Go with the flow

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Judith Jasmijn Westveer

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General introduction

The importance of freshwater streams

Freshwater streams have always provided humankind with many important resources and functions, such as drinking water, fish, irrigation of agricultural crops, hydropower and drainage of waste water (Costanza et al., 1997; Naeem et al., 2009). To increase the efficiency of these ecosystem services, many anthropogenic alterations were made (Malmqvist & Rundle, 2002). For example, streams were channelized and embanked to increase water discharge to drain urban and agricultural effluent. These alterations served specific anthropogenic purposes, but changed the natural structure and functioning of the running water ecosystems (Allan, 2004; Palmer & Febria, 2012; Meijles & Williams, 2012).

Agricultural run-off and human sewage outlets caused nutrients, organic manure, pesticides, personal health care products, pharmaceutical and hormonal pollutants to enter the waterways (Kolpin et al., 2002; Kidd et al., 2007). Furthermore, hydromorphological alterations led to a disturbed hydrograph with unfavourable conditions such as peak flow and droughts, and homogenized the physical morphology of the streambed (Furse et al., 2006). Various alterations led to a water quality decline and loss of flora and fauna and thereby degraded the whole ecosystem.

This deterioration of water quality was recognized by the EU after which the European Water Framework Directive was initiated, stating that all waters in Europe should have the ecological status of 'good' or 'very good' (European Union, 2000). To this purpose, targets were set and agreements made to improve the water quality (Kallis & Butler, 2001). Throughout Europe, subsidized projects were performed to decrease the inflow of nutrients and waste water, and to increase the migration of fish in freshwater streams and rivers many fish ladders were build. However, returning to a good ecological status does not happen overnight, and currently lowland streams in the Netherlands are still listed as some of the most deteriorated waters of Europe (EEA, 2012). Unfortunately, when it comes to the restoration of lowland streams, a lack of ecological restoration result is apparent worldwide (Leps et al., 2016; Miller et al., 2010; Palmer et al., 2010; Roni et al., 2008). At this moment, the knowledge on ecological patterns and processes underlying this limited restoration success is mostly lacking (Verdonschot et al., 2012).

Stream restoration

Stream restoration is performed at several scales, ranging from stream stretch restructuring to within-stream adjustments (Verdonschot et al., 2012). Stream stretch restoration projects are often designed by looking at pictures or maps of previous situations when conditions were considered pristine (Podolak et al., 2013), while taking legislation, budget and state of ecological degradation into account (Bernhardt et al., 2007). Morphological adjustments to the physical state of the stream channel are usually performed in order to restore strongly altered streams (Friberg et al., 2009; Eekhout et al., 2015), for example by re-meandering the main stream channel, removing embankments and artificial barriers such as weirs and dams. Smaller within-stream adjustments, focusing on ecological restoration, are performed in depleted areas that are in need of more viable populations (Palmer et al., 2010). Examples of current ecological restoration practices are the addition of woody debris to increase habitat heterogeneity (Thompson et al., 2017; Frainer et al., 2017), addition of sand to raise the stream bed and reconnect the stream and its valley, and replanting vegetation in the riparian zone (Purcell et al., 2002). At the same time, water quality improvements have been made by enforcing specific legislation regarding pollution, mainly in industrial and agricultural practices, and strong improvement of waste water treatment plants (Kallis & Butler, 2001). Both morphological and ecological measures are increasingly implemented (Didderen & Verdonschot, 2009) with the majority of them being very costly in time and money (Bond & Lake, 2003).

The assumption in all restoration efforts is that if suitable habitat is created, biota will colonize and ecological restoration will logically follow (Lake et al., 2007). This hypothesis is described as 'The field of dreams' hypothesis; if you built it, they will come (Palmer et al., 2010). However, even decades after restoration efforts have been performed, the macroinvertebrate community (Box 1) often remains impoverished in terms of species richness and abundance (Roni et al., 2008; Miller et al., 2010; Verdonschot et al., 2016) and does not meet the expectations from water authorities.

In the last decade, the realization has come that solely adjustments to the physical and chemical habitat will not lead to successful ecological restoration (Roni et al., 2008) as several, poorly understood, community assembly mechanisms are playing a role in shaping the ultimate community in restored streams (Figure 1; Lake et al., 2007). Species from the regional species pool (i.e. the total set of biota that is present in a region and could potentially inhabit a specific location), are limited by various parameters that determine the composition of the actual community in a specific habitat. If species are limited by dispersal (e.g. poor terrestrial or aquatic habitat connectivity, having unfavourable morphological features or limited flight distances) they will not easily reach new habitat and the respective local community will not represent those species. Subsequently, if these species have reached new habitat but the local habitat is unfavourable (e.g. poor suitability, heterogeneity or stability of substrata, biotic constraints or other stressors), the environmental filter will eliminate these species from the actual community. While dispersal processes have been studied extensively in terrestrial ecosystems (Clements, 1916; Egler, 1954; Odum, 1969; MacArthur & Wilson, 1967; Connell & Slatyer, 1977; Tilman, 1985; Huston & Smith, 1987; Cadotte, 2007), species in aquatic systems rely on different vectors such as water flow (Bilton et al., 2001) and therefore do not reconcile with general terrestrial theories.

I state that in order to restore the ecological structure and functioning in lowland streams we should know how biological communities in flowing water systems develop through which processes. In this thesis, I focus on the ecological mechanisms behind the dispersal of stream biota, with aquatic macroinvertebrates as model organisms for stream ecosystem functioning (Box 1).

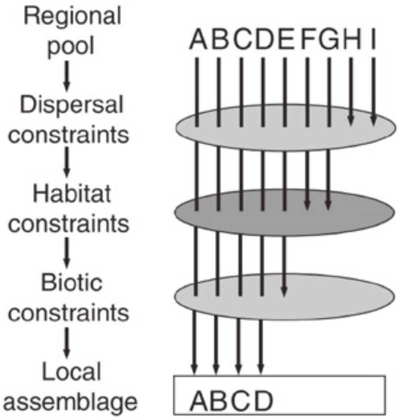


Figure 1. Theoretical framework (copied from Lake, Bond & Reich, 2007) depicting how the regional species pool encounters several dispersal, biotic and local constraints before the actual community, or local assemblage, is established.

Box 1: Aquatic macroinvertebrates

Aquatic macroinvertebrates are a very diverse group of organisms, including amongst others molluscs, crustaceans, oligochaetes and insects. The latter class contains the vast majority of species richness in most freshwater streams and has species that have aquatic as well as terrestrial life stages.

As first order consumers, aquatic macroinvertebrates are an essential part of the food web as they are eaten by fish, birds and other second order consumers (Lancaster & Briers, 2007). Macroinvertebrates graze, gather and/or predate on algae, phytoplankton, microorganisms and other macroinvertebrates during their aquatic stages (Cummins & Klug, 1979; Schmera et al., 2017).

Many aquatic invertebrate species contribute substantially to a variety of ecosystem processes (McKie et al. 2008). Important macroinvertebrate mediated ecosystem processes are bioturbation of sediments (Mermillod-Blondin et al. 2002; Jonsson & Malmqvist, 2003; Nogaro et al., 2009) and the decomposition of organic material (Gessner et al., 2010). The disappearance of these species could therefore have a considerable impact on stream ecosystem functioning.

Dispersal filter

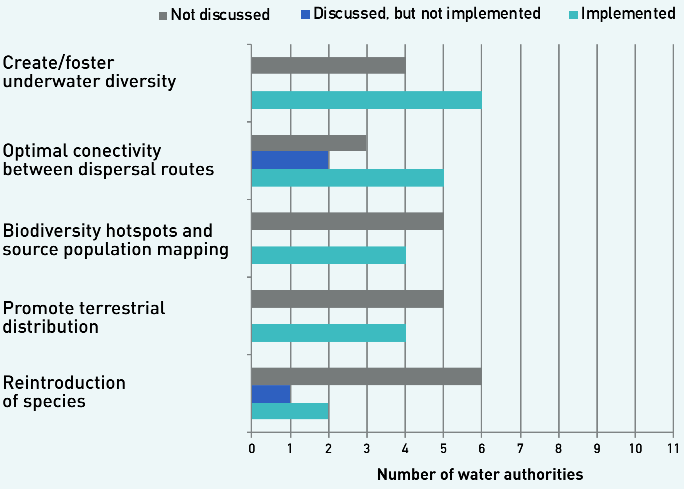
In order to restore streams not only physically and chemically but also ecologically, biota need to be able to disperse to and colonize the adjusted or newly created habitat (Smock, 2006; Parkyn & Smith, 2011). Within networks of freshwater streams, many large or small communities exist at different spatial and temporal scales (Heino et al., 2015; Leibold et al., 2016). These communities are linked by the dispersal of several, potentially interacting, species and are therefore termed 'metacommunities'. Together they form the regional species pool and are the source for all potential colonists (Sundermann et al., 2011; Tonkin et al., 2014). Unfortunately, the process of dispersal from the regional species pool to the local community is largely overlooked in restoration design (Box 2). Being unaware of ecological or anthropogenic barriers affecting habitat connectivity, or overestimating dispersal capacity of specific species, causes ecological restoration practices to be insufficient for successful recovery (Palmer et al., 2010; Roni et al., 2008).

Box 2: Considerations of water authorities regarding the dispersal of aquatic biota

To investigate how water authorities deal with specific ecological knowledge gaps in the design of stream restoration projects, we held a survey among project managers of restoration projects in the Netherlands. We focused on issues related with dispersal capacity and the measures that are currently taken to overcome these issues.

When asked: Is dispersal capacity taken into account in the design and implementation of stream restoration projects? Almost half (45%) of the water authorities (n=11) answered that they did not consider this aspect at all, 36% only considered fish dispersal and migration (e.g. fish passages) and 18% considered fish migration as well as aquatic macroinvertebrate dispersal, but none (0%) of the water authorities focused exclusively on aquatic macroinvertebrates.

When asked which measures are implemented to improve dispersal, the following measures were indicated:



These results show that stream restoration design is not always guided by the dispersal capacities of aquatic macroinvertebrates and that restoration projects are performed without having a clear image of current source population status. This could lead to an unsuccessful restoration outcome, merely by an unawareness and understanding of occurring dispersal mechanisms and subsequent limitations.

It seems that creating suitable habitat for species is essential for ecological restoration, but only once certain dispersal requirements have been fulfilled can this habitat be colonized. Therefore, I state that the ecological reasoning behind stream restoration requires an extra step that identifies how the spatial and temporal characteristics of the environment interact with an organism's dispersal biology that limits or facilitates movement between habitats; in other words, we should incorporate knowledge on how habitat connectivity and species dispersal capacity affect the distribution of (meta)communities in restored lowland streams.

Connectivity of habitats

Recent studies suggest that colonization of a restored habitat is a very slow process if the connectivity within or between streams is poor (Sundermann et al., 2011; Tonkin et al., 2014; Stoll et al., 2016). Connectivity between suitable habitats involves several scales: within and between local stream habitat(s) (e.g. connectivity within and between e.g. organic substrate patches), within catchments or stream networks (hydrological connectivity between similar environments throughout the entire stream) and between catchments (connectivity through terrestrial corridors). In other words, these scales of connectivity are often categorized as habitat, stream reach and catchment (Frissell et al., 1986).

In general, studies on the effect of disconnected habitats on species distribution are limited to specific conditions and communities (Durães et al., 2016; Sarremejane et al., 2017). Some attempts have been made to provide a conceptual synthesis of the metacommunity patterns explained by stream network connectivity and dispersal modes of inhabiting species (Brown, Wahl & Swan, 2017; Tonkin et al., 2017a). However, it remains unclear whether limited connectivity effects are merely occurring to species with specific adaptations or dispersal modes, whether the effects are barrier-dependent or whether other environmental factors even amplify potential negative effects.

Dispersal capacity of macroinvertebrates

Stream macroinvertebrates are generally considered to have high dispersal capacities given the extensive geographic distributions of some species (Bunn & Hughes, 1997). Yet, there is a lot of uncertainty regarding the underlying factors that limit or benefit their dispersal (Grönroos et al., 2013; Heino et al., 2015; Baumgartner & Robinson, 2017).

In general, four modes of dispersal are distinguished for aquatic macroinvertebrates; aerial active, aerial passive, aquatic active and aquatic passive dispersal (Smock, 2006). Active dispersal demands self-generated movements of individual organisms (e.g. through flight, swimming or walking), while passive dispersal entails movements achieved by use of an external vector (e.g. animals, wind or water) (Bilton, Freeland & Okamura, 2001). Aerial active dispersal of macroinvertebrate species is mostly upstream (Elliot, 2003). This is thought to be a trait evolved to counter downstream drift and because upstream waters tend to be of higher quality (Parkyn & Smith, 2011) and to locate optimal habitat condition for oviposition (Müller, 1982). Downstream drift events increase as disturbance events occur more frequently. Invertebrates initiate drift when local circumstances are inhospitable, such as increased siltation events (Smock, 2006) or to escape predation or overpopulation. While the majority of within stream dispersal is caused by passive drift, overland dispersal between streams mainly happens through active flight (Kovats, Ciborowski & Corkum, 1996) and sometimes crawling (e.g. crayfish).

In connected headwaters and mid-order streams, the often-described succession pattern is that aerial active generalists colonize most rapidly and weakly dispersing specialists immigrate much later (Winking et al., 2014; Li et al., 2016). Taxa of smaller sizes, with short life cycles, are expected to be abundant in early successional stages, while at advanced stages of succession, taxa with larger body sizes, longer life-cycle durations, and passive dispersal modes arrive (Miguel-Chinchilla et al., 2014).

It should be noted that life history strategies differ between species and can cause different timing of dispersal (Verberk et al., 2008a,b). Here seasonality is of great importance, since it plays a large role in the emergence en subsequent dispersal of aquatic insects (Williams & Hynes, 1976). For example, Ephemeroptera are known to be triggered by water temperature, e.g. in spring time, and emerge in huge flocks (Elliott, 1972). Coleoptera are also known to disperse rapidly after water temperature rises, to prevent drought diminishing their habitat (Miguélez & Valladares, 2008). Stochastic weather events, related to season in our temperate climate, such as heavy rainfall and wind or heath waves, could thus affect dispersal events.

Metacommunities of aquatic macroinvertebrates

To contextualize the observed patterns of distribution in catchments with varying levels of connectivity and with species with varying dispersal capacities, metacommunity structure should be taken into account. Leibold et al. (2004) and Holyoak et al. (2005) suggest that there are four main paradigms in the theory of metacommunities. These are the neutral, patch-dynamics, species sorting and mass effects model. The neutral model states that all species are ecologically equivalent and community composition is shaped by dispersal limitation, extinction or speciation. Adversative, the patch-dynamics model states that species are either good colonists or good competitors and the community is shaped by a colonist-competitor competition as succession continues. The species-sorting model assumes that species settle in their preferred environment in terms of resources and particular habitat, depending on the species dispersal capacity. The mass effect model assumes high rates of dispersal to outweigh environmental sorting, causing species to also occupy unfavourable habitats. In stream networks, with their dendritic structure, the paradigm behind community succession seems to depend on how well-connected habitats are (Durães et al., 2016; Hill et al., 2017; Sarremejane et al., 2017).

It is still unclear how community assembly mechanisms apply to a restored lowland stream with a diverse group of macroinvertebrates at various spatial and temporal scales. Additionally, we still don't know how functional traits (Box 3) and life history events relate to community development when different levels of habitat connectivity are present and hence, different community assembly mechanisms could be at play.

Environmental filter

After biota leave the regional species pool by the process of dispersal and before they enter the local assemblage (Fig. 1), local conditions will determine whether they survive and reproduce (Heino, 2013). The combination of habitat constraints (i.e., the habitat has to fit the ecological preferences of the species) and biotic interactions (predation, facilitation, competition etc.) has a profound effect on the local presence and fitness of a species (Leibold et al., 2004; Schuwirth, Dietzel & Reichert, 2016; Borgy et al., 2017). This combination is often defined as the 'environmental filter' (Poff, 1997).

