10 m^2 in restored streams following treatment resulting in the addition of thousands of eggs, given that a single mass can contain hundreds to thousands of eggs. Though individual contributions of the four insect orders varied, additions of emergent rocks increased egg mass densities of Ephemeroptera, Trichoptera, Diptera, and Coleoptera. It is notable that we were able to observe an increase in Mayfly eggs laid in restored-treated streams given we were unable to treat the streams before the start of the egg-laying season, nor census streams during the spring peak mayfly egg laying period (May). Trichoptera and Diptera responses were similar in treated streams, each order contributing a total of ten and eleven more masses per 10 m^2 than the year before. Compared to mayfly, caddisfly, and true fly masses, beetle egg masses were rare and contributed fewer eggs to the overall eggs found in streams, and this is the first time beetle egg laying has been measured in a study experimentally increasing oviposition habitat.

Not only was egg mass density increased but richness of masses as well, although the richness of eggs laid is probably an underestimate of the effects of supplemental oviposition habitat. Egg masses grouped into order or morphotype does not fully represent the degree of richness within the different orders because orders differ in number of egg cementing families and species. Ephemeroptera egg masses attached to rocks include individuals within the family Baetidae. Multiple baetid mayfly species are known to exhibit the behavior (Encalada and Peckarsky 2007, Alp et al. 2013, Lancaster and Downes 2014), egg masses characteristics of the different species are too similar to distinguish visually and were thus included in one morphotype. Likewise, the bright yellow beetle eggs are easily identifiable to the family Psephenidae, which also may include several species. Conversely, the orders Trichoptera and Diptera have multiple families that cement egg masses onto rocks (Hoffmann and Resh 2003, Reich 2004, Lancaster et al. 2010b, Alp et al. 2013, Smith and Storey 2018) and 12 Trichoptera and 7 Diptera morphotypes were found during the 2019 and 2020 censuses which represent an unknown number of species. Regardless of the uncertainty of species richness resulting from the coarseness of morphotype data, richness of egg masses increased, which is an important step for enhancing larval insect richness that is often used to assess the biological recovery or condition of streams. Though we did not measure adult insects directly, these results in the first year of treatment indicate that adult females of multiple orders were present at these sites, but they were limited by egg laying habitat.

Larval insect abundance

Preliminary results (n=3 samples processed per stream and identification to order level) suggests that the disturbance of adding rocks to these streams did not have a positive or negative effect on larval abundance. Overall, larval abundance was lower in 2020 than in 2019, however for one order, Trichoptera, larvae remained consistent restored-treated and reference streams but decreased in restored-untreated streams between years. Non cementing taxa in the orders

Ephemeroptera and Coleoptera were abundant in larval samples and could be obscuring the treatment effect in those groups or that larvae drifted out of treatment reaches. Additionally, two of the five restored streams in the treatment group had little to no rocks available prior to treatment, while egg masses increased in those streams significantly, it may take time for measurable increases in larval abundances to take place. It is also likely that in addition to egg recruitment limitation, which we alleviated, post recruitment processes are also limiting stream insect larvae. Rocks often rolled or went missing (they either rolled out of the sample reach or were buried in sediment) in restored streams. It is unclear what impact this has on eggs or larvae but we observed multiple rocks that were rolled, resting in a position that exposed masses to air which can result in egg mass mortality in as little as 2 hours (Miller et al. 2020). Lastly, restored streams may be resistant to insect recovery even with the influx of egg masses because of post-recruitment limitation, such as priority effects of the existing invertebrate community (Barrett et al. 2021).

Oviposition Habitat Stability

Restored streams behaved differently than reference streams in one major way: in high flows, rocks moved. Prior to treatment, a greater proportion of rocks rolled, went missing, or were buried in all restored streams. Two streams in particular (UT1_R4 and WUT) had the highest number and the highest proportion of rolled or missing rocks, and both streams ended up in the treatment group. However, after emergent rocks were added, the proportion of rocks rolled, missing, or buried decreased in these two sites (UT1_R4 from 21% to 16% and WUT from 22% to 5%). This improvement in the stability of rocks used for oviposition is likely due to the increase in mean size of emergent rocks added.

Implications for stream restoration

With much less physical effort compared to the physical effort restoring these five streams, we were able to increase the abundance of suitable oviposition habitat resulting in an increase in the number and richness of eggs laid in those streams. Yet, this was a small-scale experimental approach and there are important considerations to make for each stream. For instance, these were small first and second order streams where rocks of sufficient size to protrude above the water surface were also small enough to be easily maneuverable by hand. Larger streams may require heavy machinery to place rocks or other substrates suitable of oviposition. Additionally, it is not clear how long the added emergent rocks will remain in place because rocks may roll, be buried, or become embedded during high flow events. Finally, without a source of rocks upstream, added oviposition substrates that are transported out of a restored steam may not be replaced. What is clear is that incorporating sufficient egg-laying habitat into stream restoration projects can reduce one component of insect recruitment limitation, egg recruitment.

This experiment took place in western North Carolina's mountain and piedmont streams where bed composition is often naturally comprised of cobble and emergent rocks or boulders. Insects in streams with naturally sandy bed material, such as eastern piedmont and coastal streams, rely on oviposition substrates as well but would likely benefit more from woody debris and native macrophytes (Smith and Storey 2018). Woody debris is becoming more scarce in waterways overall (Wohl 2018), and its availability has been shown to limit oviposition in sand bed streams (Macqueen and Downes 2015). Although our study is most directly applicable to rocky bottomed streams, the concept of enhancing oviposition habitat is broadly applicable across all streams and regions in which different types of oviposition habitats could be added to alleviate egg recruitment limitation. Furthermore, adding diverse oviposition habitat, such as large woody debris, to rocky bottomed streams may also be beneficial because wood can provide an additional source of oviposition habitat. For instance, if the bed later fills with sediment or if rocks are not available because they are transported downstream, large logs with sufficient interstitial spaces may be used for oviposition.

