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Integrating terrestrial stages of aquatic insects into studies of dispersal and recruitment in streams

ABHANDLUNG

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DOKTORIN DER WISSENSCHAFTEN

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Spazio spazio, io voglio, tanto spazio
per dolcissima muovermi ferita:
voglio spazio per cantare, crescere,
errare e saltare il fosso
della divina sapienza.
Alda Merini

Contents

Summary

Streams have been severely degraded by human activities in the last centuries, which had dramatic effects on aquatic organisms. With the aim of recovery of healthy and diverse aquatic communities, much effort has been put lately into river restoration. However, focusing on local habitat restoration, a common approach, has often proved insufficient for achieving this goal. In fact, colonization constraints were identified as one of the principal factors limiting river restoration success. Understanding mechanisms of dispersal and recruitment of aquatic organisms has therefore become a major goal, not only for advancing ecological theory but even more importantly for improving existing watershed management approaches.

Dispersal is a major prerequisite for habitat colonization, but also for maintaining gene flow between populations and migration between different habitats. Traditionally, many dispersal studies on stream organisms have focused on processes occurring in aquatic environments. However, recently the importance of considering the entire life-cycle of aquatic organisms - including terrestrial stages of species with aquatic life-history - has been widely recognized. In the case of aquatic insects, the short-lived winged adults can play a crucial role both for completing the life-cycle and for dispersal, which for them takes place outside of aquatic environments thus bein unconstrained by water flow direction or by in-stream barriers.

The presented dissertation looked at the relevance of certain life-cycle traits for dispersal and recruitment of aquatic invertebrates, with a particular focus on adult aquatic insects. We first chose an experimental approach to investigate whether reduced substrate availability in streams with degraded morphology can become a constraint for insects with specialized oviposition behavior. In the second study, we worked at the catchment scale and compared population genetic structure of two aquatic macroinvertebrates strongly differing in their life-cycle traits and ecology. We used stable isotope analysis in the third project to study the role of emerging aquatic insects for the links between aquatic and riparian compartments of headwater stream food webs.

Our results suggest that specific biological traits, such as oviposition on certain substrate types, can set local constraints for successful reproduction in the context of modified environments. For some species, these are not only defined by local morphological factors but can also be strongly influenced by large-scale factors such as hydrological regime. However, we emphasize the importance of considering the totality of the traits relevant for population maintenance and habitat colonization. While some traits can lead to constraints in, e.g., local recruitment, others, such as strong dispersal capacity, can play a compensatory role, allowing the species to move within the landscape and access suitable habitats elsewhere. Species-specific traits also play a role for determining the magnitude of food-web links between the aquatic and riparian community. Comparing two headwater streams with strongly differing composition of benthic community, we found pronounced differences in the use of aquatic-derived subsidies by riparian spiders. Taxon-specific mode of emergence and behavior in the terrestrial environment seemed to play an important role for susceptibility to riparian predation.

Several recommendations for management can be derived from our conclusions. Simple cost-effective restoration techniques such as boulder addition can be beneficial for recruitment of many aquatic invertebrates, creating oviposition hotspots especially in channelized middle-sized streams. Furthermore, our results suggest the importance of a holistic landscape perspective when planning local river management, especially river restoration. Reach location within the stream network can play a major role, especially for species with predominantly passive dispersal: habitats downstream of nearby source populations are more likely to be colonized. Large-scale stream characteristics such as the hydrological regime can play a crucial role overriding reach-scale settings and eventually limiting local restoration success.

Zusammenfassung

In letzten Jahrhunderten wurden Flüsse stark durch menschliche Aktivitäten beeinträchtigt, mit schwerwiegenden Effekten auf aquatische Organismen. In letzter Zeit wurde versucht mit Flussrevitalisierungsmassnahmen zu gesunden und diversen aquatischen Lebensgemeinschaften zurückzukehren. Den Schwerpunkt auf lokale Wiederherstellung der Habitate zu setzen, ein weitverbreiteter Ansatz, hat sich jedoch nicht bewährt. Gewährleistung der Wiederbesiedlung von Habitaten wurde als eine der wichtigsten Voraussetzungen für das Erfolgspotential der Revitalisierungen identifiziert. Damit ist die Erforschung von Mechanismen der Verbreitung und Reproduktion aquatischer Organismen zu einem wichtigen Ziel geworden - nicht nur für die Erweiterung des Wissens über die aquatische Fauna sondern auch für die Entwicklung effizienterer Ansätze im Flussmanagement.

Die Mobilität von Organismen spielt eine wichtige Rolle für die Besiedlung neuer Habitate aber auch für den genetischen Austausch zwischen Populationen und Migration zwischen verschiedenen Lebensräumen. Traditionell haben Studien über die Verbreitung aquatischer Organismen den Fokus auf Prozesse gelegt, die im Wasser stattfinden. In der letzten Zeit wurde jedoch immer stärker erkannt, dass der gesamte Lebenszyklus aquatischer Organismen - inklusive terrestrischer Phasen bei Arten mit amphibischer Lebensweise - berücksichtigt werden muss. Bei aquatischen Insekten, spielen die kurzlebigen, beflügelten Imagines eine entscheidende Rolle, sowohl für den erfolgreichen Abschluss des Lebenszyklus als auch für ihre Verbreitung, die ausserhalb des Wassers und somit unbeeinflusst von der Richtung des Wasserstroms und Barrieren im Wasserlauf stattfinden kann.

In dieser Doktorarbeit wurde der Einfluss spezifischer Merkmale des Lebenszyklus auf die Verbreitung und Reproduktion aquatischer Makroinvertebraten untersucht. Adulte aquatische Insekten stehen im Mittelpunkt der hier präsentierten Forschung. Im ersten Kapitel haben wir mit einem Feldexperiment untersucht, ob Substratverfügbarkeit in verbauten Flüssen die Reproduktion von Insekten mit spezialisiertem Eiablageverhalten limitieren kann. Im zweiten Kapitel haben wir die populationsgenetische Struktur zweier Makroinvertebraten mit stark unterschiedlichen Verbreitungsweisen und Ökologie in einem Flussgebiet verglichen.

Mit Hilfe von Isotopenanalysen haben wir schlussendlich auch die Rolle der

emergierenden aquatischen Insekten für die Verbindungen zwischen aquatischen und Ufer-Nahrungsnetzen in Oberläufen untersucht.

Unsere Ergebnisse deuten darauf hin, dass taxon-spezifische Eigenschaften, wie z.B. Eiablage auf einem bestimmten Substrattyp, das Potential für eine erfolgreiche Reproduktion in verbauten Flüssen lokal limitieren können. Solche Limitierungen können für manche Arten nicht nur durch lokale flussmorphologische Faktoren, sondern auch durch grossräumige Faktoren, wie etwa Abflussregime, bedingt sein. Der Vergleich der Ergebnisse der ersten und der zweiten Studie zeigt aber, dass es wichtig ist, die Gesamtheit der Eigenschaften der Taxa, die für die Erhaltung der Populationen und das Kolonisationspotential der Arten relevant sind, zu berücksichtigen. Während bestimmte Eigenschaften der Taxa ihre Möglichkeiten für Reproduktion lokal stark limitieren können, können andere, sowie z.B. gute Verbreitungsfähigkeit, eine ausgleichende Rolle spielen, indem sie es Organismen erlauben, geeignete Habitate in der Flusslandschaft zu erreichen. Unterschiede in bestimmten Merkmalen, können auch die Stärke der Vernetzung zwischen aquatischen und terrestrischen Nahrungsketten beeinflussen. Beim Vergleich zweier Bergbäche mit sehr unterschiedlicher Zusammensetzung der benthischen Gemeinschaft konnten wir grosse Unterschiede im Anteil aquatischer Beute in der Diät von Uferspinnen finden. Wir erklären diese Unterschiede unter anderem durch unterschiedliches Emergenz- und Flugverhalten der jeweils dominanten aquatischen Insekten in den untersuchten Bächen, welches unterschiedliche Anfälligkeit gegenüber den Prädatoren verursachen könnte.

Auf Basis unserer Untersuchungen können wir einige praxisrelevante Empfehlungen herleiten. Einfache, kostengünstige Revitalisierungsmassnahmen wie etwa das Einbringen grosser Steine ins Flussbett können den Reproduktionserfolg vieler aquatischer Makroinvertebraten in kanalisierten Flussmittelläufen fördern. Unsere Ergebnisse deuten auch darauf hin, dass beim Flussmanagement und besonders der Planung lokaler Revitalisierungsmassnahmen, immer auch das Flusssystem grossräumig betrachtet werden sollte. Die Lage des konkreten Abschnittes im Flussnetzwerk kann für Arten mit überwiegend passiver Verbreitungsweise eine wichtige Rolle spielen: Habitate flussabwärts potentieller Quellpopulationen haben die besten Chancen kolonisiert zu werden. Auch grossräumige gewässerspezifische Faktoren wie das Abflussregime müssen berücksichtigt werden: ihre Effekte können potentiell die Effekte der lokalen Verhältnisse (wie Flussmorphologie) überlagern und dadurch den Revitalisierungserfolg begrenzen.

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General introduction

Landscape modification, dispersal and the role of adult aquatic insects

Streams have experienced a dramatic change in the last hundred years (Allan & Castillo, 2007) due to multiple anthropogenic impacts including pollution, channelization, and modification of hydrological regime. The resulting habitat destruction and fragmentation had strong effects on aquatic organisms, causing local losses of certain species and threatening the survival of many others (McKinney, 2002). The awareness of these changes was one of the driving forces that lead to substantial efforts to restore habitats in flowing waters and herewith stimulate the recovery of aquatic communities (Bernhardt *et al.*, 2005). However, the success of many restoration projects has often been found disappointingly poor (Jaehnig *et al.*, 2010; Palmer, Menninger & Bernhardt, 2010). Many studies concluded that the widespread assumption 'if we build it, they will come' (Palmer, Ambrose & Poff, 1997) is too simplistic and thus recreating stream habitat can be insufficient for restoration of healthy and diverse aquatic communities. In fact, not only habitats have been destroyed or fragmented, but also the connectivity of stream networks has been strongly disrupted, e.g. due to the construction of dams and other in-stream structures, which separated stream sections. In this context, major attention has lately been directed towards understanding colonization mechanisms for aquatic organisms and, in particular, towards studies on their dispersal (Jannson, Nilsson & Malmqvist, 2007).

Dispersal has been defined as 'the movement of individuals or propagules between spatially (or temporally) discrete localities or populations' (Bilton, Freeland & Okamura, 2001). It has a number of important ecological functions, on the one hand influencing many aspects of population biology, such as population persistence and gene flow (Bohonak & Jenkins, 2003; Hughes, 2007), and on the other hand having community-level impacts, e.g. linking different habitats by energy flow and thus directly affecting food-web interactions within and between habitats (Malmqvist, 2002). Dispersal is closely linked to the dendritic structure of stream networks and distribution of populations within them (Fig.1; Fagan, 2002; Hughes, Schmidt & Finn, 2009). Thus whether a habitat will be colonized by a certain species depends both on its specific dispersal-related traits and many factors at the landscape-scale, including the distance between the populations in the stream network, landscape structure and connectivity. Moreover, unidirectionality of water flow determines the dominant direction of dispersal and makes the location of habitats and populations within the stream network important, with downstream reaches having a higher chance to receive immigrants.

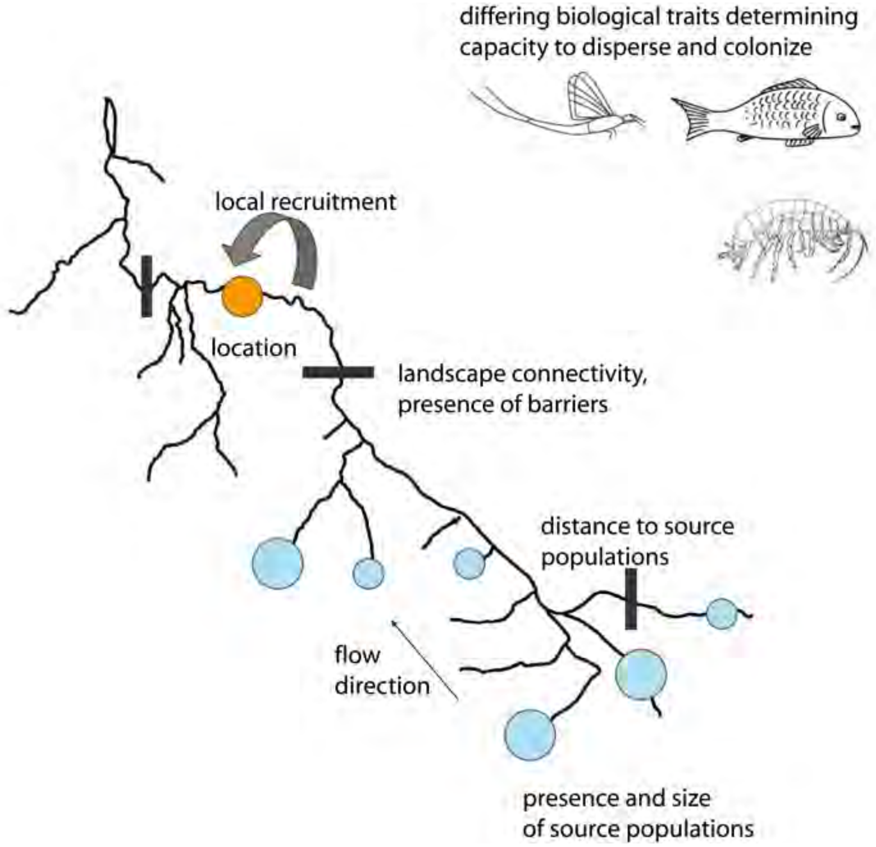

Figure 1: Overview of main factors determining colonization probability of a habitat patch.

Most studies on dispersal of stream invertebrates have focused primarily on aquatic stages of their life-cycle (Smith, Alexander & Lamp, 2009; Malmqvist, 2002). However, many aquatic insects, an important fraction of the benthic invertebrate community, also have a terrestrial phase (Fig.2). After spending the longest period of their life-cycle in the aquatic environment as larvae, they emerge into the terrestrial habitat as winged adults. They then mate and females lay eggs within or in proximity of the aquatic environment (Huryn, Wallace & Anderson, 2008). Imaginal traits, - such as their emergence path, their flight capacity, the time they spend on land, whether they feed or not as well as their mode of oviposition - can differ greatly among single taxa and strongly influence their potential to disperse and interactions with the terrestrial environment (Smith *et al.*, 2009).

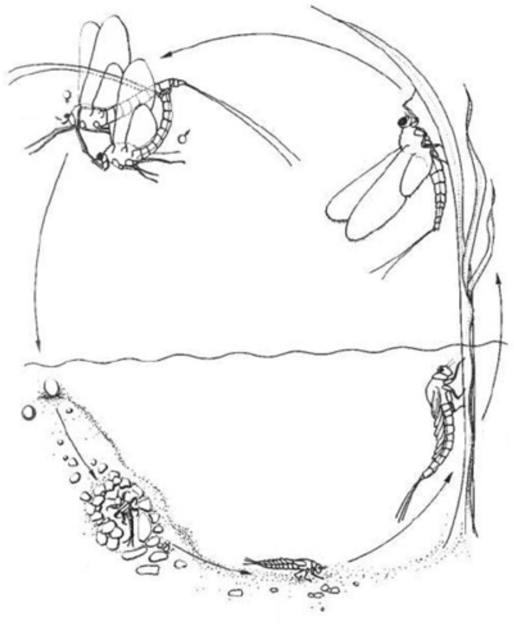

Figure 2: Life-cycle of a mayfly (from Studemann *et al.*, 1992).

Even though often very short-lived, adult stages of aquatic insects play a crucial role for several ecological processes from a population and community perspective, and even an ecosystem perspective (Smith *et al.*, 2009):

1) After mating, adult females of aquatic insects lay eggs, thus delivering often thousands of recruits to habitats, where they hatch and are able of adding whole new populations (Bunn & Hughes, 1997); 2) Flight capacity allows adult aquatic insects to disperse overland or cross in-stream barriers impassable for larvae, thus giving them considerable advantages for dispersal compared to water restricted organisms and life-stages; 3) Dispersing into the terrestrial habitat, emerged aquatic insects also represent a vector of aquatic-derived energy, potentially delivering substantial prey subsidies to a variety of riparian predators - from invertebrates, such as spiders and carabid beetles, to lizards, birds and bats (Ballinger & Lake, 2006; Baxter, Fausch & Saunders, 2005).

Thus for understanding population dynamics of aquatic insects as well as predicting their potential response to changes in stream landscapes, characteristics and requirements of terrestrial life-cycle stages must be considered together with those of the aquatic stages.

Thesis goal and outline

In the presented thesis, I studied the role of life-cycle traits for dispersal and recruitment of aquatic invertebrates, approaching different aspects of the subject with several methods and at multiple scales. Three main topics were addressed in this thesis. In **Chapter 1**, I used an experimental approach to investigate the influence of stream morphology and hydrological regime on recruitment of a range of aquatic invertebrates and focused on the effects of substrate availability on oviposition rates. **Chapter 2** describes a catchment-scale study on gene flow between populations of two aquatic invertebrates with strongly differing dispersal-related traits. In **Chapter 3**, I used stable isotope analysis to study lateral connectivity of headwaters with their riparian zones, and in particular the role of aquatic-derived subsidies (emerged insects) for the riparian predatory arthropods.

Chapter 1. Life cycle and environmental constraints on aquatic insect recruitment in human-modified streams

Oviposition is a crucial moment in the life-cycle of invertebrates, determining successful reproduction of an individual and recruitment of the next generation. Many invertebrates have developed specialized oviposition behaviours, e.g. attaching egg masses to a certain type of substrate - a costly trait explained by many authors as a benefit for reproductive success in heterogeneous landscapes with differing patch quality (Peckarsky, Taylor & Caudill, 2000; Resetarits, 1996). In human-modified streams, such traits can be a major constraint for recruitment of aquatic invertebrates, since the successful reproduction of the latter depends on the availability of specific substrates. For some aquatic insects, such substrate availability is determined not only by the presence of substrates but also by their accessibility. For instance, mayflies of the family Baetidae have to land on the protruding surface of a rock before crawling under water to attach their eggs on its submerged side (Peckarsky *et al.*, 2000).

In the field experiment presented in Chapter 1 (Fig.3) I investigated how differences in substrate availability due to human-made stream modifications as well as seasonal fluctuations of discharge influence oviposition rates of aquatic species that attach their eggs to the surface of large rocks. We added artificial oviposition substrates to the streambed in sites with contrasting morphology and followed egg deposition on them throughout the flight season of most aquatic insects. We used a model selection approach to evaluate the role of different environmental factors for determining egg mass density in each study reach.

Chapter 2. How river structure and biological traits influence gene flow: a population genetic study on two stream invertebrates with differing dispersal ability

Molecular techniques offer excellent opportunities to integrate landscape structure into

Figure 3: On the left: field experiment for the study on oviposition substrate availability. On the right: egg masses of hydropsychid caddisflies on a rock, Buenz, summer 2008.

dispersal studies. Reflecting 'effective dispersal' (which incorporates successful establishment of reproductive individuals; Nathan, 2006), gene flow plays an important role for maintaining genetic diversity of populations and influences many relevant eco-evolutionary processes such as, e.g., local adaptation (Bohonak & Jenkins, 2003). Patterns of genetic variation can be used to obtain estimates of both current and long-term average gene flow between populations (Hughes *et al.*, 2009). Consequently, molecular markers are highly effective for studying processes at different spatial and temporal scales.

In this study I used microsatellite analysis to investigate patterns of genetic structure in relation to differences in dispersal-related traits of two common invertebrate species. I conducted the study in a pre-alpine catchment, characterized by high habitat heterogeneity and the presence of both natural and man-made in-stream barriers. The studied species were *Baetis rhodani* (Pictet, 1843), a mayfly able to disperse by flight in its adult stage, and *Gammarus fossarum* (Koch, 1835), an amphipod constricted in its life-cycle to the water column. The results of this study make part of a collaborative project involving investigation of population genetic structure of three further stream-bound species conducted in the same catchment.

Chapter 3. Emerging aquatic insects as subsidies for riparian predators: when do predators care for additional snacks?

In many systems, emerged aquatic insects have been shown to contribute substantially to the diet of a variety of riparian predators (Fig.4; Baxter *et al.*, 2005). The reliance on such allochthonous food sources is assumed to depend strongly on the productivity gradient between aquatic and terrestrial habitats (Polis, Anderson & Holt, 1997) and has been shown to fluctuate seasonally due to specific phenology of organisms in both habitats (Kato *et al.*, 2003; Nakano & Murakami, 2001). In fact, it has been shown that taxon-specific differences in predator foraging behaviour, mode and timing of aquatic insect emergence as well as behaviour on land (e.g. flight behaviour) determine predator-specific prey availability (Paetzold, Bernet & Tockner, 2006).

Figure 4: Mayflies in a spider web in the riparian area of a small stream.

Studies on the importance of reciprocal subsidies between habitats often involve using stable isotope analysis. Providing time-integrated information on energy flow through food webs (Cabana & Rasmussen, 1996; Finlay, 2001), stable isotopes are a valuable tool for investigating trophic relationships. Given pronounced differences in isotopic signatures of the potential sources, their contribution to the diet of consumers can be quantified by analyzing isotopic composition of both sources and consumers. Knowledge on specific fractionation of each isotope taking place in the transition between trophic levels delivers information on trophic position of the consumers (Post, 2002). Being sensitive to dietary changes, isotopic signatures can also be used to detect temporal shifts in consumer reliance on one or another food source (Ostrom, Colunga-Garcia & Gage, 1997; Kato, Iwata & Wada, 2004).

In the study presented in Chapter 3, I used δ^{13}C and δ^{15}N signatures to investigate whether differences in the benthic invertebrate community are reflected in the magnitude of subsidy links to the riparian predator community adjacent to high-elevation headwater streams. We included a seasonal question, investigating whether the increased availability of a certain prey type during peak emergence period leads to shifts in the diets of riparian predator taxa. Furthermore I compared the isotopic signatures of the larval and adult stages of three abundant taxa of aquatic insects to test whether adult signatures remained similar to those of larvae.

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Chapter 1

Life cycle and environmental constraints on macroinvertebrate recruitment in human-modified streams

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Abstract

Successful recruitment is a prerequisite for the maintenance of viable populations. In many invertebrates (especially insects), specific traits such as specialized oviposition behaviours have evolved, e.g. attaching egg masses to a certain type of substrate - a costly trait explained by many as a benefit for reproductive success in heterogeneous landscapes with differing patch quality. Due to human activities many streams have experienced strong degradation, which reduced habitat heterogeneity substantially. In this context, specialized traits have evolved in natural landscapes and can become a major constraint for recruitment of aquatic invertebrates. The main goal of this study was to quantify, in terms of egg mass density, the response of aquatic invertebrates to changes in abiotic factors limiting oviposition substrate availability. We conducted

a field-experiment in two Swiss lowland rivers by manipulating substrate availability at sites with contrasting river morphology. Some 18 invertebrate taxa were found to use rocks for oviposition. We used egg mass density as an indicator of constraint and applied a model selection approach to compare the effects of several environmental factors on patterns in egg mass density for 2 selected aquatic insect taxa with differing recruitment-related traits. Both species showed a clear response to our experimental manipulation in one of the streams and none in the other. *Baetis* spp., a mayfly that must land on protruding rocks before ovipositing, responded strongly to factors determining rock emergence. Apparent constraints in substrate availability were found for *Hydropsyche* spp., a caddisfly with a terrestrial phase capable of diving, in one of the sites. We suggest that preference for large substrates, absent in this site, could explain this result. We attribute differences in patterns between streams to differences in hydrological regime, which was flashier in the stream where we detected strong response to our manipulation. Water level in this stream appears to be an overriding factor controlling egg mass density of both taxa. Overall, our results suggested that an interaction of reach-scale morphological and stream-scale hydrological factors determines local substrate availability. We conclude that specific traits related to recruitment determine the response of species to changes in these factors; e.g., invertebrates with specialized egg-laying behaviours are likely to be more vulnerable to multiple human pressures in streams. Our results suggest that boulder addition to stream beds can create additional oviposition structures and enhance local invertebrate recruitment. However, we note that the effect of this as well as other restoration techniques can be strongly influenced by larger-scale factors such as hydrological regime or disrupted connectivity in stream networks.

Introduction

Successful recruitment is a prerequisite for the maintenance of viable populations, and thus it is not surprising that diverse strategies of oviposition have evolved in different taxa. These strategies are commonly explained by maximizing the survival and potential hatching success of deposited eggs (Peckarsky, Taylor & Caudill, 2000; Resetarits, 1996). Thus a number of aquatic macroinvertebrates are known to attach their egg masses to wood, rocks, plants or other hard substrates in river channels (Hynes, 1979). Many of these taxa have specific preferences for oviposition substrates (Reich & Downes, 2003). For instance, factors such as rock type, size, position, substrate shape as well as whether it is embedded were shown to determine preferences of gravid females of aquatic insects that oviposit on rocks (Reich & Downes, 2003; Encalada & Peckarsky 2006; Reich *et al.*, 2011).

Even though most probably offering fitness-relevant advantages in heterogeneous landscapes, such taxon-specific preferences for oviposition sites can also make substrate availability a significant constraint for successful oviposition (Blakely *et al.*, 2006; Lancaster, Downes & Arnold, 2010a). Moreover, the ability to find and access appropriate substrates for oviposition can vary greatly among taxa. Some taxa, such as the caddisfly family Hydropsychidae, are known to have a capacity to dive and reach submerged substrates (Deutsch, 1984; Badcock, 1953). The probability for these caddisflies to find adequate substrates for oviposition are different from other taxa that must land on a protruding boulder to oviposit, a behavior recorded, e.g., for Baetidae, Rhyacophilidae, Hydrobiosidae, Chironomidae and Ceratopogonidae (Percival & Whitehead, 1928; Reich & Downes, 2003; Lancaster *et al.*, 2010a). Thus successful oviposition of many aquatic insects can be strongly linked with the hydrology and geomorphology of a stream, whereby factors such as variability in substrate size and stream depth as well as water level at the time of emergence determine whether a gravid female will be able to oviposit successfully (Peckarsky *et al.* 2000).

Many streams worldwide have been highly fragmented, modified morphologically (e.g. channelized), or impacted by flow regulation (Poff *et al.*, 1997; Allan & Castillo, 2007). In these degraded stream conditions, the availability of oviposition sites for many aquatic insects with specialized egg-laying behaviors has most probably been strongly reduced. However, few studies have investigated the implications of river degradation on substrate availability and recruitment by aquatic insects. Field experiments in urban streams (Blakely *et al.*, 2006) provided some of the first evidence that changes in oviposition substrate availability can strongly affect the distribution of aquatic insects. Considering the extent of anthropogenic impact on streams, there is an urgent need of further studies quantifying the effects of stream modification on recruitment of aquatic invertebrates.

Our main goal in this study was to identify environmental factors determining constraints for oviposition of aquatic invertebrates that attach their egg masses to rocks in streams. We manipulated substrate availability in stream reaches of contrasting morphology and used egg mass density as an indicator of constraints in substrate availability. We expected the response to substrate addition (in terms of egg mass densities on added substrates relatively to naturally present substrates) to be different depending on the local substrate availability context as well as species-specific traits in terms of oviposition behavior. We focused on the comparison of two selected aquatic insect taxa with differing biological traits. We expected *Baetis* spp., which has to land on protruded substrates in order to access habitat for oviposition, to show a stronger response to factors determining emergence of substrates (e.g. water level) than *Hydropsyche* spp. known for their capacity to dive. A model-selection approach was used to quantify the effect of our treatment as well as of several environmental variables on egg mass densities.

Methods

Description of study streams

The study was conducted in summer 2008 in two 3^{rd}-order lowland streams (Buenz, Reppisch) in Switzerland (Fig.1.1). These streams lie at similar altitudes and latitudes, thus climatic conditions at all study sites were similar. Both rivers are rather shallow (mean depth between 20 and 50 cm) and flow through a rural landscape dominated by managed forest and agriculture. The average annual discharge is 1.26 m^3/s (2005) in Buenz and 0.38 m^3/s (2008) in Reppisch, and both are characterized by a typical pluvio-nival regime with high flows in spring and fall. No major in-stream barriers (culverts, weirs, dams) were present between study sites within each stream, thus allowing for high in-stream connectivity between sites. A small hydropower station upstream of the study area in the Buenz caused occasional unnatural fluctuations of discharge due to flushing of the reservoir several times per year.

During the sampling period, water pH (mean ± SD) was 8.4 ± 0.4 (Buenz) and 8.4 ± 0.1 (Reppisch), and conductivity was 496.0 ± 188.8 μS/cm (Buenz) and 454.1 ± 39.9 μS/cm (Reppisch). Nutrient loads reflected the agricultural use in both catchments. In the Buenz, the mean phosphate concentration was 34.9 ± 24.4 μg P/L and nitrate-N was 4.8 ± 0.72 mg N/L. In the Reppisch, stream nutrient concentrations were higher in the downstream site than in the upper two sites. For instance, the mean phosphate concentration was 6.1 ± 4.1 μg P/L upstream but 37.3 ± 12.7 μg P/L downstream, and nitrate-N concentration was 1.8 ± 0.3 mg N/L upstream and 3.7 ± 1.3 mg N/L downstream.