Box 3: Trait-based freshwater ecology

Macroinvertebrates have different adaptations, or functional traits, to be able to survive, reproduce and sustain in aquatic environments (Vannote et al., 1980; Townsend & Hildrew, 1994) and as such contribute to the ecosystem functioning. The traits species have to perform their functions can be ecological (e.g. flow velocity preferences, salinity preferences) or biological (e.g. morphology characteristics, life span, feeding habits) (Schmera et al., 2015).

Previous work and current ecological legislation often focused on the relationship between biodiversity and ecosystem functioning. The more biodiverse, the more roles species can take over from each other and thus the more resilient an ecosystem is. Therefore, current research focuses more and more on the role that an organism has in an ecosystem. The roles, or so-called functions, a species possesses supports a variety of ecosystem processes, such as decomposition, production or respiration. Understanding the relationship between functional traits and the environment, helps us predict the response of an ecosystem after disturbance and subsequent restoration.

In each chapter, we use a trait-based approach to link the presence of specific species, or the entire community, to potential ecosystem processes and thereby to the functioning.

Several community assembly studies have incorporated environmental filtering in their conceptual frameworks, but since many different environmental conditions can affect biota in many different ways, these frameworks remain to be partly theoretical (Webb et al., 2010; Grönroos et al., 2013; Laughlin, 2014; Heino et al., 2015).

Habitat heterogeneity and biodiversity

As each species has specific adaptations to withstand and succeed in a specific environment, a wide range of mixed habitat patches (habitat heterogeneity) potentially leads to an increased chance of biodiversity (Brosse et al., 2003; Milesi et al., 2016). Habitat diversity (the number of types of habitats in an area), habitat configuration (the spatial arrangement of patches) and environmental variability within a habitat over time, all contribute to habitat heterogeneity (Li & Reynolds, 1995).

In terms of habitat diversity, streams naturally have many different organic and inorganic substrate types on the stream bed, such as patches of pebbles, leaf debris and dead wood trunks. These serve, amongst others, as physical refuges from larger predators and physical stress like peak flows (Lancaster & Hildrew, 1993; Bond & Downes, 2000; Lancaster, 2000), as well as a food resource for many macroinvertebrates (Reice, 1980; Arunachalam et al., 1991).

A limited habitat complexity (i.e. loss of habitat mosaics) can decrease suitable habitat and quantity of patch edges, and as such, increasing competition for resources. Furthermore, small patches can become unsuitable to sustain a population, while large distances between patches can be difficult to bridge for non-mobile species (Fahrig, 2003). Palmer (1995) describes how inter-patch movements lead to predation or dislodgment risk and how stressful resource acquisition might explain the observed negative effects in a homogenized environment.

As a last contribution to habitat heterogeneity, a disturbed hydrograph can create small, remaining, isolated patches of preferred substrate over time, which are home to many macroinvertebrate species (Tolkamp, 1980; Lake, 2000; Jähnig et al., 2009; Schröder et al., 2013). With different species inhabiting these patches, intra- and interspecific interactions are expected to occur and have a large effect on species livelihood.

To sum up, previous studies have shown that lowland stream macroinvertebrates have specific substrate and physical structure preferences, as well as the configuration of these resources in space and time (Resh et al., 1988; Lake, 2000). If these resources disappear, get fragmented or unevenly distributed, this may affect the livelihood of the associated species (Tolkamp, 1980; Townsend & Hildrew 1994; Jähnig et al., 2009; Heino 2013, Schröder et al., 2013). Unravelling the weight of specific environmental filters on the actual community would provide a more detailed insight into the distribution of aquatic macroinvertebrates. Therefore, I argue that specific studies on the effect of habitat heterogeneity and biotic interaction on species survival and fitness could elucidate the limited recolonization of restored stream reaches.

Spatial and temporal scale effects

At habitat-scale, organisms are directly affected by resources of the streambed. The availability and configuration of the resources can have a positive or negative effect on population densities, emergence success and larval biomass (Palmer, 1995; Palmer et al., 2000; Silver et al., 2000; Lancaster and Downes, 2014). Vannote et al.'s (1980) river continuum concept states that the local community of producers and consumers become established in harmony with the physical conditions of the channel, but differ per stream order.

At reach scale, organisms have the potential to disperse from one population to the next, if connectivity is sufficient. However, there is a shift in dispersal-based processes driving assembly mechanisms, from dispersal limitation in the isolated headwater streams to randomness in connected headwater and isolated mid-order streams, and to mass effects in the most connected mid-order streams (Sarremejane et al., 2017).

At catchment scale, organisms are often limited by their dispersal capacity to bridge great distances (Kovats et al., 1996). The hierarchical, dendritic structure of stream systems means that dispersal between populations is considerably more difficult than between populations living in multidirectional systems (Fagan, 2002; Lowe, 2002). A recent study showed that weak dispersers are more affected by site-specific factors, intermediate dispersers by landscape-level factors, and strong dispersers showed no discernable pattern (Cañedo-Argüelles et al., 2015).

In terms of time scale effects, it has been found that temporal environmental fluctuations can have a strong effect on biodiversity. Communities in highly seasonal environments exhibited strong shifts in community structure, whereas communities in unseasonal environments fluctuate randomly (Tonkin et al., 2017b). Temporal parameters can trigger emergence in macroinvertebrates (Corbet, 1964), have an impact on available habitat substrata and can affect substratum stability (Lancaster & Belyea, 1997). These impacts either take place in a predictable or random pattern, over the course of one year or over decades.

By combining existing knowledge of dispersal and environmental filtering on community assembly, it can be concluded that different patterns and processes occur at different scales. I state that we should consider each spatial scale separately and add the relevant temporal scale, when interpreting results on species distribution and potential dispersal or environmental limitations. This will benefit our understanding of the observed ecological patterns in restored streams at various scales.

Objectives of this thesis

Until now, it is still unclear what limits or benefits the dispersal and subsequent colonization of aquatic invertebrates under changed environmental conditions. Several studies have focused on functional traits and dispersal capacity (Kappes & Haase, 2012; Miguel-Chinchilla et al., 2014; Li et al., 2016; Winking et al., 2016), but overlooked to put these effects in an ecosystem context where other environmental filters are a major actor as well. Therefore, the aim of this thesis is to identify the mechanisms that underlie dispersal and colonization of aquatic macroinvertebrates in lowland streams at various spatial and temporal scales. We perform this study in order to better understand the limited ecological recovery of restored streams.

Thesis outline

To meet the aim of this thesis, we performed several experimental and observational studies in controlled, semi-controlled and natural systems. This combined effort allowed us to describe dispersal processes on various scales, from long-distance dispersal of adult macroinvertebrates between catchments to movements of macroinvertebrates between organic patches.

Overland dispersal of aquatic macroinvertebrates ('between catchment' –scale) is regarded as an important source of new species for isolated stream reaches and restored stream habitat. In **chapter two** the overland distances that species can cover were experimentally tested and the main drivers for dispersal were assessed. We hypothesized that long-distance dispersal events are rare and limited in range, due to fragmented terrestrial corridors and limited dispersal capacity.

At catchment-scale, both aerial and aquatic dispersal are considered to be important parameters for community development. When a stream is viewed as a combination of many small populations, it becomes clear that the entire catchment ('catchment' –scale) plays a role in community composition shifts. In **chapter three** key predictors of dispersal were studied, such as dispersal traits, distance to source pool and population abundance and answered what the most important drivers of dispersal within a catchment are. Upstream and downstream source populations are present in this 20 km stream trajectory. We tested the hypothesis that source pool properties are more decisive of effective dispersal than active dispersal traits.

In **chapter four** we looked at the process of colonization at stream stretch scale, considering the whole invertebrate community in a restored stream trajectory ('stream reach' – scale). We focused on temporal shifts, or successional patterns, in community composition related to functional traits (active vs passive dispersal), local environment and seasonality in a newly created stream reach. We hypothesized that rapid ecological succession will take place in this highly connective network, mitigated by seasonal effects and corresponding life history events.

After species have reached new habitat, the environmental conditions determine its livelihood. Species-specific habitat preferences and behaviour can determine resilience to habitat fragmentation, but how does habitat fragmentation affect survival and fitness? The effect of habitat fragmentation ('between habitats' – scale) on these parameters of two species of Trichoptera is studied in **chapter five**. Increased distances between food resources requires additional movement and increases intraspecific competition which led to the hypothesis that habitat fragmentation has negative effects for habitat-specialist species.

In **chapter six** the effects of biotic interactions within the habitat ('within habitat' –scale) were studied. As suitable habitat patches become more isolated within the matrix of substrata on the stream bottom, due to a disturbed hydrograph and lack of organic material, increasing interactions between inhabiting species are inevitable. We studied the habitat preferences of two species of shredders (Trichoptera) to answer the question of how biotic interactions affect their survival and fitness. We hypothesized that both species have adapted to live interspecifically and gain benefits from this interaction.

In **chapter seven** all our findings were put in an ecological framework. Thereafter the potential implications for stream restoration design and monitoring are discussed. I also provide an outlook for future studies related to invertebrate dispersal and stream restoration.

2

Stochasticity in long-distance dispersal of aquatic macroinvertebrates

Abstract

Stream restoration efforts are not as successful as anticipated in terms of recolonization by aquatic macroinvertebrates. Reasons for this could amongst others be related to dispersal limitations of aquatic macroinvertebrates. Dispersal and colonization are key processes in the recolonization of new habitat, but especially long-distance dispersal mechanisms are poorly understood. Long-distance dispersal can be affected by biotic and abiotic conditions. Studies on overland dispersal are often performed by using light (Malaise) traps, which do not include canopy dispersers or the stochastic effect of (extreme) weather events. We aimed to unravel spatial and temporal patterns in long-distance dispersal, with a taxonomic and functional trait approach. Over three years, we followed colonization of six outdoor mesocosms, placed along a transect with distances of 0, 2, 5, 14, 31 and 36 km from the nearest stream. The results show that active overland dispersal only occurs to the mesocosm placed in the close vicinity of a natural stream. In all other mesocosms, the colonizing rheophiles arrived randomly over the course of the years in a stochastic way. All rheophilic taxa found in the mesocosms were insects, which emphasizes the need for wings to either actively or passively disperse overland. These results indicate that communities in new stream reaches or headwaters with limited connectivity, will not develop rapidly if they can only rely on colonists reaching the habitat via overland and long-distance dispersal. Both terrestrial habitat fragmentation and weather events (storm direction) could affect these dispersal processes in a negative and positive way respectively. We conclude that water authorities should be aware of the limited overland dispersal to isolated or restored reaches. So, recolonization will be a slow process. When a faster recovery of stream communities is desired, assisted recolonization would be an option.

Keywords: Colonization, Stream restoration, Habitat fragmentation, Insects, Community Ecology

Introduction

Worldwide, tremendous efforts are being made to restore freshwater streams in order to reverse anthropogenic damage from the past centuries (Bernhardt et al., 2007; Didderen & Verdonschot, 2009). This restoration of streams is often performed by making hydromorphological adjustments to the physical habitat (Palmer et al., 2014). The assumption is that if the habitat is created, biota will colonize it and ecological restoration will follow naturally ('The field of dreams hypothesis' (Bond & Lake, 2003)). However, studies find that populations of invertebrate biota often stay depleted for decades in terms of abundance and diversity (Palmer et al., 2010; Roni et al., 2008; Miller et al., 2010; Verdonschot et al., 2016).

Recolonization by invertebrates after restoration practices appears to be limited, especially in isolated headwaters (Brederveld et al., 2011; Sarremejane et al., 2017). Reasons for this are thought to be related to dispersal limitations. Several studies have focused on dispersal mechanisms of invertebrates, mainly in upstream (by active flight or swimming) and downstream (drift) direction (Bilton et al., 2001; Smock, 2006; Heino et al., 2015). In contrast, the mechanisms of overland or long-distance dispersal of stream macroinvertebrates is poorly studied, while this is an essential process to recolonize isolated stream reaches and adjacent catchments.

Organisms have adapted to disperse actively and passively across a variety of lengths (Bohonak & Jenkins, 2003) and the act of dispersal is part of the life history of many taxa (Massol et al., 2017; Tonkin et al., 2017a). For example, adult females of Ephemeroptera and Trichoptera fly upstream prior to oviposition (Bird & Hynes, 1980; Jones & Resh, 1988) and compensate for downstream drift by larvae (Müller, 1954, 1982). Dispersal can also be triggered by environmental conditions such as unfavorable hydrological conditions (hydropeaking), pollution by chemicals, and overpopulation resulting in competition (Bilton et al., 2001; Fronhofer et al., 2015). While the majority of within-stream dispersal is caused by passive drift, the dispersal between streams (overland dispersal) happens either through active flight, or is wind- or vector-mediated (Horváth et al., 2016).

Several biotic and abiotic factors are thought to affect overland dispersal. For example, riparian vegetation and other terrestrial areas serve as corridors, resting areas, mating areas and refuge and are therefore essential structures for survival and dispersal during the, often short-lived, adult life stage. Furthermore, temperature, wind, humidity and cloud-cover also affect emergence, timing and duration of flight (Kovats et al., 1996). Each of these factors can be very random in time and space, and therefore cause random patterns in dispersal distance and frequency. Specifically, when all factors are combined, patterns in dispersal are hard to elucidate. This causes many studies to study the effect of only one parameter on a short temporal and spatial scale, which gives an incomplete picture of recolonization patterns by overland dispersal. Furthermore, most studies on overland dispersal entail only adult insects and use Malaise traps placed near natural streams over short ranges (Hoffsten, 2004; Macneale et al., 2005; Petersen et al., 1999; Petersen et al., 2004). However, this method is prone to neglect rare dispersal events (e.g. storms) and overlooks dispersal at canopy-level. At this moment, passive ways of long-range dispersal have almost never been quantified, as it is extremely difficult to capture-mark-recapture aquatic invertebrates (Bilton et al., 2001).

With outdoor mesocosms, placed far apart at different distances from natural running waters, and monitored for 3 years, we aim to find what limits or benefits overland dispersal of aquatic invertebrates. This method allows us to include any seasonal, stochastic event that affects dispersal and takes the whole invertebrate community (active and passive dispersers) into account. Our hypotheses are I) Specific traits are required for overland dispersal, related to the ability to fly actively or to use vectors, II) Invertebrate active dispersal will decrease with increasing distance, as species will not be able to fly infinitely due to the unsuitable environmental conditions that they encounter and because longer time needed to reach the destination costs more energy, III) Successful passive dispersal is stochastic, as weather events, especially wind, occur more or less random and as such will have random effects on dispersal occurrence and direction. The outcome of this study will elucidate dispersal limitation and can be used for future stream restoration purposes.

Material and methods

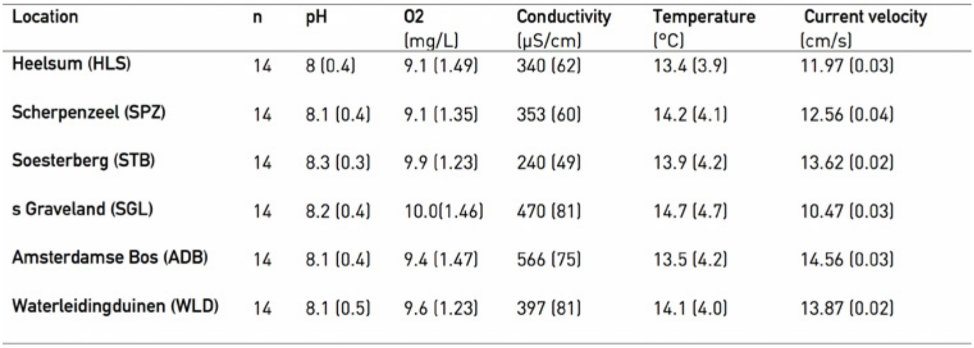
Mesocosm set-up and location

Six identical mesocosms were placed at six different outdoor locations along a transect from the western part of the Netherlands to the center of the Netherlands. Each mesocosm consisted of an oval shaped reservoir of PE material, with a partitioning wall and a pump (Aquamax Eco Premium 16000) which created a revolving water system. The mesocosm had a length of 4 m, width of 1 m, and depth of 0.5 m and contained 900 l of water. To prevent water from overflowing the mesocosm, a small hole was drilled at 2 cm below the top, covered with gauze (mesh size 250 µm). Each mesocosm was covered with a steel railing (mesh size 5 x 10 cm) excluding birds as vectors. Additionally, the railing was covered with a finer gauze net (mesh size 2 x 2 cm) during autumn, to prevent autumn shed leaves to enter the mesocosm. Each mesocosm was dug into the soil 40 cm deep and hence, had a resurrected wall of 10 cm above ground to prevent frogs and other amphibians from entering (Figure 2.1).