We increased the abundance and size of suitable rocks in gravel bed streams (restored with a median particle size (D50) < 2 mm), and an attempt was made to place rocks where they would be both stable and remain unembedded. We initially considered placing extremely large rocks in streams but ultimately were restricted to any large rock adjacent to or already in the channel that could be maneuvered by two people. Ultimately, added rocks ranged in size from large cobble to small boulders with intermediate axis, or width of 181 – 512 mm (Bunte and Abt 2001). Although added rocks were smaller than initially planned, this reduced treatment was sufficient and beneficial for multiple reasons. 1) Streams included in this study were small (0.5 – 3.5 m wide), rocks with an intermediate axis ranging from 181-512 mm were large enough to remain emergent without becoming embedded. 2) Adding more small rocks may have increased the likelihood females would find a suitable rock. Female insects have many preferences for rock locations (including fast or slow flows), size (rocks with a width between 150-224 mm were most commonly used for egg laying in our reference streams), and shape (Peckarsky et al. 2000, Bovill et al. 2013). 3) Multiple smaller rocks increase available surface area more than a few large boulders (width <1024 mm) would have increased surface area. 4) Given reasons one and two, adding many rocks decreased the likelihood that rocks with egg masses would roll and spread egg masses out over more rocks (i.e., eggs placed in multiple baskets). 5) Rocks added were found in or near the stream and were thus consistent in type and age to rocks already present in the stream. Our results demonstrate that minor, site specific modifications such as increasing the size of substrate so that there are emergent, unembedded rocks available to ovipositing adults can increase egg mass abundance and richness in restored streams.

Although locating the rocks in or near the channel had benefits and was convenient, it also limited our selection of rock sizes, shapes, and our ability to create geomorphic structures to stabilize emergent rocks. Incorporating oviposition habitat into restoration during or following construction may be a better approach to achieve a longer lasting and more stable addition of oviposition habitat. For example, a diversity of rock sizes such as particles capable of remaining stable in ten- or fifteen-year flow events increases the likelihood that oviposition substrate will remain stable and emergent during yearly high flow and can be used to shelter smaller particles. Further, larger rocks that may become embedded because of their lack of movement can be used to provide shelter from string water flows for smaller emergent rocks. Rock shape can also be used to increase stability. For instance, rocks in reference streams were often flat and leaning against one another which made them collectively resistant to movement in high flows. In fact, in reference streams many of the rocks in 2019 were in the same location throughout 2020. Rocks in restored streams were more typically block shaped or round, rarely leaning on one another and only the large, embedded rocks were recovered the following year. Large stable features made of wood or large boulders such as those used to construct cross vanes, can provide substrate for small boulders where those small boulders will remain unembedded. Finally, stream bed roughness can be very low (Manning's roughness coefficient) immediately following restoration. Therefore, restoration practiced that add oviposition habitat over multiple years may be a preferred approach to ensure that added oviposition habitats are more stable and less likely to become embedded as the additional time post construction allows the stream bed roughness to increase and fine sediments to settle.

It is common to monitor riparian plant communities and stream bed morphology up to seven years post restoration and make corrections to riparian corridors or channel morphology as necessary. Adding emergent, unembedded rocks with in the two-to-seven-year monitoring period may be more effective than immediately following construction. For example, rocks could be placed along the streamside during or directly following construction where they could be easily added into the wetted channel when the streambed stabilizes. Additionally, extending the amount of time a stream is monitored or periodically monitoring them after the 7 years elapse, may allow for later stage corrections such as oviposition habitat addition that could enhance the process of restoration. Lastly, bedload transport (movement of sand, gravel, and boulders) does and will continue to happen because of natural processes, land use changes, climate change, and as the bed settles post restoration. Restoration practices that include rocks that either remain stable during bedload transport or are replaced by bedload transport from upstream areas will be important for enhancing and sustaining insect communities.

Conclusion

Stream restoration is a multi-billion dollar industry (Bennett et al. 2013) with the objective of restoring stream structure and function, particularly of the biological communities. Yet the biological effectiveness of stream restoration is rarely assessed (Palmer et al. 2010, Wohl 2018). More often stream restoration goals center on addressing the most tractable and problematic issues such as stream bank erosion, contamination removal or flood abatement. This has undoubtedly led to marked improvements in benthic habitat for aquatic insect larvae but exploring life history strategies of these taxa in greater detail may provide multiple avenues for improvement. For instance, processes linking larval and adult life stages, such as habitat for adults to return to streams and deposit their eggs, are lesser known yet essential for many aquatic insect species (Statzner and Bêche 2010, Smith and Storey 2018). If recruitment by egg-laying adults is limited, it can be a bottleneck, restricting the establishment and persistence of aquatic insects in restored streams (Lancaster et al. 2010a, Encalada and Peckarsky 2012b, Storey et al. 2017). Increasing oviposition habitat by adding emergent rocks was an effective strategy for increasing insect egg density and richness in these streams and could be a viable management activity to aid invertebrate recruitment in these streams. More research should be done to determine what suitable rock density is sufficient to achieve a treatment effect and to assess whether degraded streams would respond to treatment. Additionally, it is not yet clear if increased eggs resulted in increases in larvae. Improving biological restoration will require interdisciplinary collaboration to assess the point or points where current practices fail to account for lesser-known habitat requirements and yield solutions and accelerate biological recovery in restored streams.

Aquatic insects play crucial roles within their aquatic and terrestrial environments and when restored streams fail to establish insect communities, these streams are unable to benefit from the functions insects provide. Our research provides a proof-of-concept test of the value of restoring stream oviposition habitat as well as a set of guidelines and informational tools that can be applied to previous and/or future stream restoration projects to accelerate biological recovery. More generally, our research tests the relative importance of pre- and post-recruitment mechanisms in regulating stream insect populations that play central roles in ecosystem structure and function.

Table 2.1. Characteristics of the restored and reference streams in western North Carolina. Restored streams were divided into 6 blocks based on proximity and reach characteristics (such as presence of an upstream culvert). One stream in each block was randomly selected to receive additional emergent rocks for insect oviposition habitat.