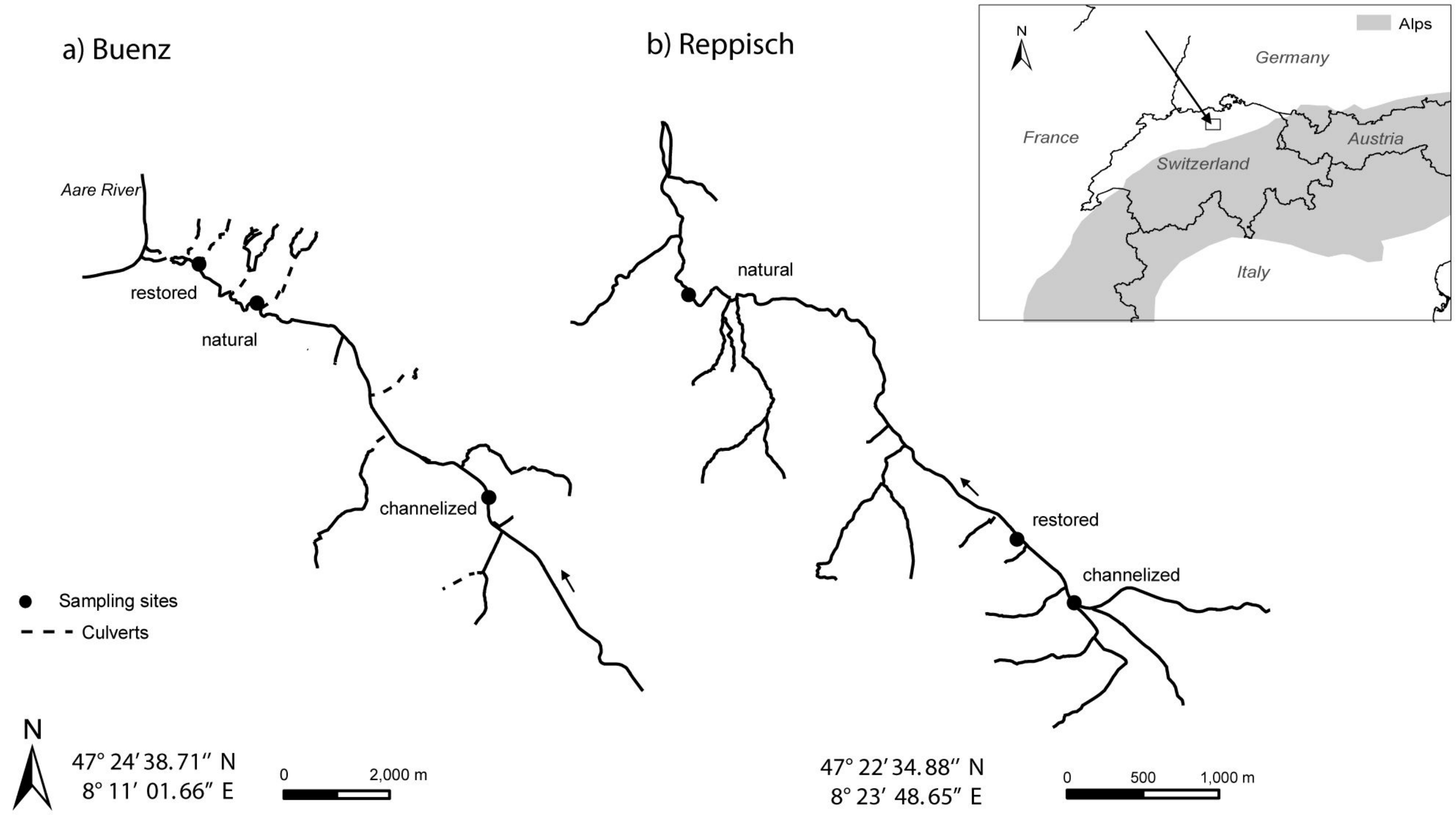

Figure 1.1: Map of sampling sites. Both streams belong to the catchment of the river Aare, a major tributary of the Rhine.

Experimental design

Within a 3.5 to 4.5 km section of each stream (Fig.1.1), we selected three sites of strongly differing riverbed morphology and categorized them as restored, channelized and near natural. The term 'restoration' in this study will be used in the sense of recent (1-year old) enhancement of morphological and hydraulic heterogeneity of a reach - whether man-made or naturally occurring, e.g. due to a particularly strong flood. Characteristic of 'restored' sites was a wide and rather shallow floodplain with high water depth variability and dynamic gravel bars with little vegetation. 'Channelized' sites represented stretches with highly monotonous profiles; channels that were modified in the 1920-30s mainly for flood protection and have remained as such since then. Sites defined as 'natural' were never channelized and at most received some minor bank reinforcements, but still maintained relatively high heterogeneity in streambed morphology. We are aware that the use of the word 'natural' might be slightly misleading, as both of our study streams flow through human-modified landscapes. We use this term simply to refer to the 'reference reach' in each stream where the morphology has remained mostly un-modified compared to the other two types of sites.

The study sites differed slightly in riparian shading. For instance, restored sites with wide, bare gravel floodplains were fully exposed to sunlight and had no arboreal vegetation, whereas channelized and near natural sites had single trees or bushes in the riparian area. Within each study section, the total channel length of restored sites was between 300 and 500 m, for natural sites between ca. 500 m (Buenz) and 2 km (Reppisch), and for channelized sites between 3 km (Buenz) and 1 km (Reppisch). The remaining channel length in both streams was a mosaic of stretches channelized to some extent with some restoration projects in progress in each catchment.

In March 2008, we installed clay bricks at each site within each stream that simulated what is considered a 'good oviposition substrate' for a wide range of species; i.e. a large, emergent, non-embedded boulder with a smooth surface, located in flowing water areas. We used a random block design, installing 3 blocks of 5 bricks at each site within a stream. The distance between single blocks within a site was between 50 and 300 m. We followed oviposition on both bricks and naturally present stones at each site, thus blocks were replicates for the experiment, while individual rocks and bricks were the sampling units.

The smooth-sided bricks were 29 cm long, 19 cm wide and either 14 or 19 cm high, and were fixed to the riverbed with metal bars. We used bricks of different height (or stacked) in order to have the bricks emerging from the water and available for insect landing throughout the experiment. All bricks were installed parallel to the direction of flow in relatively shallow but fast-flowing reaches, where the water depth made it most probable for both bricks and naturally present stones to be emergent over most of the study period. Brick installations were positioned near stream margins, except for block 1 (Reppisch) and block 3 (Buenz) at natural sites where bricks were installed towards the middle of the streambed. The sampled natural stones were chosen randomly each time according to the following criteria: they had to be at least 15 cm in their longest axis, emergent, and not embedded in the river bed. Sampling both bricks and natural stones aimed to distinguish between species-specific constraints in oviposition substrate availability. Overall, we expected the relative preference for bricks to be greater at sites where few natural stones suitable for oviposition were available.

Measurement of physical and chemical characteristics

At each study site, we assessed different factors expected to determine oviposition substrate availability, including water depth, depth variability and fluctuations during the experiment as well as substrate size distribution. We measured 10 cross-sectional profiles for depth at a frequency of every 10 meters at each site. The distance between measured points within each profile was 0.5 m. To characterize substrate size distribution, we 1) randomly sampled 100 to 150 stones at each site and recorded their size category (16 categories from 1 to over 100 cm; see Table S3.1), and 2) measured three axes (a, b, c axes) of 100 to 150 randomly chosen stones with the b-axis greater than 15 cm.

Discharge data from cantonal gauging stations (measurement frequency of 10 min) were available for both rivers. In fall 2009, during a rainy period with substantial fluctuations in discharge, we installed 3 water pressure data loggers (DL/ N 70, Sensor Technik Sirnach, Switzerland) at each of the sites. We then ran a linear regression in R 2.11.1 (R Development Core Team 2010) of water depth fluctuations at each site against respective discharges (ln-transformed values for Buenz) measured at the gauging station ($r^2 = 0.95$ - 0.99 for Buenz, $r^2 = 0.96$ - 0.97 for Reppisch). Using the acquired regression coefficients, the 2008 discharge data from the gauging stations and the data of the depth profiles, we calculated the mean water depth fluctuations at each site during the experiment.

As temperature is an important cue for aquatic insect emergence and was expected to be an important predictor for oviposition (Deutsch, 1984), we installed temperature loggers (TidbiT v2, Onset Computer Corporation, USA) on the riverbed at each of the sites for the duration of the experiment. Loggers at both restored sites were lost due to a flood during the sampling period, thus we used the temperature data from water level loggers (November 2009) to conduct a linear regression between temperature at each restored site and the nearest site upstream ($r^2 = 0.97$ for Buenz, $r^2 = 0.98$ for Reppisch). We felt confident relying on the linear regression as the distance between sites used in the calculation was <1 km and no or only minor tributaries entered each system between the sites. Using the regression coefficients, we then estimated water temperature at each restored site over the sampling period in 2008.

Field sampling of macroinvertebrates

Quantitative benthic samples ($N = 3$ per site) were collected each month in riffle/run habitats at each site with a Hess sampler (0.0045 m^2, 250 μm mesh). Flying imagos of aquatic insects were sampled using window traps (2 perpendicular transparent plexiglass windows of 43 × 50 cm) installed at each site for 48 hours ($N = 3$ per site) before sampling for eggs. Window traps were placed 0-0.5 m from the water edge at a height of 20-40 cm above the water surface (data available from June 2008). All invertebrate samples were stored in plastic bottles and preserved in the field with 70% ethanol. Macroinvertebrates were handpicked from each sample using a dissecting microscope at 10× magnification, identified to lowest practical taxonomic unit (usually genus or family), and counted.

Starting in April 2008, 5 bricks and 5 stones at each site were checked thoroughly every 3 weeks for egg masses (the two streams were sampled 1 week apart). Figure 1.2 reports the sampling dates as well as discharge and temperature conditions in the streams over the sampling period. The size of each stone as well as the surface area emerging from the water for each stone and brick were recorded when sampled. Egg masses were counted in the field, removed from the substrates, and a subsample transported to the lab for incubation and identification of hatchlings (see Supporting Information for photos of egg mass types). For some taxa (e.g. *Hydropsyche*), the counting of egg masses was not always possible because at times they would comprise aggregations of multiple egg deposits, making the distinction of individual egg masses difficult. In these cases, we measured the total area of the egg masses on the substrate and calculated the number of egg masses later based on measures taken from single egg masses of the same taxon.

Small transparent plastic containers (5.5 cm in diameter, 5 cm in height) were used for the incubation of eggs. Eggs were kept in daily-replenished, oxygenized stream water under temperature conditions similar to those in the field at the time of collection. Light conditions for incubation were held constant at a 12:12 light:dark cycle. Containers were checked regularly for hatched larvae and the hatchlings were identified to the lowest taxonomic level possible. As taxonomic keys for aquatic invertebrates rely heavily on characteristics of mature larvae, some taxa could not be identified beyond family level.

Of the taxa whose egg masses were widespread and could be reliably identified and quantified, we selected two for further statistical analysis: *Baetis* spp., a mayfly which must land on a rock before ovipositing (Peckarsky et al, 2000) and *Hydropsyche* spp., whose females can dive to reach rocks underwater (Lancaster, Downes & Arnold, 2010b; Deutsch, 1984). For these taxa, counts of egg masses were standardized to the proportion of the substrate area that had been submerged and thus available for oviposition during the 2 weeks preceding each sampling event. The mean water level measured from the cross-sectional profiles at each site was used for this calculation.

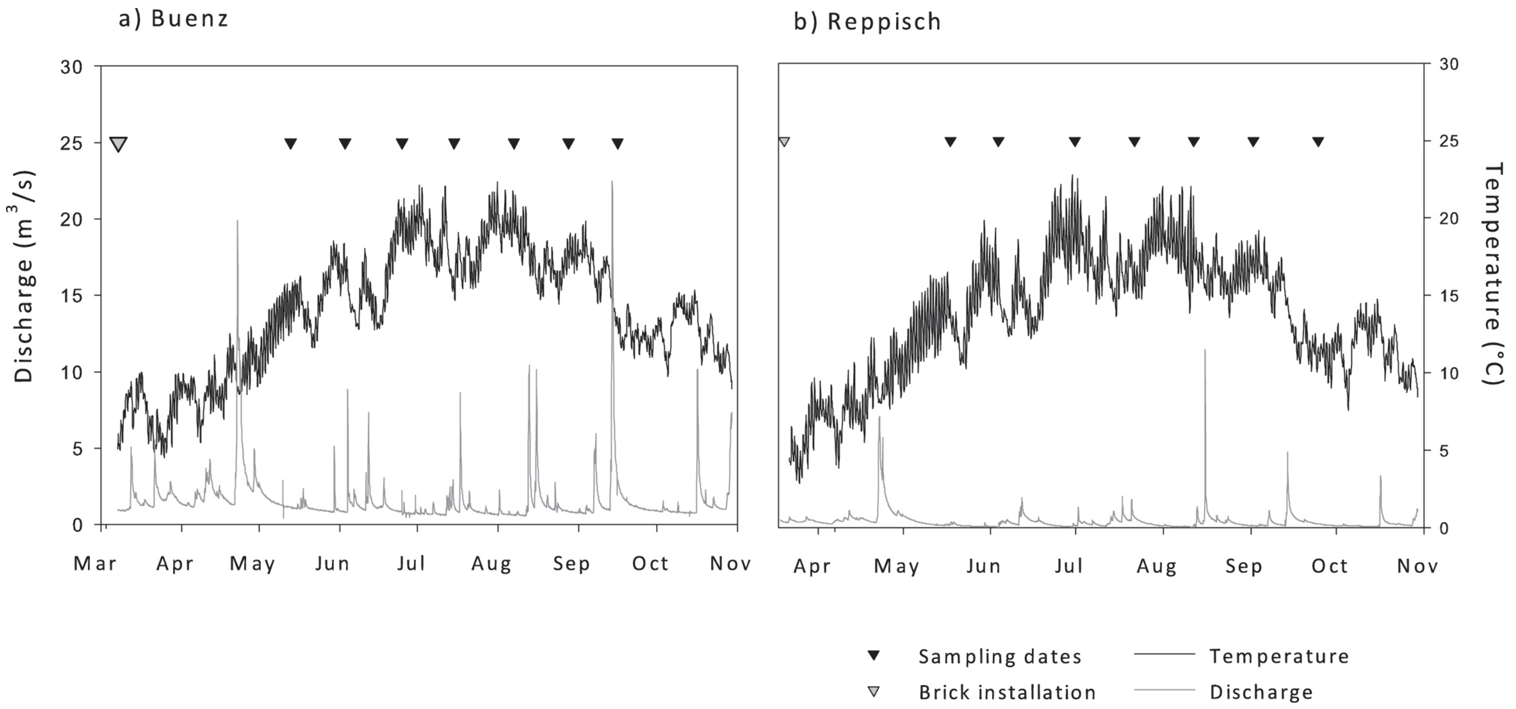

Figure 1.2: Temperature (in black) and discharge (in light grey) patterns in the rivers Buenz and Reppisch over the duration of the experiment, summer 2008. Triangles represent the beginning of the experiment (open) and single egg sampling dates (filled).

This standardized egg mass density for each taxon was then included in the statistical analysis as a response variable.

Data analysis

To characterize morphological differences between sites, we applied analysis of variance from R 2.11.1 (R Development Core Team 2010) testing the effects of among-site variation in water depth, wetted width and oviposition substrate size.

We used an information theoretic approach to identify factors that best explain egg mass density variation in the study streams (Burnham & Anderson, 2002). We formulated 28 candidate models, based on the literature and our personal expertise (Table 1.2) and included the factors 'site morphology', 'substrate type', 'water level', and 'temperature' (Table 1.1). For 'temperature' and 'water level' quadratic terms were included into the analysis to check for a potential optimum effect. We also tested several interactions of single variables. Quadratic and interactive effects were not simultaneously used in the same model due to the low number of degrees of freedom.

The effect of our experimental manipulation was tested in the models which included interaction of factors 'substrate type' (which tested difference in egg mass density between natural and experimentally added substrates) and factors 'site morphology' and 'water level'. Thus finding support for the interaction 'substrate type' and 'site morphology' would mean that the response of brick addition in terms of relative egg mass density (compared to natural stones) was different depending on the 'site morphology' context. Statistical support for the interaction 'substrate type' and 'water level' would correspond to a difference in 'water level effect' for egg mass densities on natural and added substrates. We fitted the candidate models as linear mixed effects models using the function *lme* from the package '*nlme*' (Pinheiro *et al*, 2009) in R (V. 2.11.1) (R Development Core Team 2010), separately per species and stream. We included 'sampling day' and 'block' as random effects in all models to consider multiple error terms determined by the block design and multiple sampling occasions. A model with only random effects was also included in the analysis, reflecting the case that random effects might best explain the data.

We used AICc for model ranking, a variant of AIC (Akaike Information Criterion) corrected for small sample size (Burnham & Anderson, 2002). Akaike weights (w; range: 0 to 1) were calculated for each model. Model weights allow the assessment of the relative strength of evidence in the data for alternative models. The model with the highest Akaike weight is best supported by the data. Evidence ratio ($w_{best\ estimated\ model}$ / w_j) was calculated for each model in the set to judge its relative likelihood (Burnham & Anderson, 2002). If several models had substantial support, a confidence set of models was reported, with the cut-off value for the evidence ratio at 8 (Burnham & Anderson, 2002). Confidence intervals were calculated for the estimates of the factors included in the best-supported models (slopes for continuous variables, differences in

Table 1.1: Overview of explanatory variables included in statistical analyses.

Factor (code)	***Factor type***	***Fixed/Random***	***Levels***	***Explanation***
Block	Categorical	Random	3	A factor comprising parameters specific to location of each experimental unit and not included into the study; each block consisted of 5 sampled brick or stones.
Substrate	Categorical	Fixed	2	Sampled substrate; factor levels: brick or stone
Day	Continuous	Random		Day in the season.
Site	Continuous	Fixed	3	A categorical factor comprising morphological condition of the site; factor levels: channelized, restored, natural
Water level (Hydro)	Continuous	Fixed		Mean depth [cm] at each site in the 2 week period preceding to each sampling.
Temperature (Temp)	Continuous	Fixed		Mean temperature (°C) in the 2 week period preceding to each sampling.

intercept for comparisons between factor levels of categorical variables). All continuous explanatory factors were Z-standardized. Residual plots were used to check for normality and response variables were log (response+1) transformed to meet the assumptions of normality and homogeneity of variances.

Results

Variation in environmental variables

Streambed profiles separated relatively shallow, wide restored sites from deeper, narrower channelized and natural sites. An important characteristic of channelized sites was a low variability in water depth and width (Table 1.3). The overall proportion of large stones (>15 cm in b-axis) was two-fold greater at the restored site at Buenz and natural site at Reppisch than the other sites (Table 1.3). The natural site at Buenz had a particularly low presence of large substrates.

In the Buenz, water-level increased most steeply with increasing discharge at the channelized site (Fig.1.3) regression slope: 0.17), followed by the natural (slope: 0.12) and the restored site (slope: 0.10). In the Reppisch, the highest regression slope of this relationship was found at the natural site (0.24), followed by the channelized and restores site with very similar slopes (0.194, 0.191, respectively).

Availability and use of sampled substrates for oviposition

Over 90% of the introduced bricks protruded the water surface at the median discharge prior to each sampling event at all sites in both rivers and were thus available for adult insects. Stones were more often submerged or fully dry than bricks: e.g. 65%-74% of the sampled stones were emerged at channelized sites at median discharge.

Egg masses of different taxa detected during the experiment were found on both bricks and stones, indicating that bricks effectively increased the local availability of oviposition substrates. The proportion of substrates with egg masses attached was generally higher in the Buenz (overall 84%) than in the Reppisch.

Egg masses of different taxa detected during the experiment were found on both bricks and stones, indicating that bricks effectively increased the local availability of oviposition substrates. The proportion of substrates with egg masses attached was generally higher in the Buenz (overall 84%) than in the Reppisch (overall 75%). The restored site in the Buenz and the channelized site in the Reppisch had the highest percentage of non-colonized substrates (26.4% and 35.6%, respectively).

A total of 13 identifiable egg mass types of insect and non-insect taxa were found attached to stones and bricks in both rivers during the experiment (Fig.1.4; see description and photos of egg masses in supplement). These taxa made up 85-90% of the

Table 1.2: Full set of candidate models used for quantifying egg-mass density of insect taxa ovipositing on stones and bricks. 'Hydro'= water level, 'Sub'= substrate type, 'Temp'= temperature, 'Site'= site morphology

Model	***Factors***										
	fixed										*random*
1	Site	+ Sub	+ Hydro		+ Temp		+ Hydro×Temp	+ Site×Hydro	+ Sub×Hydro	+ Site×Sub	+ Day + Block
2	Site	+ Sub	+ Hydro	+ Hydro^2	+ Temp	+ Temp^2					+ Day + Block
3	Site	+ Sub	+ Hydro								+ Day + Block
4	Site	+ Sub	+ Hydro	+ Hydro^2							+ Day + Block
5	Site	+ Sub	+ Hydro					+ Site×Hydro	+ Sub×Hydro	+ Site×Sub	+ Day + Block
6	Site	+ Sub	+ Hydro					+ Site×Hydro			+ Day + Block
7	Site	+ Sub	+ Hydro						+ Sub×Hydro		+ Day + Block
8	Site	+ Sub	+ Hydro							+ Site×Sub	+ Day + Block
9	Site				+ Temp	+ Temp^2					+ Day + Block
10	Site				+ Temp						+ Day + Block
11			+ Hydro	+ Hydro^2	+ Temp	+ Temp^2					+ Day + Block
12			+ Hydro		+ Temp		+ Hydro×Temp				+ Day + Block
13			+ Hydro		+ Temp						+ Day + Block
14	Site		+ Hydro	+ Hydro^2							+ Day + Block
15	Site		+ Hydro								+ Day + Block
16	Site		+ Hydro								+ Day + Block
17	Site	+ Sub						+ Site×Hydro			+ Day + Block
18	Site	+ Sub								+ Site×Sub	+ Day + Block
19		+ Sub	+ Hydro								
20		+ Sub	+ Hydro	+ Hydro^2							
21		+ Sub	+ Hydro						+ Sub×Hydro		
22	Site										+ Day + Block
23			+ Hydro	+ Hydro^2							+ Day + Block
24			+ Hydro								+ Day + Block
25					+ Temp	+ Temp^2					+ Day + Block
26					+ Temp						+ Day + Block
27											
28											+ Day + Block

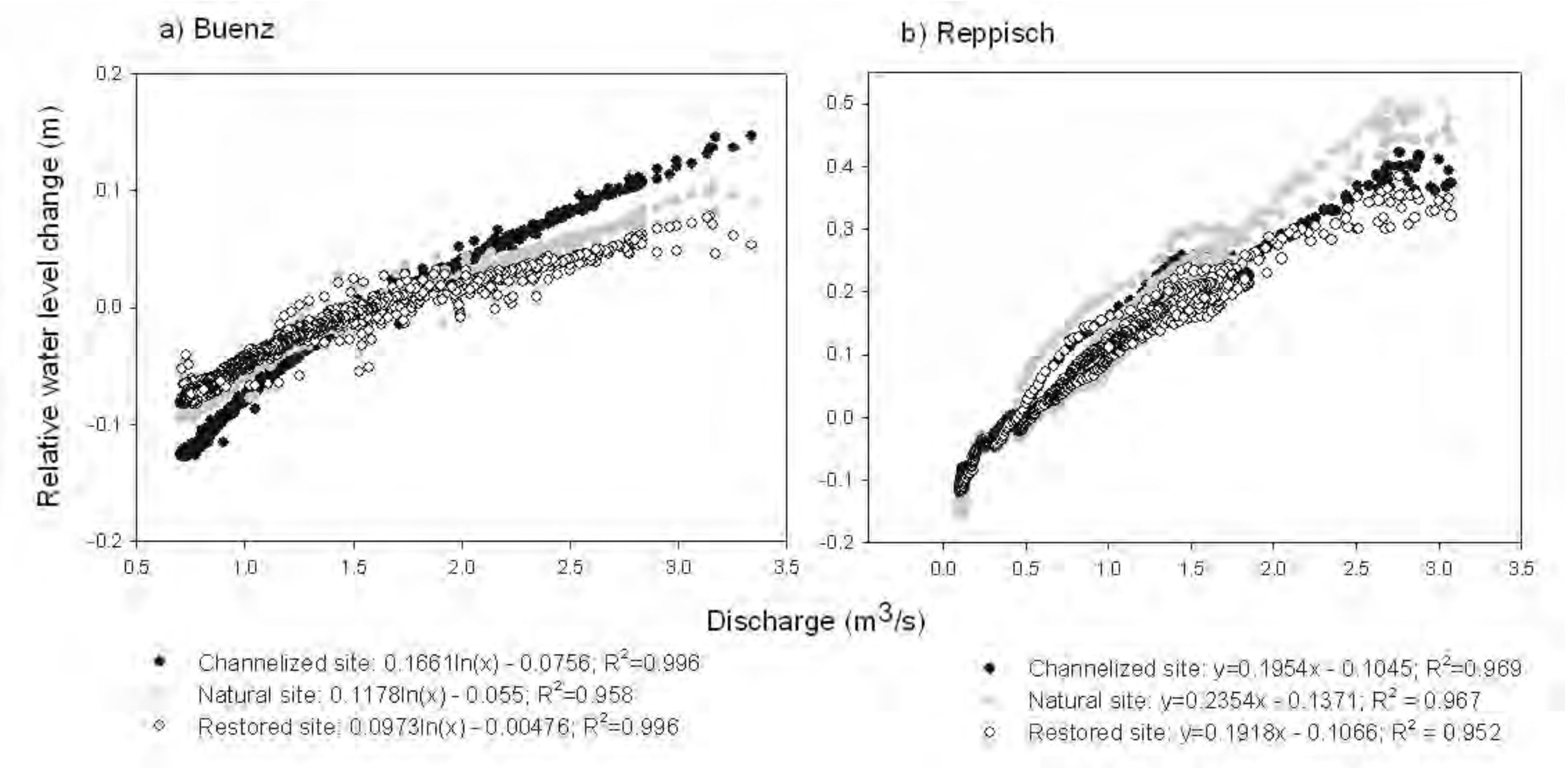

Figure 1.3: Water level change in response to discharge at the sampling sites. Water level change is reported relative to the average water level during the sampling period (set as '0').

Table 1.3: Overview of main morphological parameters for all sites in the two study streams.

	channelized			***natural***			***restored***				
	Mean	***SD***	***CV***	***Mean***	***SD***	***CV***	***Mean***	***SD***	***CV***	***F***	***P***
Buenz											
Depth	0.45	0.2	0.33	0.45	0.14	0.45	0.23	0.11	0.58	89.2	<0.001
Wetted width at average discharge	6.61	0.66	0.1	9.25	2.61	0.28	11.1	3.7	0.33	20.9	<0.001
Coarse substrate (>15cm)	27.4	22.2	0.81	20.9	7.18	0.34	21.6	7.2	0.33	5.84	0.04
% of coarse substrate	4.86			4.62			10.5				
Reppisch											
Depth	0.36	0.12	0.37	0.4	0.12	0.44	0.31	0.04	0.57	2.39	0.09
Wetted width at average discharge	6.25	0.96	0.15	6.86	1.3	0.19	8.39	3.2	0.38	2.26	0.13
Coarse substrate (>15cm)	26	17.2	0.66	26.2	11.8	0.45	23.7	9.21	0.39	0.25	0.78
% of coarse substrate	5.96			8.43			4.49				

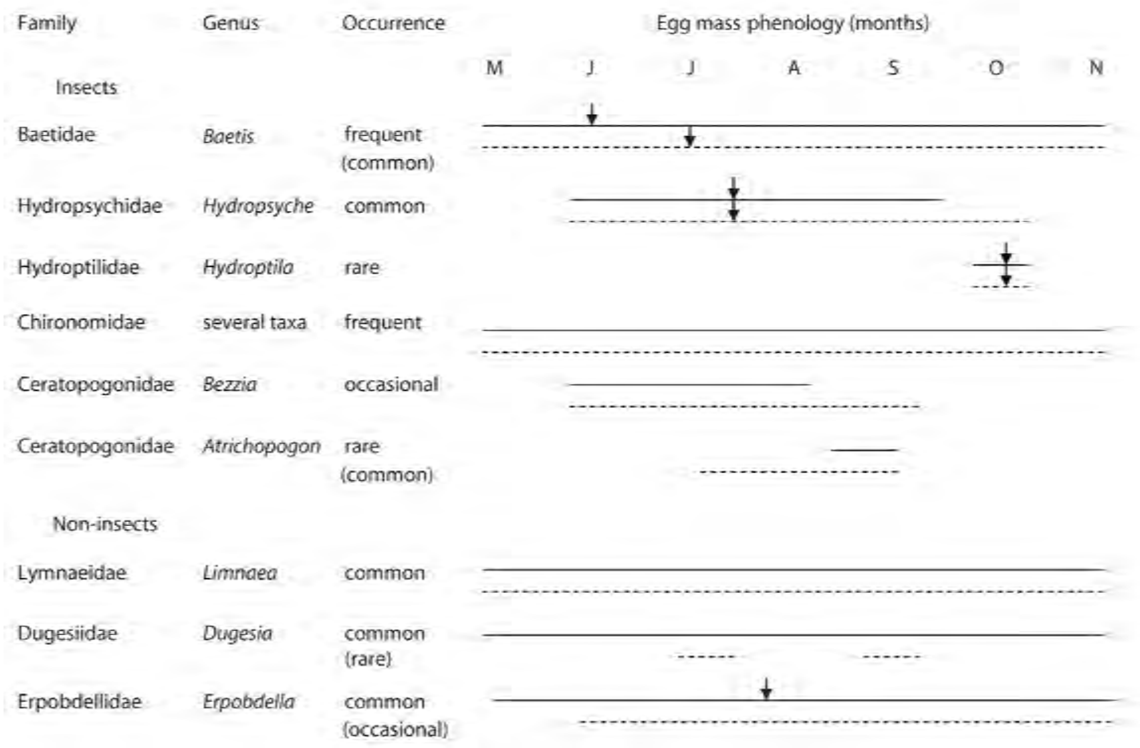

Figure 1.4: Overview of taxa ovipositing on bricks and stones across rivers in the summer 2008. The phenology of oviposition shows the presence of egg masses during the sampling period per taxon in the studied streams (Buenz in solid, Reppisch in dashed lines). Arrows indicate the timing of peak oviposition (if detected). Occurrence gives the highest frequency of each egg mass type being found on single sampling occasions (both substrate types included). Frequency categories mean: 1) 'frequent' - egg mass type was found on at least 50% of sampled substrates in all sites; 2) 'common' - egg mass type present in all sites on overall at least 25% of sampled substrates; 3) 'occasional' - egg mass type found; 4) 'rare' means that the egg mass type was only observed on a few occasions.

benthic abundance in the 2 study rivers. Occasionally, other taxa were found among the collected egg masses, including Polycentropodidae, Simuliidae, *Rhyacophila* spp. and 2 dipteran taxa (probably Empididae and Tabanidae). We lack reliable data on these taxa because they were either difficult to identify, e.g. their egg masses were mixed with those of other taxa or they were very rare.