Figure 2.1. Left: top down view of the stream mesocosm with organic and inorganic patches to mimic a natural streambed environment, blue arrows indicate water flow direction. Right: picture of the actual mesocosm. Each mesocosm was placed at an outdoor location in the summer of 2014 and monitored regularly until the summer of 2017.

The pump had a capacity of 15600 l/hr which created an average stream velocity of 12 cm/s. Current velocity, conductivity, pH, temperature and dissolved oxygen concentration were measured at each sampling moment (Table 2.1). The bottom of the stream consisted of 15 cm of sand (grain size 1-3 mm, origin IJsselmeer, certified non-polluted quality, approx. 600 kg per mesocosm) and several patches of pebbles (pebble size 1-4 cm, 75 kg per mesocosm). Furthermore, each mesocosm had six small cages with leaves (abscised oak *Quercus robur*, total volume of 3.7 m^3), aquatic plants (*Berula erecta*) and six stone bricks (20 cm x 10 cm x 8 cm). All substrates in the artificial stream had been chosen to mimic the organic and inorganic substratum of a natural stream. This provided food and refuge for aquatic macroinvertebrates. The artificial streams had been filled with tap water, which was replaced by rain water over time. Additional tap water was added in one specifically hot summer in 2016. The mesocosms were never completely frozen during wintertime.

Table 2.1 Mean (sd) of hydrological, physical and chemical characteristics of the mesocosms. Each mesocosm was sampled 14 times over the course of three years.



Location	n	pH	O2 (mg/L)	Conductivity (µS/cm)	Temperature (°C)	Current velocity (cm/s)
Heelsum (HLS)	14	8 (0.4)	9.1 (1.49)	340 (62)	13.4 (3.9)	11.97 (0.03)
Scherpenzeel (SPZ)	14	8.1 (0.4)	9.1 (1.35)	353 (60)	14.2 (4.1)	12.56 (0.04)
Soesterberg (STB)	14	8.3 (0.3)	9.9 (1.23)	240 (49)	13.9 (4.2)	13.62 (0.02)
s Graveland (SGL)	14	8.2 (0.4)	10.0(1.46)	470 (81)	14.7 (4.7)	10.47 (0.03)
Amsterdamse Bos (ADB)	14	8.1 (0.4)	9.4 (1.47)	566 (75)	13.5 (4.2)	14.56 (0.03)
Waterleidingduinen (WLD)	14	8.1 (0.5)	9.6 (1.23)	397 (81)	14.1 (4.0)	13.87 (0.02)

The mesocosms were installed approximately 30 km apart (Table 2.2) in a shaded, wind-sheltered yet unhidden spot. The mesocosms were placed along a transect, perpendicular to the elevated eastern part of the Netherlands where natural streams are located (Fig. 2.2B), to increase the distance to the largest source of dispersing macroinvertebrates. Furthermore, this transect was chosen to give each mesocosm a similar position with regard to the prevailing wind direction, which is generally south-west. No mesocosm had a downwind source population of macroinvertebrates and therefore a more advantageous geographical position in comparison to any other mesocosm.

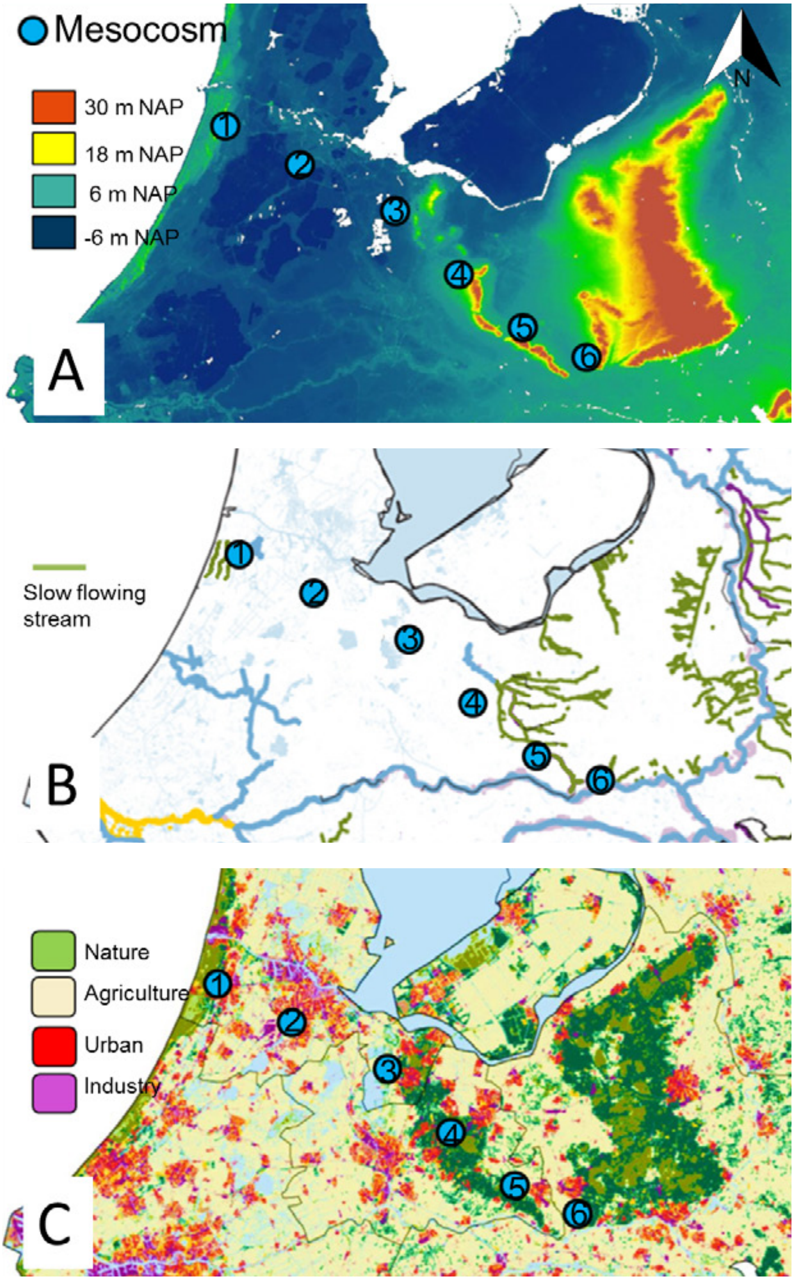
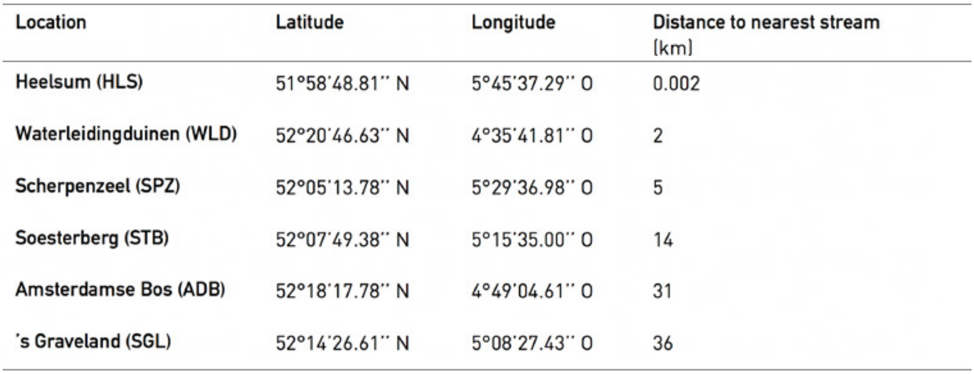


Figure 2.2. Map of the study area (the center of the Netherlands) with in the east and south-east a region with natural streams and in the west some artificial flowing waters in the Amsterdam water dunes. From west to east: 1 = Waterleidingduinen, 2=Amsterdamse Bos, 3='s Graveland, 4=Soesterberg, 5= Scherpenzeel, 6= Heelsum. A) Elevation map of the region, with most of the region being flat (blue colors), except the higher east (De Veluwe area leveling up to about 30 meters above average sea level) and the dunes in the west). B) Stream networks (green lines). C) Land-use map divided into four categories: nature, agriculture, urbanization and industry.

Table 2.2. Coordinates of mesocosm locations and distance to the nearest stream. Each mesocosm was placed approximately 30 km (25-37 km) apart and at varying distances from adjacent streams.



Location	Latitude	Longitude	Distance to nearest stream (km)
Heelsum (HLS)	51°58'48.81'' N	5°45'37.29'' O	0.002
Waterleidingduinen (WLD)	52°20'46.63'' N	4°35'41.81'' O	2
Scherpenzeel (SPZ)	52°05'13.78'' N	5°29'36.98'' O	5
Soesterberg (STB)	52°07'49.38'' N	5°15'35.00'' O	14
Amsterdamse Bos (ADB)	52°18'17.78'' N	4°49'04.61'' O	31
's Graveland (SGL)	52°14'26.61'' N	5°08'27.43'' O	36

Sample collection and processing

During the first six months, the mesocosms were sampled each month. After this period, the mesocosms were sampled each season. Invertebrate samples were taken from each substratum according to an identical method at each sampling moment: three scoops of sand were taken with the micro-macrofauna shovel (with standardized volume, Tolkamp 1980), three scoops of pebbles with the same shovel, two cages with leaves were collected using an underlying 250 µm net, similarly one brick was collected using an underlying net and one driftnet sample was taken by carefully stirring the sediment and all substrata throughout the mesocosm. This sampling procedure ensured to include all substrata and therefore all habitats present in the mesocosm. The samples were pooled and stored in a bucket. All tools used for sampling were rinsed with hot water after sampling a location.

Samples were transported to the laboratory, rinsed and stored in ethanol (70%) within 12 hours. In the laboratory, each individual was identified up to species-level if possible (Crustacea, Gastropoda, Hirudinae, Insecta: Trichoptera, Ephemeroptera, Plecoptera, Coleoptera, Simuliidae). Juveniles were identified up to genus-level. Oligochaeta and Arachnida were excluded from the analyses due to limited identification at species-level. Care was taken to ensure that taxonomic resolution was sufficient according to Haase et al. (2006). Identification was performed with the use of a dissecting microscope (120 x magnification) and a light microscope (300 x magnification).

Functional trait data

After identification, all rheophilic species were classified based on the Dutch autecology list (score 4 and 5 were listed as rheophiles, Verberk et al. 2012). Data on functional traits was provided by freshwaterecology.info, a database with taxonomic and ecological information on freshwater macroinvertebrates (Schmidt-Kloiber & Hering, 2015), combined with the trait-database EKOO (Verdonschot, 1990) and Tachet's et al. database (2010). All trait data was transformed to a binary point system, with score 1 for possession and 0 for lack of the trait. We used the following three trait categories for our analyses, based on the level of importance for colonization and presence of existing knowledge for our community: dispersal mode, time of emergence/flight and voltinism.

Data analysis

One-way ANOVA was performed with Tukey post-hoc test to find significant differences of taxa richness and abundance over time (n=14) and space (n=6). Significant differences between groups were indicated with different letters in the ancillary figures. No test results are shown for data that entail single individual observations. To compare the presence of traits between rheophilic and non-rheophilic species, all data was transformed into percentages. All data was processed in SPSS, IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA).

Results

Taxonomic differences per mesocosm

In total, 21 rheophilic taxa were identified, belonging to 5 different orders of insects. Abundances varied per taxa and mesocosm (Figure 2.3). After three years, the mesocosm in Heelsum (HLS, at 0 km from stream) was home to 17 different rheophilic taxa belonging to 8 families of aquatic invertebrates. Most other mesocosms only held taxa of the families Chironomidae and Simuliidae, apart from one observation of a Dryops larvae (Coleoptera) in Scherpenzeel (SPZ, 5 km distance to the nearest stream) and a Baetidae larvae (Ephemeroptera) in the Amsterdamse Bos (ADB, 31 km distance to the nearest stream).

Incorporating taxa abundance in rheophilic species comparisons showed that in each mesocosm Simuliidae made up the majority of the rheophilic community (SPZ: 99.5%; STB: 71.3%; SGL: 69.1%; ADB: 95.8%; WLD: 71.5%). Apart from

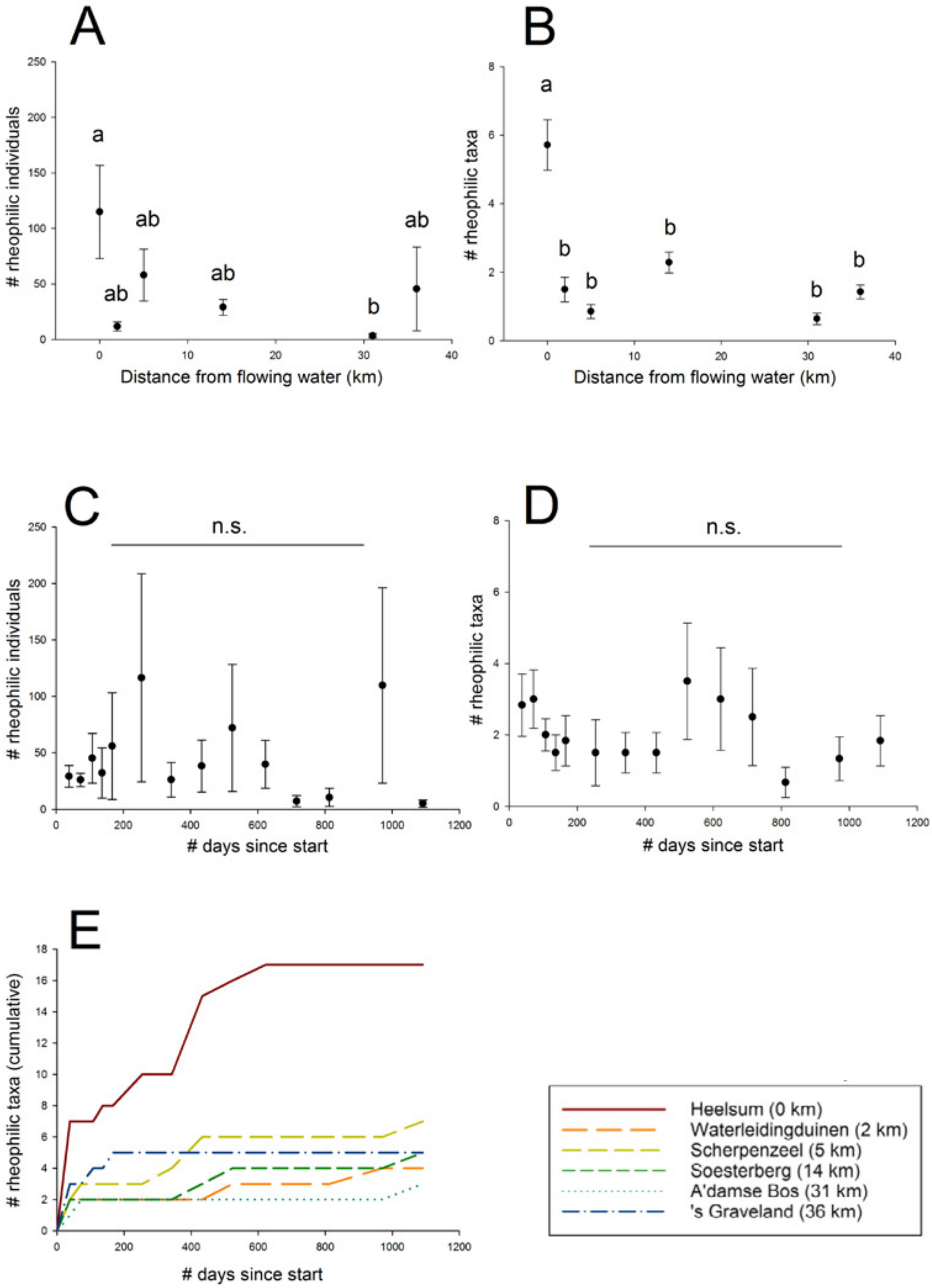


Figure 2.3. Number of rheophilic individuals and taxa plotted against distance to the nearest stream (panel A and B) and day since start of the experiment (panel C and D). Different letters over the bars indicate significant differences in ANOVA with post-hoc tests (Tukey post-hoc, P<0.05). Cumulative curves of number of colonizing rheophilic taxa over time for each mesocosm (Panel E).

the community in the Heelsum mesocosm (HLS: 2,1%), where rheophilic Chironomidae made up the largest part. Not only rheophilic taxa inhabited the mesocosms. The percentage of non-rheophiles of all aquatic invertebrates per mesocosm was HLS: 75%, SPZ: 88.5%, STB: 93.6%, SGL: 84.3%, ADB: 99.5%, WLD: 96.8%, respectively.