Stream Type	Stream name	Block	Mean Width (m)	Survey Area (m^2)	Lat Long (bottom of reach)	Drainage km^2	Land-use type	Restoration type	Years since restoration	River Basin
Restored-Untreated	Morgan Creek - Main Up	1	2.76	138.23	35.5787, -82.0330	4.87	1	PR1-4	11	Broad
Restored-Untreated	Morgan Creek - MUT2	3	0.47	23.70	35.5781, -82.0327	0.10	1	RP1/2	11	Broad
Restored-Untreated	Morgan Creek - MUT3	2	0.68	34.14	35.5791, -82.0346	0.13	1	RP1/2	11	Broad
Restored Untreated	Pinch Gut - Candiff_M3	4	2.85	142.35	36.2815, -80.5915	7.10	3	RP1/2	7.5	Yadkin
Restored-Untreated	Warrior Creek - Mtn	5	2.94	147.2	36.0514, -81.2688	4.58	1	RP2	14	Yadkin
Restored-Treated	Morgan Creek- Main Down	1	2.52	126.00	35.5806, -82.0352	4.87	1	PR1-4	11	Broad
Restored-Treated	Morgan Creek - MUT1	2	1.02	50.80	35.5778, -82.0315	0.36	1	RP1/2	11	Broad
Restored-Treated	Morgan Creek - MUT5	3	0.49	24.60	35.5806, -82.0371	0.13	1	RP1/2	11	Broad
Restored-Treated	Pinch Gut - UT1_R4	4	2.46	122.85	36.4557, -80.4258	3.08	2	RP1/2	11	Roanoke
Restored-Treated	Warrior Creek - WUT	5	1.57	78.60	36.0526, -81.2673	1.29	1	RP2	14	Yadkin
Reference	Morgan Creek - Beaverdam Creek	6	2.12	106.20	35.5514, -81.7400	1.09	1	Reference	NA	Broad
Reference	Pinch Gut - Indian Creek	4	2.70	135.15	36.4117, -80.2530	2.23	1	Reference	NA	Roanoke
Reference	Warrior Creek - BooneFork	5	3.54	176.85	36.0069, -81.6144	4.40	1	Reference	NA	Catawba

Notes: Land-use numbers correspond to: 1= deciduous/mixed forest, 2=hay/pasture, 3=agriculture.

Table 2.2. Availability of emergent rocks suitable for oviposition before (2019) and after (2020) treatment in restored and reference streams. Rocks were added by hand into a stratified random subset of half the restored streams in early May 2020, with readjustments for sunken or embedded rocks to maintain elevated oviposition habitat availability throughout the egg laying season (May-October).

		2019	**2020**	
Stream Type	**Stream name**	**Mean number of emergent rocks/10 m^2**	**Number of emergent rocks added per stream**	**Mean number of emergent rocks/10 m^2**
Restored-Untreated	Morgan Creek - Main Up	0.17	0	0.09
Restored-Untreated	Morgan Creek - MUT2	1.83	0	5.30
Restored-Untreated	Morgan Creek - MUT3	0.94	0	0.94
Restored-Untreated	Pinch Gut - Candiff_M3	1.03	0	0.43
Restored-Untreated	Warrior Creek - Mtn	0.49	0	0.32
Restored-Treated	Morgan Creek- Main Down	0.47	63	2.72
Restored-Treated	Morgan Creek - MUT1	2.08	33	5.12
Restored-Treated	Morgan Creek - MUT5	1.36	44	10.57
Restored-Treated	Pinch Gut - UT1_R4	3.57	84	10.01
Restored-Treated	Warrior Creek - WUT	4.26	59	10.56
Reference	Morgan Creek - Beaverdam Creek	7.52	0	13.50
Reference	Pinch Gut - Indian Creek	6.70	0	8.35
Reference	Warrior Creek – Boone Fork	3.91	0	7.97