Baetis spp.: *Larval and egg mass densities*

We found larvae of two common species of *Baetis* in both streams: *B. rhodani* (Pictet) and *B. scambus* (Eaton). The range in mean benthic densities (over all sampling occasions) was between 916.67 ± SE 252.21 per m^2 (channelized) and 1113.85 ± SE 341.05 per m^2 (natural) in the Buenz, and 518.22 ± SE 132.04 per m^2 (channelized) and 1117.93 ± SE 294.32 per m^2 (natural) in the Reppisch (high variance of data reflects seasonal variation).

Overall, *Baetis* egg mass density was highest on both types of substrates at the deepest sites (Fig.1.5), with the exception of the channelized site in the Reppisch. In the Buenz, egg mass density of *Baetis* was similar on bricks and stones (Table 1.4): mean over all sampling dates and sites for bricks 346.45 ± SE 101.99, for stones 414.85 ± SE 116.27, whereas it was higher on stones in the Reppisch: 19.06 ± SE 5.31 for bricks, 169.06 ± SE 53.78 for stones. For instance, egg mass density in the Reppisch was up to 60 times higher on stones than bricks at the natural site in the peak oviposition period (Fig.1.5).

Baetis spp.: *Model selection results*

Models 7 and 8 including factors 'site morphology', 'substrate type' and 'water level' as well as interactions 'water level' x 'substrate type' and 'site morphology' × 'substrate type' explained the most variation in egg mass density of *Baetis* at Buenz (Table 1.5). In the Reppisch, model 17 including only factors 'site morphology' and 'substrate type' received most substantial support (Table 1.6). The confidence intervals for the estimates of intercepts (means of egg mass density) for single levels of categorical factors suggested that egg mass densities differed between sites and substrates in both streams (Tables S1.2 - S1.4). No such difference was detected for the comparison between 'channelized' and 'restored' sites in the Reppisch, where the confidence intervals included zero.

A negative slope for the relationship between the 'water level' and egg mass density in the Buenz suggests that with increasing water level, egg mass density of *Baetis* decreased. Support for the interaction terms suggests that the difference in egg mass density on bricks and stones varied depending on 'site morphology' and the effect of 'water level' was different for the two substrate types.

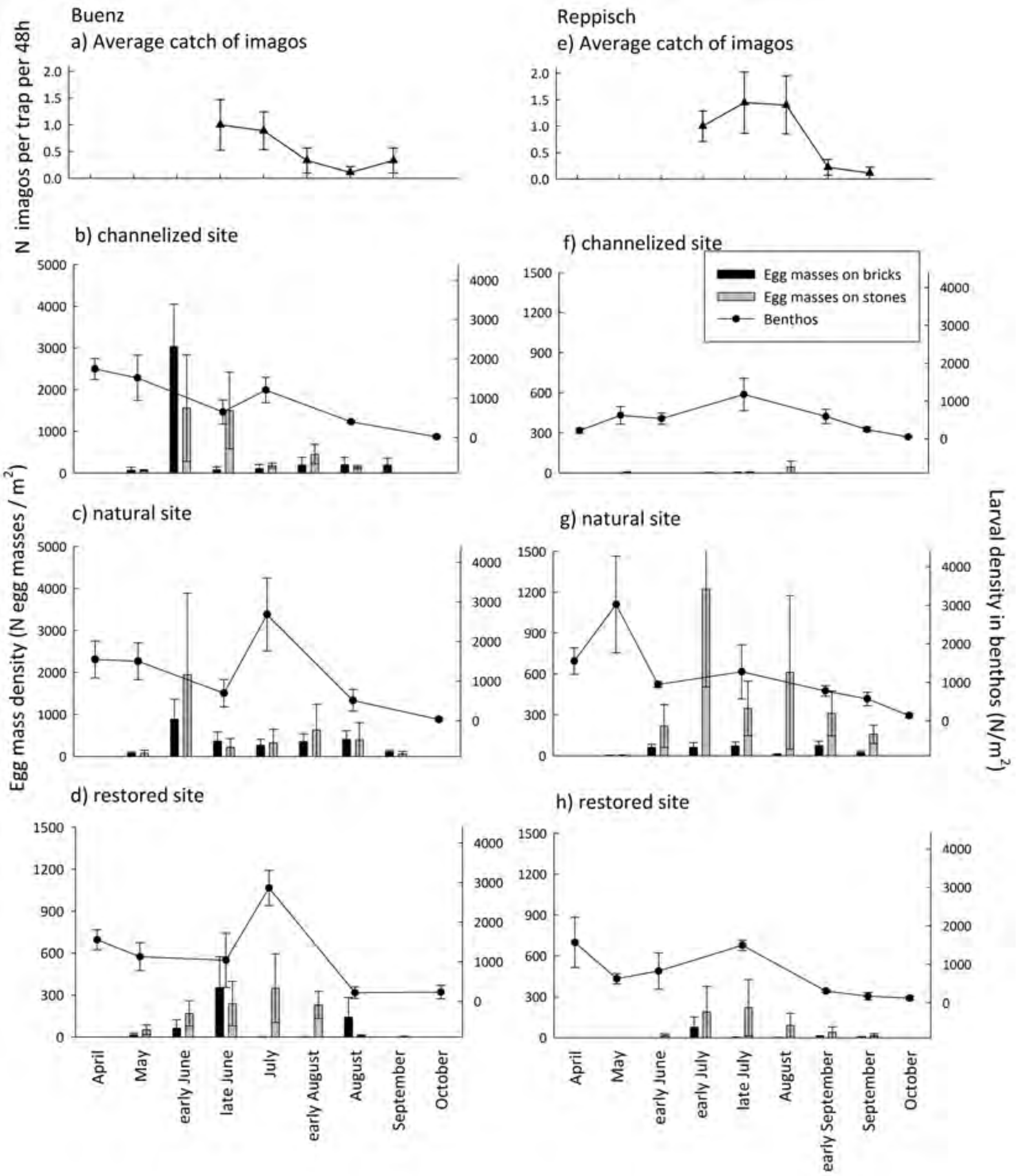

Figure 1.5: *Baetis* spp. egg mass density on bricks and stones in the Buenz and Reppisch, summer 2008. Error bars indicate standard errors.

Hydropsyche spp.: *Larval and egg mass densities*
Several species of the genus *Hydropsyche* were identified in benthic samples. *H. siltalai* (Dohler) was clearly dominant in both streams, with *H. dinarica* (Marinkovic) and *H. instabilis* (Curtis) co-occuring occasionally. Mean benthic abundances of *Hydropsyche* varied between sites and streams (Fig.1.6). In the Buenz, densities ranged between 133.63 ± SE 53.45 per m^2 in the restored and 558.15 ± SE 124.55 per m^2 in the channelized site. In the Reppisch, mean larval density in the downstream natural site was an order-of-magnitude higher than in the two upstream sites: 502.74 ± SE 207.15 per m^2 versus 57.04 ± SE 21.44 per m^2 in the channelized and 45.63 ± SE 12.81 per m^2 in the restored sites.

In the Buenz, egg mass density of *Hydropsyche* was slightly higher at the channelized site than the other sites (Fig.1.6). More egg masses were laid on bricks than stones at the natural site (Table 1.4). The opposite trend was observed in restored and channelized site, where mean egg mass density was at least 3 (and on some occasions up to 45) times higher on stones than bricks. Data from Reppisch showed highest egg mass concentrations in the natural site. A slight tendency of higher egg mass density on bricks relative to stones was found in the restored site.

Hydropsyche spp.: *Model selection results*
In the Buenz, models 18 and 8 including factors 'site morphology', 'substrate type', 'water level' and interaction between 'substrate type' and 'site morphology' explained the patterns in *Hydropsyche* egg mass densities best (Table 1.5). 'Site morphology' as a separate factor was found to be an unreliable predictor as the estimates for differences between sites included zero in their confidence intervals. The slope for the relationship between 'water level' and egg mass density was negative. In the Reppisch, models 28 and 26 received the highest support (Table 1.6) thus suggesting that only random factors and 'temperature' (with a positive slope) contributed to explaining the variation in *Hydropsyche* egg mass densities in this stream.

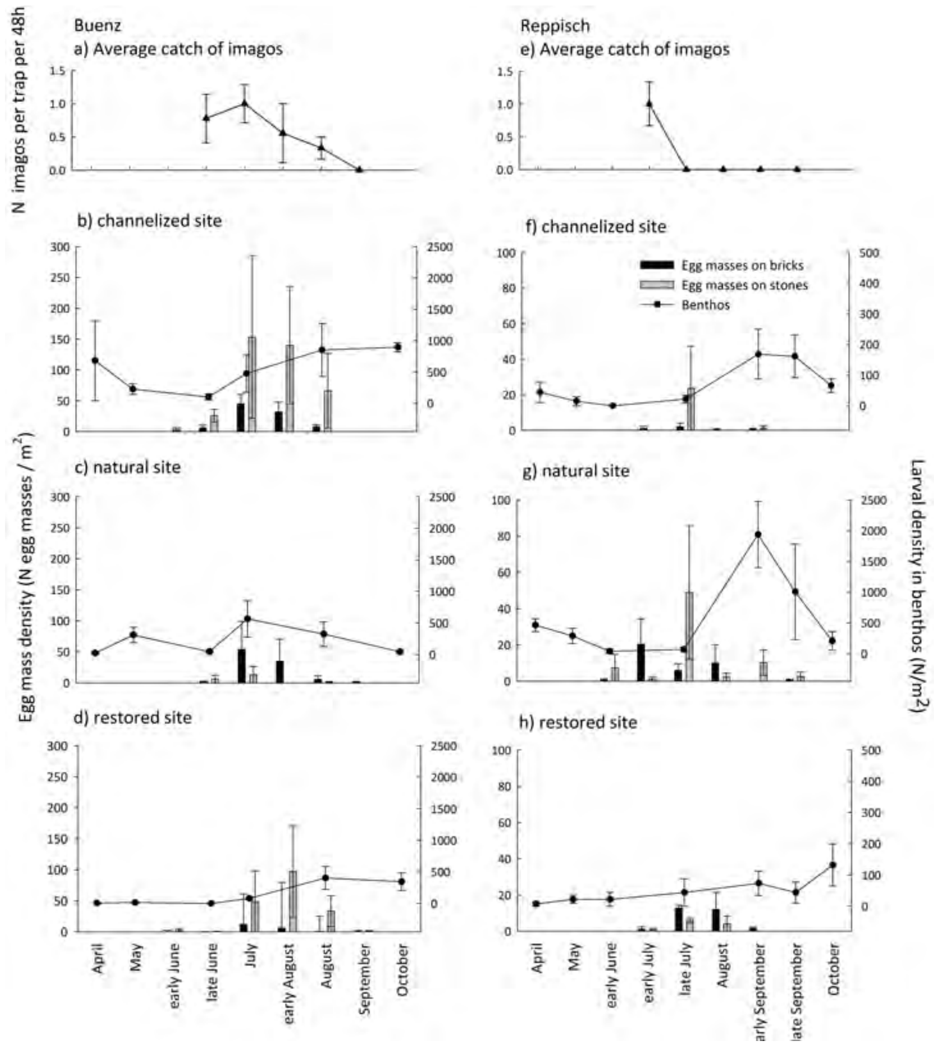

Figure 1.6: *Hydropsyche* spp. egg mass density on bricks and stones in the Buenz and Reppisch, summer 2008. Error bars indicate standard errors. Note the scale difference between graphs for the two rivers.

Table 1.4: Mean egg mass densities at Buenz and Reppisch.

	channelized		***natural***		***restored***	
	bricks	***stones***	***bricks***	***stones***	***bricks***	***stones***
Buenz						
Baetis	592.10 ± SE 277.86	584.72± SE 247.18	348.74 ± SE 92.04	518.11 ± SE 242.44	86.13 ± SE 43.91	149.82 ± SE 47.44
Hydropsyche	22.70 ± SE 6.93	96.85 ± SE 40.14	24.40 ± SE 13.89	5.10 ± SE 3.49	11.51 ± SE 2.97	45.23 ± SE 22.21
Reppisch						
Baetis	0.71 ± SE 0.38	8.43 ± SE 6.64	43.15 ± SE 9.73	409.84 ± SE 142.55	13.33 ± SE 10.76	81.28 ± SE 39.95
Hydropsyche	1.20± SE 0.71	7.88 ± SE 7.88	12.10 ± SE 5.51	17.42 ± SE 13.31	8.69 ± SE 3.36	3.67 ± SE 1.49

Table 1.5: Model selection results for egg mass density in the Buenz. Factors in bold did not include zero in their confidence intervals.

Model	***Factors***	***K***	***LogLik***	***AICc***	***ΔAICc***	***Akaike weight***	***Evidence ratio***
Baetis							
7	**Site** + **Substrate** + **Hydro** + **Hydro×Substrate**	9	-1428.05	2890.46	0.00	0.84	1.00
8	**Site** + Substrate + **Hydro** + **Site×Substrate**	10	-1426.14	2894.27	3.82	0.13	6.74
Hydropsyche							
18	Site + **Substrate** + **Site×Substrate**	9	-1039.16	2112.69	0.00	0.68	1.00
8	Site + **Substrate** + **Hydro** + **Site×Substrate**	10	-1036.69	2115.37	2.68	0.18	3.82

Table 1.6: Model selection results for egg mass density in the Reppisch. Factors in bold did not include zero in their confidence intervals.

Model	***Factors***	***K***	***LogLik***	***AICc***	**Δ*AICc***	***Akaike weight***	***Evidence ratio***
Baetis							
17	Site + Substrate	7	-1239.52	2501.66	0.00	0.88	1.00
Hydropsyche							
28	(Day + Block)	4	-821.76	1654.02	0.00	0.58	1.00
26	Temp	5	-820.73	1655.45	1.44	0.28	2.06

Discussion

The wide range of human modifications to river morphology represents a serious threat to aquatic organisms but also an opportunity to address key questions in biology (Malmqvist, 2002). We used human-caused differences in stream morphology as a context for an experimental field study and explored variation in egg mass density of two taxa of aquatic insects in relation to environmental factors influencing oviposition substrate availability. Our results suggest that reduced specific substrate availability can become a constraint for reproduction of aquatic insects. However, differences in the patterns found in the two studied streams indicate that some catchment-scale factors can have an overriding effect in determining such local constraints.

Environmental constraints on oviposition

Overall, both for *Baetis* and *Hydropsyche*, we found indications of environmental constraints and a strong response in terms of egg mass density to our experimental manipulation in the Buenz, whereas no similar patterns were detected in the Reppisch. We first discuss the differences between the two species found in the Buenz and then suggest some possible explanations for the differences in the results between the two streams.

Model selection results detected indications of environmental constraints in terms of substrate availability as well as an overall negative effect of ’water level’ for both taxa in the Buenz. The water level effect was much more pronounced for *Baetis*, being included in both best supported models (with total Akaike weight = 0.97), whereas in *Hydropsyche* this factor was only present in the second-best model (Akaike weight = 0.18). Moreover, support for the effect of interaction of ’water level’ and ’substrate type’ suggests that changes in egg mass density of *Baetis* in response to water level fluctuations were different between bricks and stones. In fact, the slope of the relationship was steeper for stones (for details see Table S1.2), which means that with increasing discharge, egg mass density decreased less rapidly on bricks (which were emergent on more sampling occasions) than on stones. These results lie in line with our initial expectation that *Baetis* might show a stronger response to factors determining substrate emergence, as for oviposition baetids need to first land on a protruding rock surface and then attach the eggs on the submerged side of the rock (Percival & Whitehead, 1928; Encalada & Peckarsky, 2006).

The relative egg mass density on bricks (compared to stones) changed depending on the site morphology in the Buenz. This effect was clearly more pronounced for *Hydropsyche*: total Akaike weight of the models including the interaction of ’site morphology’ and ’substrate type’ was 0.96 (compared to 0.13 for *Baetis*; Table 1.5). The nature of this effect was different for the two taxa. In *Hydropsyche*, a strong change in the relative use of bricks occurred in the natural site, the only site where egg mass density was consistently higher on bricks than on stones (Fig.1.6, Table 1.4). In contrast, for *Baetis* a somewhat elevated use of bricks for oviposition was detected in the channelized site (Fig.1.5, Table 1.4). This suggests that different types of constraints, specific for the sites, should be acting on oviposition of the two species determining the relative use of bricks and stones. A set of morphological parameters assessed during the experiment (Table 1.3) indicate that the mean size of coarse substrate was the most pronounced difference in factors determining substrate availability in the natural site compared to the other sites.

In fact, 95% of coarse substrates in the natural site in the Buenz were smaller than bricks. Thus a possible explanation for the preferential use for bricks by *Hydropsyche* in this site could be a preference for large substrates (available as stones in other sites). Such preference would not be surprising as it has already been observed for several taxa of aquatic insects and interpreted as a strategy to lay eggs in a habitat less prone to stranding and desiccation (Peckarsky *et al.*, 2000; Encalada & Peckarsky, 2006; Reich & Downes, 2003). In contrast, the channelized site, where a similar but weaker effect was found for *Baetis*, is most distinguished from the other two by its deep and very monotonous profile, reflected in a more rapid response to discharge fluctuations (Fig.1.3). In fact, in this site the lowest proportion of sampled natural stones (66%) was available (protruding) during the period of the experiment. Thus elevated relative use of the bricks in the channelized site by *Baetis* suggests once again that it responds specifically to the availability of protruding substrates.

Overall, we find indications for strong environmental constraints in terms of substrate availability for both species in the Buenz. In this stream, in line with our initial expectations, *Baetis* responded primarily to factors determining substrate emergence, whereas *Hydropsyche* showed a very weak response to 'water level', suggesting that its capacity to dive (Deutsch, 1984) makes it much less vulnerable to this factor. However, another factor, possibly low availability of large substrates, appeared to constrain oviposition of *Hydropsyche* in one of the sites.

No similar patterns were found in the Reppisch. None of the species showed a statistically detectable response to the addition of bricks. Thus no apparent constraints in substrate availability were detected in this stream in contrast to the Buenz. We offer several explanations for this result. First, some catchment scale relevant environmental factor could cause an overriding constraint, enhancing the effects of local stream morphology in the Buenz but not in the Reppisch. Hydrology could potentially be such a factor. Buenz has a ca. fourfold higher discharge and is somewhat deeper than the Reppisch (Table 1.3). Moreover due to the activity of a small hydropower plant, its discharge is distinctly flashier than that of the Reppisch (Fig.1.2). This would be a feasible explanation for *Baetis*, which we expected to be especially sensitive to water level fluctuations, as it has to look for protruding substrates for oviposition.

The overall low densities of ovipositing adults could have lead to low densities of egg masses due to a lack of 'crowding' on the substrates, even if their overall availability in the reach was low. This could be a feasible scenario for *Hydropsyche* whose benthic abundances in the two upstream sites of Reppisch are an order-of-magnitude lower than in the natural site. In fact, very low catches of imagos of *Hydropsyche* in the two upstream sites lie in line with this explanation. In contrast both benthic and egg mass abundances in the natural site in the Reppisch were comparable to those found in the Buenz. No clear patterns in terms of substrate type preference in this site for *Hydropsyche* and low use of bricks for *Baetis* indicate that also here that substrate availability is not a constraint for oviposition of none of the species at all sites.

The role of local oviposition constraints for regional recruitment: outlook

Conducted on a reach scale, this experimental study provided insight into the local and catchment-scale factors that determine species-specific hotspots for recruitment of aquatic insects. Even in a natural heterogeneous landscape, such hotspots are distributed patchily (Lancaster, Downes & Reich, 2003; Peckarsky *et al.*, 2000). Linked to other (sink) patches by dispersal, they play a role of sources of egg supply relevant both for local and regional recruitment and follow the classical scenario of source-sink dynamics (Pulliam, 1988; Hixon, Pacala & Sandin, 2002; Palmer, Allan & Butman, 1996). In a modified landscape exposed to multiple pressures from stream channel degradation to unnatural hydrological regime, not only the number of available 'hotspots' must have decreased dramatically (both in space and time), but also the connectivity between 'sources' and 'sinks' must have been strongly reduced. Multiple evidence suggests that the scale of human impacts overrides the scale of dispersal of most aquatic organisms (Hughes, 2007; Zwick, 1992). Thus for a better understanding of implications of local recruitment constraints for regional population dynamics a further step would be targeting a larger scale perspective. How are such hotspots distributed in the stream landscape? How well are they linked for species with different dispersal capacities? What is the critical hotspot availability necessary to provide a sufficient supply of recruits for maintaining a regional population? These would be the exciting further questions to ask, especially in the context of modified landscapes. Combining experimental approaches with landscape mapping and studies on species dispersal might deliver at least some of the answers to these questions.

Implications for management

Based on our results, we can make some recommendations for river management. For example, we suggest that the introduction of large boulders into rivers, a simple low-cost technique often applied for small-scale river restoration (Roni, Hanson & Beechie, 2008), could enhance recruitment potential for many invertebrate taxa, in particular aquatic insects. We expect this measure to be most efficient in channelized middle-sized streams with gravel riverbeds. Creation of such 'hotspots' could have local as well as regional effects, as they could play the role of a source for regional supply of recruits. However the success of such restoration measures can be constrained by drastic unnatural fluctuations in discharge, such as from hydropeaking (Bretschko & Moog, 1990), which could limit availability of emergent substrates during high flow (and also potentially cause desiccation of egg masses during low flow). Thus we re-emphasize the importance of a natural flow regime for achieving the goals set for river restorations.

Acknowledgments

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Supplement

Table S1.1: List of substrate size categories.

Substrate size
1-2 cm
2-3 cm
3-4 cm
4-5 cm
5-6 cm
6-7 cm
7-8 cm
8-9 cm
9-10 cm
10-12 cm
12-15 cm
15-20 cm
20-25 cm
25-30 cm
>30 cm
>100 cm

Table S1.2: Parameter estimates for *Baetis* spp., Buenz, model 7 (AIC weight = 0.85). Confidence intervals of estimates for variables in bold do not contain zero. Response variable was log (1+y) transformed.

Variable	***Type of variable***	***Estimated parameter***	***Contrasts***	***LCL***	***Estimate***	***UCL***
		intercept	(site channelized,bricks)	2.631	3.450	4.269
Site	categorical	difference in intercept	channelized - natural	0.661	1.415	2.169
Site	categorical	difference in intercept	channelized - restored	-6.142	-4.431	-2.719
Site	categorical	difference in intercept	restored - natural	3.828	5.846	7.863
Substrate	categorical	difference in intercept	bricks - stones	0.108	0.495	0.882
Hydro	continuous	slope	(bricks)	-2.226	-1.303	-0.381
Hydro×Substrate	continuous	difference in slope	Hydro (bricks - stones)	-1.239	-0.851	-0.462

Table S1.3: Parameter estimates for *Baetis* spp., Buenz, model 8 (AIC weight = 0.13). Confidence intervals of estimates for variables in bold do not contain zero. Response variable was log (1+y) transformed.

Variable	***Type of variable***	***Estimated parameter***	***Contrasts***	***LCL***	***Estimate***	***UCL***
		intercept	(site channelized, bricks)	2.730	3.590	4.450
Site	categorical	difference in intercept	channelized - natural	0.818	1.706	2.594
Site	categorical	difference in intercept	channelized - restored	-6.936	-5.153	-3.370
Site		difference in intercept	restored - natural	4.777	6.859	8.940
Substrate	categorical	difference in intercept	bricks - stones	-0.462	0.218	0.899
Hydro	continuous	slope	(bricks)	-2.609	-1.703	-0.797
Site×Substrate	categorical	difference in intercept	natural: bricks-stones	-1.572	-0.626	0.320
Site×Substrate	categorical	difference in intercept	restored: bricks-stones	0.536	1.495	2.453

Table S1.4: Parameter estimates for *Baetis* spp., Reppisch, model 17 (AIC weight = 0.88). Confidence intervals of estimates for variables in bold do not contain zero. Response variable was log (1+y) transformed.

Variable	*Type of variable*	*Estimated parameter*	*Contrasts*	*LCL*	*Estimate*	*UCL*
		intercept	(site channelized, bricks)	-0.526	-0.042	0.441
Site	categorical	difference in intercept	channelized - natural	1.636	2.186	2.736
Site	categorical	difference in intercept	channelized - restored	-0.148	0.402	0.953
Site	categorical	difference in intercept	restored - natural	1.240	1.783	2.327
Substrate	categorical	difference in intercept	bricks - stones	0.392	0.672	0.952

Table S1.5: Parameter estimates for *Hydropsyche* spp., Buenz, model 18 (AIC weight = 0.68). Confidence intervals of estimates for variables in bold do not contain zero. Response variable was log (1+y) transformed.

Variable	*Type of variable*	*Estimated parameter*	*Contrasts*	*LCL*	*Estimate*	*UCL*
		intercept	(site channelized, bricks)	0.187	0.761	1.336
Site	categorical	difference in intercept	channelized - natural	-0.455	0.067	0.589
Site	categorical	difference in intercept	channelized - restored	-0.978	-0.450	0.079
Site	categorical	difference in intercept	restored - natural	-0.012	0.517	1.046
Substrate	categorical	difference in intercept	bricks - stones	0.044	0.391	0.739
Substrate×Site	categorical	difference in slope	bricks - stones (natural)	-1.541	-1.058	-0.576
Substrate×Site	categorical	difference in slope	bricks - stones (restored)	-0.264	0.226	0.716

Table S1.6: Parameter estimates for *Hydropsyche* spp., Buenz, model 8 (AIC weight = 0.18). Confidence intervals of estimates for variables in bold do not contain zero. Response variable was log (1+y) transformed.

Variable	***Type of variable***	***Estimated parameter***	***Contrasts***	***LCL***	***Estimate***	***UCL***
		intercept	(site channelized, bricks)	0.548	1.110	1.671
Site	categorical	difference in intercept	channelized - natural	-0.216	0.345	0.906
Site	categorical	difference in intercept	channelized - restored	-2.952	-1.772	-0.593
Site	categorical	difference in intercept	restored - natural	0.735	2.118	3.500
Substrate	categorical	difference in intercept	brick - stone	0.046	0.393	0.741
Hydro	continuous	slope	(bricks)	-1.370	-0.762	-0.153
Substrate×Site	continuous	difference in intercept	bricks - stones (natural)	-1.543	-1.060	-0.578
Substrate×Site	continuous	difference in intercept	bricks - stones (restored)	-0.267	0.223	0.713

Table S1.7: Parameter estimates for *Hydropsyche* spp., Buenz, model 26 (AIC weight = 0.28). Response variable was log (1+y) transformed.

Variable	***Type of variable***	***Estimated parameter***	***Contrasts***	***LCL***	***Estimate***	***UCL***
		intercept	(site channelized, bricks)	0.083	0.299	0.515
Temperature	continuous	slope	(bricks)	0.022	0.227	0.432

Egg mass types observed on rocks in rivers Buenz and Reppisch in summer 2008, photographs by Maria Alp

Ephemeroptera, Baetidae

Baetis spp Found from May to October. Very rarely found as single egg masses, most often densely aggregated and overlapping. Fresh egg masses very light-colored, older ones - darker.

Figure S1.1: Baetidae, egg masses. Scale bar: ca. 1 cm.