Colonization over time and space

Rheophilic species colonized each mesocosm within the first month after installing them in the field. The mesocosm located right next to a near-natural stream (Heelsumse beek) had the most biodiverse community of rheophilic species from the early onset and maintained a higher level of biodiversity during the experiment compared to the other mesocosms. New species kept arriving to the mesocosms throughout the duration of the experiment (Figure 2.3E).

The number of rheophilic individuals per mesocosm differed significantly between the location closest to a stream and the location at 31 km from the nearest stream, in the west of the Netherlands (Figure 2.3A. One-way ANOVA: $F_{(5,78)}=2.569$, $p=0.033$), with the highest number of individuals in the mesocosm close to the nearest stream. The number of rheophilic taxa differed significantly between the same mesocosm closest to the nearest stream and all other mesocosms (Figure 2.3B. One-way ANOVA:$F_{(5,78)}=24.049$, $p<0.001$).

The occurrence of all rheophilic individuals of all taxa summed per sampling moment showed no significant differences (Figure 2.3C. Individuals: One-way ANOVA: $F_{(1,13)}=0.703$, $p=0.754$; Figure 2.3D. Taxa: $F_{(1,13)}=0.738$, $p=0.720$)

Seasonal effects

Most taxa arrived in the summer of year 1, just after the start of the experiment, with a maximum of seven new species in the mesocosm in Heelsum. The lowest number of new colonizers appeared in wintertime, however, differences in total numbers of colonizers between seasons were not significant (one-way ANOVA: $F_{(3,23)}=0.587$, $p=0.530$). no clear seasonal or yearly pattern was found for any of the rheophilic taxa that colonized and inhabited any of the mesocosms (Figure 2.4).

All five taxa that colonized more than one mesocosm, did so randomly over the course of three years (Figure 2.5). None of these taxa showed significant differences

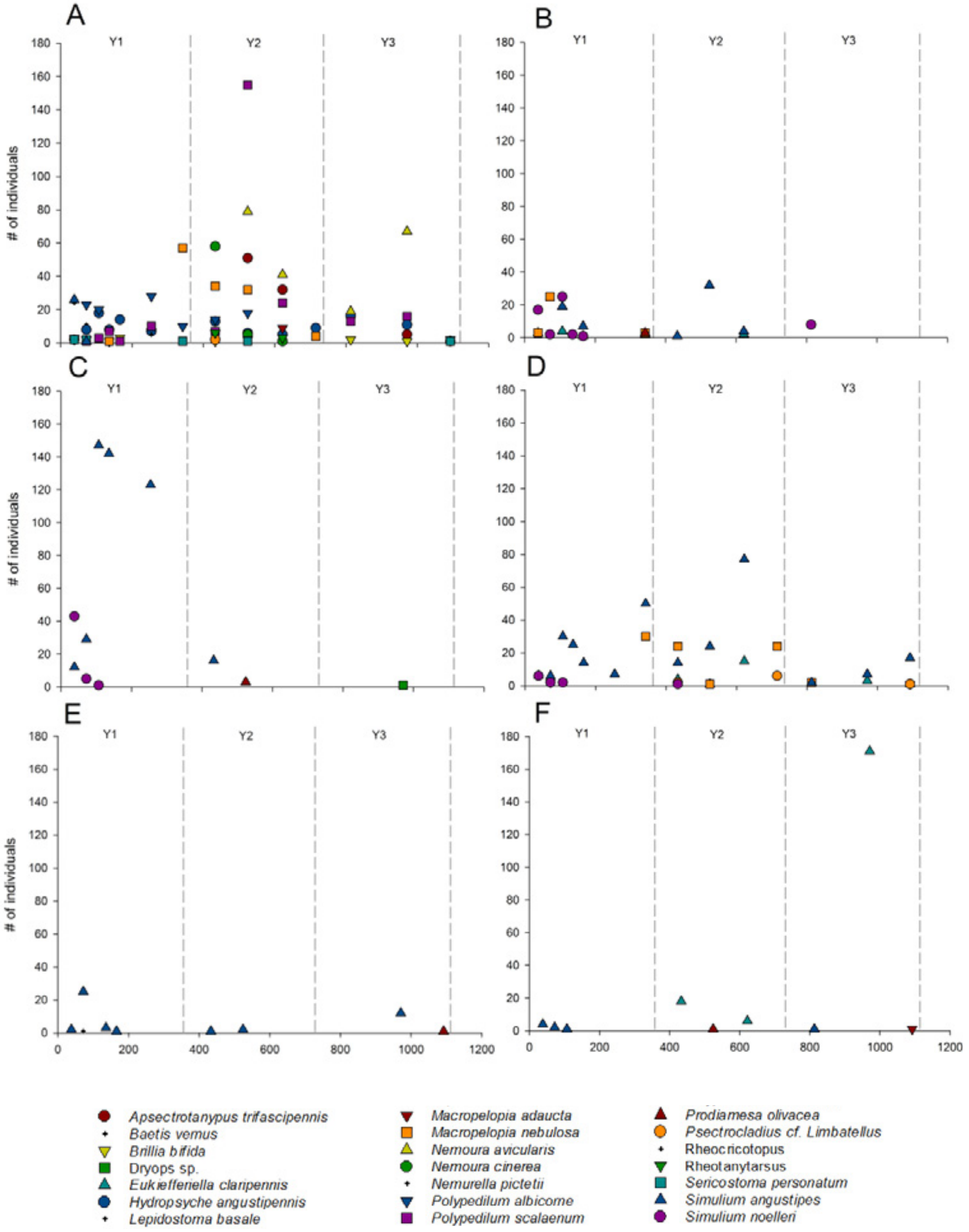


Figure 2.4. The abundance of each rheophilic taxon collected per season. Dashed lines separate the three consecutive years (Y1, Y2, Y3). Panel A) Heelsum. B) Waterleidingduinen. C) Scherpenzeel. D) Soesterberg. E) Amsterdamse Bos. F) 's Graveland. Three outliers were removed for visual clarity but were incorporated in the data analyses: 591 individual *Polypedilum albicorne* were sampled in the spring of year 1 in Heelsum, 394 individuals of *Simulium angustipes* were sampled in the spring of year 3 at 's Graveland and 291 individuals of *Simulium angustipes* were sampled in the first winter in Scherpenzeel.

in occurrence over seasons per year: *Simulium noelleri* (Figure 2.5A. One-way ANOVA: $F_{(4,13)}=1.760$, $p=0.221$), *Simulium angustipes* (Figure 2.5B. One-way ANOVA: $F_{(10,3)}=0.856$, $p=0.582$), *Macropelopia nebulosa* (Figure 2.5D. One-way ANOVA: $F_{(7,13)}=0.689$, $p=0.683$), *Eukiefferiella claripennis* (Figure 2.5E. One-way ANOVA: $F_{(8,13)}=0.404$, $p=0.872$). Statistical results are absent for *Prodiamesa olivacea* since most observations entailed single individuals (Figure 2.5C). Notable is that the Simuliidae (*Simulium noelleri* and *Simulium angustipes*) arrived in five out of six mesocosms within the first month of the experiment.

Functional traits

In terms of dispersal mode, all of the found colonizing rheophilic taxa had aerial active and aquatic passive dispersal abilities (Fig. 2.6). Few taxa scored as aerial passive dispersers as well as aerial active dispersers, though all active dispersers can also be moved passively by wind. The majority of all rheophilic taxa emerged in spring or summer (86 and 90 % respectively), while autumn also appeared to be common (76 % of taxa) for emergence and flight in these colonizers. Moreover, the majority (43) of all colonizing taxa showed a univoltinistic life cycle, while bivoltinism, trivoltinism and multivoltinism also occurred for 57, 48 and 33 % of all taxa respectively (Fig 2.6).

For part of the 124 non-rheophilic taxa that were found in the mesocosms, information on the same trait categories as selected for the rheophilic species was present in the trait databases. When comparing the traits between non-rheophilic taxa and rheophilic taxa, we found that rheophilic taxa were less aerial passive than non-rheophilic taxa (14 vs 44 % % of all taxa respectively) and more aerial active (100 vs 76 %). Furthermore, rheophilic taxa had no semivoltine life cycles, while 60% of the non-rheophiles did. In contrast, 48% of the rheophilic taxa had trivoltine life cycles and 33% was multivoltine, while none of the non-rheophilic taxa possessed these traits.

Discussion

Our results show that overland dispersal took place in close proximity of the stream but was already strongly limited at approximately 2 km from the nearest stream. Such limited dispersal distance was also described by multiple other studies (Kovats et al., 1996; Petersen et al., 1999, 2004; Macneale et al., 2005; Masters et al., 2007; Kureck & Fontes, 1996; Collier & Smith, 1998), where light traps were used as a

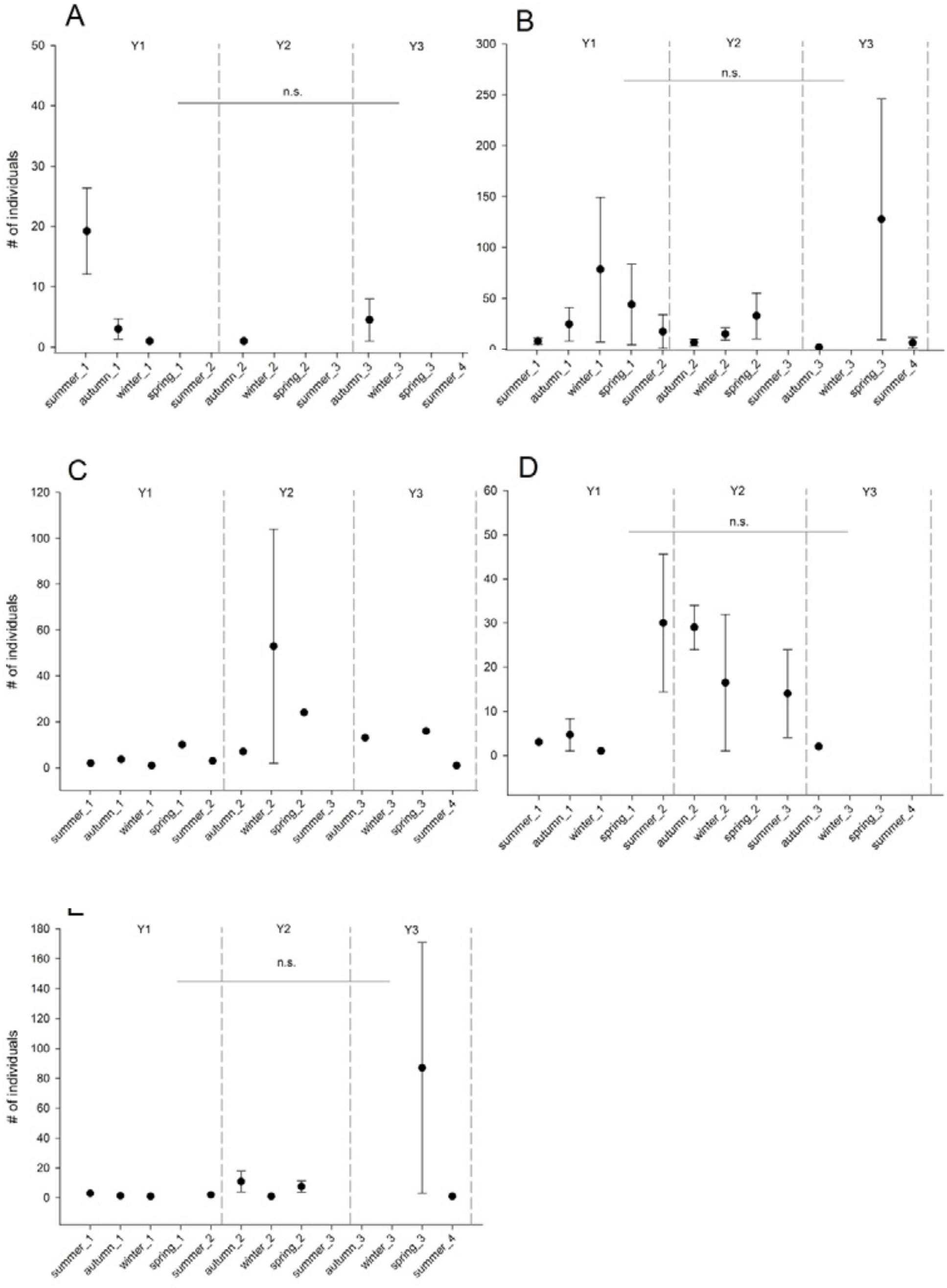


Figure 2.5. Number of individuals of the five rheophilic taxa (# of individuals) that were found in more than one mesocosm plotted against season. Dashed lines separate three consecutive years (Y1, Y2, Y3). A. *Simulium noelleri*, *B. Simulium angustipes*, *C. Prodiamesa olivacea*, *D. Macropelopia nebulosa*, *C. Eukieferriella claripennis.* No significant differences were found in abundance over time.

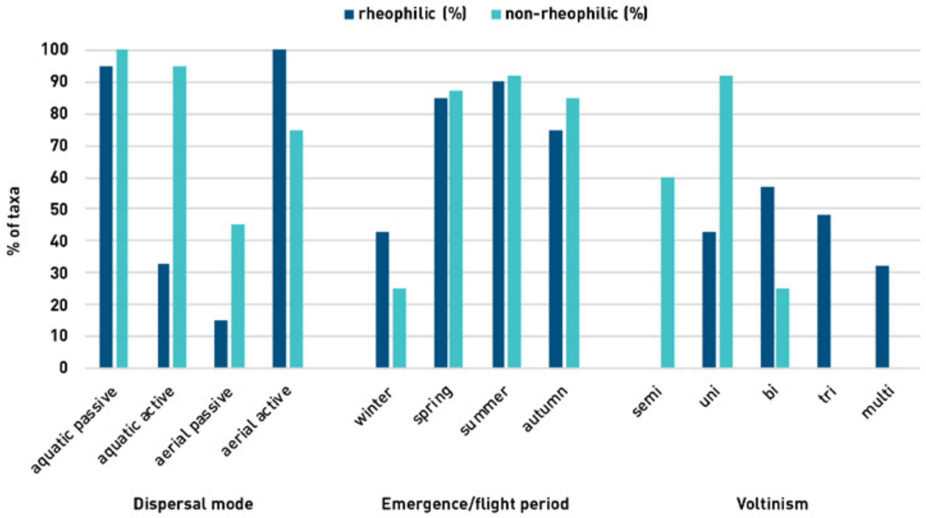


Figure 2.6. Colonizing rheophilic (n=21) and non-rheophilic taxa (n=25) possessed specific traits, categorized by dispersal mode, emergence/flight period and voltinism. Traits were scored binary, with possession of the trait as 1 and absence of trait as 0. Since each taxon can possess several traits from one category, the maximum percentage of all trait categories combined exceeds 100%.

method to estimate dispersal distance. In general, many species only disperse over short distances and stay as close as possible to the location of emergence, in other words stay within a so-called 'home-range' (Verdonschot & Besse-Lototskaya, 2014). However, long-distance flight occurs and is needed for the colonization of new, often isolated, habitats.