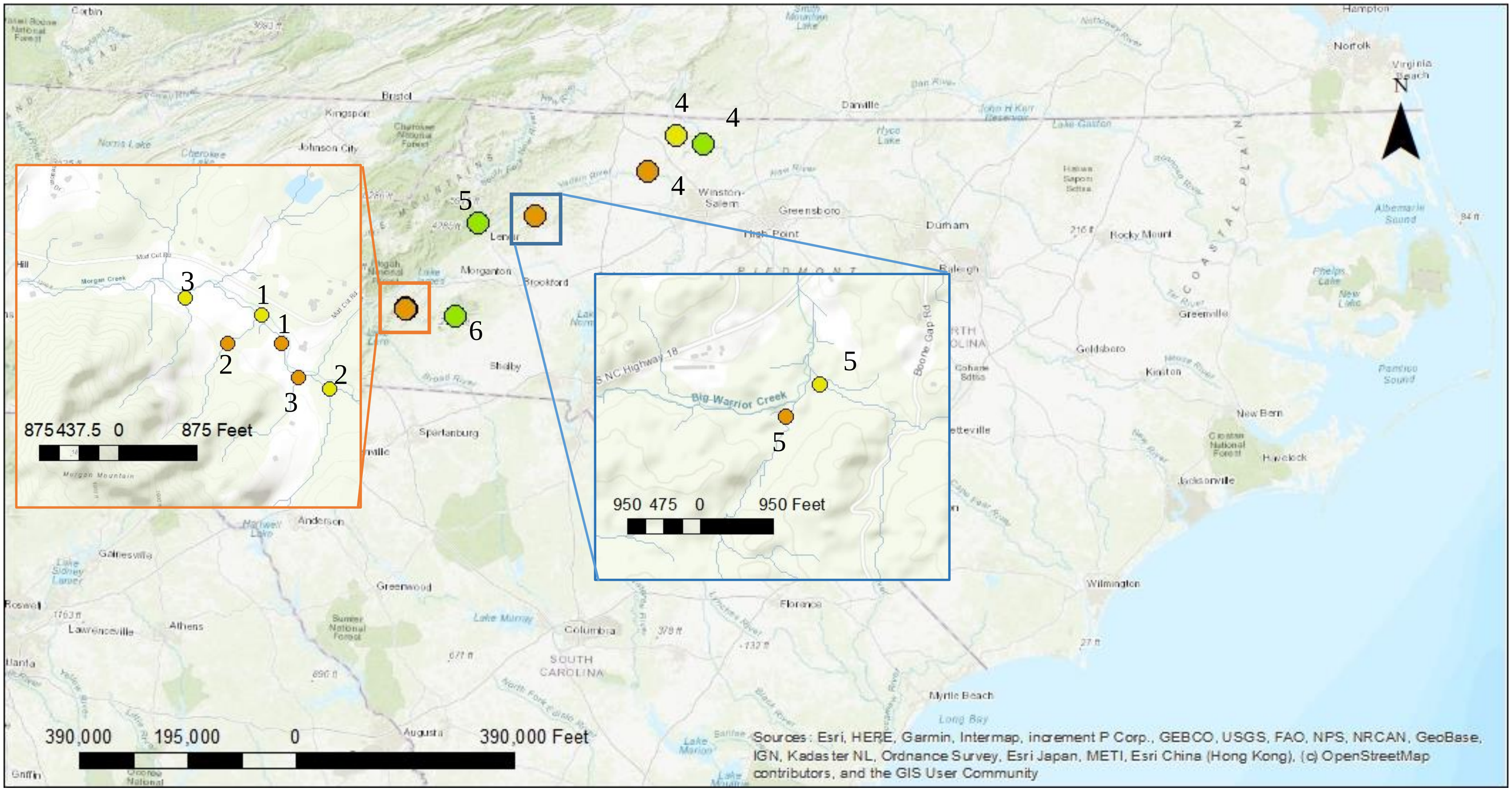

Figure 2.1. Map of study area located in western North Carolina. Restored-untreated sites are indicated in orange, restored-treated sites are yellow, and reference sites are green. Numbers indicate blocks. The northern most sites comprise block 4 while, sites located in the blue box and reference site to the east are block 5, the orange box along with the reference site to the east include blocks 1-3 and 6.

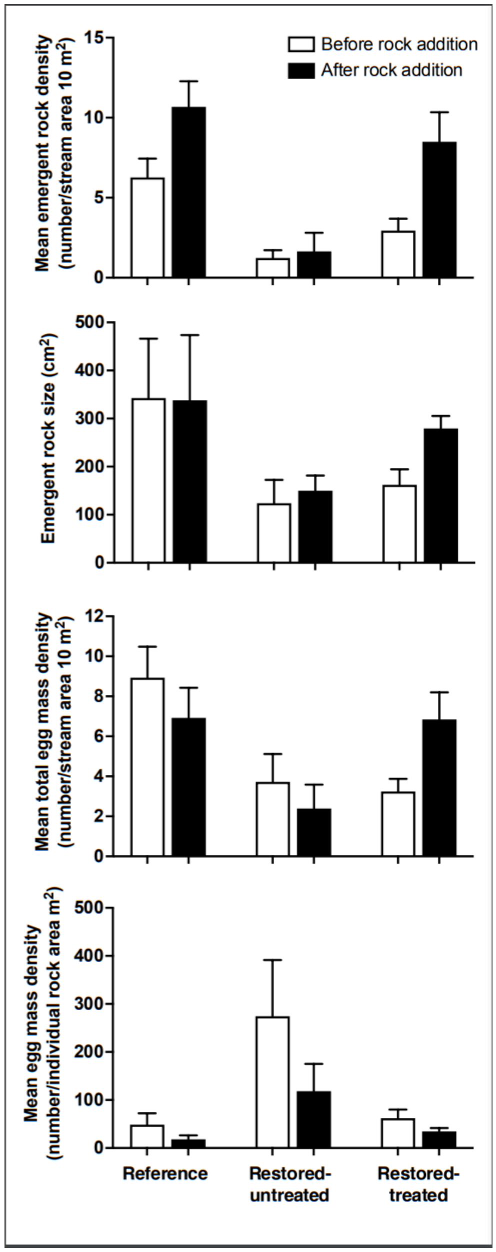

Figure 2.2. a) The average number of suitable/emergent rocks found during multiple rock and egg mass census visits in restored and reference streams located in western North Carolina. Censuses occurred throughout the 2019 and 2020 insect oviposition seasons. 283 emergent rocks were added to five (restored-treated) of the ten restored streams in 2020, prior to the start of census visits. The remaining restored streams (restored-untreated) were unmodified. b) Average emergent rock size increased in treatment streams following the addition of rocks. c) Mean egg mass density, averaged across the insect egg laying season (June-October), responded to increases in oviposition habitat compared to untreated and reference streams. d) Egg masses were more densely aggregated on available substrate in streams where oviposition habitat was limited.

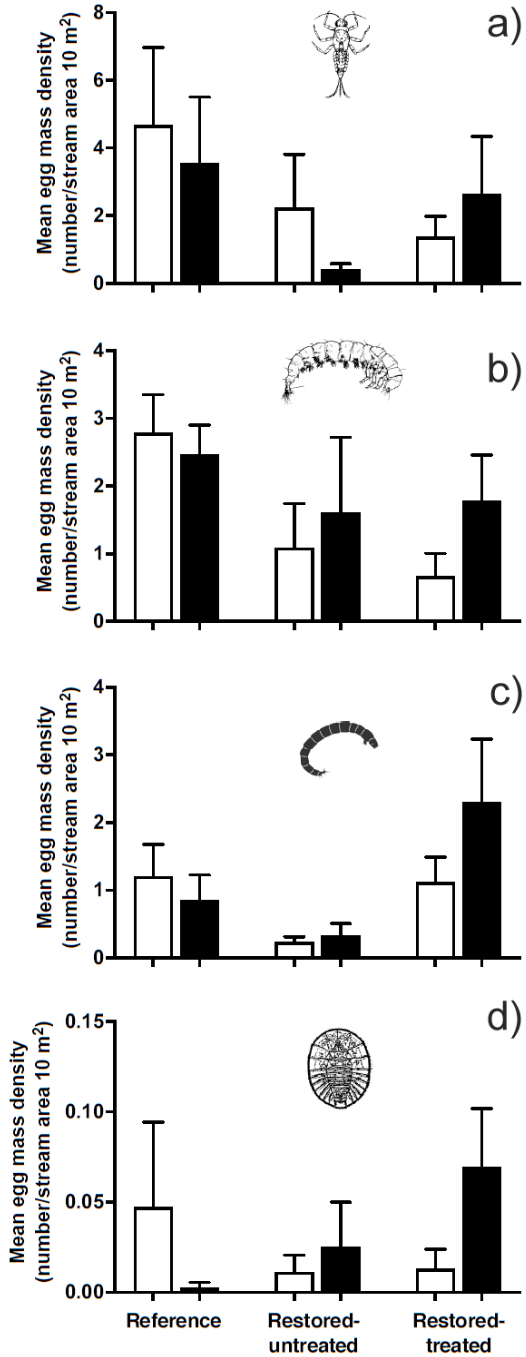

Figure 2.3. Mean egg mass density averaged across the insect egg laying season (June-October) in three reference streams and ten restored streams in western North Carolina. Emergent rocks were added to five (restored-treated) of the ten restored streams in 2020. The remaining restored streams (restored-untreated) were unmodified. Responses of individual orders were not significant: a) mean ephemeropteran egg mass density b) mean trichopteran egg mass density c) mean dipteran egg mass density d) and mean coleopteran egg mass density.