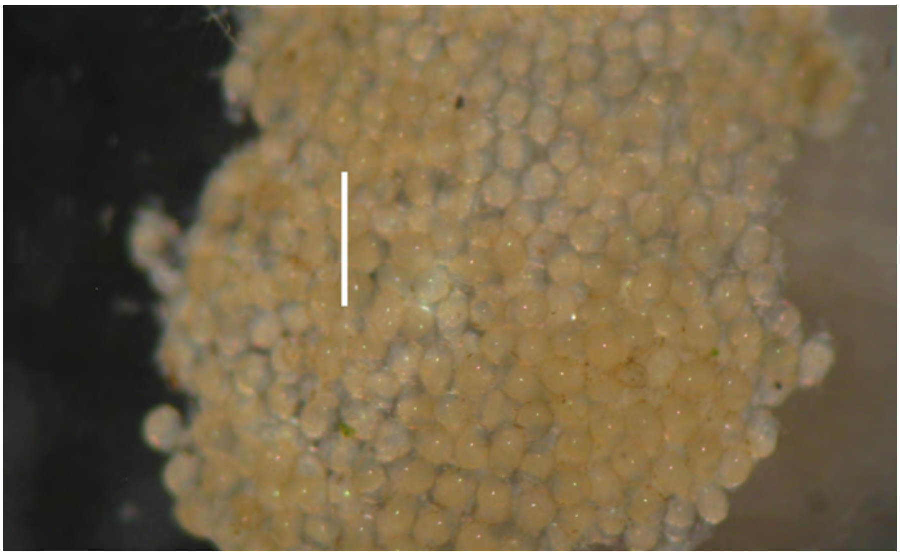

Figure S1.2: Baetidae, egg masses. Scale bar: ca. 2 mm.

Figure S1.3: Baetidae, egg masses. A densely packed rock.

Figure S1.4: A female of *Baetis* (supposedly *rhodani*) laying eggs on the brick surface.

Trichoptera, Hydropsychidae

Hydropsyche spp. Found primarily in July and August. Egg masses flat and grainy, reddish. Often found aggregated and sometimes overlapping.

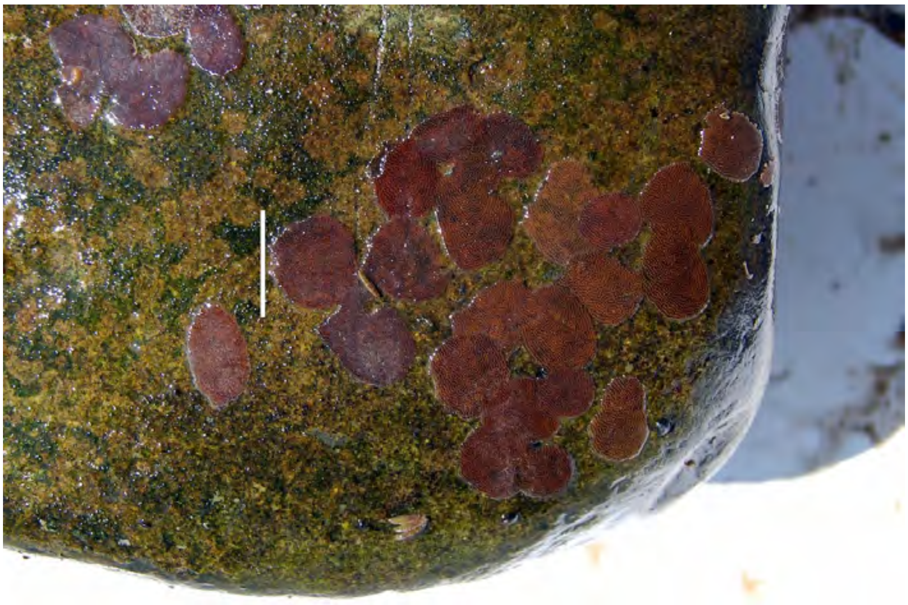

Figure S1.5: Hydropsychidae, egg masses. Scale bar: ca. 15 mm.

Figure S1.6: Hydropsychidae, egg masses.

Trichoptera, Hydroptilidae

Hydroptila spp. Egg masses found in October as single dense and 'grainy' spherical egg masses. Never aggregated. Yellowish. Single eggs visible in the field with naked eye.

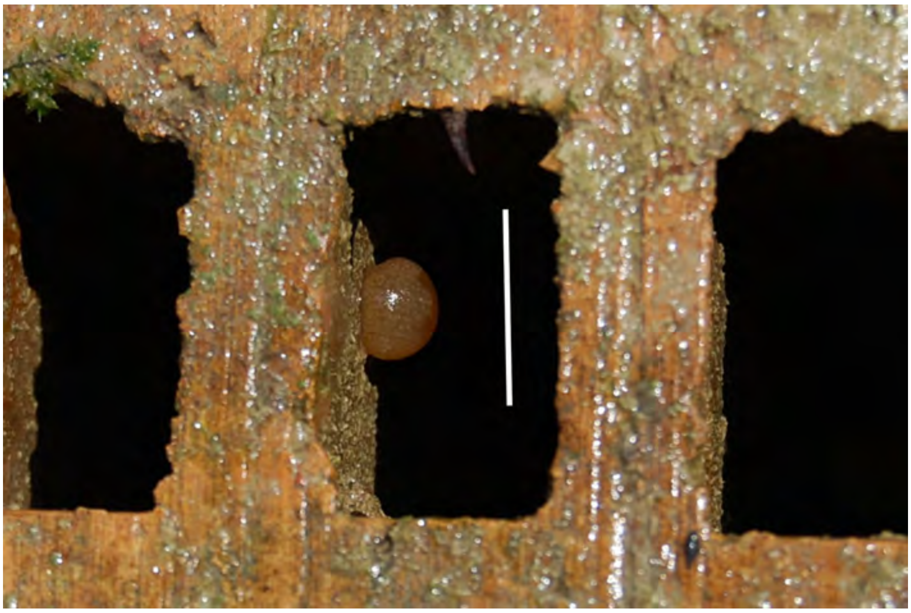

Figure S1.7: Hydroptilidae, an egg mass attached to a brick. Scale bar: 13 mm

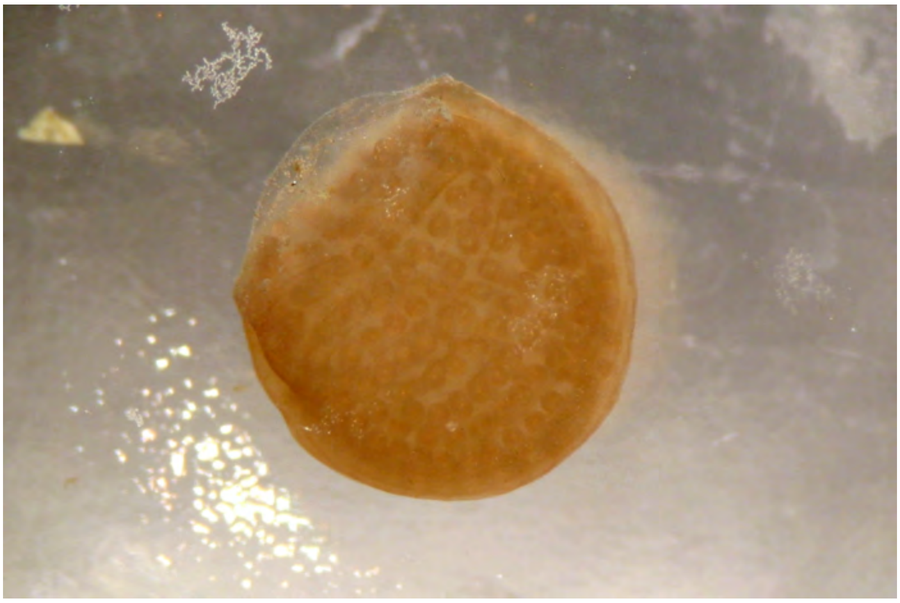

Figure S1.8: Hydroptilidae, an egg mass.

Diptera, Ceratopogonidae

Bezzia sp. Found in June and August. Laid at the border of water and air, often aggregated. Round and flat on the side, where attached to the substrate. Single egg masses seem a pack of radiating needles. Fresh egg masses greyish, older ones nearly black.

Figure S1.9: Ceratopogonidae, *Bezzia*, old and fresh egg masses on a rock in the field.

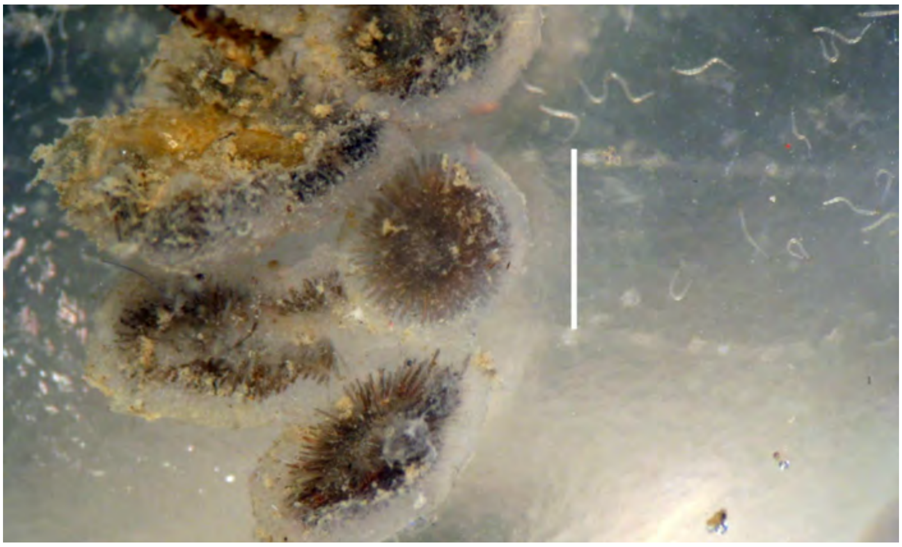

Figure S1.10: Ceratopogonidae, *Bezzia*, egg masses. Scale bar: ca. 3 mm.

Figure S1.11: Ceratopogonidae, *Bezzia*, egg masses laid at the border of water and air.

Atrichopogon sp. Tiny dry egg masses, each egg looking like a black dash. No gelatinous cover. Found on smooth surfaces in late summer.

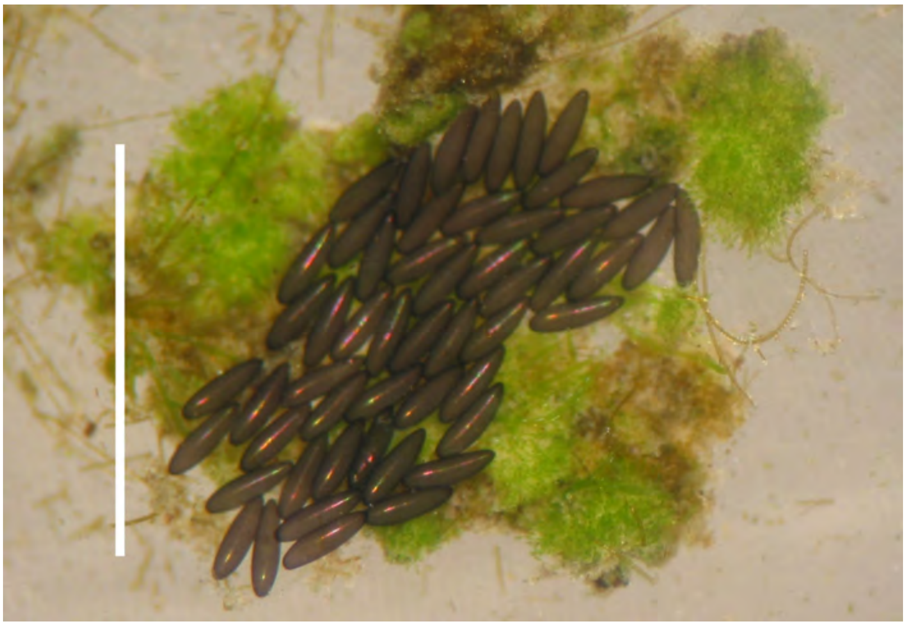

Figure S1.12: Ceratopogonidae, *Atrichopogon*, an egg mass. Scale bar: ca. 2 mm.

Figure S1.13: Larva of *Atrichopogon*. Scale bar: ca. 1 mm.

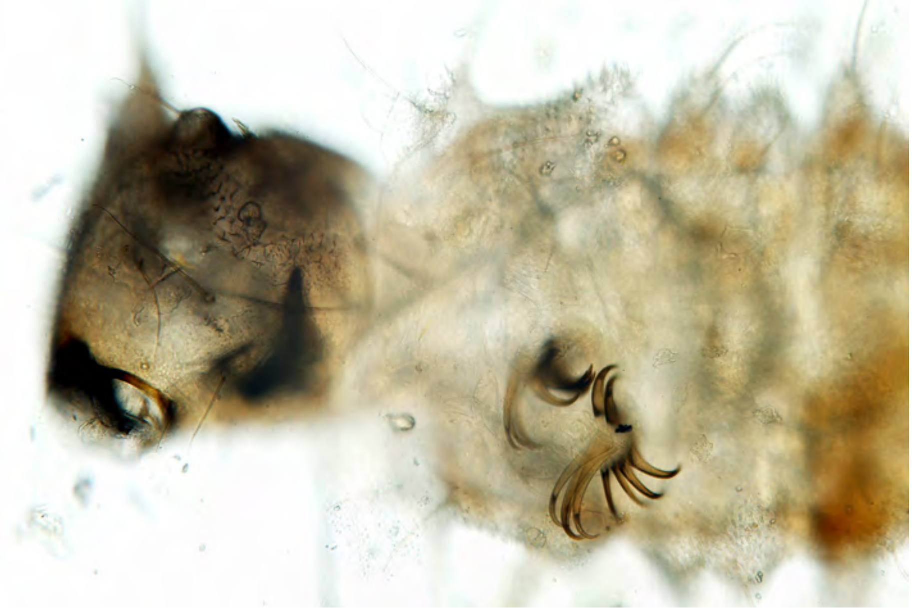

Figure S1.14: Larva of *Atrichopogon*, a close-up.

Diptera, Chironomidae

A variety of egg mass types usually highly aggregated: from very gelatinous roundish egg masses to long threads. When fresh, light-coloured, whitish or transparent. Older egg masses darker.

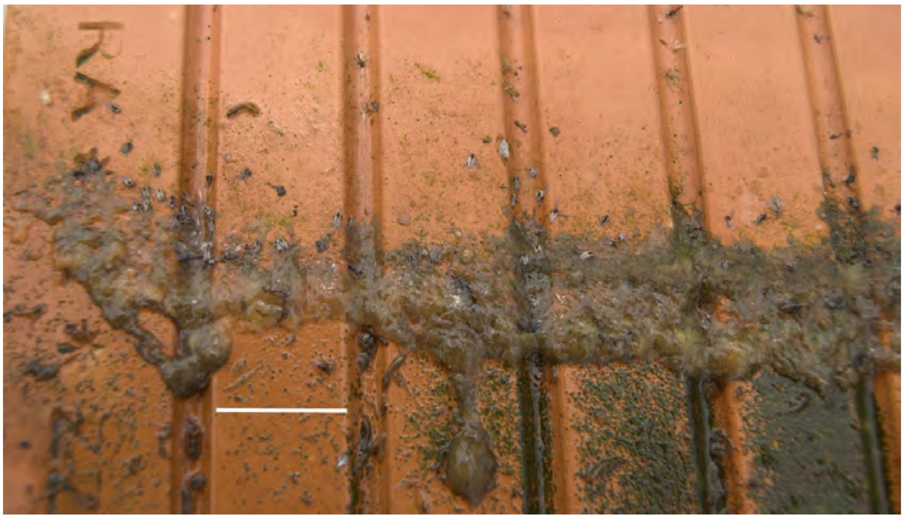

Figure S1.15: Chironomidae, egg masses of type 1. Highly aggregated gelatinous egg masses. Single egg masses hardly distinguishable. Scale bar: ca. 15 mm.

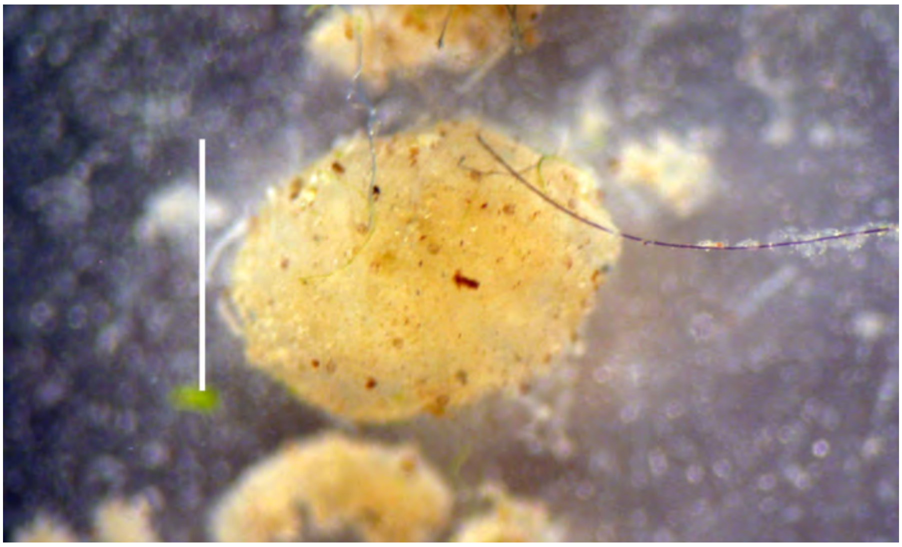

Figure S1.16: Chironomidae, egg mass of type 1. A single gelatinous roundish egg mass, non-transparent, no single eggs visible. Scale bar: ca. 3 mm.

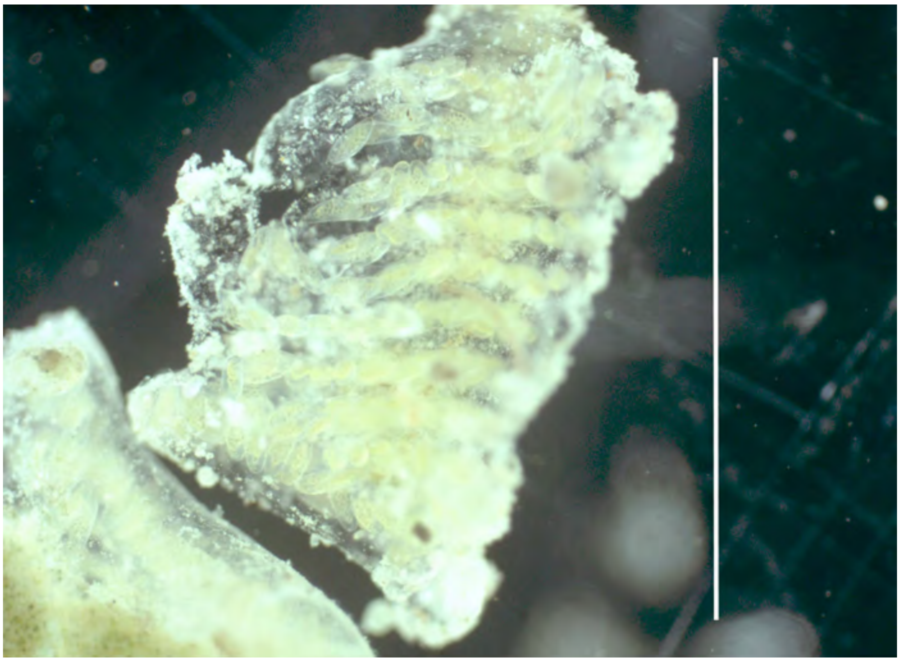

Figure S1.17: Chironomidae, egg mass of type 2. A rather rectangular egg mass with 'zig-zag' shaped threads of eggs visible in the transparent gelatinous matrix. Scale bar: ca. 5 mm.

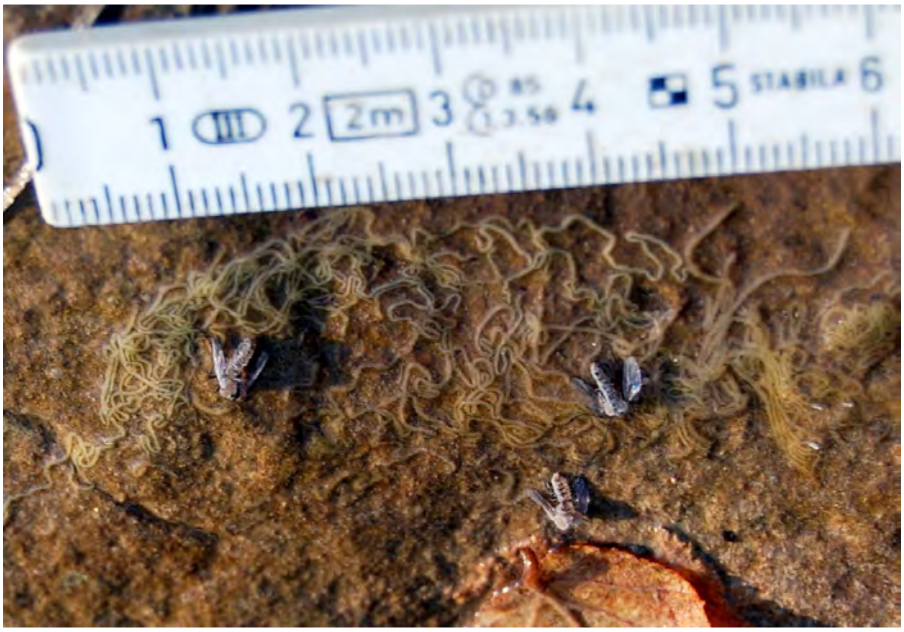

Figure S1.18: Chironomidae, egg masses of type 3. Egg masses in the shape of long thin threads. Units on the pictured ruler: cm.

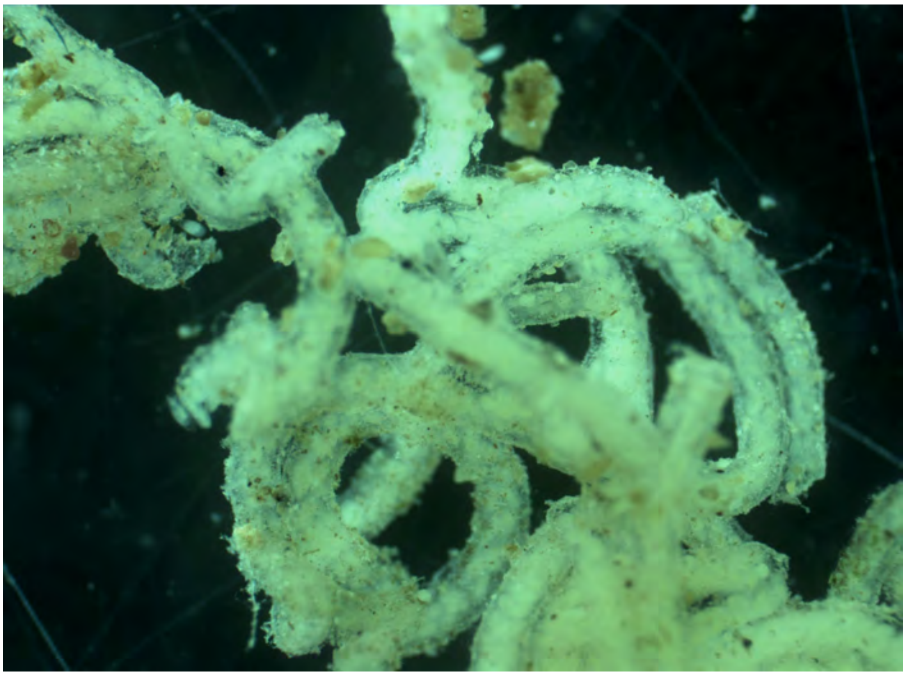

Figure S1.19: Chironomidae, egg mass of type 3, a close-up.

Figure S1.20: Chironomidae, egg masses of type 4. Elongated, in the field transparent egg masses. In contrast to type 3 not a long thread but distinguishable short single egg masses. Scale bar: ca. 6 mm.

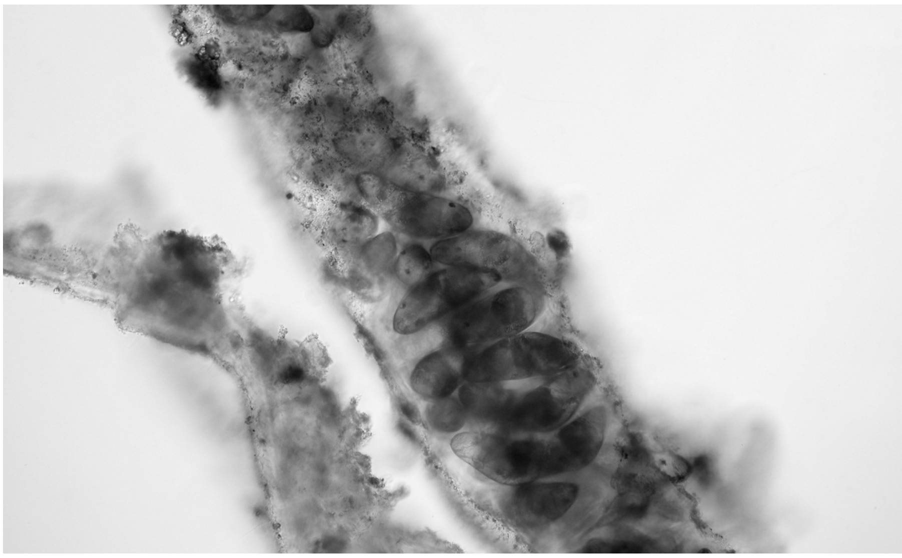

Figure S1.21: Chironomidae, egg mass of type 4. A close-up, single eggs are visible within the egg mass.

Planariidae

Dugesia spp. Found from April to October. Laid as single eggs attached to the substrate by a fine thread. Sometimes several found on the same substrate, but never aggregated.

Figure S1.22: Planariidae, *Dugesia*, a single egg. Scale bar: ca. 2 mm

Mollusca, Limnaeacea

Limnaea spp. Found from April to October. Gelatinous egg masses in a prolonged shape. Mainly found as single egg masses. Easy to detach from the substrate, quite dense and compact. Sometimes several found on the same substrate, but never aggregated.

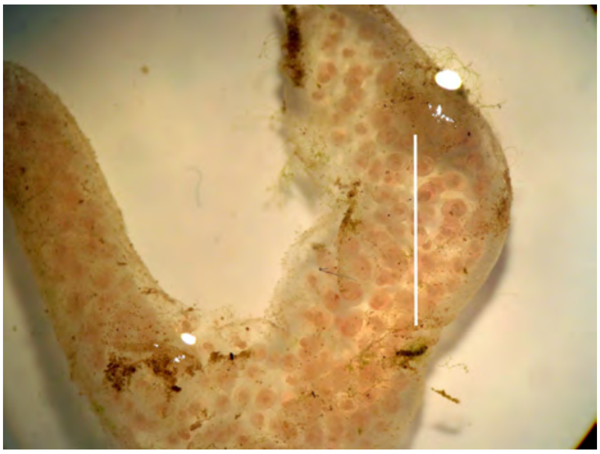

Figure S1.23: Mollusca, *Limnaea spp.*, egg mass. Scale bar: 5 mm.

Hirudinea, Erpobdellidae

Erpobdella octoculata Egg masses found from April to October in flat yellowish cocoons with hard cover. Older cocoons dark-coloured. Size of a single cocoon ca. 0.5-1 cm. Found both single per substrate and aggregated.

Figure S1.24: Hirudinea, *Erpobdella octoculata*, a cocoon with already hatched eggs. Scale bar: ca. 5 mm.

Chapter 2

How river structure and biological traits influence gene flow: A population genetic study on two stream invertebrates with differing dispersal ability

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In review in Freshwater Biology

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Abstract

Few populations are isolated in space and time. Rather, most are connected to some extent to other populations. These connective links can influence various aspects of population biology, ranging from population persistence to genetic structure and diversity. Dispersal abilities are often largely determined by life-cycle traits and may differ significantly between species. We investigated the population genetic structure of two

ecologically important aquatic invertebrates: an amphipod (*Gammarus fossarum*) with a purely aquatic life cycle and a mayfly (*Baetis rhodani*) with a terrestrial adult stage. Samples were collected in a pre-alpine catchment in Switzerland characterized by high habitat heterogeneity and the presence of both natural and man-made barriers. We found very low genetic differentiation between populations of *B. rhodani* both within the studied catchment and in a larger scale comparison. *G. fossarum* showed strong genetic differentiation even on spatial scales of a few kilometres and a clear pattern of isolation by distance. Genetic diversity decreased from downstream towards upstream populations of *G. fossarum*. In-stream barriers were not consistently associated with increased genetic divergence in either of the two species. Our results suggest high rates of gene flow in *B. rhodani* across large geographical scales. In *G. fossarum*, gene flow appears spatially restricted and more common in the direction of water flow. We attribute these interspecific differences primarily to the flight capacity of *B. rhodani* in the terrestrial adult stage. *G. fossarum* may be more prone to local adaptation which could further reduce effective dispersal between populations. These two very common European freshwater species probably respond quite differently to ongoing habitat destruction and fragmentation. The high dispersal capacity and lower habitat specialization of *B. rhodani* may give this species a considerable advantage in surviving anthropogenic disturbances and colonizing newly available habitats. In contrast, we expect *G. fossarum* to be more vulnerable to environmental change; the presence of nearby source populations may be a necessary prerequisite for this species to be able to colonize new sites.