Long distance dispersal

In the mesocosm nearest to a natural stream, all colonizing taxa were insects belonging to the order of Diptera, Coleoptera, Trichoptera, Plecoptera and Ephemeroptera. In the mesocosms at further distances, all colonizing rheophiles belonged to the order of Diptera, except for two observations of two species of Coleoptera and Ephemeroptera, respectively. Two species of Simuliidae, that are known to be fast colonizers (Pegel, 1980), colonized the majority of all mesocosms within the first month of the experiment. These observations indicate that no taxonomic orders, except for Diptera, have the capacity to disperse over distances of 2 km or more within the three years of observation.

For several taxa, it is reported that females disperse relatively far to new habitat to deposit eggs as the final act of their life cycle (Vander Vorste et al., 2016). To actively do so, their life span has to be sufficiently long in order to reach these areas, as they are generally weak flyers and do not take up any resources after emergence (Jannot et al., 2007). However, while active aerial dispersal, by winged adults is generally seen as the most important mechanism for overland dispersal, passive aerial dispersal can be a successful dispersal mechanism as well to reach areas far away (Baumgartner & Robinson, 2017).

Passive aerial dispersal occurs when organisms are transported outside of waterways through wind or animal vectors (Cáceres & Soluk, 2002). At high wind speeds, long distances can be travelled quickly, causing also passive aerial dispersers or taxa with a shorter life span to reach areas far away (Corkum, 1987). Aquatic organisms can also be transported to other water bodies by other animals, such as mammals, birds, amphibians and adult insects by carrying eggs, larvae or adults on their body or by carrying egg propagules or even resting stages in their stomach. Although we did not find any hololimnic (organisms with a fully aquatic life cycle) rheophile taxa in our mesocosms, the presence of non-rheophilic hololimnic invertebrates indeed suggests that the mesocosms were suited for such passive (aerial or terrestrial) transport. In addition, new taxa continuously reached, colonized and survived in the mesocosms over time, even after 3 years while the habitat remained similar in the semi-controlled mesocosms. No clear seasonal or yearly patterns were found for any of the taxa that colonized and inhabited each mesocosm and all taxa that colonized more than one mesocosm, did so randomly over the course of three years. This all indicates that long-distance dispersal, possibly mediated by vectors such as wind or animals, is merely a stochastic process (Cáceres & Soluk, 2002; Cohen & Shurin 2003), and that passive dispersal over long-distances is an important mechanism structuring invertebrate stream communities in remote areas.

Recent studies already showed that terrestrial habitat fragmentation is often also a key problem for re-colonizing newly restored areas (Brederveld et al., 2011; Delettre & Morvan, 2000; Kimer et al., 2008; Parkyn & Smith, 2011; Soons et al., 2006). The observed limited dispersal range of aquatic invertebrates in this study could also be caused by habitat fragmentation. The mesocosms were located in predominantly urbanized and agricultural areas (as is the majority of the surface of the Netherlands), with small patches of nature (forest, heathland and dunes) predominantly near the most eastern situated mesocosms. The landscape between sites might lack the necessary bushy or wooded vegetation or has barriers such as highways and housing. Therefore, also landscape and habitat fragmentation could limit stream restoration success (Heino et al., 2015; Lake et al., 2007; Palmer et al., 2007; Smith et al., 2009).

Functional traits

Macroinvertebrates have a set of functional traits that determine how they survive in and interact with their environment. We found that 100% of all colonizing rheophiles appeared to be aerial active dispersers. Aerial active dispersers become aerial passive ones when taken up by the wind and transported over longer distances. Corkum (1987) suggested that the wings of some mayfly are adapted to utilize wind for long-distance dispersal, but e.g. Johnson (1969) rarely observed mayflies and caddisflies flying at heights above 60 m. In general, take-off could be inhibited by high wind velocities (Wolfenbarger et al., 1974). Still, our results indicate that passive aerial dispersal could be an important pathway. Furthermore, small propagules also can undergo long-distance dispersal via wind or rain (Green & Figuerola, 2005). Whether that was also the case in our study is unknown as we did not sample small propagules, nor was our sampling intensity high enough. In general, large thoraces and fully developed wings maintain a greater investment in flight apparatus than small thoraces and reduced wings (Denno et al., 1989). In our study, the majority of dispersers belonged to the Diptera, especially chironomids which are species with large thoraxes though reasonably sized wings. Maybe wing size and speed of wing movement could provide these species an advantage.

The fast colonizing simulids must possess special traits but it has not previously been found what trait makes them this exceptionally successful. Pegel (1980) showed that several simulid species did colonize new substrates within a few hours. Furthermore, densities could reach sometimes several hundred individuals per 100 cm2 within a few days (e.g. Kiel, 1996; Matthaei et al., 1996). Such features point out that simulids can be early colonizers with a fast dispersal capacity and high numbers of eggs. The way of positioning themselves onto the substrate does not elucidate their successful dispersal methods, but could enhance their abundance and hence, chance of colonizing new habitat in high numbers.

Management implications

Our findings indicate that isolated stream reaches can only be recolonized by overland dispersal if they are in close proximity to the nearest source pool.

Distances between restored stream trajectories and more natural streams, or source populations, often exceed 2 km. Therefore, managers should not consider recolonization to happen quickly. If overland dispersal is the only recolonization pathway, the community will be depleted in terms of abundance and diversity.

Terrestrial habitat fragmentation most likely will have a profound effect on the limited dispersal between mesocosms and therefore on overland dispersal between catchments. Terrestrial habitat fragmentation is often listed as one of the biggest issues macroinvertebrates face (Parkyn & Smith, 2011; Bond & Lake, 2003). Agricultural areas that no longer contain woody vegetation will prevent some species from crossing from one stream or catchment to another. Urban areas also pose a potential threat to successful dispersal, since species for example use reflection of polarized light to find new waterbodies (Smith et al., 2009; McIntyre, 2000; Blakely et al., 2006) and houses and roads are a hostile environment with often unsuited micro-climatic conditions. By creating of suitable terrestrial habitat patches, such as vegetation corridors, in stream restoration projects, the dispersal of aerial dispersers could be enhanced by extending their home-range, resulting in higher colonization rates of restored stream reaches. Unfortunately, while it seems logical to include the riparian zone in stream restoration design, this is not always the case. An alternative option would be to reintroduce a viable, fitting invertebrate community to kick-start the ecological restoration in isolated stream reaches.

Conclusion

By performing a long-term, spatially extensive, outdoor mesocosm study, we showed that species were able to colonize the mesocosm that was located right next to a natural stream, but significantly less species managed to disperse to the mesocosms at 2, 5, 14, 31 and 36 kilometers from flowing water habitat. This suggests that most taxa have a limited home-range in which they mate, rest and seek refuge after they emerge from the water, and that only few taxa are able to disperse beyond that home-range. The taxa that did manage to disperse and develop in the mesocosms beyond the home-range showed no clear relationship with time (season or year) or distance of dispersal. The pattern in which the long-distance dispersers colonized the mesocosms appeared to be stochastic. This could be the result of random weather events (storms), as wind could be a vector that drives long-distance dispersal. All rheophilic taxa found in the mesocosms were insects, which emphasizes the need for an adult terrestrial winged stage to either actively or passively disperse overland. Two species of Simuliidae managed to disperse to all mesocosms in a very limited time, but the traits that make these species so successful remain elusive.

Our findings can be applied in stream restoration purposes as we now have solid evidence that only a limited number of taxa will disperse beyond their home-range and long-distance dispersal is random in time and space. To improve the success rate of restoration projects, suitable terrestrial habitat patches, such as vegetation corridors, could be created enhancing the dispersal of aerial dispersers by extending their home-range. In addition, when a faster recovery of stream communities is desired, assisted recolonization would also be an option.

Acknowledgements

We would like to thank Ton van Haaren en Ralf Verdonschot for assisting in the identification of the aquatic macroinvertebrates and many others for a helping hand during the installation of the outdoor mesocosms. Family 't Hoen, family Van der Peut, family Van der Laak, Waternet, Gemeente Amsterdam and Stichting 't Utrechts landschap have all been tremendously hospitable in allowing us to set up the mesocosms on their property for which we are grateful.

3

Dispersal distance and population abundance drive colonization of aquatic invertebrates in restored lowland streams

Abstract

Dispersal and colonization of aquatic invertebrates are key processes in the ecological restoration of lowland streams. However, drivers of these processes are still debated. Habitat connectivity and species-specific dispersal capacities are known variables to determine successful dispersal, but it is unclear to which extent they affect the observed patterns in dispersal and colonization in new communities. Metacommunity theory helps us understand how communities develop in environments with differing levels of habitat connectivity. We aim to unravel the relative importance of dispersal drivers in new mid-order lowland stream communities by performing a field study during first phase colonization over a period of 2,5 years.

Our results show that successful dispersal depends on the distance between the new habitat and the regional species pool, as well as the abundance of the population within that pool. This indicates that the community in restored streams is driven by mass effects. Taxa that colonized new habitat came from nearby sources and had a significantly lower chance of colonization as distance increased. Furthermore, a random forest analysis showed that regional species pool properties (distance, distribution and population abundance) had a more profound effect on colonization success than any functional trait regarding dispersal capacity. However, combinations of specific traits regarding life cycle, locomotion and feeding were found to increase the probability of successful colonization.

The outcome of this study indicates that stream restoration projects should focus on limiting distances between source population and denuded areas, establish stepping stones with suitable habitat throughout whole catchments and reinforce existing source populations in terms of abundance and diversity to increase recolonization of habitats after restoration practices.

Keywords: ecosystem resilience, functional traits, macroinvertebrates, metacommunity theory, random forest analysis, stream restoration

Introduction

Invertebrate dispersal and subsequent colonization are key processes in the ecological restoration of disturbed sections of streams (Smock, 2006; Parkyn & Smith, 2011). Restoration activities are usually limited to the enhancement of the physical environment and water quality, it being anticipated that stream organisms will recover rapidly and spontaneously following stream restoration (Kail & Hering, 2009; Palmer et al., 2010). However, after restoration practices, the local faunal community is often depleted for decades in terms of abundance and diversity (Miller et al., 2010; Roni et al., 2008; Verdonschot et al., 2016).

Stream invertebrates are generally considered to have high dispersal capabilities given the extensive geographic distributions of some species (Bunn & Hughes, 1997). Yet, there is a lot of uncertainty regarding the underlying causes that limit or benefit dispersal and with that community development (Grönroos et al., 2013; Heino et al., 2015; Baumgartner & Robinson, 2017). Understanding the patterns and processes of community development in mid-order lowland streams helps to prioritize when and where to apply restoration efforts.

Leibold et al. (2004) and Holyoak et al. (2005) suggest that communities are shaped by a combination of regional and local factors (environmental filtering through habitat suitability and species interaction), dispersal factors (dispersal capacity and connectivity of habitats) and stochastic events (e.g. extinction, speciation). The four main paradigms in the theory of metacommunities, defined as a set of local communities that are linked by dispersal of multiple potentially interacting species (Gilpin & Hanski, 1991; Wilson, 1992), are the neutral model, patch-dynamics, species sorting and mass effects model (Leibold et al., 2004). The neutral model states that all species are ecologically equivalent and community composition is shaped by dispersal limitation, extinction or speciation.

Adversative, the patch-dynamics model states that species are either good colonists or good competitors and the community is shaped by a colonist-competitor competition as succession continues. The species-sorting model assumes that species settle in their preferred environment in terms of resources and particular habitat, depending on the species dispersal capacity. The mass effect model assumes high rates of dispersal to outweigh environmental sorting, causing species to also occupy unfavourable habitats. In stream networks, with their dendritic structure, the paradigm behind community succession seem to differ per level of habitat connectivity (Durães et al., 2016; Hill et al., 2017; Sarremejane et al., 2017). This suggests that aquatic invertebrate metacommunity research should focus on the paradigm that relates best to the spatial structure of the aquatic network (Heino et al., 2015).

In connected headwaters and mid-order streams, the often-described succession pattern is that aerial active generalists colonize most rapidly and weakly dispersing specialists immigrate much later (Winking et al., 2014; Li et al., 2016). Taxa of smaller sizes, with short life cycles, are expected to be abundant in early successional stages, while at advanced stages of succession, taxa with larger body sizes, life-cycle durations, and passive dispersal modes arrive (Miguel-Chinchilla et al., 2014). The question remains how functional traits are related to community development when different levels of habitat connectivity are present and hence, different metacommunity assembly mechanisms are at play.

Recent findings of scale-dependent effects on invertebrate community development show that colonization depends on the distance to the nearest source pool and the taxon pool occupancy rate (Stoll et al., 2016; Tonkin et al., 2014). If the source population is further than 5 km, there is a very limited chance of colonization, regardless of dispersal mode (Sundermann, Stoll, & Haase, 2011). These findings lead to think that the mass effects model represents stream community development most accurately, where abundant neighbouring populations act as a source to colonize new areas independent of dispersal capacities or habitat quality.

The aim of this study is to unravel which metacommunity paradigm represents mid-order lowland stream community development best, in order to understand invertebrate community recovery. We test the relative importance of key predictors of effective dispersal in mid-order lowland streams after restoration practices. To do so, we performed a field study where we monitored the macroinvertebrate community for 2,5 years after restoration and compared this to the surrounding species pools.

We expect high dispersal from multiple connected stream tributaries will override environmental selection and that the mass effect model is the main assembly mechanism in mid-order streams within one catchment. Our major hypotheses are therefore 1) the closer the regional species pool the more effective dispersal will be, 2) abundant species in the regional species pool will colonize more effectively, 3) active and passive dispersers will be equally successful in reaching new habitat.

This study will elucidate the effects of the regional species pool and functional trait characteristics for macroinvertebrate distribution in lowland streams after restoration practices. Since limited dispersal by macroinvertebrates negatively affects the ecological recovery of freshwater streams, the outcome of this study is important to prioritize restoration efforts.

Material and methods

Study area

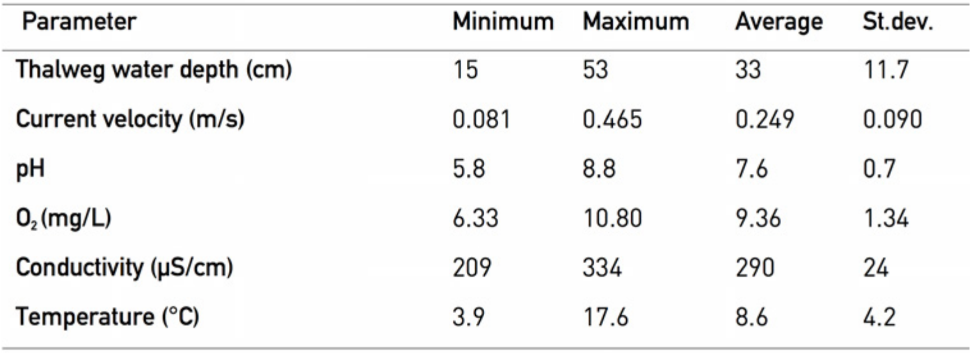
The Leuvenumse stream (52°18'55.17"N; 5°42'33.63"E) is located in the province of Gelderland, the Netherlands. It is a slow-flowing, meandering lowland stream (flow velocities ranged from 8 cm/s in winter up to 46 cm/s during spates in summer) of approximately 20 km long. The catchment consists of a main channel with 21 tributaries, with a draining area of approximately 5000 ha. The first 7 km of the stream flows on top of a clay layer and is fed by superficial ground water, the middle part of the stream is mainly fed by precipitation and the most downstream part receives both precipitation and groundwater from deeper sediment layers (Higler, 1980). The headwater is surrounded by agricultural land use area. Further downstream, the channel flows through deciduous and coniferous woodland. After this forested area, the stream continues through urban and agricultural areas and private estates before it flows into the Veluwe lake. The streambed substrate consists of sand with patches of gravel and coarse particulate organic matter. On average, the studied stream is about 4 meters wide. The deepest part of the channel (thalweg) varies between 15-53 cm, depending on season and location. Throughout the monitoring period the water temperature shifted seasonally from a minimum of 4 °C in winter up to 18 °C in summer. Dissolved oxygen, pH and conductivity were measured at each sampling moment (Table 3.1).