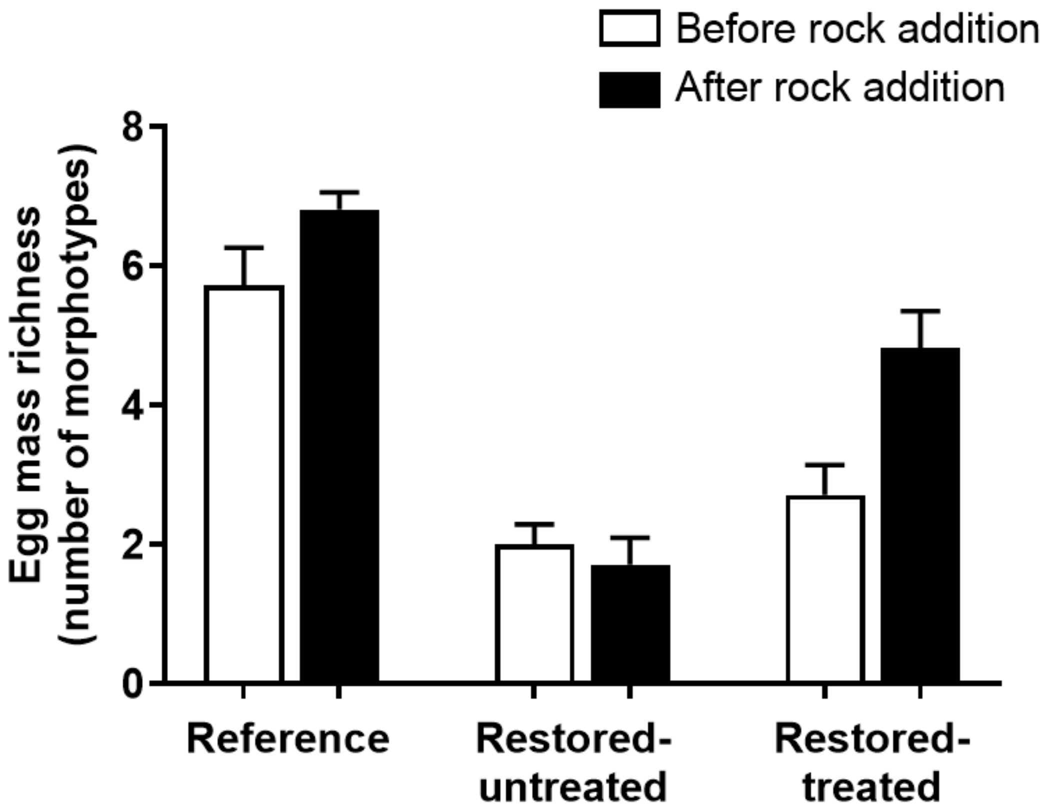

Figure 2.4. The average number of egg mass morphotypes found during multiple rock and egg mass census visits in restored and reference streams located in western North Carolina. Censuses occurred throughout the 2019 and 2020 insect oviposition seasons. Emergent rocks were added to five (restored-treated) of the ten restored streams in 2020, prior to the start of census visits. The remaining restored streams (restored-untreated) were unmodified.

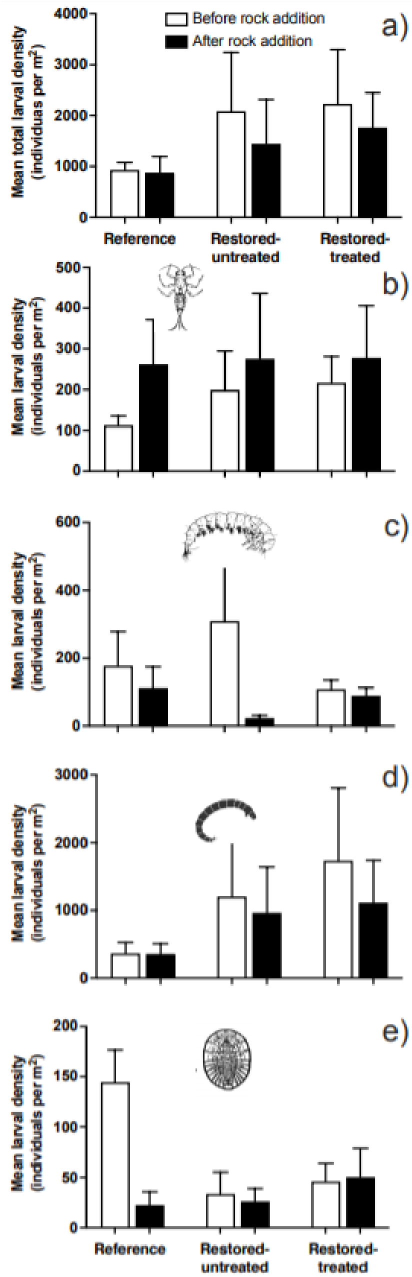

Figure 2.5. Mean larval density averaged across the insect egg laying season (June-October) in three reference streams and ten restored streams in western North Carolina. Emergent rocks were added to five (restored-treated) of the ten restored streams in 2020. The remaining restored streams (restored-untreated) were unmodified. a) Total larval density did not respond to treatment, but two of the four individual insect orders with egg cementing taxa did respond to treatment. b) Mean ephemeropteran larval density c) mean trichopteran larval density d) mean dipteran larval density e) and mean coleopteran larval density.