Introduction

In recent decades, stream ecologists have increasingly recognized the importance of looking at species population dynamics from a coarser-scale perspective (Lowe, 2002; Bohonak, 1999). Few populations are isolated in space and time. Rather, most are connected to some extent to other populations (Malmqvist, 2002). These connective links can influence various aspects of population biology, ranging from population persistence to genetic structure and diversity (Bohonak & Jenkins; 2003, Hughes, 2007). Estimating the degree of connectivity between populations is central in conservation and management, for example for estimating extinction probabilities and recolonization potential. The extent of gene flow between populations is furthermore an important determinant of the potential for local adaptation (Lenormand, 2002). Connectivity between populations within a species is determined by several important factors, including the spatial position and distance of populations in the stream network, and species-specific life history and dispersal traits (Hughes, Schmidt & Finn, 2009).

Potential modes of dispersal, in fact, differ greatly between different organisms and, in particular, aquatic invertebrates (Hughes, 2007). For instance, non-insect aquatic invertebrates have a life-cycle constrained to the water column, where they disperse either passively (e.g. by passive drift) or actively (e.g. swimming, crawling, or entering the drift actively). These dispersal modes are assumed to be strongly influenced by the unidirectional flow of rivers, and dispersal is thought to take place mostly in a downstream direction (Haenfling & Weetman, 2006). In contrast, aquatic invertebrates that have a terrestrial life stage, such as aquatic insects that emerge as winged adults, can avoid this downstream bias and are, in principle, able to disperse in all directions during the terrestrial stage. Indeed, these winged adults have been documented to disperse upstream, downstream and laterally (e.g. Winterbourn *et al.*, 2007; Griffith, Barrows & Perry, 1998; Hershey *et al.*, 1993), and being capable of movements even between drainages overland (Schmidt, Hughes & Bunn, 1995).

Knowledge on such species traits and conclusions gained from observational and experimental studies (e.g. mark-recapture experiments) lead to the widespread assumption that many aquatic invertebrates are characterized by high dispersal rates (Bohonak & Jenkins, 2003). However, these approaches estimate primarily 'dispersal potential' and not the 'effective dispersal' (which comprises successful establishment of reproductive individuals; Nathan, 2006). Based commonly on data from a limited time-frame and often restricted to a certain life-stage of aquatic invertebrates, these methods cannot provide estimates of the long-term and coarse-scale patterns of dispersal and also do not incorporate resulting gene flow crucial for eco-evolutionary processes (Koenig, Van Vuren & Hooge, 1996). Moreover, to understand the factors shaping dispersal patterns in different species, knowledge on landscape structure and connectivity should be incorporated into dispersal studies (Lowe, 2002; Wiens, 2002; Fagan, 2002).

Molecular techniques offer excellent opportunities to integrate landscape structure into dispersal studies and avoid some of the limitations of other methods (Malmqvist, 2002; Hughes, 2007; Koenig *et al.*, 1996). Patterns of genetic variation can be used to obtain estimates of both current and long-term average gene flow between populations, and consequently molecular markers are highly effective for studying processes at different spatial and temporal scales. In fact, in the last decade, such population genetic analyses have been performed for a range of aquatic organisms and habitats (Hughes, 2007), but most of them have been conducted in natural streams with unimpeded connectivity (Watanabe *et al.*, 2010). Ongoing human-caused habitat destruction and fragmentation require a better understanding of how landscape change can affect species with different dispersal capacities. Up to now, few studies have investigated the effects of barriers on gene flow and dispersal of aquatic invertebrates (Monaghan *et al.*, 2001) and even fewer have compared several species in their response to fragmentation (Watanabe *et al.*, 2010; Hoehn, Sarre & Henle, 2007; Monaghan *et al.*, 2002; Sato *et al.*, 2008).

In this study, we examined the influence of landscape structure and fragmentation on two aquatic invertebrates with different dispersal-related traits. Specifically, we quantified the genetic divergence between populations of an amphipod (*Gammarus fossarum*) and a mayfly (*Baetis rhodani*) within a pre-alpine catchment, characterized by high habitat heterogeneity and the presence of both natural and man-made in-stream barriers. While in their aquatic stage both species have similar dispersal capabilities (Elliott, 2003), *B. rhodani* in contrast to *G. fossarum* possesses a winged adult stage allowing for overland dispersal. We therefore expected populations of *B. rhodani* to show overall lower genetic differentiation than *G. fossarum*, whose dispersal is limited to the water column throughout its lifetime. Furthermore, we expected elevated differentiation between populations of *G. fossarum* separated by in-stream barriers, while these barriers would have little influence on dispersal and thus gene flow between populations of the mayfly.

Study site

The study was conducted from spring to autumn 2009 at the river Sense, a pre-alpine 4^{th} order stream in western Switzerland. Having a total length of 35.7 km, the Sense drains a watershed of 432 km^2 and flows into the Saane (Rhine catchment) near Laupen. Along most of its length, the river has a relatively natural morphology with open, wide, gravel-dominated floodplains characterizing the mainstream and various tributaries flowing through forested and agricultural landscapes. About 15 km of the stream flow through a natural canyon (between sites Se06 and Se08, Fig.2.1) confined by steep sandstone walls. No in-stream barriers disrupt the continuity of the natural stretch of the Sense. The river contains major engineering measures for flood protection downstream of Thoerishaus (site Se02, Fig.2.1) to the confluence with the Saane, where its channel has been straightened and banks fortified with rip-rap. This river stretch is also characterized by man-made drops and ramps. Connectivity of tributaries to the mainstream varies throughout the catchment with some being detached by natural (e.g. waterfalls) or man-made (mainly ramps and drops) barriers.

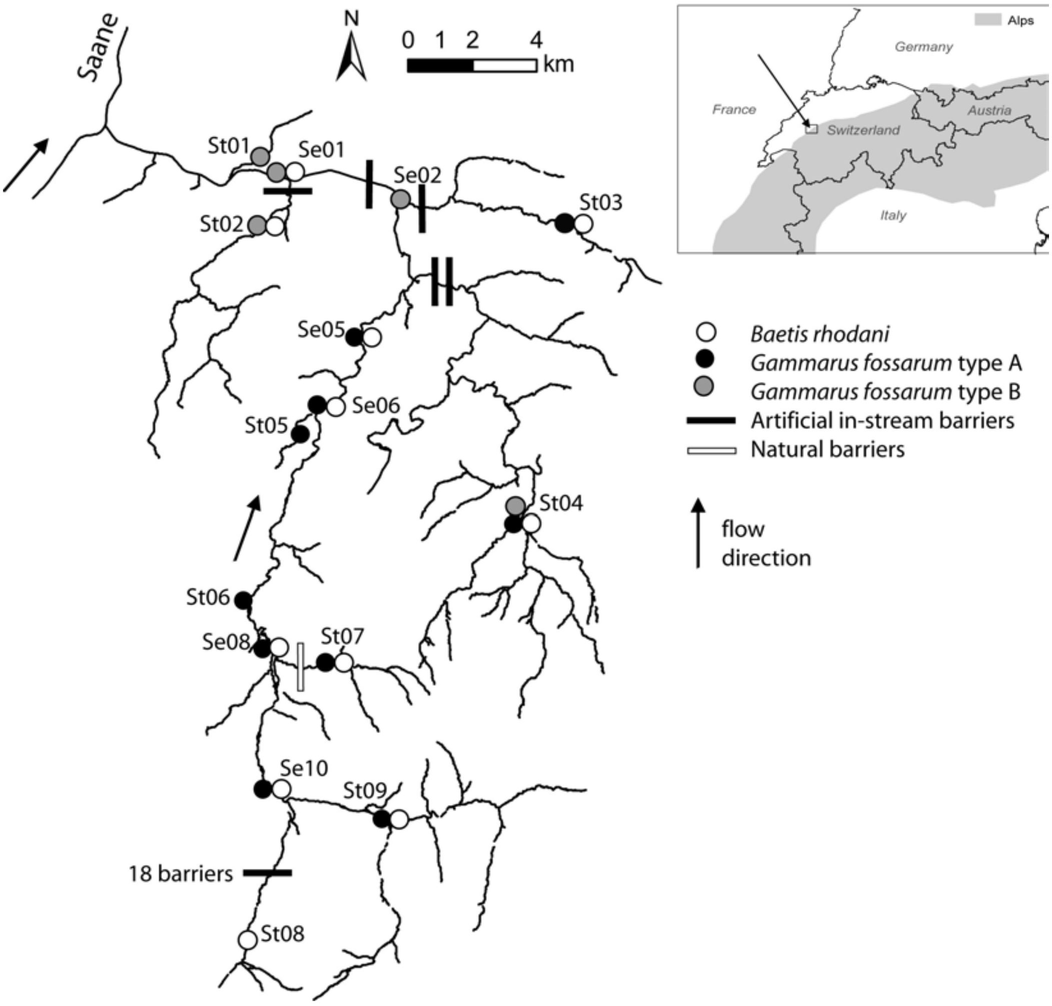

Figure 2.1: Sampling sites for *Baetis rhodani* and *Gammarus fossarum*, Sense, 2009.

Study organisms

Baetis rhodani (Pictet, 1843) and *Gammarus fossarum* are widespread Central European freshwater species (Williams, Ormerod & Bruford; 2006, Humpesch, 1979; Boxshall, 2005). Mayflies of the genus *Baetis* are grazers and show little habitat specialization, in the Sense being found throughout the catchment. *Baetis* spends most of its life cycle as aquatic larvae, able to disperse both actively (crawling, swimming) and passively (by drift). It has been shown that the most significant long-distance dispersal of baetid mayflies (including overland dispersal between neighbouring drainages) takes place during the winged terrestrial stage (Schmidt *et al.*, 1995).

During this stage, they mate and disperse distances up to several km in the search of habitat suitable for oviposition (Peckarsky, Taylor & Caudill, 2000). *Baetis rhodani* is reported to be bivoltine (Studemann *et al.*, 1992), although periods of emergence and flight are typically asynchronous and extend over several months (Humpesch, 1979).

Gammarus fossarum is a shredder, feeding mainly on leaf litter. Consequently, its habitat is more patchily distributed than that of *B. rhodani* and, in larger river systems, it is found primarily in forested tributaries and headwaters (Siegismund & Muller, 1991). In contrast to *B. rhodani*, the amphipod *G. fossarum* spends its entire life cycle in the water column dispersing through active swimming, crawling and passive drift (Elliott, 2003).

Both *Baetis rhodani* and *Gammarus fossarum* have been described as complexes of cryptic species with several strongly differentiated lineages (Müller, 2000; Williams, Ormerod & Bruford, 2006).

Methods

Field sampling

We collected individuals of the two study species in 2009 using kick-nets. We sampled a total of 15 sites located either in the mainstream (Se-named sites) or in the tributaries (St-named sites) of the Sense (Fig.2.1, Table S2.1 in Supplement). *Gammarus fossarum* was rarely found directly in the mainstream and was collected mostly at the inflows of small tributaries offering the typical habitat with leaf-inputs. All benthic samples were stored in 90% ethanol. For morphospecies identification, we used species-specific traits described in Elliott & Humpesch (2010) for *Baetis rhodani* and Eggers & Martens, 2001 for *Gammarus fossarum.*

As preliminary molecular analysis showed substantially higher levels of genetic differentiation in *Gammarus* than *Baetis* in the Sense catchment, more *Gammarus* populations were included in the molecular analysis (for detailed overview of populations see Table S2.1). Sample size of each sampled population was between 13 and 28, resulting in a total of 294 individuals for *Gammarus* and 293 individuals for *Baetis.* To investigate genetic differentiation at a larger geographical scale, we included an outgroup population from the Rhine drainage for both species (situated at a waterway distance of at least 100 km from the lowest Sense site).

Genotyping

DNA of both species was extracted following the HotSHOT method (Montero-Pau, Gomez & Munoz, 2008). We used available molecular markers for both species - 7 microsatellite loci reported by Williams *et al.* (2002) for *B. rhodani* (*Brh*-1, *Brh*-2, *Brh*-3, *Brh*-4, *Brh*-5, *Brh*-6, *Brh*-7) and 9 microsatellite loci reported by (Westram, Jokela & Keller, 2010) for *G. fossarum* (gf08, gf13, gf18, gf19, gf21, gf22, g24, gf27, gf28). In *B. rhodani*, the primer pairs to amplify *Brh*-2, *Brh*-3, *Brh*-4 and *Brh*-6 loci (of Williams *et al.* 2002) were newly designed based on the published sequences (EMBL accession numbers AY081169, AY081170, AY081171, AY081173; for details see Table S2.2).

For *G. fossarum*, we amplified the markers following the protocol described in Westram *et al.* (2010). For *B. rhodani*, the markers were amplified in one multiplex PCR reaction in a final volume of 12.5 μl. Each reaction contained 6.25 μl multiplex PCR mastermix (Qiagen), 1.25 μl primer mix, 1.25 μl Q-Solution, 2.75 μl deionized water and and 1 μl of DNA. Individual primer concentrations in the primer-mix are reported in Table S2.2. An initial denaturation step of 15 min at 95°C was followed by 35 cycles of 30 s at 94°C, 90 s at 57°C, 60 s at 72°C, and a final elongation step of 10 min at 60°C. The amplified fragments were diluted 1:10 in water and mixed with GeneScan LIZ500 size standard. Subsequently, they were run on a 3730x DNA Analyzer (ABI) and peaks were scored in the program GeneMarker Version 1.8 (Softgenetics). Due to difficulties with scoring (presumably due to unspecific amplification), the locus *Brh*-5 was excluded from further analysis.

Statistical analyses

The same statistical analyses were preformed for both species. We tested all loci in all populations for deviations from Hardy-Weinberg equilibrium (HWE) in FSTAT version 2.9.3.2 (Goudet, 2001). P-values were estimated based on 1560 randomisations. The frequency of null alleles was estimated for all loci in FREENA (Chapuis & Estoup, 2007). We tested for deviations from linkage equilibrium between all pairs of loci using ARLEQUIN version 3.1 (Excoffier, Laval & Schneider, 2005) with 10,000 permutations and 2 initial conditions for the EM algorithm. Significance levels were adjusted using Bonferroni correction.

Population structure

For assessing overall genetic differentiation within the Sense catchment as well as in comparison to the outgroup, we conducted an AMOVA in ARLEQUIN version 3.1 (Excoffier *et al.*, 2005) with the significance tests based on 10,100 permutations. We then calculated pairwise F_{ST} values between all samples in ARLEQUIN version 3.1. Significance was assessed based on 10,000 permutations. Significance levels were adjusted using Bonferroni correction. As F_{ST} is dependent on marker variability (Hedrick, 2005, Jost, 2008), we additionally calculated pairwise D_{est} (Jost, 2008), an alternative measure of genetic differentiation formulated to avoid this problem. The online application SMOGD (Crawford, 2010) was used for this calculation.

For investigation of the population genetic structure within the Sense, we also used the clustering method implemented in the software STRUCTURE version 2.3.3 (Pritchard, Stephens & Donnelly, 2000). Without considering the geographical origin of samples, STRUCTURE estimates the most probable number of groups (clusters, K) for all analyzed individuals. We assessed the optimal number of clusters using the approach of Evanno, Regnaut & Goudet (2005). We investigated values of K from 1 to 7 for *Baetis* and 1 to 10 for *Gammarus*, with a burn-in period of 10,000 followed by 100,000 iterations and 10 runs for each K. We assumed an admixture model and correlated allele frequencies.

Testing for effects of geographical distances on population differentiation

Waterway and straight-line geographical distances between all pairs of populations were measured from 1:50,000 maps of the region with Swissmap50 (2000, Wabern, Switzerland). Information on the presence of in-stream barriers was taken from the study of Junker (2010), which mapped all natural and anthropogenic in-stream barriers of over 0.5 m height within our sampled area in the Sense catchment. Linearized pairwise F_{ST} ($F_{ST}/(1\text{-}F_{ST})$) was used as a measure of genetic distance (Rousset, 1997), and either the distance along waterways or straight-line distance as a measure of geographical distance. We also used a partial Mantel test to assess the effect of in-stream barriers after accounting for waterway distance. All analyses were carried out using the online software IBD version 3.16 (Jensen, Bohonak & Kelley, 2005), and reduced major axis regression was used to estimate the slope and intercept of the isolation by distance relationship.

Patterns of genetic diversity

As a measure of genetic diversity, we calculated allelic richness per population based on 15 diploid individuals for both *Baetis* and *Gammarus* (the lowest sample size available) in Fstat. To test for patterns in genetic diversity along the course of the Sense, we first calculated the waterway distance of each population from the point where the Sense enters the next higher order stream (Saane). This distance gives an estimate of how far upstream a population is located. We then conducted a linear regression of mean allelic richness (across all loci) within each population against this distance in R Version 2.11.1 (R Development Core Team 2010).

Results

Baetis rhodani

B. rhodani was found in all sites sampled in the Sense catchment (Fig.2.1). All analyzed loci were highly polymorphic, with a total number of alleles between 12 (*Brh*-4) and 63 (*Brh*-7). Three Sense populations (St07, Se06 and St02) and the outgroup (Bu) showed significantly elevated F_{IS} values across loci (F_{IS} 0.1-0.2; Table S2.3). However, in these populations, not all loci showed heterozygote deficits, and statistically significant deviations from Hardy-Weinberg equilibrium were observed only at two (*Brh*-1 and *Brh*-7 in St07, Se06) or three loci (*Brh*-1, *Brh*-3 and *Brh*-7 in St02, Bu). The estimated average frequency of null alleles was 4.7%, with the highest values at *Brh*-7 (average of 7% across populations) and *Brh*-1 (10%). We found no systematic pattern of linkage disequilibrium: only 5 out of 165 tests were significant and these involved several pairs of loci.

In the comparison of the Sense populations with the outgroup, one locus (*Brh*-4) showed substantially higher differentiation ($F_{ST} = 0.24$) than the other loci (maximum $F_{ST} = 0.096$). In fact, at this locus 92 % of individuals from the Sense showed only a limited subset (3 alleles) of the total allelic pool found in this study (overall 12 alleles including those from the outgroup population; Fig.S2.1). This locus was excluded from further analysis of genetic differentiation as it is potentially under selection. However, pairwise F_{ST} values calculated with and without this locus were highly correlated (Pearson correlation = 0.86, $p < 0.001$).

Low but statistically significant genetic differentiation was observed between the Sense and the outgroup (AMOVA, between-group differentiation: $F_{CT} = 0.0174$, $p = 0.042$), while no significant substructure was detected within the Sense (AMOVA, within-group differentiation: $F_{SC} = 0.0053$, $p = 0.054$). Among the pairwise comparisons between Sense populations, only 7 of the 55 F_{ST} values were significantly different from zero and these never exceeded 0.019. In contrast, all the pairwise F_{ST} values between the Sense and the outgroup population were significant and reached values up to 0.039 (for full summary see Table S2.5). D_{est} values showed the same pattern. The STRUCTURE analysis based only on samples from the Sense detected no evidence of substructure within the catchment (best support for K = 1).

Mantel tests with linearized F_{ST} as a measure of genetic distance suggested a significant increase of genetic differentiation in *B. rhodani* with straight-line distance (Fig.2.3a, Table 2.1). Partial correlation tests revealed no relationship with waterway distance but elevated genetic differentiation in response to the number of barriers between populations. However, these results were not confirmed in the analyses based on D_{est} which showed no association of genetic differentiation with any of the tested geographical variables (Table 2.1; Fig.S2.2).

Genetic diversity of *B. rhodani* measured as allelic richness showed no consistent

Table 2.1: Results of Mantel tests for *Baetis rhodani* and *Gammarus fossarum* type A using two measures of genetic differentiation, linearized F_{ST} and D_{est}. Partial Mantel tests were used to investigate the effect of waterway distance and dispersal barriers (first two rows given for each species), and a Mantel test to investigate the effect of straight line distance on genetic divergence (third row). In *G. fossarum*, the analyses were repeated without population St04 (see text for details). Significant p-values are in bold.

	F_{ST}-based analysis				***D_{EST}-based analysis***			
	Slope	***SE slope***	***R***	***p-value***	***slope***	***SE slope***	***R***	***p-value***
Baetis rhodani								
waterway distance	0.001	0.0001	0.147	0.220	0.0019	0.0003	0.165	0.169
barriers			**0.422**	**0.013**			0.007	0.518
straight line distance	0.002	0.0002	**0.302**	**0.029**	0.0035	0.0005	0.207	0.089
***Gammarus fossarum* type A**								
waterway distance	0.024	0.003	**0.470**	**0.007**	0.005	0.001	**0.377**	**0.018**
barriers			-0.320	0.060			-0.062	0.417
straight line distance	0.054	0.006	**0.644**	**0.001**	0.010	0.001	**0.705**	$<$ **0.001**
***Gammarus fossarum* type A (excluding the St04 population)**								
waterway distance	0.032	0.004	**0.658**	**0.002**	0.006	0.001	**0.798**	**0.001**
barriers			-0.018	0.457			0.194	0.217
straight line distance	0.051	0.006	**0.685**	**0.002**	0.009	0.001	**0.829**	$<$ **0.0010**

pattern within the catchment and was not significantly correlated with the distance from the confluence (Fig.2.4a, $R^2 = 0.026$, $p = 0.63$). Allelic richness in the outgroup was slightly elevated compared to Sense (11.61 versus 10.53).

Gammarus fossarum

G. fossarum was found throughout the Sense catchment, missing only from one of the sampling locations - St08. Two genetically highly distinct groups of *G. fossarum* were found in the Sense catchment (Fig.2.1), corresponding to the previously described types A and B (Müller, 2000) that most likely represent two distinct but morphologically cryptic species. The two types can be easily distinguished based on our microsatellite marker gf27, which is fixed for allele '205' in type B, while it never shows this allele in type A (Westram, unpublished data). Being reproductively isolated (Westram, 2011; Müller, 2000), these two types of *G. fossarum* must be analyzed separately. The species were geographically sorted, with type B occurring predominantly at sites close to the confluence with the Saane (i.e., more downstream sites; Fig.2.1). As the number of type B populations (5) was too small to investigate geographical patterns in more detail, we report only results for *G. fossarum* type A.

All of the genotyped loci were polymorphic within the type A Sense populations, with the lowest total number of alleles found for gf22 (3) and highest for gf21 (18). No deviations from Hardy-Weinberg equilibrium across loci were detected in any of the populations (Table S2.4). The estimated null-allele frequencies were low with an average of 1% across all loci and populations, and a maximum value of 4% (averaged over populations) reached at gf28. No significant linkage disequilibrium was detected for any of the locus pairs.

The overall genetic differentiation of *G. fossarum* type A both within the Sense catchment (AMOVA, within-group differentiation:$F_{SC} = 0.234$, $p < 0.001$) and in comparison to the outgroup (AMOVA, between-group differentiation:$F_{CT} = 0.381$, $p < 0.001$) was highly significant. Pairwise F_{ST} values ranged from 0.05 to 0.55 within the Sense and reached up to 0.7 in comparisons with the outgroup (Table S2.6). The analysis with STRUCTURE supported 5 major clusters within the Sense catchment; geographically nearby populations were also genetically similar (Fig.2.2). Both in F_{ST} values and STRUCTURE results, population St04 in the headwaters of the largest tributary of the Sense (Schwarzwasser) revealed highest genetic similarity to the nearest overland populations. In fact, Mantel tests including this population showed the highest correlation between genetic distance and straight-line geographical distance (Table 2.1). Without St04, nearly equal (and statistically significant) correlations were observed for the two geographical distance measures (Table 2.1) although it has to be noted that, in this case, waterway and straight-line distance themselves were highly correlated (Pearson correlation = 0.98, $p < 0.001$). No significant barrier effect was found (Table 2.1; Fig.2.3d).

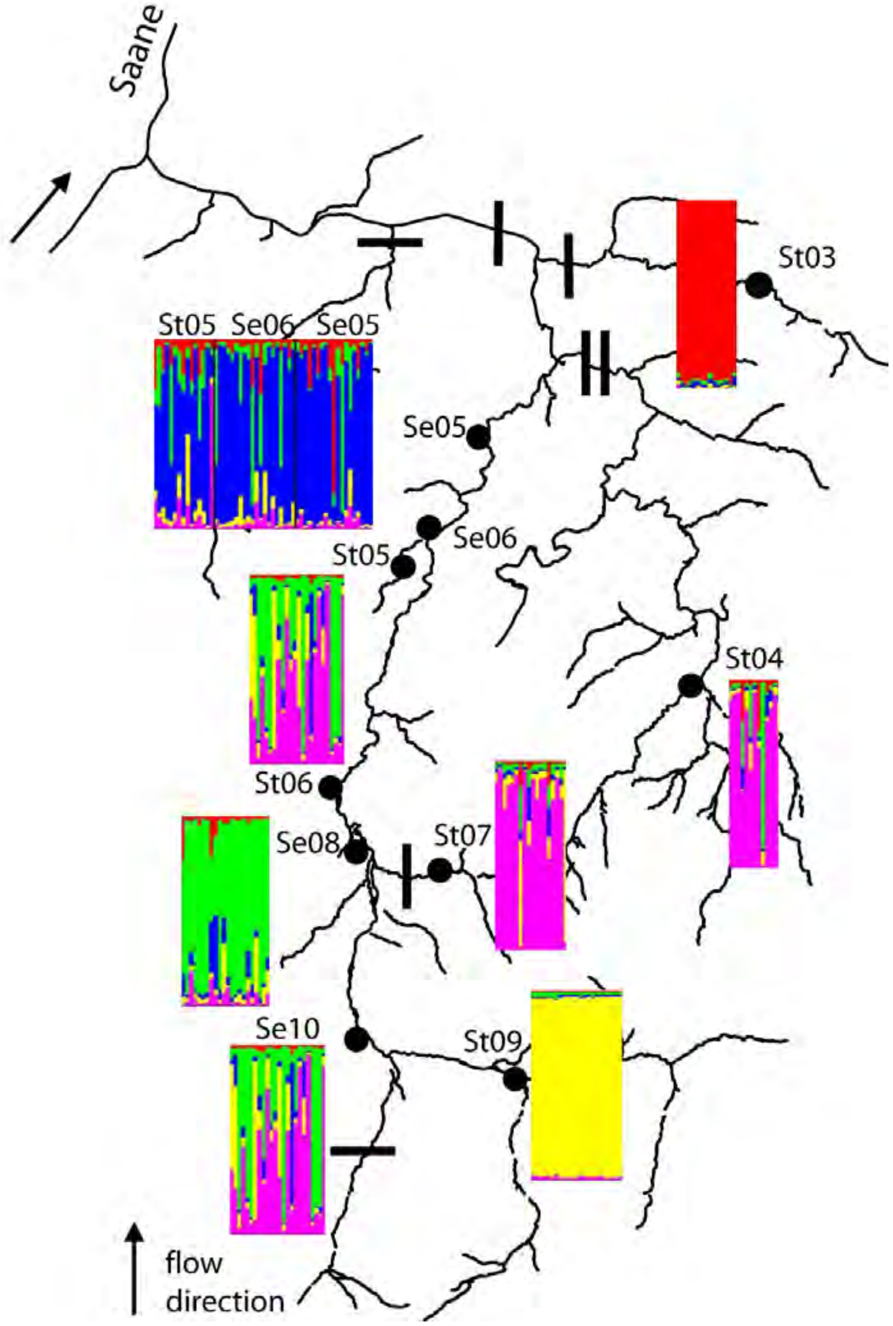

Figure 2.2: Results of STRUCTURE analysis for *G. fossarum* type A. STRUCTURE identified five genetic clusters within the Sense catchment, represented by the five different colors. Each bar represents a single individual, with each color representing the contribution of a given cluster to an individuals genotype.