In the autumn of 2014, three former stream trajectories were reconnected to the existing stream by blocking parts of the main channel with sand. This caused all stream water to flow through the reconnected trajectories which had been dry forest floor without any pre-restoration communities up till then.

Sample collection and processing

In total, each newly reconnected stream trajectory was sampled 18 times over the course of 2 years and three months. After this period, no new taxa were sampled which led us to assume that community saturation slowed down the colonization rate and communities were comparable to regional species pool communities in terms of developmental level. Hester-Dendy multiplate samplers (Acorn

Table 3.1. Morphological, physical and chemical characteristics of three reconnected stream trajectories in the Leuvenumse stream, the Netherlands. These parameters were measured 18 times from September 2014 till November 2016 within each reconnected trajectory, adjacent to the streambed surface where the invertebrate samples were taken.



Parameter	Minimum	Maximum	Average	St.dev.
Thalweg water depth (cm)	15	53	33	11.7
Current velocity (m/s)	0.081	0.465	0.249	0.090
pH	5.8	8.8	7.6	0.7
O_2 (mg/L)	6.33	10.80	9.36	1.34
Conductivity (µS/cm)	209	334	290	24
Temperature (°C)	3.9	17.6	8.6	4.2

Naturalist™, Tustin, CA, USA) were placed on the stream bottom of the three new stream trajectories as soon as water started flowing. To sample the entire invertebrate community, we took three multiplate samples (total sampling area is 0.10 m2 per sampler) and two handnet samples (mesh size 250 µm, width 30 cm). The net samples consisted of a multi-habitat sample composed of 1 m of sand and 1 m of detritus habitat per sampling moment in each trajectory.

All sampled invertebrates were transported to the laboratory, where all organisms were rinsed and stored in ethanol (70%) within 12 hours after being taken from the stream. In the laboratory, each individual was identified up to species-level if possible (Crustacea, Gastropoda, Hirudinae, Insecta: Trichoptera, Ephemeroptera, Plecoptera, Coleoptera, Simuliidae). Juveniles and some invertebrate-groups were identified up to genus-level (Bivalvia, Insecta: Chironomidae). Oligochaeta and Arachnida were excluded from the analyses due to limited identification at species-level. Care was taken to ensure that taxonomic resolution was sufficient according to Haase et al. (2006). Identification was performed with the use of a dissecting microscope (120 x magnification) and a light microscope (300 x magnification).

Regional source pool

Water authority Vallei & Veluwe monitors all faunal groups in the catchment regularly since 1995 to track community development (Cuppen, 2006) and to provide data for EU Water Framework Directive reports. Monitoring was done according to a set protocol (STOWA, 2010). This protocol describes a 1-10 meter multi-habitat handnet sample per location, at least once a year in either spring or autumn. Taxa were identified up to species level. We used all monitoring data, excluding Oligochaeta and Arachnida, from 10 years prior to our field study (2005-2014). 75 separate monitoring events took place during this time span throughout the catchment, with an average of 9 monitoring moments per year (in 2006 and 2013 the area was not monitored). In total, 6 downstream and 21 upstream locations were included in this study to establish the regional source pool (Fig. 3.1A). Bray-Curtis community similarities (Fig. 3.1B), differ between the reconnected stream trajectory communities and the monitored regional source pool communities, representing the habitat variability in the catchment. Euclidean source pool distances differed from 0.5 km up to 10 km. The total length of the monitored catchment encompasses 18 km.

Functional trait data

Data on functional traits was provided by freshwaterecology.info, a database that contains taxonomic and ecological information on freshwater macroinvertebrates (Schmidt-Kloiber and Hering, 2015), combined with trait-database EKOO (Verdonschot, 1990) and Tachet's et al. database (2010). All trait data is fuzzy coded using a 10 point system or was transformed to fit a 10 point system. We used the following six trait categories for our analyses, based on the level of importance for colonization and presence of existing knowledge for our community: feeding type, locomotion type, dispersal mode, rarity-score, number of reproductive life cycles per year and r/K-strategy.

Data analysis

Chi-square tests were performed to compare the relative community composition between the regional species pool and the new habitat. Both communities were grouped per number of taxa per taxonomic class and dispersal modes. A logistic model was fitted to evaluate the effect of Euclidean distance to colonist source and taxon abundance on colonization success. Due to the non-linear effect of abundance this variable was ln-transformed. Euclidean distances were used since overland distance was highly correlated with river network distance in this area. Additionally, no clear differences between overland and river network distance approaches were found in several other studies (Grönroos et al., 2013; Stoll et al., 2016).

Random forest analyses were performed to identify key predictors, taking minimum, maximum and average Euclidean distance into account, as well as standard distance

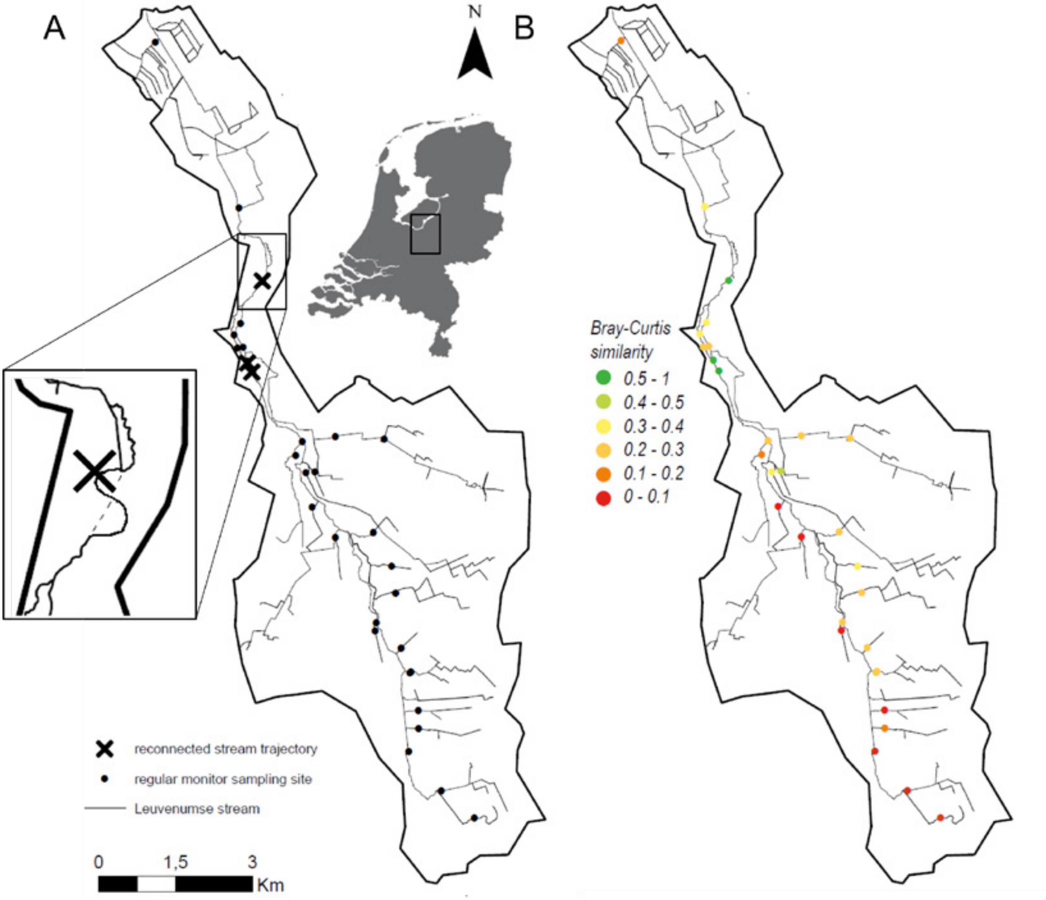


Figure 3.1. Map of the Leuvenumse stream catchment. Water flows in northern direction. A) Dots indicate sample sites that are monitored by water authority Vallei & Veluwe over the last 10 years and are used to determine the regional species pool in this catchment. Crosses indicate the location of three reconnected meanders which were sampled for 2.5 year to monitor the colonizing community. The inset shows the blocked main a with the newly created meanders and the monitoring site. B) Bray-Curtis similarities were calculated between the reconnected trajectories (in green) and the regularly monitored sampling sites (e.g. regional species pool).

(a measure for how wide spread each taxa is in the catchment), average abundance and 34 functional traits. Medians were calculated for missing trait values (Ishwaran et al., 2008; Ding & Simonoff, 2010). Data was tested against a null-model with 1000 random permutations (Raes & Ter Steege, 2007).

Decision trees (Breiman, Friedman, Olsen, & Stone, 1984) were calculated for each dispersal mode (active/passive aerial/aquatic) to show which combination of traits are present in taxa with different dispersal modes. Each branch represents a division in presence of absence of traits that ultimately leads to successful or unsuccessful colonization. AUC and p-values validated the models. All statistical analyses were performed using R version 3.4.1, provided by the R Foundation for Statistical Computing, Vienna, Austria.

Results

Effect of population abundance and distance on colonization

To calculate the combination of distance to source pool and abundance within source pool as an effect on colonization probability, we used this equation:

$$\mathrm{P(colonization)} = \frac{e^{0.30+0.24\,distance-0.10\,\ln(abundance)}}{1+e^{0.30+0.24\,distance-0.10\,\ln(abundance))}}$$

All parameters were highly significant (<0.001). The interaction between distance and ln(abundance) appeared to be not significant (Fig. 3.2, Table 3.2). Nonlinear relations were tested with a generalized additive model and not found (Chi-square: X^2(4.7)=8.99, p=0.09).

Taxa with high abundances are more likely to disperse, as well as taxa that are present at close distances to the reconnected stream trajectories.

To unravel the relative effect of all key predictors, a random forest analyses calculated and listed the most important variables to predict successful colonization. The most important predictor is average distance between the regional source pool and the new habitat, with minimum distance being almost as important to predict if taxa are able to colonize new habitat. Third variable to predict successful colonization is the level of distribution (spread) of the taxa across the entire catchment. Abundance of

Table 3.2. Results of deviance analysis of the generalized linear model to evaluate the effects of predictor variables on colonization success.

Predictor	Resid. df	Resid. dv	P	AIC
Distance	2601	3510.9	<0.001	3514.9
ln (abundance)	2601	3473.0	<0.001	3477
Distance + ln (ab)	2600	3431.4	<0.001	3437.4
Distance * ln (ab)	2599	3429.8	0.198	3437.8

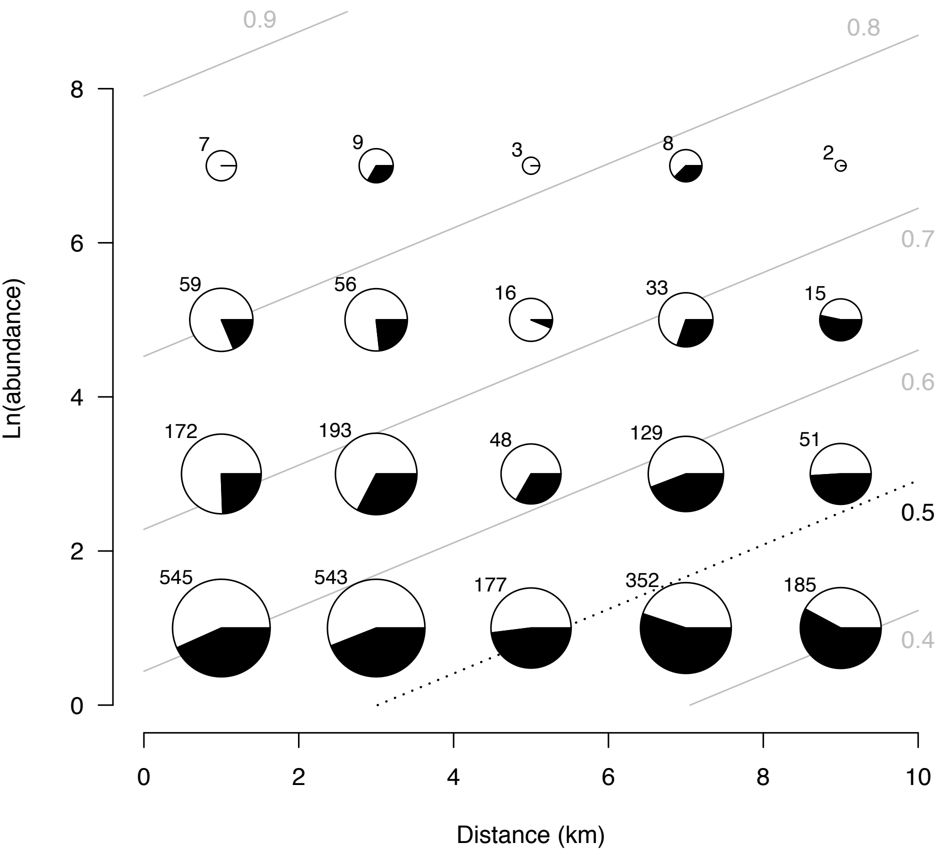


Figure 3.2. The pie-charts give the fraction of species, within the respective distance of abundance class (specified on x- and y-axes), that did or did not colonize the new area (white and black sections in the pie charts respectively). The number at each pie chart specifies the total number of species within the respective distance and abundance class – this total number is also reflected by the size of the pie charts. The lines underlying the pie-chart specify the probability that a species with a given abundance and at a given distance from the new area will colonize this new area. The numbers at the right and top margins specify the probability levels for these lines. The 0.5 probability-contour is highlighted with a dotted line: species with values above this line can be expected to be colonists.

taxa is less fit to predict chances of successful colonization than distance variables, but still more fit than any functional trait.

Specific functional traits related to feeding, number of reproductive cycles and locomotion that are important predictors of successful colonization are listed in table 3.3.

Table 3.3. Random forest predictors sorted on level of importance to predict successful colonization. Area under the curve: 0.719, p-value < 0.001.

Predictor	Importance	Predictor correlation >0.3 (p<0.05)
Distance (average)	13.53	Distance (min), distance (max), standard distance
Distance (minimum)	12.73	Distance (average)
Standard distance (spread)	11.99	Distance (average), distance (max)
Abundance (average)	10.99	
Distance (maximum)	7.24	Distance (average), standard distance
Grazer	5.46	
Univoltine	4.40	Bivoltine
Bivoltine	4.03	Univoltine
Aquatic passive	3.57	
Active walking	3.46	

Relative dispersal success

Relative community proportions of the regional species pool (number of species observations: n=2603) and the new habitat (n=2233) show the diversity of taxa that successfully dispersed (Fig. 3.3). No significant differences were found for the dispersal mode (active/passive aerial/aquatic, Fig. 3.3A) of taxa, nor for the taxonomic diversity per community (Fig. 3.3B, 3C) between the regional species pool and the reconnected trajectories (Chi-square: X2(3)=0.26, p=0.97 for dispersal modes , X2(5)=2.61, p=0.82 for taxonomic diversity per class, X2(10)= 22, p=0.108 for number of taxa per insect order). It should be noted that 85 % of the colonized community were insects, and the remaining 15% consisted of other taxonomic classes (Fig. 3.3B). The majority of insect taxa were Diptera, Trichoptera and Coleoptera (Fig. 3.3C). In total, 95 taxa colonized the reconnected trajectories throughout the 2.5 years of monitoring out of 307 taxa that were identified in the regional species pool.