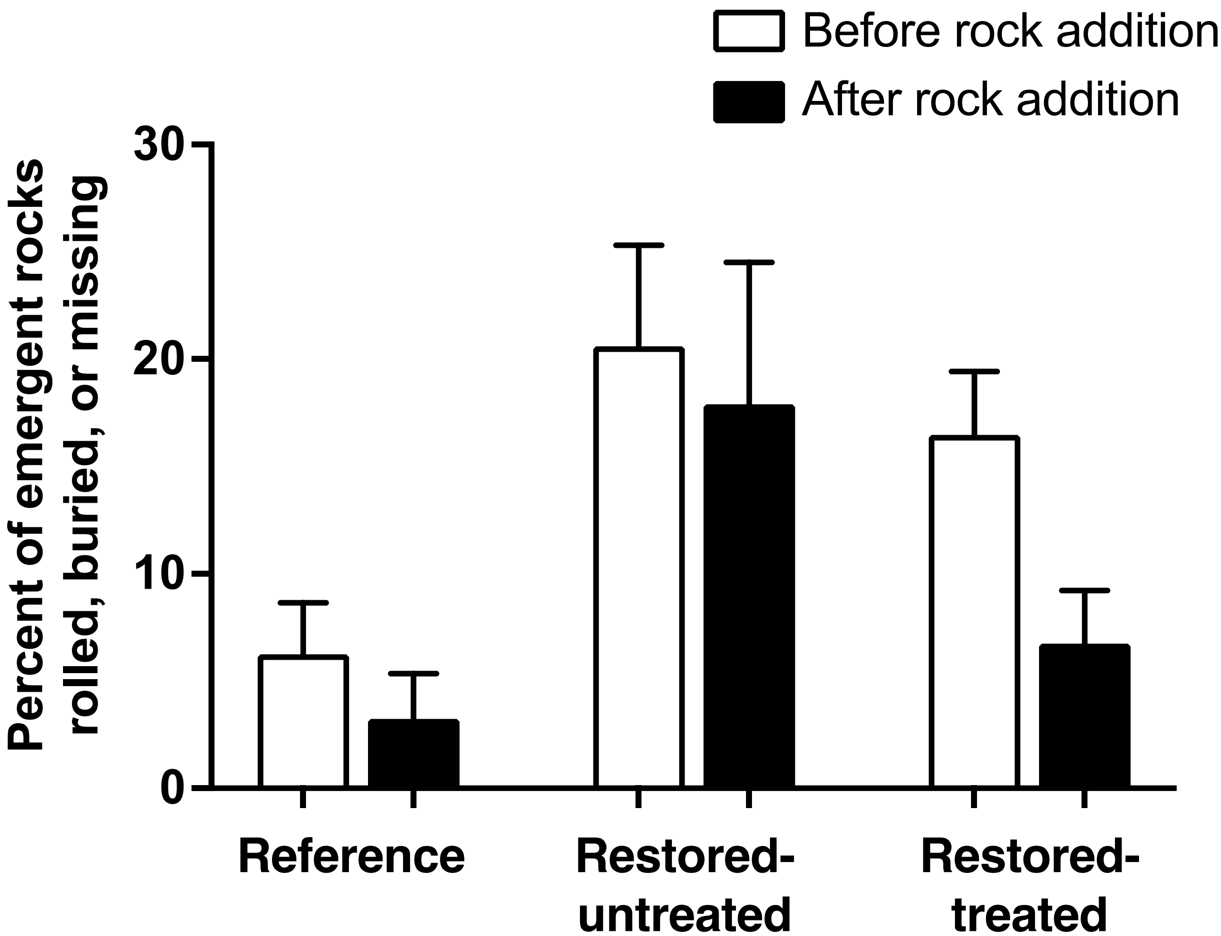

Figure 2.6. Percent of emergent rocks rolled, buried, or missing in restored and reference streams located in western North Carolina. Rocks were marked with a nontoxic paint pen and censused for insect egg masses throughout the 2019 and 2020 insect oviposition seasons. Rolled rocks were identified by noting the orientation of their paint pen identification number. Missing rocks either rolled out of the study reach, were buried, or otherwise unrecoverable. 283 emergent rocks were added to five (restored-treated) of the ten restored streams in 2020, prior to the start of census visits. The remaining restored streams (restored-untreated) were unmodified.