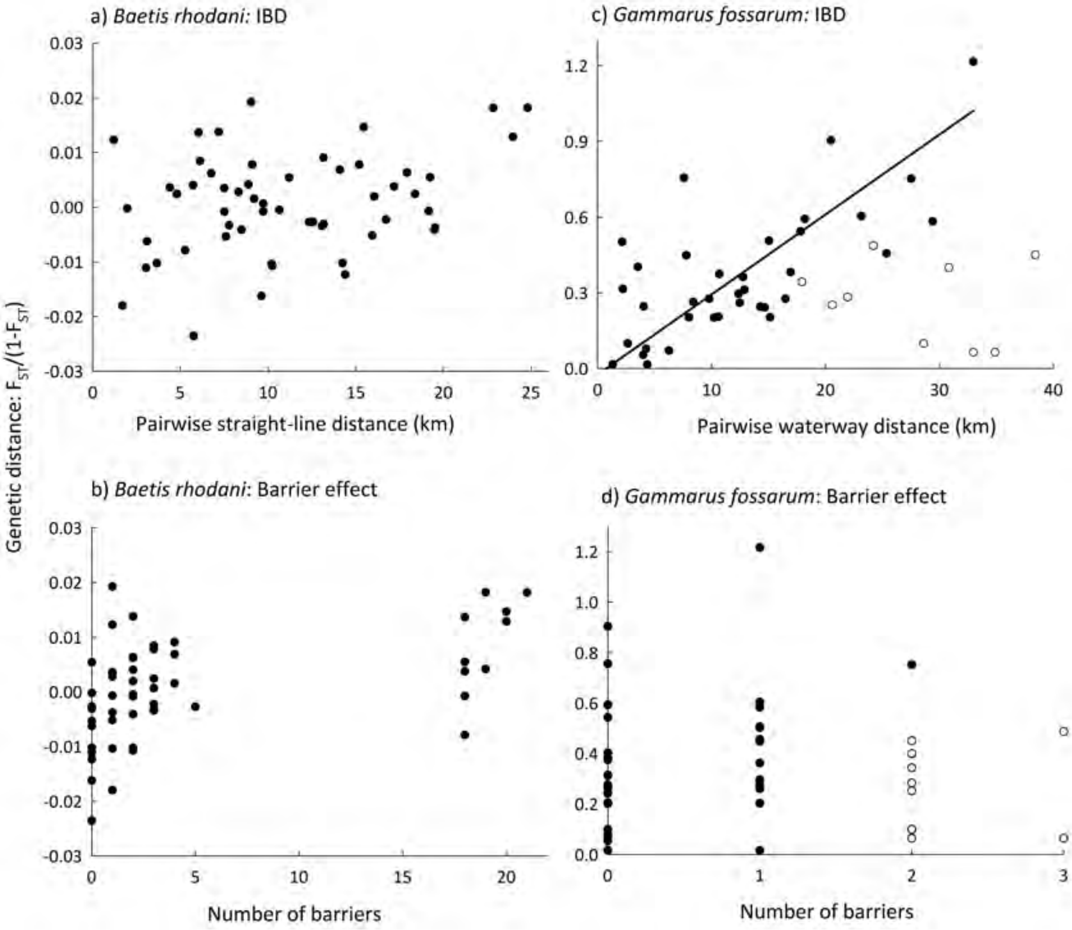

Figure 2.3: Isolation by distance and the effect of barriers: *Baetis rhodani* (a,b) and *Gammarus fossarum* (c,d) in the Sense catchment. Trendlines plotted according to reduced major axis regression and only when the Mantel test was significant both with F_{ST} and D_{est}. For *G. fossarum* open circles represent pairwise comparisons involving population St04, filled circles the remaining pairwise comparisons within the Sense. Trendline plotted for *G. fossarum* is based on linear regression excluding St04 values.

Allelic richness in the Sense catchment was overall low, with an average over all loci and populations of 2.89 ± SE 0.23 in the Sense versus 3.58 in the outgroup population. Within the Sense catchment, it decreased significantly with waterway distance from the Saane confluence (Fig.2.4b; $R^2 = 0.51$, $p = 0.02$).

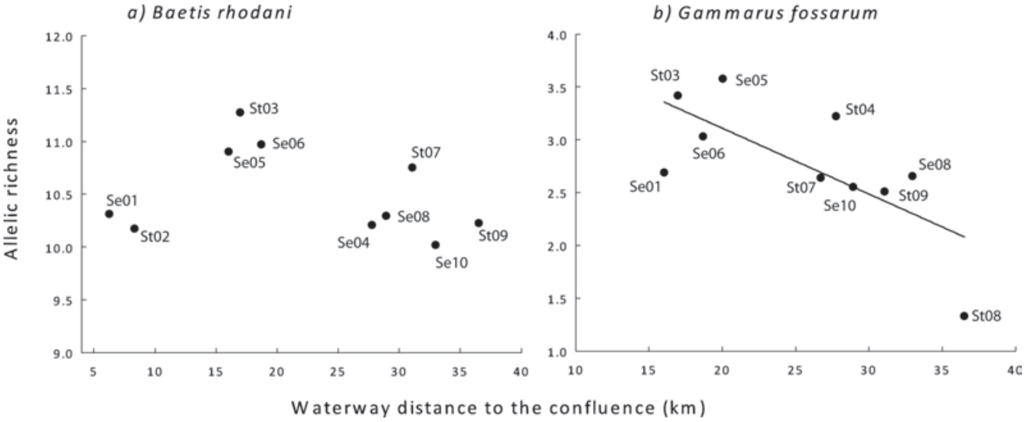

Figure 2.4: Mean allelic richness in populations of *Baetis rhodani* (a) and *Gammarus fossarum* (b) against waterway distance from the Sense-Saane confluence. A line was fitted only if the regression was significant.

Discussion

Studying *Baetis rhodani* and *Gammarus fossarum* within the same catchment offered the opportunity to directly compare the extent of genetic differentiation between populations of two aquatic invertebrates strongly differing in dispersal-related traits. Extensive sampling in the catchment and the use of highly polymorphic microsatellites allowed us to investigate these patterns at the scales of a few kilometers. The two species showed highly pronounced differences in genetic structure that probably reflect different patterns of dispersal and resulting gene flow. These interspecific differences are likely to lead to different responses to human-induced habitat fragmentation and disturbance.

Baetis rhodani

Consistently significant and elevated genetic differentiation of all Sense populations of *B. rhodani* with the outgroup indicates an increase of genetic divergence with geographical distance at a larger geographical scale (over a hundred kilometers), while such a pattern could not clearly be identified on the small scale (within Sense; Table 2.1;

Fig.2.3a). Of course, some within-catchment genetic structure might still be present but so weak that more molecular markers are needed to statistically detect it.

The low genetic differentiation observed in this species probably results from high rates of gene flow, both locally as well as at larger spatial scales, rather than from, e.g. a very recent origin of the studied populations. Specifically, we are not aware of any reports in the literature of a recent range expansion, and some temporal stability of population sizes is also suggested by the observed homogeneity in genetic diversity across populations. Our findings support our initial expectations and are highly consistent with the few existing studies on baetid mayflies that also suggest high levels of gene flow at similar geographic scales (Schmidt *et al.*, 1995; Monaghan *et al.*, 2001).

Our conclusion of high dispersal capacity of *B. rhodani* seems at odds with the significant effect of barriers on population divergence detected by the F_{ST} -based Mantel tests. However, this result is strongly influenced by negative F_{ST} values (Fig.2.3b), none of which is individually significant, and no significant effect of barriers is observed when using D_{est} an alternative measure of genetic distance (Table 2.1). Thus, we cannot confidently conclude that in-stream barriers reduce gene flow between populations of *B. rhodani* in the Sense catchment.

Deviations from Hardy-Weinberg equilibrium have been reported to be common in baetid mayflies (Schmidt *et al.*, 1995; Monaghan *et al.*, 2001; Hughes *et al.*, 2003). Several researchers have suggested that such a pattern could emerge if the sampled individuals were the offspring of a small number of matings ('patchy recruitment hypothesis' (Schmidt *et al.*, 1995; Monaghan *et al.*, 2001; Bunn & Hughes, 1997). Under such a scenario, systematic deviations from HWE are expected across loci. Such a pattern is not observed in our study, where global F_{IS} was significantly elevated only in four out of 12 populations and, more importantly, the results were driven by a subset of the loci. This suggests that the deviations are more likely due to locus-specific effects like null alleles, an explanation supported by the presence of potential null homozygotes (i.e. missing genotypes) at these loci.

Alternatively, the unrecognized presence of multiple cryptic species in a given sample could produce deviations from Hardy-Weinberg equilibrium (i.e. a Wahlund effect). Several distinct lineages of *B. rhodani* exist in Europe but their distribution in northern Switzerland is unknown (Williams, Ormerod & Bruford, 2006). When such diverged lineages come into secondary contact and interbreed, the rates of gene flow can be highly heterogeneous across the genome (Barton & Bengtsson; 1986, Kruuk *et al.*, 1999). Selectively neutral loci may introgress freely while other loci can show steep allele frequency clines (Kruuk *et al.*, 1999). Under such a scenario, genetic signals of admixture (i.e. heterozygote deficits) could potentially be restricted to a subset of the loci.

An interesting pattern in this context is the strongly elevated genetic divergence between Sense and outgroup observed at locus *Brh*-4, which is also reflected in distinct allele frequency spectra (Fig.S2.1). An intriguing but, at present, highly speculative interpretation of this result could be that *Brh*-4 is diagnostic of different *B. rhodani* lineages, with one lineage dominating in the Sense and another in the Buenz. Elevated divergence at *Brh*-4 (but not necessarily at other loci) could be maintained if the marker is located in a genomic region involved in genomic incompatibilities between these lineages.

Alternatively, higher differentiation at *Brh*-4 might also indicate that this locus is linked to a genome region under divergent selection between environments. Rare individuals carrying alleles more typical of the outgroup populations could be immigrants into the Sense catchment - an explanation that requires overall high dispersal rates of *Baetis*, as these individuals were found throughout the Sense catchment and not only in proximity to the confluence.

Gammarus fossarum

The pattern of isolation by distance and the results of the cluster analysis indicate that most gene flow in the Sense occurs locally, primarily between neighboring populations, supporting our expectation of higher genetic differentiation in *G. fossarum* compared to *B. rhodani*.

Levels of genetic differentiation between populations of *G. fossarum* type A within the Sense catchment were remarkably high. F_{ST} values detected at the spatial scale of 10-20 km in this study are comparable to those reported (using the same microsatellite loci) for populations at distances an order of magnitude greater (Westram, 2011). The results of Westram (2011) were generated from populations often located in direct proximity to major waterways, equivalent to being directly linked to 'dispersal highways'. In contrast, our study allowed smaller-scale detection of genetic patterns within a catchment with the sampling sites located at different distances from major waterways. This scale could be analogous to changing from 'highways' to smaller (and slower) 'off-roads'.

The unusually high differentiation both within Sense but also in relation to other Swiss populations of *G. fossarum* type A (Westram, 2011) might be due not only to the limited dispersal capacity of the species but also to the isolation of the Sense catchment from other fluvial networks. Although *G. fossarum* was found throughout the catchment, sampled individuals in the four most downstream populations were exclusively of type B. The Rhine drainage is suspected to be undergoing a recent invasion of *G. fossarum* type B, primarily from drainages to the west of it (Westram, 2011). An invasion of type B might also be occurring in the Sense catchment, potentially resulting in isolation of the remaining *Gammarus* type A populations in the river from other populations in the Rhine drainage. In contrast, type A population further east in the Rhine drainage (i.e. most of those studied by Westram *et al.*, 2011) are surrounded by other type A populations with which gene exchange is possible. The isolation of the Sense from other type A populations may be a reason for the overall high differentiation of the Sense from the rest of the drainage as well as for the lowered allelic richness compared to other populations in Switzerland: 2.89 ± SE 0.23 in the Sense versus 4.13 ± SE 0.21 in populations studied by Westram (2011).

The apparent sign of 'overland' dispersal between the population St04 and populations closest to it by straight-line distance seems counterintuitive, as physiological constraints makes terrestrial dispersal of *G. fossarum* highly improbable. However, it is not the first time that indications for overland dispersal of this species have been found (Westram, 2011). We see several explanations for the patterns of overland genetic similarity in the Sense catchment.

Current population structure of *G. fossarum* could reflect historical rather than contemporary gene flow, a common pattern found in areas affected by recent geological changes (Waters *et al.*, 2001). The landscape where the Sense flows today was nearly entirely covered by the ice of two bordering major glacier massifs Rhone and Aare-glaciers, during the last major alpine glaciation in the Quaternary (Würm, 10-25,000 years ago; Rutsch, 1947). Indeed, the catchment was formed during and after this glacial period and could have experienced further changes during the glacier retreat - both in the connectivity of single tributaries and in the general levels and direction of water flow (Grosjean, 1961; Rutsch, 1947). Thus, a possible scenario is that St04, St07, St06 and Se10 populations were connected in the past and genetic similarities have persisted even though the hydrological connectivity patterns have changed. Similar patterns have been found in other entirely aquatic species reflecting drainage rearrangements due to recent geological processes (Waters *et al.*, 2001; Vonlanthen *et al.*, 2007). However, in our case, an important role of such historical events seems difficult to reconcile with the low genetic divergence observed between St04 tributary and the neighboring populations overland ($F_{ST} = 0.06$). Thus we assume that independently of historical patterns some gene flow and thus dispersal must still be taking place today.

Waterfowl have been shown to be a potential vector facilitating dispersal of many aquatic invertebrates (Figuerola & Green, 2002). However, it seems difficult to envisage that this type of dispersal could be frequent enough to contribute substantially to gene flow given that the relevant vector species (e.g. ducks) are typically not found in *G. fossarum* habitat. Moreover, it is unclear why such dispersal events would not be observed in other parts of the catchment. Non-facilitated dispersal overland is a further possibility for ongoing gene flow. The shortest overland distance between the headwaters of Schwarzwasser (St04) and Laubbach (St07), the closest site overland, is about 150 meters. One could imagine that, e.g. during major rain events, some dispersal between the two tributaries is possible by crawling overland.

Detecting no in-stream barrier effect as well as finding *G. fossarum* in habitats difficult to access (such as St06, a tiny and extremely steep tributary of Sense within the canyon area) also indicates that the small-scale dispersal capacity of *G. fossarum* could have been underestimated. Contrary to our expectations, the results of this study suggest that in-stream barriers present in the Sense catchment (recent man-made ones as well as old natural ones, such as the waterfall separating St07 from the mainstream) can still be passable for *G. fossarum.* Most probably this might happen by crawling up the wetted surface of the barrier.

While local gene flow may be seemingly unimpeded by barriers, our study also offers examples of pronounced genetic differentiation. In most cases, such genetic divergence is observed between more distant sites, also within the unfragmented river section (e.g. Figs 2.2 and 2.3). Low gene flow at the catchment scale might be due to several reasons: 1) physiological constraints: large-scale dispersal would involve extensive movement in the main-stream with much rougher flow conditions as in the tributaries (this explanation however would contradict our assumption that larger waterways play a role of dispersal highways, offering faster transport between habitat patches); 2) behavioral constraints: *Gammarus* might, e.g. avoid dispersing across long distances in the mainstream (itself a poor habitat for *Gammarus*) when suitable habitat can be found locally; 3) local adaptation in a heterogeneous environment: gene flow could be primarily taking place between populations adapted to similar habitats, whereas reproductive success of immigrants adapted to other conditions could be low, in this case long-distance dispersal could take place but be undetectable in gene flow estimates.

Finally, we observed a decrease in genetic diversity from the headwaters towards the more downstream sites. This pattern is consistent with the unidirectional diversity hypothesis, and suggests that dispersal at the catchment scale is primarily in downstream direction (Markwith & Scanlon, 2007) and probably mainly through drift. A similar decrease in genetic diversity from headwaters towards the lower sections of the Sense was observed in bullheads, a fish species with limited dispersal ability (Junker, 2010). In contrast, when comparing allelic richness in tributaries to nearest mainstream populations of *G. fossarum*, we found no consistent pattern for such a decrease, which may be an indication of active small-scale dispersal in the upstream direction: from mainstream into tributaries.

Overall conclusions

Our findings supported our initial hypotheses about the influence of life-cycle traits on gene flow. The dispersal capacity of *G. fossarum* and aquatic larvae of *B. rhodani* are known to be quite similar (Elliott, 2003) and, consequently, we attribute the differences in gene flow between these species primarily to the terrestrial flight capacity of *B. rhodani* in the adult stage. However, differences in habitat availability also are likely to be contributing to the observed patterns in genetic divergence. Habitat suitable for *B. rhodani* is available throughout the catchment and the distribution of the species is largely continuous. *G. fossarum*, on the other hand, is primarily found in forested tributaries, thus the species is naturally more patchily distributed. Furthermore, we cannot exclude that dispersal behavior (due to, e.g. a possible avoidance of entering the main-stream by one of the species) of the two species might also differ, however this was not examined in the present study.

Implications for aquatic invertebrate dispersal in human-altered streams

Major habitat destruction as well as disruptions of connectivity took place in streams due to human activities primarily in the past 150 years (Malmqvist, 2002; Allan & Castillo, 2007). Against this background, knowledge on the dispersal ability of species is gaining additional importance and is crucial for understanding the consequences of habitat alteration and fragmentation on aquatic organisms. Indeed, reduced dispersal due to disrupted connectivity has been pointed out by many researchers (Lake, Bond & Reich, 2007; Palmer, 2009) as one of the most important constraints for the success of river restoration.

Even though they are both very common aquatic species, *B. rhodani* and *G. fossarum* will probably respond quite differently to habitat destruction and fragmentation. We expect that the high dispersal capacity of *B. rhodani* and its lower habitat specialization give this species a considerable advantage in surviving anthropogenic disturbances. In natural systems, it has already been shown to be a typical post-disturbance colonizer (e.g. Robinson & Uehlinger, 2007). Based on our conclusions on its high long-distance dispersal capacity, we also expect it to be a fast colonizer of newly available habitats in human-dominated streams (e.g. restored river sections).

Not knowing the mechanistic reasons for the low rates of gene flow we detected for *G. fossarum* in a predominantly natural catchment, we cannot yet make firm predictions on the response of this species to habitat fragmentation. If observed low dispersal rates were due to its actual low dispersal capacity or behavioral constraints, distances on the scale of a few kilometers could become impossible to cross for this species. In this case, management measures targeting *G. fossarum* (a keystone species in aquatic food web), such as habitat restoration, would necessarily need to be located in close (< 5 km) proximity to potential source populations.

Alternatively, dispersal rates may be much higher than the rates of gene flow if, for example, strong local adaptation prevented successful establishment of immigrants. In this case, the species could be able to colonize, e.g., newly available and yet non-occupied habitats, even across larger distances. Future studies are needed to assess if the extensive neutral divergence observed in *G. fossarum* type A also reflects adaptive differentiation. If this is the case, local extinctions might result in substantial losses of adaptive genetic diversity.

Finally, our results suggest that long-distance dispersal of *G. fossarum* happens primarily in downstream direction, thus location of a habitat patch within the river network strongly influences its probability to be colonized by this species. Importantly, due to its affinity to habitats with high leaf-input (primarily present in forested tributaries and headwaters), *G. fossarum* is most probably impacted not only by in-stream modifications, but also by modifications of riparian areas, e.g. clear-cutting. Thus for maintaining and restoring populations of this species, stream management must necessarily be coupled with careful planning of riparian areas maintenance.

Acknowledgments

We thank Lara Pfister for help with laboratory analysis, Julian Junker for data on the in-stream barriers in the Sense, and Jukka Jokela for fruitful discussions at different stages of the study. Data analyzed in this paper were generated at the Genetic Diversity Centre of ETH Zurich. This project was partially funded by an Action Field Grant from Eawag and Federal Department of the Environment (FOEN).

Supplement

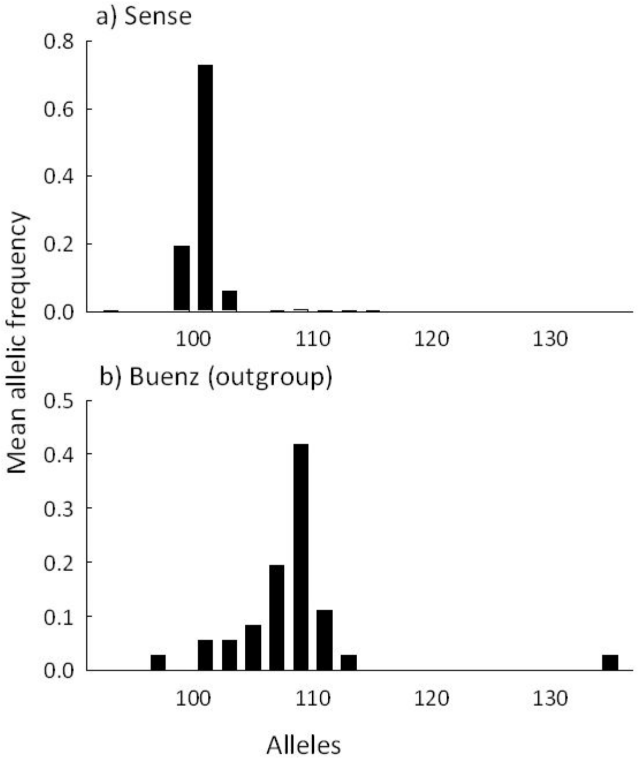

Figure S2.1: *B. rhodani*, allelic frequencies at locus Brh-4 in a) the Sense catchment and b) a population from the river Buenz.

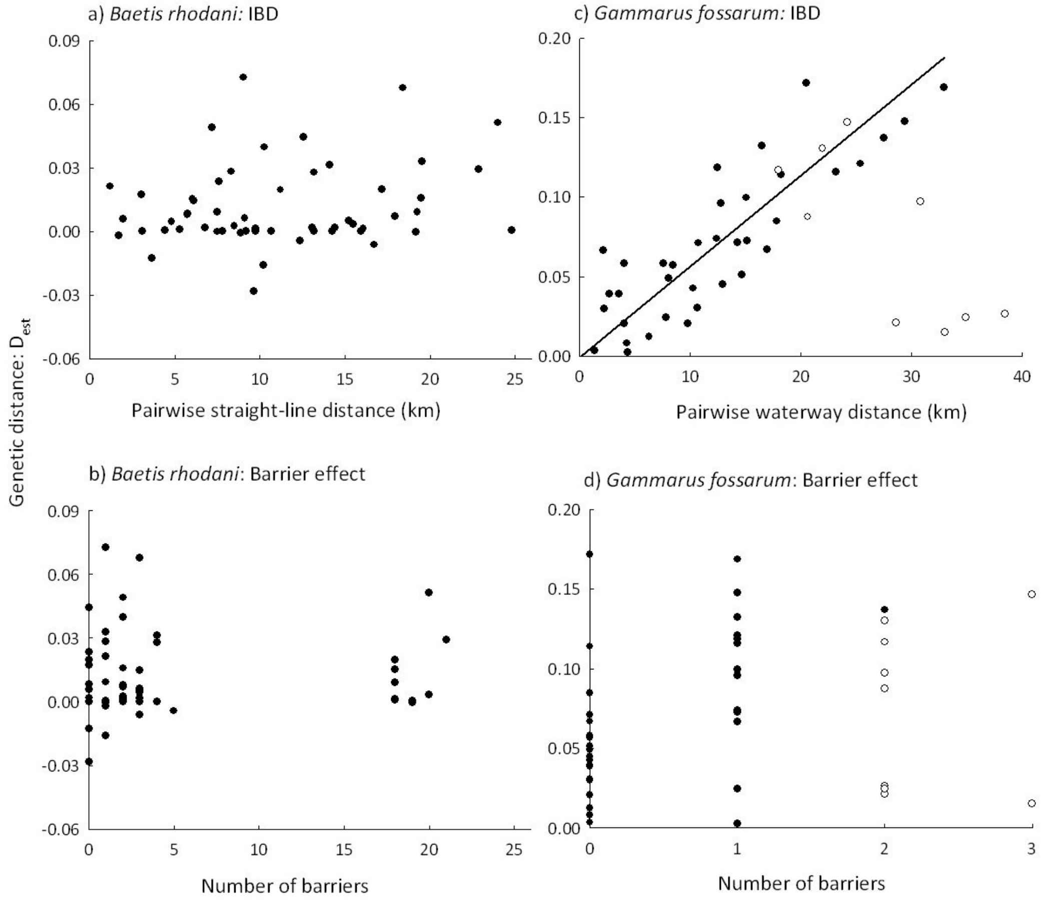

Figure S2.2: Isolation by distance and barrier effect with D_{est} as a measure of genetic distance. Trendlines plotted according to the reduced axis regression and only when the Mantel test was significant with both F_{ST} and D_{est}. For *G. fossarum* open circles represent pairwise comparisons involving population St04, filled circles the remaining pairwise comparisons within Sense. Trendline plotted for *G. fossarum* is based on linear regression excluding St04 values.

Table S2.1: Overview of sampling sites. A = *G. fossarum* type A; B = *G. fossarum* type B.

Site	***Catchment***	***mainstream/tributary***	***latitude***	***longitude***	***elevation (m)***	***N individuals***	
						B. rhodani	***G. fossarum***
St09	Sense	tributary (Kalte Sense)	46°42.974’	7°19.743	912	27	23(A)
St08	Sense	tributary (Warme Sense)	46°40.530’	7°17.507’	1030	28	-
Se10	Sense	mainstream	46°43.874’	7°17.732’	827	28	24 (A)
St07	Sense	tributary (Laubbach)	46°45.302’	7°19.064’	818	26	18 (A)
Se08	Sense	mainstream	46°45.731’	7°18.090’	760	27	22 (A)
St06	Sense	tributary	46°46.273’	7°17.529’	744	-	23 (A)
St05	Sense	tributary (Sodbach)	46°49.393’	7°18.746’	671	-	20 (A)
Se06	Sense	mainstream	46°49.608’	7°19.294’	646	27	15 (A)
Se05	Sense	mainstream	46°50.762’	7°20.229’	611	28	20 (A)
St04	Sense	tributary (Schwarzwasser)	46°47.651’	7°24.045’	753	26	13 (A), 22 (B)
Se02	Sense	mainstream	46°53.082’	7°21.202’	554	-	16 (B)
St03	Sense	tributary (Scherlibach)	46°52.783’	7°25.322’	712	26	15 (A)
Se01	Sense	mainstream	46°53.484’	7°18.480’	531	23	19 (B)
St02	Sense	tributary (Tafersbach)	46°52.867’	7°18.085’	653	27	20 (B)
St01	Sense	tributary (Oelibach)	46°53.682’	7°17.327’	519	-	24 (B)
Outgroups							
Bu	Buenz		47°18.277’	8°19.667’	387	24 -	
Ja	Aare		46°50.988’	7°41.448’	899	-	24 (A)

Table S2.2: Primer description for *B. rhodani.* A pig-tail (in bold) was added to the 5' end of the newly designed reverse primers.

Locus	***Primer sequence (5'-3')***	***fluorescent dye***	***concentration in primer mix (μM)***	***size range (bp)***
Brh-1	(Williams, Wilcock & Bruford, 2002)	6-FAM	2	165-265
Brh-2-IK-F	TTTCTTGGCAAACACGCAG			
Brh-2-IK-R	**GTTTCTT**ACAGAGATTGGTACCGCTATAA	6-FAM	2	162-184
Brh-3-IK-F	GGCGTAAAATTAGAATTTACCTCAT			
Brh-3-IK-R	**GTTTCTT**ATCACGCACTCGCCGTTTA	PET	2	130-170
Brh-4-IK-F	GAAAAGGAGCGAGCGAGAG			
Brh-4-IK-R	**GTTTCTT**GAAGAACAAGAGCGGCCAAC	6-FAM	2	93-135
Brh-5	(Williams *et al.*, 2002)	PET	2	199-225
Brh-6-IK-F	CGCCCACAAAAGCAAACC			
Brh-6-IK-R	**GTTTCTT**TGCGGTGGCTTAATCAAAGT	NED	2	160-228
Brh-7	(Williams *et al.*, 2002)	VIC	6	149-315

Table S2.3: *Baetis rhodani*: Allelic richness based on all loci and without *Brh*-4, and F$_{IS}$ values. F$_{IS}$ values in bold are significantly different from zero.

Population	***Allelic richness all loci***	***Allelic richness (Brh4-excluded)***	***F_{IS} all loci***
St09	9.25	10.70	**0.087**
St08	9.42	10.23	0.096
Se10	8.83	10.02	0.065
St07	9.49	10.75	**0.149**
Se08	9.26	10.30	0.127
Se06	9.70	10.97	**0.115**
Se05	9.64	10.90	0.048
St04	9.20	10.21	0.079
St03	10.42	11.27	0.09
Se01	9.76	10.31	0.14
St02	9.31	10.17	**0.2**
Bu	11.01	10.70	0.109

Table S2.4: *Gammarus fossarum* type A: Allelic richness andF_{IS} based on 9 microsatellite loci. F_{IS} values in bold are significantly different from zero.

Population	***Allelic richness***	***F_{IS}***
St09	1.33	0.061
Se10	2.66	0.05
St07	2.51	-0.022
Se08	2.55	-0.076
St06	2.64	0.043
Se06	3.03	0.042
St05	3.58	-0.057
Se05	3.99	0.037
St04	3.22	0.044
St03	3.42	-0.048
JA	3.59	-0.004

Table S2.5: F_{ST} (below diagonal) and D_{est} (above diagonal) values for *Baetis rhodani* calculated based on 5 loci (*Brh*-4 excluded). F_{ST} values in bold are significantly different from zero. No p-values were delivered by SMOGD software for D_{est}.

Population	**St09**	**St08**	**Se10**	**St07**	**Se08**	**Se06**	**Se05**	**St04**	**St03**	**Se01**	**St02**	**Bu**
St09	0	0.0009	0.0174	0.0004	0.0083	0.0444	0.0019	0.0397	0.0328	0.0158	0.0677	0.0824
St08	-0.0079	0	0.0154	-0.0005	0.0015	0.0198	0.0090	0.0034	0.0003	0.0512	0.0292	0.0834
Se10	-0.0112	0.0135	0	0.0000	-0.0126	0.0196	0.0000	0.0001	-0.0004	0.0071	-0.0063	0.0266
St07	0.0036	0.0042	-0.0063	0	-0.0021	0.0282	-0.0160	0.0002	0.0012	0.0049	0.0314	0.0006
Se08	-0.0241	-0.0008	-0.0104	-0.0183	0	0.0236	-0.0283	0.0027	0.0000	0.0001	0.0017	0.0255
Se06	-0.0027	0.0038	0.0054	0.0028	-0.0054	0	0.0058	0.0017	0.0726	0.0491	0.0146	0.0778
Se05	-0.0125	0.0054	-0.0031	-0.0105	-0.0165	-0.0002	0	-0.0001	0.0093	0.0078	0.0045	0.0240
St04	-0.0108	0.0144	-0.0005	-0.0033	-0.0041	0.0062	-0.0008	0	0.0001	0.0278	-0.0043	0.0013
St03	-0.0038	**0.0179**	-0.0007	0.0020	-0.0052	**0.0189**	0.0035	0.0007	0	0.0061	0.0000	0.0144
Se01	-0.0041	0.0127	0.0063	0.0077	-0.0104	0.0136	0.0040	0.0090	0.0078	0	0.0213	0.1030
St02	0.0024	0.0178	-0.0023	0.0068	-0.0034	0.0084	0.0024	-0.0028	0.0016	0.0121	0	0.0200
Bu	**0.0304**	**0.0385**	**0.0248**	0.0127	0.0132	**0.0343**	**0.0197**	**0.0158**	0.0103	**0.0328**	0.0173	0

Table S2.6: F_{ST} (below diagonal) and D_{est} (above diagonal) values for *Gammarus fossarum* (type A). F_{ST} values in bold are significantly different from zero. No p-values were delivered by SMOGD software for D_{est}.