Combinations of functional traits

Decision or classification trees showed that for each mode of dispersal different trait combinations exist and lead to successful colonization. Based on the fuzzy-coded functional trait information regarding dispersal capacity, several pathways within these decision trees lead to successful and unsuccessful colonization of taxa in the reconnected trajectories. Life cycle characteristics (uni- and bivoltinism) were the first branch (decision) in three out of four trees to determine successful colonization. In subsequent branches, feeding strategies and additional modes of

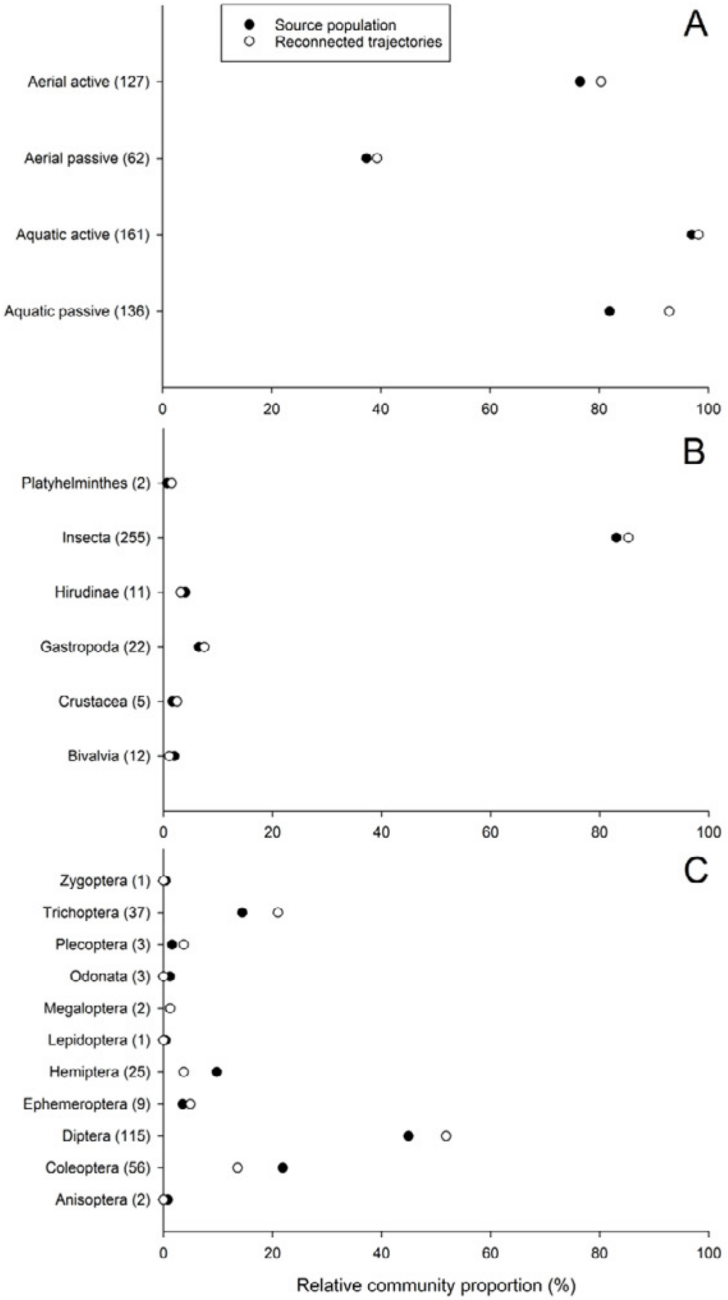


Figure 3. Relative community proportions (in %) of the source population and the reconnected trajectories after 2.5 years for each A) dispersal mode, B) taxonomic class and C) insect order. The total number of taxa per group is listed on the y-axes. The total community consisted of 307 taxa, of which most taxa possessed more than one mode of dispersal. Hence, the relative community proportion of all dispersal modes combined exceeded 100 percent.

dispersal were decisive of successful colonization. The AUC-value of the decision trees based on aerial active and aquatic active and passive dispersers was found to have a good model accuracy (e.g. AUC>0.7, Raes & Ter Steege, 2007). However, further testing showed that none of the fitted classification trees, representing each mode of dispersal, had acceptable reliability (p<0.05) to correctly predict colonization success based on specific trait combinations (Fig. 3.4).

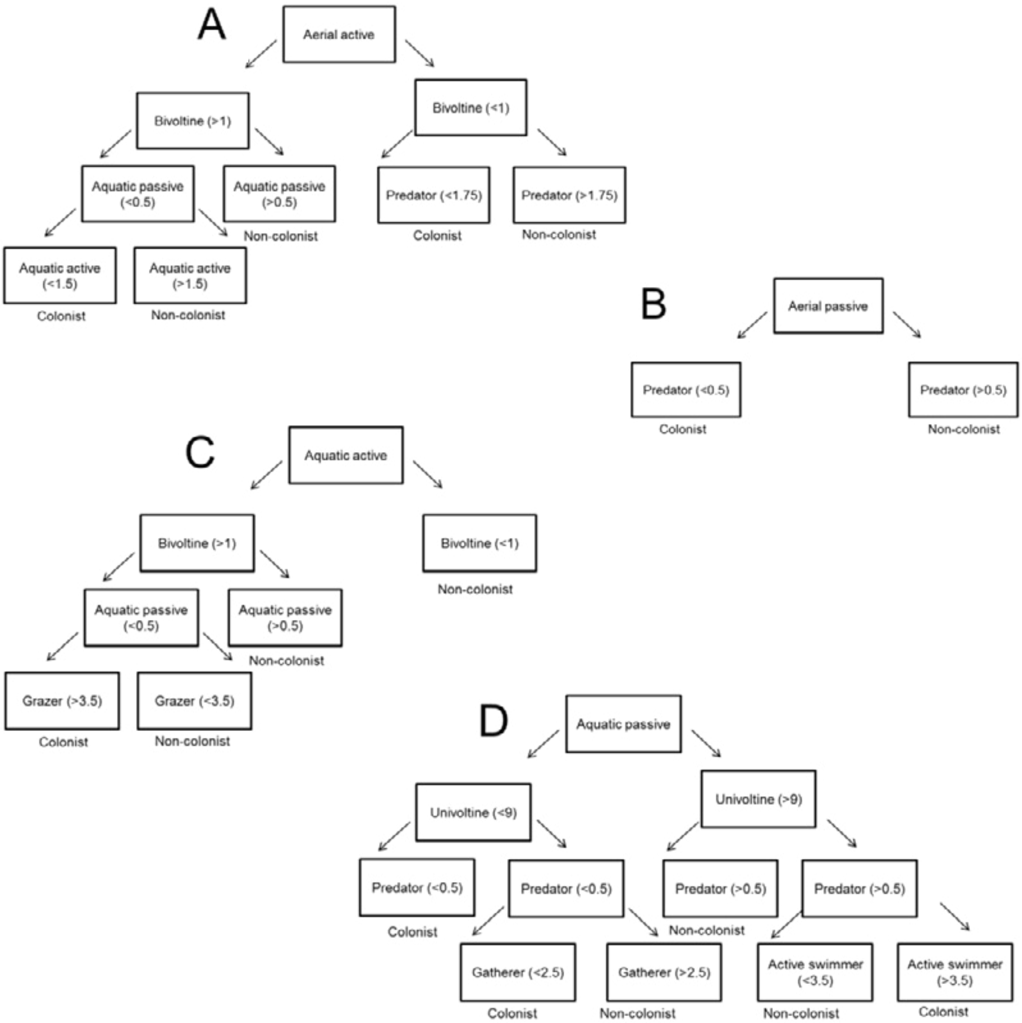


Figure 3.4. Classification trees predicting the successful (colonist) or unsuccessful colonization (non-colonist) of taxa in the reconnected trajectories, grouped per mode of dispersal: A) Aerial active dispersers (AUC=0.803, p<0.09, Correctly Classified Instances(CCI) = 99/127), B) Aerial passive dispersers (AUC=0.665, p<0.839, CCI= 45/62), C) Aquatic active (AUC=0.783, p<0.159, CCI=124/161), D) Aquatic passive dispersers (AUC=0.748, p<0.502, CCI=103/136).

Discussion

Effect of population abundance and distance on colonization

The distance between the source pool and the reconnected trajectories showed to have a great effect on colonization success. Additionally, the distribution of taxa across the catchment appeared to have a profound effect on the chances of taxa to successfully reach new habitat. This indicates that colonization success does not just depend on the distance to the nearest source, but depends on the distance of multiple sources or rather distribution of each taxa throughout the catchment. This supports the theory that mass effects are active in mid-order lowland streams with multiple metapopulations continuously acting as sources and sinks simultaneously (Stoll et al., 2016; Sarremejane et al., 2017). The mass effects model states that the net flow of individuals is created by differences in population size (or density) in different patches (Shmida & Wilson, 1985) and that high rates of dispersal override environmental filtering (Leibold et al., 2004; Leibold et al., 2016).

Our results also revealed that species with high abundances within the source population have a higher chance of successful colonization. However, a random forest analysis showed that species abundance predicts successful dispersal less effectively than the minimum distance of the source pool to new habitat. It was found that even species that had previously been sampled with one individual could disperse successfully to the new habitat. This could be the result of stochasticity associated with births, deaths, immigration and emigration (Hubbell, 2001) or a random chance of collection.

It has been recognized that colonizer abundance is a key driver for population dynamics, but it remains unclear how colonizer phenotype and abundance interact (Burgess & Marshall, 2011). Reproducing a high number of offspring (r-strategy) is seen as a typical colonizer trait. This phenotypic trait is found to be present in mainly flightless and poorly-flying organisms (Miguel-Chinchilla et al., 2014) and can have large effects on dispersal when stochastic drift events occur.

In highly connective lowland streams, seasonal spates are common and stochastic drift events occur (Poff & Ward, 1989). Aquatic invertebrates are susceptible to drift during high flow, which accounts for 42% (Williams & Hynes, 1976) to 82% (Townsend & Hildrew, 1976) of the macroinvertebrate redistribution within streams. When mass effects take place, as occurs during stochastic drift events, the chances of dispersing to new habitat and settling there are higher for abundant taxa than for rare taxa.

Relative dispersal success

Results show that species from each taxonomic class, and more specifically from each insect order, had their way to disperse successfully, since relative community proportions were equal between the regional species pool and the reconnected trajectories. Similarly, each dispersal strategy gave individuals equal chances of arriving in the new habitat during first phase colonization. We found that in our case active dispersal was not more effective than passive, which contradicts the patch-dynamic metacommunity theory where species are either good colonizers or good competitors (Tilman, 1990, 1994). Neither can we conclude that habitat patches were identical (Bray-Curtis similarities differed between metacommunities, Fig 3.1B) or that local species diversity is limited by dispersal, as is the case with the patch-dynamics perspective (Leibold et al., 2004).

Interestingly, hololimnic species (with a fully aquatic life cycle) are generally thought to be weak dispersers (Kappes & Haase, 2012) but arrived during first phase colonization in the newly reconnected trajectories. While several studies show that passive dispersing specialists arrive much later during succession (Winking et al., 2014; Winking et al., 2016), this effect was not confirmed in our study.

Once more, this leads us to think that in highly connected mid-order streams the community is assembled by mass effects (Sarremejane et al., 2017) rather than shaped by dispersal capacity. During mass effects, high rates of dispersal allow species to occur in localities with suboptimal environmental conditions (Leibold et al., 2004; Shmida & Wilson, 1985). This indicates that abundant neighbouring populations are able to maintain local populations even when habitat heterogeneity or suitability is low (Stoll et al., 2016).

Functional trait effects

Recent advances have been made in viewing organisms as a combination of traits instead of contributing fitness and survival to one specific adaptation (Mondy et al., 2016; Mondy & Usseglio-Polatera, 2013; Poff et al., 2006; Verberk et al., 2008). The adaptive value of a particular trait may depend on the other traits possessed by the species. Because of this context-dependence, trait-based approaches should take into account the way combinations of traits interact and are constrained within a species (Verberk et al., 2013).

For each mode of dispersal, fitted classification trees were not reliable enough to show with which functional traits interacted to ultimately lead to successful colonization. It should be noted that 85 % of the colonized community were insects, and the remaining 15% consisted of other taxonomic classes. This biased our results, and our interpretation, towards functional traits that are possessed by insects.

However, information on key functional traits that were derived by the random forest model show that successful colonizers possess the ability to graze as well as gather food more often than unsuccessful colonizers. It has previously been reported that grazers and scrapers that consume periphyton, and shredders and gatherers that feed on conditioned coarse organic matter are expected to be early colonizers (Gore, 1982; Hershkovitz & Gasith 2013). Additionally, species with univoltine and bivoltine life cycles appear to be more successful in dispersing to and colonizing new habitat than species that do not have these traits. These species reproduce more often than semivoltine species and thereby have an increased occurrence of dispersal events of juveniles (Usseglio-Polatera et al., 2000). Active walking increases the chance of successful colonization, more than other traits associated with locomotion. As stated by Townsend & Hildrew (1994) recolonization potential depends on aspects of mobility and reattachment capability, in which active walkers are often also capable of attaching themselves to substratum and successfully establish themselves in preferred habitat. In conclusion, it indicates that active walkers with uni- or bivoltine life cycles, that graze or gather their food, were the best colonisers in our case study.

Implications for stream restoration

This study shows that both distance to and abundance of species in the regional species pool affects the chance to successfully disperse. This indicates that restoration projects have a higher chance of obtaining a diverse community of desired species if the regional species pool is nearby and has high abundances of species (Stoll et al., 2016; Baumgartner & Robinson, 2017). Additionally, a wide distribution of taxa throughout the catchment benefits colonization success (Tonkin et al., 2014) which indicates that knowledge of the entire regional species pool will aid with spatial prioritization of restoration projects.

The results of this study also indicate that stream restoration can benefit from many small within-stream projects, such as habitat diversification, instead of one large hydro-morphological project, as this decreases the distance between two suitable habitat patches. The inclusion of 'stepping-stones' with suitable habitat providing food and refugia, could facilitate dispersal in a very effective way (Phillipsen & Lytle, 2013; Cañedo-Argüelles et al., 2015).

Another implication for future restoration projects is to consider the stochastic dispersal events of juvenile invertebrates. The seasonal timing, corresponding to peak flows after heavy rainfall, of major drift events can lead to a rapid recolonization of new habitat. Regardless of dispersal traits, this could increase the dispersal rate of all species.

Conclusions

To conclude, this study shows that aquatic invertebrate dispersal depends on the proximity of the regional species pool, as well as the distribution and abundance of taxa within that pool. The regional species pool properties are better predictors than functional traits whether taxa will be able to successfully disperse and colonize new habitat. This indicates that mass effects account for the community development in mid-order streams. Active and passive, aerial and aquatic dispersers have equal chances of colonization.

Understanding the underlying metacommunity mechanism during community development in mid-order lowland streams, helps to make decisions on when and where to apply restoration efforts. Distance between source pool and restored habitat, the inclusion of stepping stones with suitable habitat, timing of restoration practices and reinforcing existing source populations in terms of abundance and diversity are parameters that drive restoration success.

Stream restoration projects should therefore focus on limiting distances between source population and denuded areas, establish stepping stones with suitable habitat throughout whole catchments and reinforce existing source populations in terms of abundance and diversity to increase recolonization of habitats after restoration practices.

Acknowledgements

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Connectivity and seasonality cause rapid taxonomic and functional trait succession within an invertebrate community after stream restoration

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Abstract

General colonization concepts consent that a slow process of microhabitat formation and subsequent niche realization occurs during early stages after new habitat is released. Subsequently, only few species are able to colonize new habitat in the early onset of succession, while species richness increases steadily over time. Although most colonization studies have been performed in terrestrial ecosystems, running water ecosystems are equally or even more prone to colonization after disturbance due to their dynamic nature. We question how invertebrate succession patterns reconcile with general colonization concepts. With this study, we provide insight into the colonization process in newly created lowland stream trajectories and answer how within-stream bio- and functional diversity develops over time. Our results show a rapid influx of species, with a wide range of functional traits, during the first season after water flow commenced. During more than two years of regular monitoring, immigration rates were highest in autumn, marking the effects of seasonality on invertebrate dispersal. Biodiversity increased while abundance peaks of species alternated between seasons. Moreover, also days since start of the experiment explains a considerable part of the variability for taxa as well as traits. However, the relative trait composition remained similar throughout the entire monitoring period and only few specific traits had significantly higher proportions during specific seasons. This indicates that first phase colonization in freshwater streams can be a very rapid process that results in a high biodiversity and a large variety of species functional characteristics from the early onset of succession, contradicting general terrestrial colonization theory.