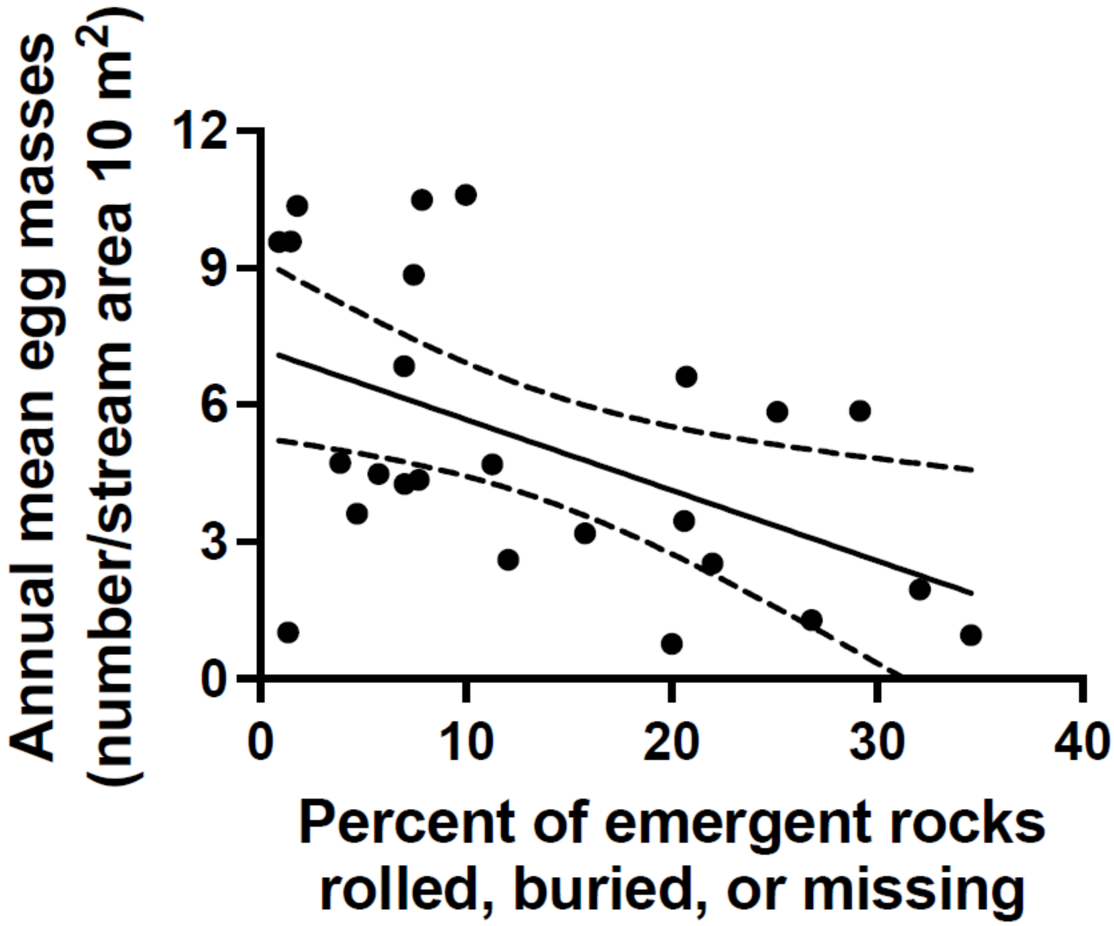

Figure 2.7. Linear regression of the relationship between the percent of emergent rocks rolled, missing, or buried and the number of egg masses cemented to rocks. Center line is the best-fit line and upper and lower dashed lines are 95% confidence bands. Note the y axis is the natural log scale and the x axis is the logit scale.

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APPENDICES

Appendix A

Supporting Information. Jordt, S., and B.W. Taylor. 2021. A rolling stone gathers no eggs: the importance of stream insect egg laying natural history for stream restoration. Ecology.

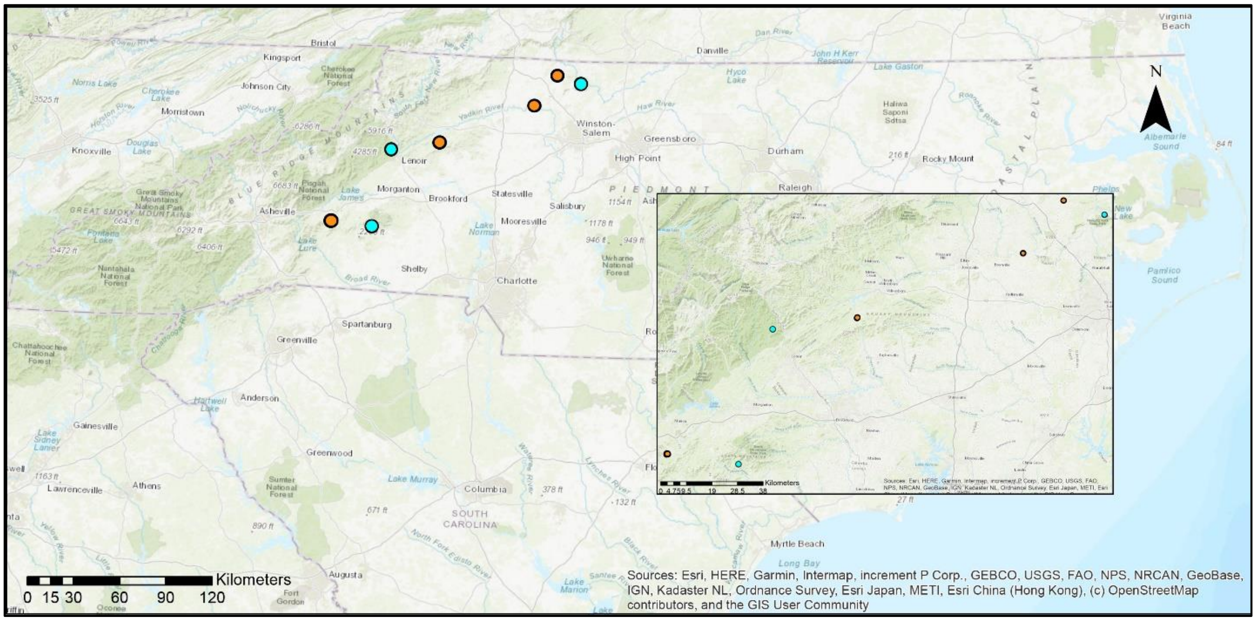

Figure A1. Study streams were located in western North Carolina, USA. Aqua dots indicate the three reference streams and orange dots indicate the location of one or more restored streams (n=10 total). We were limited to three reference sites because of property access and a general lack of reference quality streams near restored sites. The three reference sites were located in either North Carolina State Parks or the Pisgah National Forest, and one was a State of North Carolina water-quality reference stream.

Table A1. Characteristics

of the restored and reference stream sites in western North Carolina.

Stream type	**Project name**	**Stream site name**	**Restoration type, age (y)**	**Drainage area (km^2)**	**Width (m)**	**Stream area (m^2)**	**Latitude, longitude**	**Elevation (m)**	**Dominant watershed land use, %**	**Project ID†**
Restored	Morgan Creek	Main Creek Upper	PR1-4, 11	0.029	2.76	138.22	35.578243, -82.032735	372	deciduous/mixed forest, 74%	92527
Restored	Morgan Creek	Main Creek Lower	PR1-4, 11	0.029	2.52	126	35.578970, -82.032950	373	deciduous/mixed forest, 74%	92527
Restored	Morgan Creek	UT1	RP1/2, 11	0.0014	1.02	50.8	35.578040, -82.031697	373	deciduous/mixed forest, 91%	92527
Restored	Morgan Creek	UT2	RP1/2, 11	0.0004	0.47	23.7	35.579560, -82.033347	373	deciduous/mixed forest, 97%	92527
Restored	Morgan Creek	UT3	RP1/2, 11	0.0005	0.68	34.14	35.579138, -82.034904	374	deciduous/mixed forest, 100%	92527
Restored	Morgan Creek	UT5	RP1/2, 11	0.0005	0.49	24.6	35.580582, -82.037076	369	deciduous/mixed forest, 100%	92527
Restored	Pinch Gut Tributary	Main Creek (UT1)	RP1/2, 11	0.0119	2.46	122.85	36.456090, -80.425094	323	hay/pasture, 54%	92518
Restored	Candiff Creek	None	RP1/2, 7.5	0.0274	2.85	142.35	36.279194, -80.587942	246	agriculture, 38%	92767
Restored	Warrior Creek	Mountain Creek	RP2, 14	0.0177	2.94	147.2	36.050993, -81.268820	334	forest, 93%	412
Restored	Warrior Creek	Unnamed Trib	RP2, 14	0.005	1.57	78.6	36.052604, -81.267709	333	mixed forest, 61%	412
Reference	*Reference Morgan Creek*	Beaverdam Creek	NA	0.0075	2.12	106.2	35.551718, -81.740325	391	deciduous/mixed forest, 88%	NA
Reference	*Reference Pinch Gut & Candiff*	Indian Creek	NA	0.0155	2.703	135.15	36.411828, -80.253455	294	forest, 94%	NA
Reference	*Reference Warrior Creek*	Boon Fork UT	NA	0.0039	3.53	176.85	36.006676, -81.613931	403	forest, 100%	NA