Population	***St09***	***Se10***	***St07***	***Se08***	***St06***	***Se06***	***St05***	***Se05***	***St04***	***St03***	***Ja***
St09	0	0.0393	0.0244	0.0582	0.0206	0.0848	0.1139	0.1718	0.0265	0.1687	0.5266
Se10	**0.2872**	0	0.0082	0.0582	0.0123	0.0713	0.0512	0.0669	0.0244	0.1475	0.4486
St07	**0.3096**	**0.0727**	0	0.0665	0.0025	0.0739	0.0958	0.0996	0.0152	0.1369	0.4895
Se08	**0.4305**	**0.1979**	**0.3344**	0	0.0299	0.0427	0.0305	0.0449	0.0973	0.1209	0.5979
St06	**0.2167**	**0.0664**	0.0163	**0.2398**	0	0.0489	0.0570	0.0711	0.0212	0.1158	0.5037
Se06	**0.3521**	**0.1971**	**0.2289**	**0.1680**	**0.1690**	0	0.0034	0.0389	0.0875	0.0726	0.4264
St05	**0.3722**	**0.1949**	**0.2662**	**0.1705**	**0.2089**	**0.0169**	0	0.0206	0.1303	0.1322	0.3834
Se05	**0.4749**	**0.2765**	**0.3363**	**0.2378**	**0.2726**	**0.0903**	**0.0512**	0	0.1167	0.1186	0.3786
St04	**0.3107**	**0.0610**	**0.0610**	**0.2858**	**0.0904**	**0.2012**	**0.2208**	**0.2558**	0	0.1468	0.4243
St03	**0.5488**	**0.3684**	**0.4293**	**0.3134**	**0.3766**	**0.1691**	**0.2168**	**0.2068**	**0.3275**	0	0.5759
Ja	**0.6312**	**0.5048**	**0.5284**	**0.5331**	**0.5055**	**0.4033**	**0.3885**	**0.3860**	**0.4341**	**0.4424**	0

Chapter 3

Emerging aquatic insects as subsidies for riparian predators: when do predators care for additional snacks?

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Abstract

Streams and their riparian zones are strongly linked by reciprocal energy flows. The importance of such resource subsidies for the recipient habitat is assumed to depend to a large extent on the productivity gradient between the two habitats and can vary strongly in space and time. Emergent aquatic insects have been shown to subsidize riparian predators, many of which feed on both aquatic and terrestrial prey. Seasonality of life cycles of both terrestrial and aquatic organisms can lead to considerable temporal fluctuations in prey availability in both habitats. Due to different predation behaviours and different target prey, gradients in subsidy availability and sensitivity to resource shifts can also be strongly predator-specific. We hypothesized that differences in productivity and composition of benthic invertebrate communities between streams

would be reflected in the availability of aquatic prey and thus affect the predator-specific reliance on aquatic subsidies. Using natural δ^{13}C and δ^{15}N signatures, we investigated the contribution of aquatic and terrestrial prey to the diet of riparian predatory arthropods in two high-elevation headwater streams in western Colorado, USA, with contrasting benthic composition. We also included a seasonal aspect, investigating whether the enhanced availability of a certain prey type during its peak emergence leads to shifts in riparian predator diets; and we compared the isotopic signatures of the larval and adult stages of three abundant taxa of aquatic insects to test whether adult signatures more closely resembled those of larvae or of terrestrial prey taxa. Our results suggest strong differences in reliance by riparian predators on aquatic and aquatic-derived subsidies between two headwater streams. Density (activity) of ground-dwelling lycosid spiders in pitfall traps was significantly higher in the more productive, chironomid-dominated stream than in the other stream. δ^{13}C signature of adult chironomids was undistinguishable from terrestrial prey; however δ^{15}N values suggested that adults of this aquatic taxon constitute an important part of diet for most of the riparian predator taxa. Moreover, a slight depletion in δ^{13}C of one spider taxon relative to terrestrial prey in that stream indicated that other aquatic prey also contribute to its diet. We found very little evidence for seasonal shifts in the use of aquatic prey by riparian predators. Furthermore, no response to peak mayfly emergence was detected in the stream where mayflies are the predominant taxa in the benthos. Linyphiid spiders were the only taxon that showed a slight seasonal diet shift towards consuming more aquatic prey later in the summer. Adult isotopic signatures of grazing mayfly and caddisfly species were similar to larval signatures, whereas those of a common shredding stonefly showed pronounced differences from larvae in both δ^{13}C and δ^{15}N, with adult signatures resembling those of terrestrial prey. This result suggests that some species of aquatic-derived prey can be difficult to distinguish in their imaginal carbon and nitrogen signatures from terrestrial prey. Overall we conclude that benthic productivity and composition can influence the strength of food-web interactions between headwater streams and their riparian zones. Further studies are needed to understand how specific prey production and identity affect aquatic subsidies of riparian predators.

Introduction

In natural streams, aquatic and terrestrial environments are strongly coupled (Polis, Anderson & Holt, 1997). Energy flow in both directions links them together, and such subsidies can influence the recipient system to a certain extent (Polis, Anderson & Holt, 1997; Baxter, Fausch & Saunders, 2005). For example, leaf litter and terrestrial insect input into streams can be major food resources across all trophic levels in aquatic food webs (Wallace *et al.*, 1997; Nakano, Miyasaka & Kuhara, 1999). Similarly, emergence and stranding of aquatic insects is an important vector for the flow of aquatic-derived energy into riparian habitats (Jackson & Fisher, 1986; Gratton, Donaldson & Zanden, 2008). A variety of riparian consumers, including birds (Murakami & Nakano, 2002), lizards (Sabo & Power, 2002) and predatory arthropods (Sanzone *et al.*, 2003; Hering & Plachter, 1997), have been shown to rely to a certain extent, if not fully (Paetzold, Schubert & Tockner, 2005), on aquatic prey subsidies.

The importance of such resource subsidies for the recipient habitat is assumed to depend to a large extent on the productivity gradient between systems (Ballinger & Lake, 2006; Burdon & Harding, 2008; Polis & Hurd, 1996), and can vary strongly in space and time. One study relating stream geomorphology to the output of emergent insects found that densities of riparian predatory arthropods were elevated in proximity to pools from which more aquatic insects emerged (Iwata, 2007). Furthermore, seasonality of life cycles of both terrestrial and aquatic organisms can lead to considerable temporal fluctuations in productivity gradients. For example, in forested streams, seasonal peaks in aquatic insect emergence as well as of input of leaf litter with attached prey into streams can cause considerable shifts in the gradient in prey availability for both aquatic and riparian predators (Nakano & Murakami, 2001; Kato, Iwata & Wada, 2004).

Due to different predation behaviours and different target prey (Paetzold & Tockner, 2005; Collier, Bury & Gibbs, 2002; Sanzone *et al.*, 2003), gradients in subsidy availability and sensitivity to resource shifts can be strongly predator-specific. For instance, caddisflies and stoneflies that emerge by crawling on land (Hynes, 1979) are typical prey for nocturnal ground-dwelling predators such as beetles and riparian lycosid spiders (Paetzold & Tockner, 2005). Many chironomids and some families of mayflies (e.g. Baetidae), in contrast, make a more rapid transition from nymph to subimago emerging directly on the water surface (Hynes, 1979). These taxa are less vulnerable to most ground-dwelling predators, while being more susceptible to predation by web-building spiders. Moreover, strong differences exist in the temporal patterns of aquatic insect emergence, which can take place over several months or be strongly synchronized and result in substantial yet short-term resource pulses (Corbet, 1964). These taxa-specific differences in predator foraging behaviour, mode and timing of aquatic insect emergence as well as behaviour on land (e.g. flight behaviour) determine predator-specific prey availability.

Natural stable carbon and nitrogen isotope ratios are widely applied to provide time-integrated information on energy flow through food webs (Cabana & Rasmussen, 1996; Finlay, 2001). Stable carbon isotope ratios (δ^{13}C) can differ strongly among primary producers, reflecting specific differences in CO_2 uptake (Rounick & Winterbourn, 1986). Moving up the food chain, carbon isotope ratios remain relatively stable, thus δ^{13}C ratios of consumers reflect those of their food, with average fractionation of 0.4‰ (Vander Zanden & Rasmussen, 1999; Rounick & Winterbourn, 1986; Post, 2002). Therefore, given several distinguishable types of basal resources available (e.g. aquatic- and terrestrial-derived carbon), δ^{13}C signatures can be used to follow the paths of resources through the food web. Nitrogen stable isotope ratios behave differently, accumulating in the 'food chain' on average 3.4‰ from one trophic level to another and thus providing information on the trophic position of consumers (Post, 2002). Furthermore, nitrogen signatures also can differ between basal resources and, in this case, become an additional marker for derived energy source. Both isotopes have been shown to be sensitive to dietary changes at short time scales (days; Ostrom, Colunga-Garcia & Gage, 1997; Kato *et al.*, 2004).

In the following study, we applied stable isotope methodology to investigate whether differences in benthic invertebrate communities are reflected in the magnitude of subsidy links to the riparian predator community adjacent to high-elevation headwater streams in the western Colorado Rocky Mountains, USA. Using natural δ^{13}C and δ^{15}N signatures, we assessed the relative contribution of aquatic- and terrestrial-derived subsidies for a variety of riparian predatory arthropods (primarily spiders) in two streams with contrasting benthic invertebrate communities. The benthos of Marmot Creek was comprised mostly of mayflies (Ephemeroptera) and stoneflies (Plecoptera), which were present at much lower densities in Whiterock Creek, being primarily dominated by dipterans (Chironomidae) and caddisflies (Peckarsky, B. L, unpublished data). We also included a seasonal question, investigating whether the enhanced availability of a certain prey type (here, baetid mayflies) during the peak emergence period leads to shifts in the diets of riparian predator taxa. More specifically, we tested for: 1) stream-specific differences in the extent of reliance on aquatic-derived subsidies between different riparian predators, and 2) an enhanced aquatic signal in riparian predators collected after the expected peak mayfly emergence in Marmot Creek, and no such shift in Whiterock Creek.

Methods

Study area

The study was conducted in 2009-2010 in two west-facing headwater streams, Marmot and Whiterock, draining the East River catchment on the Western slope of the

Rocky Mountains in Gunnison County, Colorado, USA (latitude 38°959' N, longitude 106°989' W). The riparian zones of both streams have dense willows , and Marmot also has conifers growing along the edges of the stream channel. Similar in size and discharge, the two streams differed in elevation, channel gradient, nutrient availability and geomorphological stability (Table 3.1), which was reflected in pronounced differences in productivity and composition of benthic communities.

Table 3.1: Sampling site description (Peckarsky, unpublished data). BOM = benthic organic matter.

Stream	***Marmot***	***Whiterock***
Discharge (m^3/s)	0.015-0.08	0.015-0.05
Elevation (m a.s.l.)	2949	3216
Slope	15.6	4.8
Median substrate size, D_{50} (mm)	43.5	20
Stream width (m)	0.92	1.25
Bankfull depth (m)	0.18	0.25
Pfankuch channel stability rating (mean)	102	59
Index of geomorphologic instability	51	7.0
Mean specific conductivity	256	212
BOM chl *a* (mg/m^2)	3.76	56.26
NO_3 (mg/L)	0.232	0.459
Mean P (μg/L)	1.0	1.5
Limiting nutrients (NDS experiment)	N and P	P

Dominant primary producers in Marmot were diatoms that covered 93% of the rocks and gravel, whereas aquatic moss was a co-dominant primary producer in Whiterock (27% of rock cover vs. 50% cover by diatoms). Overall benthic invertebrate abundance was 2917.3 ± SE 860.3 (N/m^2) in Marmot and nearly 2× higher (5373.1 ± SE 4908.4 N/m^2) in Whiterock. A largest proportion of the benthic community in Marmot was comprised of mayflies (26.2% of benthic abundance) and stoneflies (19.5%). Caddisflies made up only a small fraction (3.8%), whereas the remaining aquatic insects were dipterans (37.8%), primarily chironomids. In Whiterock, the aquatic community was primarily dominated by chironomids (68.1%) and caddisflies (10.8%). In addition, mayfly and stonefly abundances were much lower (3.2 and 6.0%, respectively) in Whiterock than in Marmot (Peckarsky, B. L, unpublished data).

Macroinvertebrate sampling

We sampled riparian predators and aquatic and terrestrial prey on 19 June and 16 July 2009. Based on previous studies, we expected the June sampling to be before and the

July sampling after the main emergence pulse of baetid mayflies, which are typically observed in the East River catchment around the beginning of July (Peckarsky, Taylor & Caudill, 2000). We used pitfall traps (transparent plastic cups, depth = 7 cm, top diameter = 9 cm, bottom diameter = 5.3 cm) for the collection of ground-dwelling predators and terrestrial as well as aquatic-derived prey (e.g. chironomid adults). Traps were installed for 24 h in the riparian zone along 50-m reaches immediately adjacent to each stream. They were filled with soapy water and randomly distributed within the reach at one trap per every 5 m of the study reach (on both sides of the stream). Additional samples of terrestrial invertebrates (herbivore prey and predators) were collected with a sweep net within 5 m of the stream bank. Benthic invertebrate samples were collected with a D-net and common taxa were identified and preserved for isotope analysis. We used Ubick *et al.* (2005) for identification of spiders, and Merritt, Cummins & Berg, (2008), Baumann, Gaufin & Surdick (1977), Peckarsky, Dodson & Conklin (1985) to determine aquatic insect larvae and imagos. 'Aquatic prey' isotopic signatures in the discussion refer to values averaged over aquatic prey (grazers and predators) with isotopic signatures distinctly different from terrestrial prey in both life-stages ('distinctly aquatic' signatures). We use the term 'aquatic-derived prey' for terrestrial adults of taxa of aquatic insects whose adult isotopic signature is undistinguishable from terrestrial prey. 'Terrestrial prey' will further be used for prey with entirely terrestrial life cycles.

To compare potential differences in isotopic signatures between larvae and imagos as well as to investigate annual variation in values, additional samples of aquatic prey were collected in July 2010. Here, three taxonomically distant aquatic species that we expected to differ in their imaginal feeding biology were sampled as larvae and imagos: *Baetis bicaudatus* (Ephemeroptera), *Allomyia gnathos* (Trichoptera) and *Zapada haysii* (Plecoptera).

Statistical analysis

We conducted a two-way nested analysis of variance (ANOVA) with 'stream × time' interaction to test the differences in abundance of riparian predators between streams and sampling occasions. Abundance in single pitfall traps was used as replicates. We used R Version 2.11.1 (R Development Core Team 2010) for this calculation. ANOVA assumptions were tested with diagnostic graphs. Abundance of lycosid spiders was box-cox transformed to meet the assumptions.

Isotope analysis

All samples were frozen as soon as possible after sampling, and then oven-dried for 48 h at 40°C. Several individuals (10 - 15 for smaller species, at least 3 for larger species, except *Megarcys signata* (Plecoptera) with 1 individual per sample) were sampled per taxon to achieve a mean isotope distribution for each species after homogenization. Homogenized samples of 0.2 - 0.7 mg were placed into 4 × 6 mm cylindrical tin cups and weighed to a precision of 0.001 mg for stable isotope analysis. Each sample was run in triplicate. Nitrogen and carbon isotope composition were determined simultaneously using a ThermoFisher Flash-EA 1112 coupled with a Conflo IV interface to a ThermoFisher Delta V isotope ratio mass spectrometer (IRMS). Samples were combusted in the presence of O_2 in an oxidation column at 1030°C. Combustion gases were passed through a reduction column (650°C), and the produced N_2 and CO_2 gases were separated chromatographically and transferred to the IRMS via an open split for on-line isotope measurements. Isotope ratios are reported in the conventional δ-notation with respect to atmospheric N_2 (AIR) and V-PDB (Vienna Pee Dee Belemnite) standards, respectively. The methods were calibrated with IAEA-N1 ($\delta^{15}N = 0.45$), IAEA-N2 ($\delta^{15}N = +20.41$) and IAEA N3 ($\delta^{15}N = +4.72$) reference materials for nitrogen, and NBS22 ($\delta^{13}C = -30.03$) and IAEA CH-6 ($\delta^{13}C = -10.46$) for carbon. Reproducibility of the measurements was $< 0.15‰$ for both $\delta^{13}C$ and $\delta^{15}N$.

Results

Riparian predator community

Riparian predators represented on average 41% (Marmot) to 51% (Whiterock) of pitfall catches, the rest being both terrestrial and aquatic-derived (primarily dipteran) non-predatory arthropods. Ants, and lycosid and linyphiid spiders were most abundant among predators caught with pitfalls at both streams. Mean predator abundance per pitfall (per 24 h) was significantly different: 5.16 ± SE 0.91 in Whiterock versus 2.95 ± SE 0.52 in Marmot (ANOVA: $F = 4.73$, $p = 0.04$), which indicates a higher activity-abundance of ground-dwelling predators at this stream (Nentwig, 1982). However, this result was primarily attributed to lycosid spiders, which were significantly more abundant in pitfall traps adjacent to Whiterock than Marmot during both collection times (ANOVA: $F = 18.22$, $p = 0.0001$; Fig.3.1). A significant stream × time interaction was detected for the remainder of the ground-dwelling predators caught by pitfall traps, mostly due to Formicoidae, which increased near Marmot and decreased near Whiterock between June and July (ANOVA: $F = 4.23$, $p = 0.05$; Fig 3.1).

Six additional spider families were collected with the sweep nets. Three spider families were only found near Whiterock (Tetragnathidae, Liocranidae and Theridiidae), whereas myriapods and Thomisidae were collected only near Marmot. Thus, a total of 10 taxa of riparian predators, both web-weaving and ground-dwelling, were included in the isotope analysis (Table 3.2).

Table 3.2: Overview of aquatic and riparian invertebrates included in the isotope analysis. In parentheses, taxa that were detected on the sampling occasion but could not be analyzed due to small sample size. Spider typology taken from Collier *et al.*, 2002; Wise, 1993; Sanzone *et al.*, 2003

Presence of taxon				***sampling method***	***Taxon***	***Comments***
Marmot		**Whiterock**				
June	**July**	**June**	**July**		**Riparian predators**	
+	+	+	+	pitfall	Linyphiidae	Araneae, sheet-web weaving spiders
	+	(+)	+	pitfall	Dictynidae	Araneae, spiders which build irregular webs close to or directly on the ground
	+			sweep net	Thomisidae	Araneae, sit-and-wait spiders (on vegetation)
			+	sweep net	Tetragnathidae	Araneae, horizontal orb-weaving spiders
			+	sweep net	Theridiidae	Araneae, spiders which build tangle space webs
		+	+	pitfall/sweep net	Liocranidae	Araneae, wandering spiders
+			+	pitfall/sweep net	Gnaphosidae	Araneae, wandering or sit-and-wait spiders (on the ground)
+	+	+	+	pitfall	Lycosidae	Araneae, wandering and hunting spiders
+	+	+	+	pitfall	Formicoidae (2 taxa)	Hymenoptera, wandering omnivores
+	+			pitfall	Myriapoda	wandering predator
					Aquatic grazers	
+	+		+	kick sampling	*Baetis bicaudatus*	Ephemeroptera, grazer
+	+			-	*Cinygmula* spp.	Ephemeroptera, grazer
		+	+	-	*Allomyia gnathos*	Trichoptera, grazer
		+		-	*Zapada haysii*	Plecoptera, shredder
+	+			-	*Megarcys signata*	Plecoptera, predator
		+	+	-	*Neothrema alicia*	Trichoptera, grazer
			+	-	*Rhyacophila alberta*	Trichoptera, predator
	+			-	*Arctopsyche grandis*	Trichoptera, mixed diet
					Terrestrial prey	
+	+			sweep net	Heteroptera	Hemiptera, phytophag
+	+	+	+	-	Phylloidea	Sternorrhyncha, phytophag
	+			-	Aphidae	Hemiptera, phytophag
		+	+	-	Auchenorrhyncha	Hemiptera, phytophag

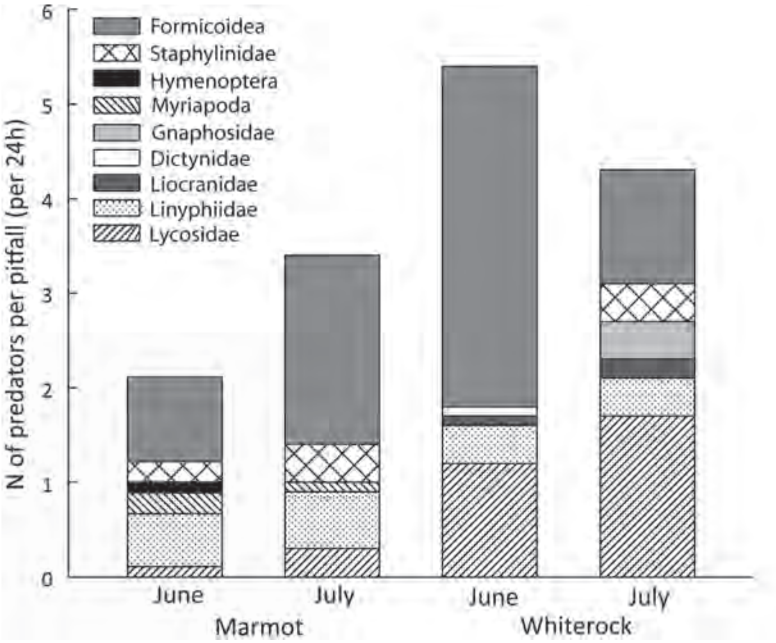

Figure 3.1: Mean abundance of riparian predatory taxa per pitfall per 24 h.

Isotope analysis

In both streams, δ^{13}C values differed strongly between aquatic (larval signatures) and terrestrial prey (on average by 7.0‰ in Marmot and 8.6‰ in Whiterock), delivering a clear separation in carbon source signatures (Fig.3.2; for isotopic values for single taxa see Tables S3.1 and S3.2). However, some aquatic taxa sampled in 2009 as adults (Chironomidae and *Zapada haysii*) showed δ^{13}C signatures indistinguishable from those of terrestrial prey.

Marmot

In Marmot, δ^{13}C values of most riparian predators overlapped with respective terrestrial prey (Fig.3.2a). Overall, carbon signatures for all groups changed little between the two sampling occasions indicating no major diet changes within any taxa. For riparian predators, the maximum signature shift was a δ^{13}C depletion of 0.88 in myriapods. Nitrogen isotope signatures of aquatic prey in Marmot were overall substantially elevated compared to terrestrial prey (Fig.3.2a). δ^{15}N values of riparian predators were 3.6‰ higher than those of terrestrial prey and 2.7‰ lower than those of aquatic prey averaged (and 0.22‰ lower than grazers). There were no systematic differences in isotopic signatures of individual riparian predator taxa relative to the different hunting strategies (Fig.3.2a).

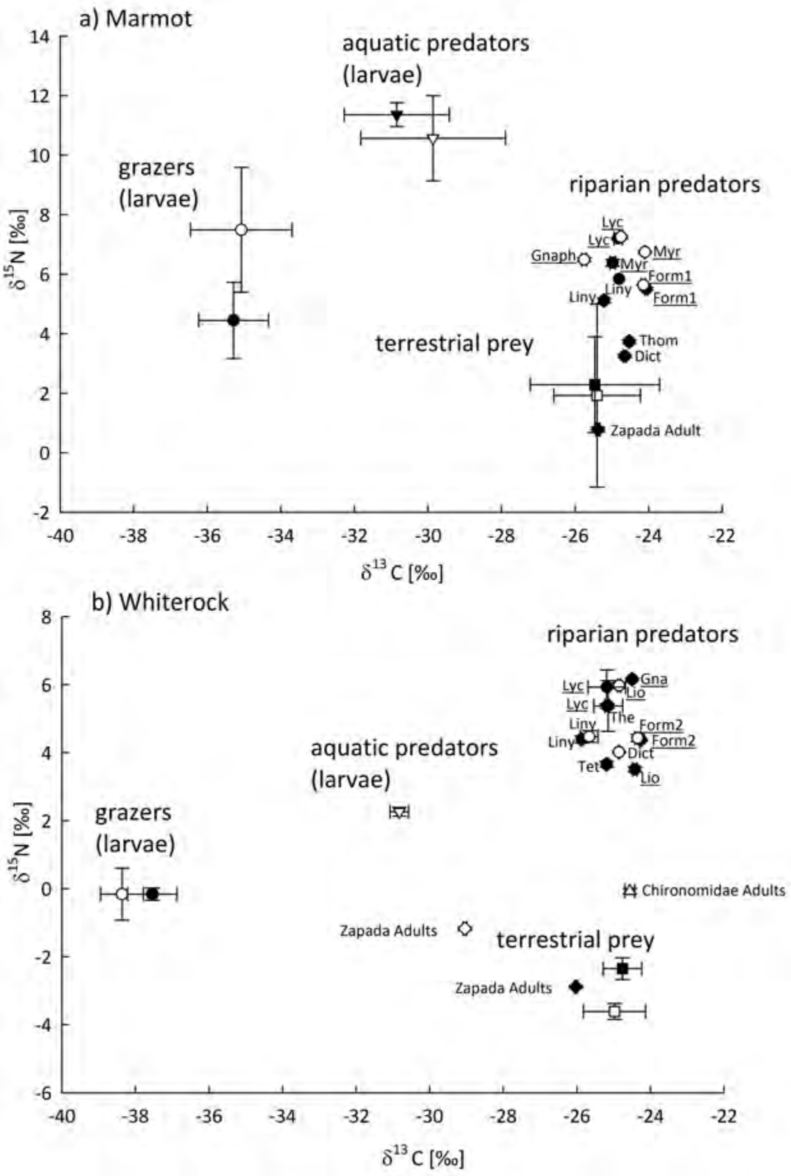

Figure 3.2: δ^{15}N and δ^{13}C of riparian and aquatic predators and prey at Marmot (a) and Whiterock (b) in summer 2009. Larval isotopic signatures are shown for aquatic insects. Open symbols correspond to June samples, filled symbols to July samples. Error bars show standard deviation. Note that scales for δ^{15}N data differ between the two streams. Theridiidae and Lycosidae (June) overlap on the graph for Whiterock. Letter codes indicate different predator taxa: Lycosidae (Lyc), Thomisidae (Thom), Dictionidae (Dict), Linyphiidae (Liny), Liocranidae (Lio), Gnaphosidae (Gnaph), Theridiidae (The), Tetragnathidae (Tet), Formicoidae (Form), Myriapoda (Myr). Labels for ground-dwelling predators are underlined.

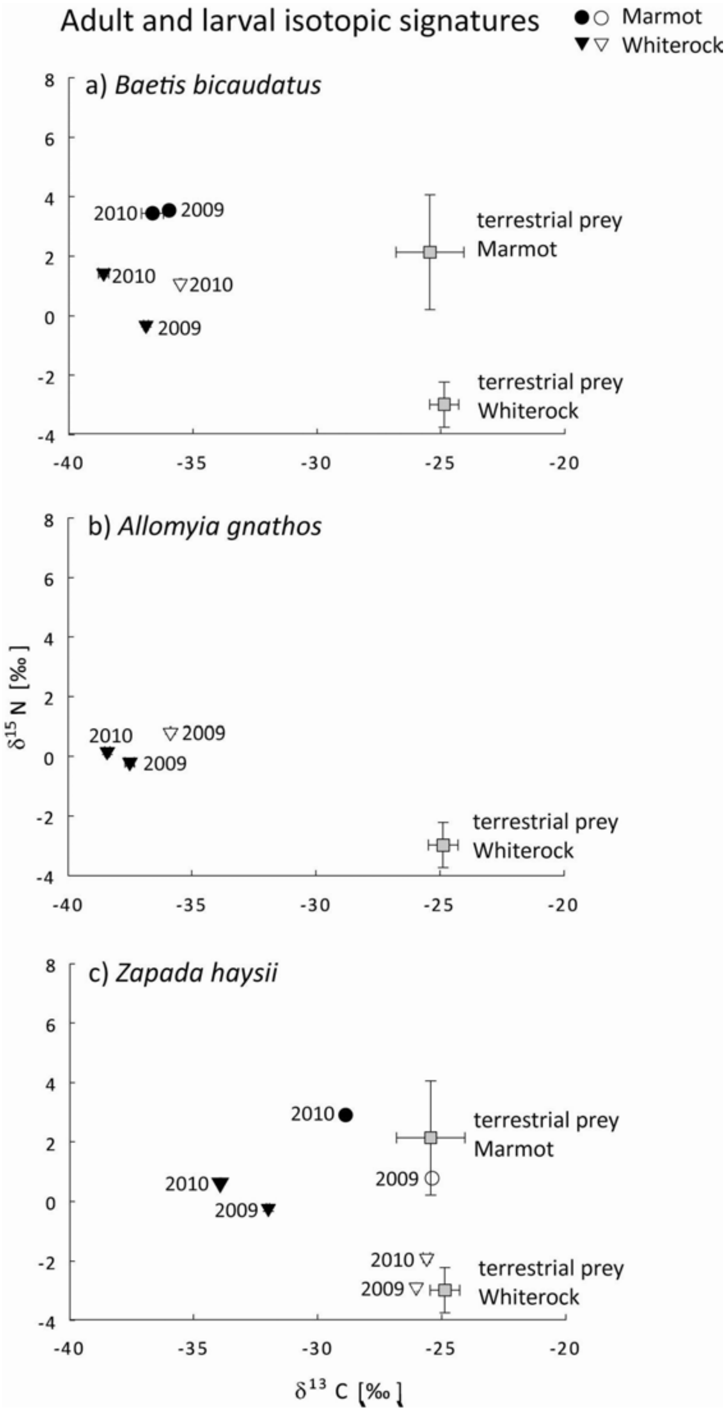

Figure 3.3: Comparison of the isotope composition between larvae and adults sampled in the same month in 2009 and 2010 in Marmot and Whiterock: *Baetis bicaudatus* (a), *Allomyia gnathos* (b), *Zapada haysii* (c). Filled symbols represent larvae, open symbols - imagos. Grey squares represent mean values for terrestrial prey measured in 2009.

Whiterock

As in Marmot, carbon isotope signatures of riparian predators in Whiterock were very similar to those of terrestrial prey (Fig.3.2b). However, in one spider family, the web-weaving Linyphiidae, we found on both sampling dates some depletion in δ^{13}C compared to terrestrial prey (Table S3.3). In linyphiid spiders, we also detected a small seasonal change in the carbon signature (Table S3.3). For instance, they were -0.421‰ more depleted in δ^{13}C relative to terrestrial prey in July than in June, a value comparable to a shift by one trophic level (Post, 2002); this result suggests a higher similarity to aquatic prey in July than in June.

δ^{15}N values of all potential prey types in Whiterock were substantially lower than in Marmot, whereas they were nearly the same in the two streams for riparian predators (Fig.3.2). The differences in nitrogen enrichment of the aquatic prey between the two streams are consistent with previously observed differences in nitrogen signatures of both primary producers and detritus, both of which were lower in Whiterock than Marmot (Moslemi, J. M., unpublished data). Thus, in contrast to Marmot, riparian predators in Whiterock were more enriched in δ^{15}N than both prey types with δ^{15}N being on average 7.84‰ higher than terrestrial prey (for single taxa see Table S3.4) and 5.03‰ higher than aquatic prey. Notably, while the δ^{13}C of aquatic-derived imagines of Chironomidae (Diptera) was indistinguishable from that of terrestrial prey, their δ^{15}N was elevated by about 3.6‰ (Fig.3.2b). In most predator groups, nitrogen signatures shifted little between the two sampling occasions, the only exception being Liocranidae in Whiterock, which were 2.46‰ more depleted in δ^{15}N in July.

Shifts between larval and adult isotopic signatures

In general, annual variation in larval and adult isotopic signatures of aquatic species sampled both in 2009 and 2010 was low (Fig.3.3). However, there were some patterns of variation between the life stages of the three common species with respect to δ^{13} C and δ^{15}N enrichment. For *Baetis bicaudatus* and *Allomyia gnathos*, those shifts were moderate, and their carbon isotope signatures remained distinctly different from that of terrestrial prey (more depleted by 10.75‰ for *B. bicaudatus* and by 11.11‰ for *A. gnathos*). In contrast, both δ^{13}C and δ^{15}N of *Zapada haysii* changed dramatically between life stages with adults becoming more difficult to distinguish from terrestrial prey in samples from July 2009 and 2010.

Discussion

Our study suggests that differences in abundance and composition of benthic community can have a strong effect on the use of aquatic subsidies by riparian predators.

Predator activity and presence of aquatic prey in the diet of different predator taxa

The activity-abundance of lycosid spiders, cursorial predators, which in other studies have been shown to opportunistically feed on aquatic prey and respond with elevated densities to its emergence pulses (Power *et al.*, 2004; Paetzold, Bernet & Tockner, 2006), was significantly higher in Whiterock. Moreover, Tetragnathidae, typical riparian specialists (Power *et al.*, 2004), as well as some other spider taxa, were only found at Whiterock.

Similarly, the isotopic signatures showed pronounced differences between the two streams. In Marmot the values for both δ^{13}C and δ^{15}N (the latter higher in aquatic prey than in predators) suggested that neither aquatic prey nor the aquatic-derived terrestrial stages contributed to the diet of the riparian predators collected. In contrast, in Whiterock, the more productive and stable stream, δ^{13}C signatures of one web-building spider taxon (Linyphiidae) deviated from terrestrial prey towards the δ^{13}C values of aquatic insects, suggesting inclusion of some aquatic carbon in their diet. Chironomids, the dominant benthic taxon in this stream showed a 'terrestrial' carbon signature; however, their nitrogen values were distinctly elevated compared to terrestrial prey. Consumption of chironomids could thus potentially explain the wide gap between δ^{15}N of riparian predator and terrestrial prey in Whiterock, which cannot be attributed solely to trophic fractionation (reported to lie in the range of 0.5 - 5.5‰ for δ^{15}N (Post, 2002). This scenario is very realistic, as small size and weak flight capacity make chironomids a typical target prey for many web-weaving riparian spider taxa (Linyphiidae, Theridiidae and Tetragnathidae; Nentwig, 1980). Furthermore, the capture of chironomid adults in pitfall traps suggests habitat overlap with riparian predators, which could increase their probability of capture. Also lycosids have been reported to actively catch insects emerging directly from the water (Paetzold *et al.*, 2006). We cannot, however, rule out possible predation on other terrestrial prey of higher trophic levels, which were not observed in this study.

Feeding on chironomids cannot explain the slight depletion of carbon detected for a single spider family. Hence we suspect that some aquatic prey taxon must be contributing to the diet of this taxon. We speculate that predation on adult caddisflies (dominant grazers in Whiterock), who emerge after crawling out of the stream and are available for both ground-dwelling and web-building taxa, could explain the observed deviation of predatory δ^{13}C signatures towards those of aquatic prey.

Seasonality of subsidies

We found no evidence that riparian predators responded to peak mayfly emergence in Marmot Creek, which had a higher abundance of mayflies (baetids), detecting no seasonal shifts in their isotopic signatures (Fig.3.2a). We suggest several potential explanations for this result. First, overall productivity and specifically baetid abundance of Marmot could be low relative to the terrestrial prey production in the riparian area. Such a scenario would correspond to the contention that the relative importance of allochthonous subsidies is determined by productivity gradients between the terrestrial and aquatic habitats (Polis, Anderson & Holt, 1997). Second, mayfly swarms in the East River catchment have been observed at great distances away from streams (Peckarsky *et al.*, 2002), as were the locations where mayflies were collected at Whiterock for this study. Thus mayfly swarming behaviour could result in no habitat overlap with riparian predators. Third, peak emergence of baetids in 2009 may not have occurred between the timing for our two sampling dates, which would have affected the abundance of aquatic prey of this taxon available for riparian predators.

Similarly to Marmot, most predatory taxa in Whiterock sampled on both occasions did not show a pronounced shift in δ^{13}C that would reflect enhanced predation on aquatic prey. Linyphiid spiders were the only exception. Building elaborate nets these spiders have been reported to be stationary, maintaining their location once the net is in place (Power *et al.*, 2004). Thus their signature is a 'passive' reflection of available aerial prey, in contrast to e.g. lycosids which hunt actively and thus to some extent select their prey. The detected shift in their carbon signature from June to July could reflect an overall increase in aquatic insect emergence with the progression of the warm season or be due to an emergence pulse of a single taxon, e.g. caddisflies, the dominant aquatic insect with a distinctly aquatic carbon signature.

Changes in isotopic signatures between aquatic larvae and terrestrial adults

In contrast to the mayflies and caddisflies, comparison of adult and larval isotopic signatures revealed a pronounced change in both δ^{13}C and δ^{15}N of a common leaf-shredding stonefly (*Zapada haysii*). We attribute that shift to terrestrial feeding of *Zapada* imagos. A terrestrial carbon signature was also detected for adults of chironomids, although no comparisons were made to the larval signatures. Most chironomids are known to spend several days on land (Pinder, 1986) and nectar feeding has been reported in the literature (Burtt, Perry & McLachlan, 1986). In contrast, finding no pronounced shift in *Baetis* and *Allomyia* reflects the lack of adult feeding by those taxa in terrestrial environments.

Results of this study suggest that some species of aquatic-derived prey in the predator diet can go undetected, because they are isotopically indistinguishable in their imaginal carbon and nitrogen signatures from terrestrial prey. Therefore, we could have potentially underestimated the contribution of aquatic subsidies to the diet of riparian predators in the studied streams. This is especially valid for Marmot, where stoneflies (mainly *Zapada haysii*) represent 19% of benthic abundance and where we obtained no data for chironomids.

Conclusions and implications

Most studies that detected a high contribution of aquatic subsidies to the diet of riparian arthropods were conducted in habitats with very low terrestrial productivity, e.g. streams with exposed dry gravel bars (Collier, Bury & Gibbs, 2002; Hering & Plachter, 1997; Paetzold, Schubert & Tockner, 2005) or desert streams (Sanzone *et al.*, 2003), where the gradient of prey availability was clearly shifted towards the aquatic environment. In forested headwater streams, the importance of aquatic subsidies for riparian environments is probably lower and seems to strongly depend on the relative productivity of the stream compared to the riparian zone (Burdon & Harding, 2008). Studies of Nakano & Murakami (2001) and Kato, Iwata & Wada (2004) showed that the productivity gradient between aquatic and terrestrial environments in headwaters is in a seasonally shifting balance, determined by the phenology in relative productivity in the two environments.

In this study, we aimed to understand whether differences in the abundance and composition of benthic communities influence the strength and phenology of aquatic subsidies to the riparian predator community in more open-canopied, high elevation headwater streams. Our results suggested very different patterns for two such streams strongly differing in the taxonomic composition of their benthic community and overall productivity. In spite of some aquatic prey being difficult to distinguish from terrestrial prey, combining the information on two isotopes and on activity of ground-dwelling predators, we conclude that in the more productive stream (Whiterock) aquatic prey play a substantially larger role in subsidizing the riparian predator community than in the less productive stream (Marmot). Future research should focus on understanding the mechanism causing the observed differences: whether the subsidies were influenced by the overall higher stream productivity or by specific contributions of individual taxa more vulnerable to predation.

Acknowledgements

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Supplement

Table S3.1: Marmot, isotope signatures (‰). AP= aquatic prey, APR= aquatic predator, RPR= riparian predator, TP= terrestrial prey

Feeding type	***Taxon***	$\delta^{13}C$	$\delta^{15}N$	***total C %***	***total N %***
June 2009					
AP	*Baetis bicaudatus*	-36.05	6.01	49.92	9.23
AP	*Cinygmula* spp.	-34.10	8.97	44.38	9.05
APR	*Megarcys signata*	-29.86	10.57	60.09	13.36
RPR	Formicoidae 2	-24.15	5.64	47.13	11.10
RPR	Formicoidae 3	-24.71	5.80	48.25	12.11
RPR	Gnaphosidae	-25.76	6.49	49.80	-
RPR	Linyphiidae	-24.80	5.84	48.34	12.14
RPR	Lycosidae	-24.75	7.25	47.40	11.50
RPR	Myriapoda	-24.10	6.74	50.17	11.35
TP	Heteroptera	-24.57	4.09	50.21	11.16
TP	Phylloidea	-26.23	-0.25	53.21	9.89
July 2009					
AP	*Baetis bicaudatus*	-35.95	3.54	48.30	9.54
AP	*Cinygmula* spp.	-34.61	5.35	41.79	8.31
APR	*Arctopsyche grandis*	-29.84	11.08	47.17	10.10
APR	*Megarcys signata*	-31.86	11.65	46.76	9.83
RPR	Dictynidae	-24.65	3.25	47.05	11.13
RPR	Formicoidae 2	-24.06	5.51	47.40	11.08
RPR	Linyphiidae	-25.21	5.11	49.62	11.00
RPR	Lycosidae	-24.83	7.20	49.01	11.40
RPR	Myriapoda	-24.98	6.38	50.84	10.64
RPR	Thomisidae	-24.53	3.74	47.72	11.48
TP	Aphidae	-24.81	1.58	48.11	10.56
TP	Heteroptera	-24.12	4.13	49.00	10.63
TP	Phylloidea	-27.451	1.148	51.37	10.48

Table S3.2: Whiterock, isotopic signatures (‰). AP= aquatic prey, APR= aquatic predator, RPR= riparian predator, TP= terrestrial prey.

Feeding type	***Taxon***	$\delta^{13}C$	$\delta^{15}N$	***total C %***	***total N %***
June 2009					
AP	*Allomyia gnathos*	-37.95	-0.70	48.60	8.52
AP	*Zapada haysii*	-32.00	-0.24	47.69	10.24
AP	*Neothrema alicia*	-38.77	0.38	53.41	8.42
APR	*Rhyacophila alberta*	-30.83	2.26	45.87	10.69
RPR	Formicoidae 3	-24.35	4.43	46.06	11.43
RPR	Linyphiidae	-25.66	4.47	48.73	12.41
RPR	Liocranidae	-24.85	5.97	49.73	10.45
RPR	Lycosidae	-25.15	5.37	47.07	11.74
TP	Auchenorrhyncha	-24.38	-3.44	50.83	10.57
TP	Phylloidea	-25.57	-3.78	52.50	9.78
July 2009					
AP	*Allomyia gnathos*	-37.51	-0.20	53.74	8.06
AP	*Baetis bicaudatus*	-36.88	-0.32	52.16	10.66
AP	*Neothrema alicia*	-38.23	0.03	56.18	7.39
RPR	Dictynidae	-24.86	4.02	49.01	11.69
RPR	Formicoidae 3	-24.27	4.37	49.13	11.99
RPR	Gnaphosidae	-24.50	6.16	50.09	12.77
RPR	Linyphiidae	-25.87	4.39	50.74	11.30
RPR	Liocranidae	-24.42	3.51	50.04	11.13
RPR	Lycosidae	-25.18	5.93	47.58	12.15
RPR	Tetragnathidae	-25.19	3.66	51.26	11.18
RPR	Theridiidae	-25.17	5.37	50.86	11.41
TP	Auchenorrhyncha	-24.39	-2.58	51.88	10.48
TP	Phylloidea	-25.13	-2.12	53.10	9.93

Table S3.3: Difference in $\delta^{13}C$ values (in ‰) between riparian predator taxa and terrestrial prey sampled on the same occasion (values not corrected for fractionation). Negative values indicate a depletion of predators in $\delta^{13}C$ compared to terrestrial prey, which indicates, that aquatic prey contributes to some extent to the diet. Average trophic fractionation of $\delta^{13}C$ is reported to be 0.4 ± 1.3‰ (Post, 2002). While some taxa (other than linyphiids) showed small shifts in the mean $\delta^{13}C$ toward an aquatic signal, variation around those means suggest the shifts may not be significant. Thus only Linyphiidae are discussed.

	Marmot		***Whiterock***	
Taxon	***June***	***July***	***June***	***July***
web-weaving predators				
Linyphiidae	0.599	0.247	**-0.688**	**-1.109**
Dictynidae		0.811		-0.098
Thomisidae		0.932		
Tetragnathidae				-0.431
Theridiidae				-0.416
ground-dwelling predators				
Liocranidae			0.125	0.337
Gnaphosidae	-0.354			0.261
Lycosidae	0.648	0.627	-0.174	-0.426
Formicoidae 1	1.256	1.399		
Formicoidae 2		0.691	0.630	0.492
Myriapoda	1.300	0.483		

Table S3.4: Difference in δ^{15}N values (in ‰) between riparian predator taxa and terrestrial prey sampled on the same occasion (values not corrected for fractionation). Average trophic fractionation of δ^{15}N is reported to be 3.4 ± 1‰ (Post, 2002).

	Marmot		***Whiterock***	
Taxon	***June***	***July***	***June***	***July***
web-weaving predators				
Linyphiidae	3.922	2.826	8.084	6.742
Dictynidae		0.960		6.366
Thomisidae		1.448		
Tetragnathidae				6.007
Theridiidae				7.722
ground-dwelling predators				
Liocranidae			9.582	5.860
Gnaphosidae	4.568			8.507
Lycosidae	5.330	4.912	8.984	8.277
Formicoidae 1	3.717	3.218		
Formicoidae 2	3.884		8.041	6.721
Myriapoda	4.819	4.097		

General conclusion and outlook

General conclusions

Using a variety of approaches, I investigated how specific life-cycle traits influence patterns of dispersal and recruitment of aquatic invertebrates, and, in particular, aquatic insects. I showed that these traits determine both taxon-specific colonization constraints and advantages in different environments and also influence the magnitude of the links between aquatic and riparian communities. One of the central conclusions of this work is on the importance of considering the totality of the traits relevant for a biological process, in this case, colonization. Based on the experimental study (Chapter 1), one would expect baetid mayflies to be very vulnerable to stream habitat degradation, as they can be strongly constrained in reproduction by their specialized oviposition behaviour and hence availability of substrates for oviposition. In spite of this trait, baetids are still very widespread in European streams, many of which have been strongly degraded. In line with this fact, our molecular study (Chapter 2) detected very high gene flow between populations of *Baetis rhodani* not only on the catchment scale but also on a larger geographic scale. Hence, though the absence of protruding rocks during the emergence period can constrain recruitment of *Baetis rhodani* locally, its high mobility in the adult stage seems to enable this species to avoid this bottleneck, i.e. by dispersing to other areas where appropriate rocks are available. Low larval habitat specialization of *Baetis rhodani* must be further facilitating successful dispersal, as no long distances have to be covered to get from one suitable habitat patch to another (Fig.1).

In contrast, life-cycle traits of *Gammarus fossarum* are very different. It typically carries its eggs around till they hatch, and thus has no specialized requirements for oviposition. Due to its feeding habits, its habitat is more patchily distributed, whereas dispersal is restricted to the water column, and on the large scale occurs predominantly in a downstream direction. The very high genetic differentiation we detected between populations of *G. fossarum*, even on spatial scales of few kilometers, suggests that whether due to very low dispersal capacities or due to enhanced local adaptation of this species, very little effective dispersal happens even between neighboring populations. Thus bottlenecks for populations of *G. fossarum* are determined by very different traits

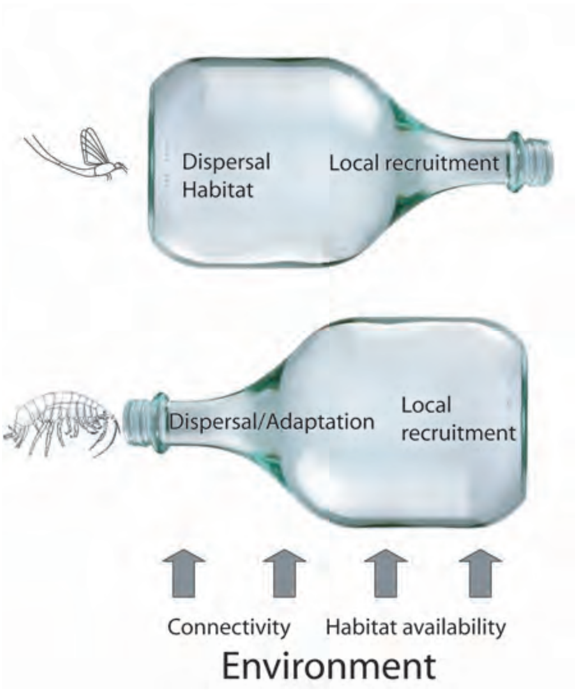

Figure 1: Different life-cycle traits determine taxon-specific bottlenecks for colonization emerging in the interaction with the environment.

than for *B. rhodani* and their response to anthropogenic pressures can be expected to differ (Fig.1). Even in strongly fragmented and modified landscapes, it will be much less probable for *B. rhodani* to reach a population bottleneck. Moreover, this species can also be expected to be a quick colonizer of newly available habitats. *G. fossarum*, in contrast, can be expected to be very vulnerable to habitat loss, which can be caused not only by in-stream modifications but also by, e.g., clearing of riparian forests delivering litter to streams. Moreover, our results suggest that colonization by *G. fossarum* will most probably require source populations within the distance of few kilometers. These two examples demonstrate how strongly species-specific life-cycle traits and ecology can influence species potential to withstand landscape modification and fragmentation.

The results of Chapter 3 suggest that species-specific traits also influence susceptibility of emergent insects to predation by riparian predators, thus having an effect on the magnitude of energy transfer between aquatic and terrestrial environments. Many riparian predators (lizards, birds, bats, arthropods; reviewed in Ballinger & Lake, 2006; Baxter, Fausch & Saunders, 2005) have been shown to rely on such subsidies, often to a high extent. Taxon-specific timing, the path of insect emergence as well as their flight behaviour (e.g. swarming) can strongly influence the phenology and magnitude of such links. Thus changes in stream benthic communities can be reflected in quantity, specific quality and timing of the available subsidy and thus potentially have strong effects on the riparian predator community.

The three studies described in this thesis add pieces to different parts of the same puzzle - one being explored in the never-ending wish to understand processes governing populations and communities as well as the links between them. Some key processes such as oviposition may be concentrated locally on few habitat patches and still have major impacts on a larger scale, providing a pointed source of recruits, important not only for local but also regional populations (Peckarsky, Taylor & Caudill, 2000; Lancaster, Downes & Arnold, 2010). Dispersal, on the other hand plays a role of glue, linking together different habitat patches (sources and sinks), connecting populations in a landscape (Palmer, Allan & Butman, 1996) as well as creating links between communities and even ecosystems (Malmqvist 2002). An integrated approach, combining different pieces - different life-stages, different spatial and temporal scales - is a prerequisite of finally getting the picture complete.

Management implications

Several recommendations for management can be derived from this work. Based on the results of the experiment with brick addition, I conclude that simple cost-effective restoration techniques such as addition of boulders to the stream bed in channelized middle-sized streams is a promising tool for enhancing recruitment potential for many invertebrate taxa. Creation of such 'hotspots' could have not only local but also regional effects, establishing a source for regional supply of recruits.

My results also suggest the importance of keeping a landscape perspective when developing river management plans including river restoration. Reach location within the stream network can be determinant for the probability of a habitat to be colonized - particularly for species with predominantly passive dispersal, strongly biased in the direction of the flow (such as *G. fossarum*). For instance, habitats downstream of nearby source populations are more likely to get colonized. Also large-scale factors, such as hydrological regime, have to be necessarily taken into account when, e.g., deciding on prioritization of sites to be restored. Pronounced unnatural water level fluctuations (e.g. due to hydropeaking) can become an additional constraint, e.g., for oviposition of many species.

Outlook: Areas for future research

Many questions, however, have yet to be answered. An important emerging area of ecological research investigates the capacity of organisms with certain life-cycle traits to adapt to the new characteristics of the environment. In this context, an interesting question in continuation of our work would be whether invertebrates with specialized oviposition traits can adapt their behavior to the lack of natural oviposition substrates and use substrates not typical for natural environments - e.g. objects introduced into streams by humans: from bridge supports to persistent rubbish (e.g. plastic bottles). Some anecdotal records (e.g. from our own observations in the field) deliver evidence for such use, however, we are not aware of any study that thoroughly investigates this phenomenon and its implications for population recruitment in modified landscapes. Such studies on adaptation capacity would be of interest both from scientific and management points of view, allowing better predictions of species resilience in the context of new environmental pressures. Also little is known about requirements of adult stages of aquatic insects towards terrestrial environments and the aquatic-terrestrial interface. Emergence, flight efficiency, swarming behavior, vulnerability to predation can be strongly influenced for instance by the presence, specific type and structure of riparian vegetation (Delettre & Morvan, 2000; Winterbourn *et al.*, 2007). Anthropogenic ecological traps (e.g. urban lights in riparian areas) also have been shown to have a major impact on terrestrial stages of aquatic insects, attracting them and increasing their mortality (Hölker *et al.*, 2010). Classifying structures in riparian zones in respect to their effect on terrestrial stages of aquatic insects and mapping them on the catchment scale could help understand large scale consequences of the riparian zone management for aquatic populations.

Based on the research in the presented chapters I also highly encourage combining several methods in studies of dispersal. Though delivering a lot of valuable information, results based on molecular techniques are sometimes difficult to interpret if no parallel studies are conducted with other techniques. In our study, direct approaches, such as mark-recapture experiments, would help to understand which interpretation of the lack of barrier effect is most feasible and test specifically whether a certain species (such as e.g. *G. fossarum*) actually are capable of crossing the studied types of barriers.

Curriculum Vitae

Personal information

Name	Alp
First name	Maria
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Education

11/2007 - 06/2011	PhD at the Department of Aquatic Ecology of Eawag: Swiss Federal Institute of Aquatic Science and Technology, Zurich, Switzerland. 'Integrating terrestrial stages of aquatic insects into studies of dispersal and recruitment in streams' Supervisor: PD Dr. Christopher T. Robinson
4/2005 - 05/2006	Master thesis at the Department of Applied Ecology, Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland and Humboldt-University to Berlin, Germany. 'Feeding ecology of brown trout in alpine streams impacted by hydropeaking' Supervisors: Dr. Armin Peter (Eawag), PD Dr. Rolf Schneider (Humboldt-University to Berlin)

10/2000 - 4/2006	Undergraduate education in biology at the Humboldt-University to Berlin, Germany. Specialization in zoology, aquatic ecology and animal physiology.
7/1996 - 6/1999	Tallinn Humanitarian Gymnasium, Tallinn, Estonia

Professional experience

06/2009 - 07/2009	Visiting student at the lab of Dr. Barbara L. Peckarsky at Rocky Mountain Biological Lab, Colorado, USA. A collaborative project on aquatic subsidies in high-elevation streams.
07/2007 - 09/2007	Scientific assistant at Fish Ecology & Evolution Department, Eawag, Kastanienbaum, Switzerland. Literature research, report compilation.
12/2004 - 05/2005	Student assistant at the Institute of Comparative Zoology, Humboldt-University to Berlin, Germany. Assisting at the practical lessons of animal anatomy, tending the zoological collection.
04/2004 - 07/2004	Student assistant at the institute of Geobotany, ETH Zurich, Switzerland. Fieldwork, working with databases.
12/2003 - 02/2004	Student assistant at the Institute of Applied Entomology, ETH Zurich, Switzerland. Insect breeding.
12/2002 - 08/2003	Student assistant at the Institute of Systematic Zoology, Humboldt-University to Berlin, Germany. Literature research.

Publication list

Alp, M., Westram, A.M., Keller, I. & Robinson, C.T. How river structure and biological traits influence gene flow: a population genetic study on two stream invertebrates with differing dispersal capacity (in press in *Freshwater Biology*).

Gostner, W., Alp, M., Schleiss, A. & Robinson. C.T. A new hydro-morphological index of diversity for assessing the physical potential for river health (in review in *Hydrobiologia*).

Alp, M., Karpati, T., Werth, S., Gostner, W., Scheidegger, C. & Peter, A. (2011) Erhaltung und Förderung der Biodiversität von Fliessgewässern. *Wasser, Energie, Luft*, 103 (3), 216-223.

Werth, S., Weibel, D., Alp, M., Junker, J., Karpati, T., Peter, A. & Scheidegger, C. (2011) Lebensraumverbund Fliessgewässer: Die Bedeutung der Vernetzung. *Wasser, Energie, Luft*, 103 (3), 224-234.

Professional activities

Revision of manuscripts for journals Aquatic Sciences, Freshwater Science (former J-NABS) and Aquatic Ecology

Presentations

2011	Symposium for European Freshwater Sciences (SEFS), Girona, Spain
2011	River Corridor Restoration Conference (RCRC), Ascona, Switzerland
2010	North American Benthological Society (NABS/ALSO joint meeting) in Santa Fe, USA
2010	Achieving Ecological Outcomes: aquatic ecological responses to catchment management, FBA, Windermere, UK
2009	North American Benthological Society (NABS), Grand Rapids, USA
2009	Biology09, Bern, Switzerland
2008	Deutsche Gesellschaft für Limnologie (DGL), Constance, Germany

Other commitments

2010	Member of the Eawag Committee on Gender Equity and Equal Opportunities
2008	PhD ombudsperson, Eawag

Further training

2010	Mixed-effect models in R, University of Zurich, Switzerland
2009	Environmental Politics, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland
2009	Ecosystem Management, ETH, Zurich, Switzerland
2009	Advanced Topics in Stream Ecology, University of Coimbra, Portugal
2009	Generalized linear models in R, University of Zurich, Switzerland
2005	Taxonomie und Ökologie. Teil 1: Trichoptera. Eawag, Switzerland

Language skills

Russian	mother tongue
Estonian	fluent
German	fluent
English	fluent
Italian	fluent
French	very good
Spanish	basic

Computer skills

Microsoft Office
R
Adobe Illustrator
SigmaPlot
GeneMarker
Arlequin
Fstat
STRUCTURE
StreamTree