PR1-4 as defined by the Natural Channel Design method of restoration. Priority 1 re-establishes bankfull stage at the historical floodplain elevation, Priority 2 creates a new floodplain and stream pattern at the current elevation. Priority 3 widens the floodplain at the existing bankfull elevation. Priority 4 stabilizes the existing streambank. Dominant watershed land use was obtained from the: North Carolina National Land Cover Database, 2016. Elevation was obtained using latitude and longitude and the USGS The National Map TNM Elevation viewer.
NA is not applicable.
†https://deq.nc.gov/about/divisions/mitigation-services/dms-projects

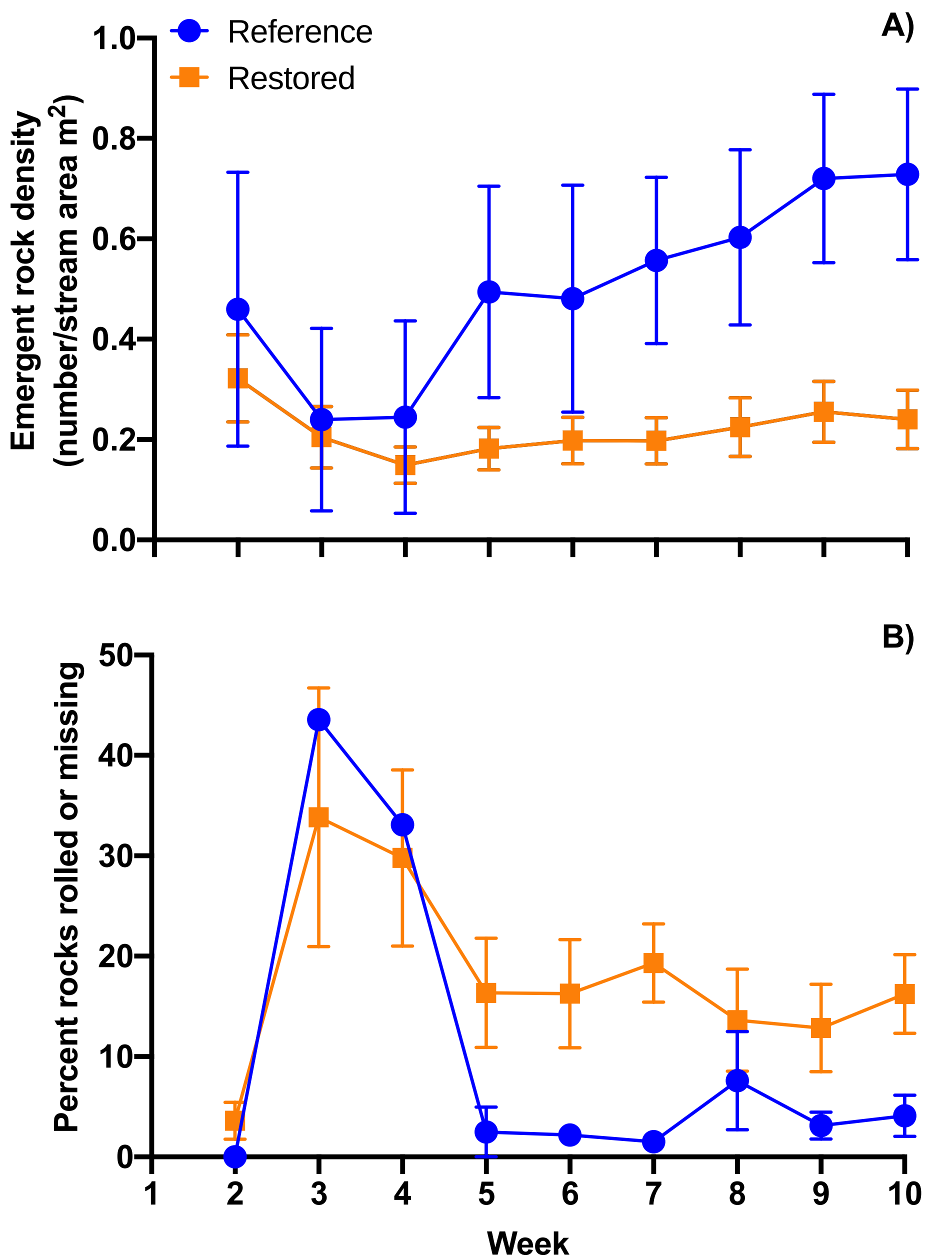

Figure A2. Reference (blue circles) and restored (orange squares) streams differed in (A) number of emergent rocks and (B) percent of emergent rocks that rolled or were not recovered over the sampling period from early 29 May (week 1) to 13 October 2019 (week 10). Data are means weighted by the number of reference and restored streams sampled each week.

Section A3: Statistical Analyses

Data in Figure 2 panels A-D were natural log(x+1) transformed to achieve normality and homogeneity of variance prior to repeated measures analysis of variance (ANOVA) with an autoregressive covariance structure (AR1). The percent rocks rolled in Figure 2 panel F were analyzed using a generalized linear model with an exponential distribution and log link function. Statistical analyses were performed using SAS 9.4 and RStudio 1.2.5042.