Keywords: biodiversity, colonization, lowland streams, ecosystem functioning, disturbance, resilience

Introduction

Colonization is a key concept in community ecology and its study has revealed many mechanisms by which ecological communities commence and develop during ongoing succession (Clements, 1916; Egler, 1954; MacArthur & Wilson, 1967; Odum, 1969; Connel & Slayter, 1977; Tilman, 1985; Huston & Smith, 1987; Cadotte, 2007). Most theories describe either facilitating, tolerating or inhibiting interactions between species during succession. The main resemblance in each theory, however, is that the early successional stage is characterized by a slow sequence of microhabitat formation and houses a species poor community. Successive to the colonization of vegetation is the appearance of invertebrates, as plants provide nutrients and refuge (Southwood et al., 1979; Schowalter, 2016). While substantial redundancy exists among theories on succession, there are still many knowledge gaps. Especially when it comes to identifying key parameters that determine the recolonization of fauna (Pulsford et al., 2016).

Remarkably, most colonization studies have been performed in terrestrial ecosystems, even though disturbance and subsequent succession is common in lotic ecosystems due to their dynamic nature (Townsend, 1989; Lake, 2000). Aquatic invertebrates have traits to quickly disperse to recently released habitat (Waters, 1964; Gore & Milner 1990; Gustafsson et al., 2013; Winking et al., 2014; Baumgartner & Robinson, 2017), but previous studies on stream colonization have shown that succession does not merely depend on species characteristics or time itself (Bilton et al., 2001). Invertebrate succession is rather shaped by a combination of 1) the distance between the regional species pool and new habitat (Gore, 1982; Lake et al., 2007; Sundermann et al., 2011), 2) the presence of a suitable habitat for settling and colonization (Jähnig et al., 2009; Heino, 2013), 3) dispersal capacity and life history traits of present species (Winking et al., 2014; Tachet et al., 2010; Van Leeuwen et al., 2013; Heino et al., 2015; Li et al., 2016)], and 4) the timing of dispersal (Peckarsky, 1986; Miguélez & Valladares, 2008).

Unconnected terrestrial habitat patches rely on wind-mediated dispersal, along with animal and sometimes even human vectors (Maguire, 1963; Bullock & Clarke, 2000; Havel & Shurin, 2004; Horváth et al., 2016). However, freshwater streams are often connected throughout a catchment and water flow is regarded as the number one facilitator of dispersal in streams (Li et al., 2016). As general colonization concepts seem to match terrestrial colonization more than flowing water colonization, we aim to unravel invertebrate successional patterns in newly created trajectories in temperate lowland streams.

In order to do this, we monitored three restored stream trajectories that have been connected to an existing stream channel. As previous studies have shown, the recolonizing community is shaped by the distance and composition of the surrounding species pool (Lake et al., 2007; Sundermann et al., 2011) and habitat formation is affected by the inflow of allochtonous material providing a fast formation of refugia (Lancaster & Hildrew, 1993a, b). This leaves us to address:

1) how does taxonomic and functional trait diversity develop over time in a new stream trajectory, 2) which traits regarding dispersal capacity and life history are important during early succession, and 3) how dispersal success is affected by timing. Our expectation is a rapid increase in taxonomic as well as functional diversity right after water flow commences. New species, with different suits of functional traits, will arrive throughout the first years while the habitat is developing and more niches become available.

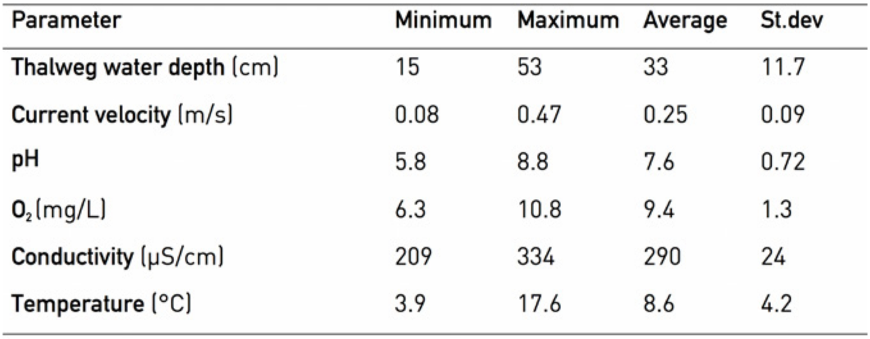
Metacommunity studies often neglect temporal patterns (such as ecological colonization and succession) in the interpretation of community composition, since it requires long-term and extensive monitoring. While the outcome of many metacommunity studies have led to an increased understanding of how spatial dynamics and local interactions structure communities, more empirical evidence is needed in order to fully comprehend the composition patterns in newly created habitats (Winegardner et al., 2012). Therefore, we focus on temporal shifts in community composition in a restored stream. The observed temporal shift cannot be viewed independently of larger scale spatial context, but the outcome will support a mechanistic explanation for the speed at which stream communities develop after disturbance and lead to a clearer understanding of early succession patterns in lotic systems.

Material and methods

Study area

The area studied is located in the Leuvenumse stream (52°18′55.17″N; 5°42′33.63″E), in the province of Gelderland, the Netherlands. This is a slow-flowing, meandering lowland stream (flow velocities ranged from 8 cm/s in winter up to 46 cm/s during spates in summer), fed by both precipitation and ground water. On average, the studied stream is about 4 meters wide. The deepest part of the channel (thalweg) varies between 15-53 cm, depending on season and location. Parts of the catchment are used by agriculture. The stream itself alternates through open pasture and deciduous and coniferous woodland. The streambed substrate consists of sand and clay with patches of gravel and coarse particulate organic matter. Throughout the monitoring period, the water temperature shifted seasonally from a minimum of 3.9 °C in winter up to 17.6 °C in summer. Besides water temperature, dissolved oxygen, pH and conductivity were measured at each sampling moment (Table 4.1). In the autumn of 2014, three former stream trajectories were re-connected to the

Table 4.1. Morphological, physical and chemical characteristics of three re-connected stream trajectories in the Leuvenumse stream, the Netherlands. These parameters were measured 18 times from September 2014 until November 2016 within each trajectory, adjacent to the streambed surface where the invertebrate samples were taken.



Parameter	Minimum	Maximum	Average	St.dev
Thalweg water depth (cm)	15	53	33	11.7
Current velocity (m/s)	0.08	0.47	0.25	0.09
pH	5.8	8.8	7.6	0.72
O_2 (mg/L)	6.3	10.8	9.4	1.3
Conductivity (µS/cm)	209	334	290	24
Temperature (°C)	3.9	17.6	8.6	4.2

existing stream by blocking parts of the main channel with sand (Fig 4.1). This caused all stream water to flow through the re-connected trajectories that had been dry forest floor without any pre-restoration communities up until then. All data regarding invertebrate community and morphological, physical and chemical characteristics derive from these three trajectories.

Permission to enter the national park and sample biota in the re-connected trajectories was provided by local authority NGO Natuurmonumenten with permit number 035-2014-430.

Sample collection and processing

Within the first 6 months after water flow commenced (September - March 2014), the invertebrate community was sampled every two weeks in all three re-connected stream trajectories. After 6 months, samples were taken once per season as we assumed that community saturation slowed down the colonization rate. To sample the entire invertebrate community, three multiplate samples and two handnet samples were taken at each trajectory and each sampling moment. Hester-Dendy multiplate samplers (total sampling area is 0.10 m2 per sampler) were used to exclude habitat variation during colonization and placed on the stream bottom of the three new stream trajectories as soon as water started flowing (t=0). Net samples (mesh size 250 µm, width of net is 30 cm) consisted of a multi-habitat sample composed of 1 m of mineral and 1 m of organic habitat, and were included to cover all habitat variation present at each sampling moment.

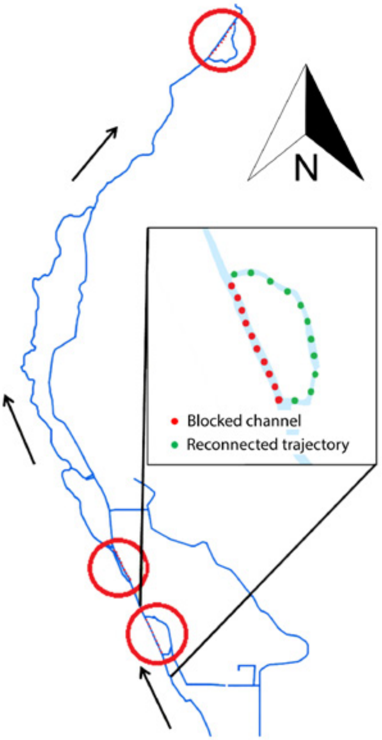


Figure 4.1. Simplified map of the Leuvenumse stream catchment with the three reconnected former stream trajectories (indicated with red circles). The arrows show the direction of the water flow. The inset shows the most upstream location; the existing channel was blocked with sand (red dots), after which the water took its natural course through the former streambed (green dots).

Samples were transported to the laboratory, where they were rinsed and the invertebrates picked out and stored in ethanol (70%) within 12 hours after being collected from the stream. In the laboratory, each individual was identified up to species-level if possible (Crustacea, Gastropoda, Hirudinae, Insecta: Trichoptera, Ephemeroptera, Plecoptera, Coleoptera, Simuliidae). Juveniles and some invertebrate-groups were identified up to genus-level (Bivalvia, Insecta: Chironomidae). Oligochaetes and Arachnida were excluded from the analyses due to limited identification at species-level. Care was taken to ensure that taxonomic resolution was sufficient according to Haase et al. (2006). Identification was performed with the use of a dissecting microscope (120x magnification) and a light microscope (300x magnification).

Functional trait data

Data on functional traits was provided by freshwaterecology.info, a database that contains taxonomic and ecological information on freshwater macroinvertebrates (Schmidt-Kloiber & Hering, 2015), combined with trait-database EKOO (Verdonschot, 1990) and Tachet's et al. database (2010). All trait data is number coded using a 10-point system or was transformed to fit a 10-point system. We used the following six trait categories for our analyses, based on the level of importance for colonization and presence of existing knowledge for our community: feeding type, locomotion type, dispersal mode, rarity-score, number of reproductive life cycles per year and r/K-strategy. Selected functional trait data was available for respectively 94%, 92%, 68%, 65%, 41% and 23% of the 96 taxa that were found in the new trajectories during the sampling period.

Statistical analyses

The multi-habitat and multiplate samples were pooled since the combination of taxa found by both methods represented the local community at each sampling moment. It appeared that habitat variation did not affect the data composition. No aquatic plants were present in any of the sampling sites, and streambed substrate coverage remained largely similar over time (data not included). The abiotic parameters (Table 4.1) did not shift over time, apart from some specific and well-known relationships between season and parameter (temperature, water depth) and were therefore not included in the analysis as an explanatory factor.

Samples of each of the three trajectories were pooled due to high community similarities (Jaccard similarity coefficient = 0.80). This enhanced the robustness of the analyses, and averaged out any local and small-scale effects.

The development of diversity at the species level is explored by clustering omnipresent species abundance patterns over time, with a non-metric multidimensional scaling (NMDS) ordination plot (Kenkel & Orlóci, 1986) and a distance based redundancy analysis (McArdle & Anderson, 2001). To unravel community shifts in functional trait composition over time, community weighted trait means included taxa abundance per sampling moment. The explained variance in the NMDS by seasonality as well as days since the start of the experiment were evaluated. Next, the partial contribution by season and days since start were evaluated by a distance based RDA and tested by a permutation-based ANOVA at a 0.05 significance level.

To test if specific functional traits were of increasing or decreasing importance during succession, linear regressions of functional traits over time were calculated. Only taxa presence/absence data was considered in this case, multiplied with specific trait values and divided by the total number of species present per sampling moment.

All analyses were conducted in R, using functions from the package vegan for NMDS and distance-based redundancy analysis.

Results

Species composition

In total, 96 different taxa were found in the new trajectories during the entire monitoring period of 735 days. Species diversity ranged between 23 and 45 taxa per sampling moment (Fig 4.2).

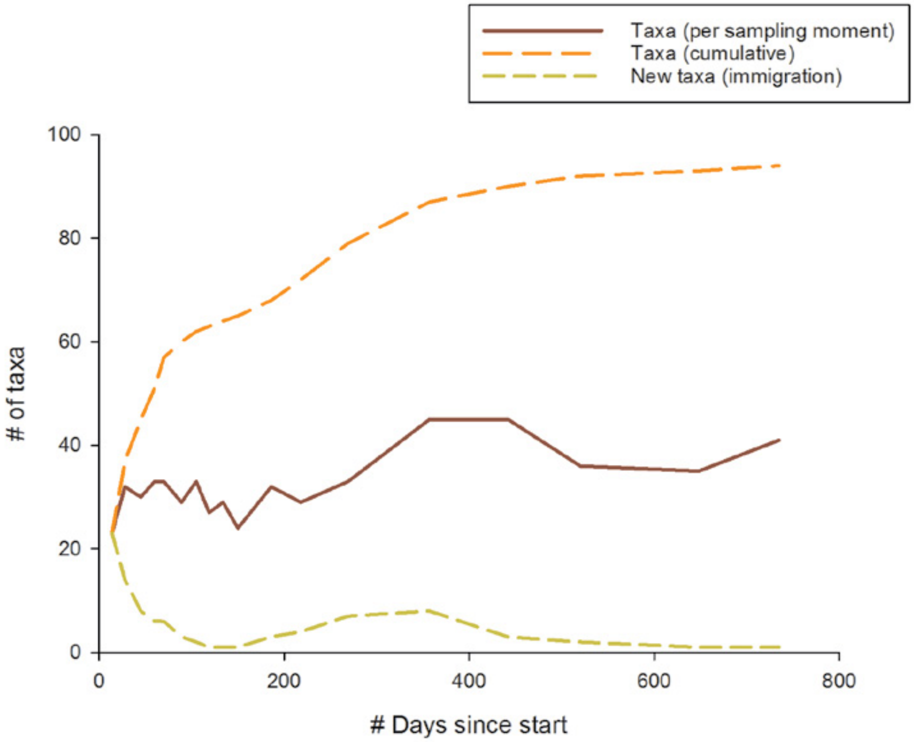


Figure 4.2. Number of taxa over time, measured 18 times from the moment water started flowing through the reconnected trajectories of the Leuvenumse stream. All three trajectories were pooled due to community similarities (Jaccard Jij=0.8). Number of taxa is plotted per sampling moment (absolute), # new taxa arriving (immigration) and # of total taxa (cumulative) over time (days since start).

The colonization process started with a rapid increase in taxonomic diversity, with 23 taxa being present within the first 14 days after water flow commenced (t=1). After 60 days (t=4), already more than 50% of all taxa collected was present. The highest numbers of newly identified immigrating taxa is at the start in autumn (t=1, 2 and 3) and subsequent autumn sampling moment (t=14), exactly one year later. Towards the end of the experiment, a decreasing amount of new colonizers was observed. A linear model was used to predict differences in immigration rate based on season and time since start. A significant regression was found (F4,13=5.12, p=0.01, R2 = 0.61), with more taxa arriving in autumn than winter.

Insects were most abundant throughout the study, ranging from 141 to 949 individuals per sample moment (average 640 ± sd 211, Fig 4.3). Bivalves showed abundance peaks with high numbers of individuals at the start of the colonisation (max. 642 individuals), but low abundances towards later sampling moments (average 105 ± sd 158 individual bivalves per moment). Crustaceans steadily became more abundant over time, ranging from 17 at t=1 to 407 individuals at t=13 (average of 44 ± sd 98 individual crustaceans per moment). The depression in bivalve and crustacean abundance at t=6 is probably due to the occurrence of a heavy spate at the moment of monitoring.

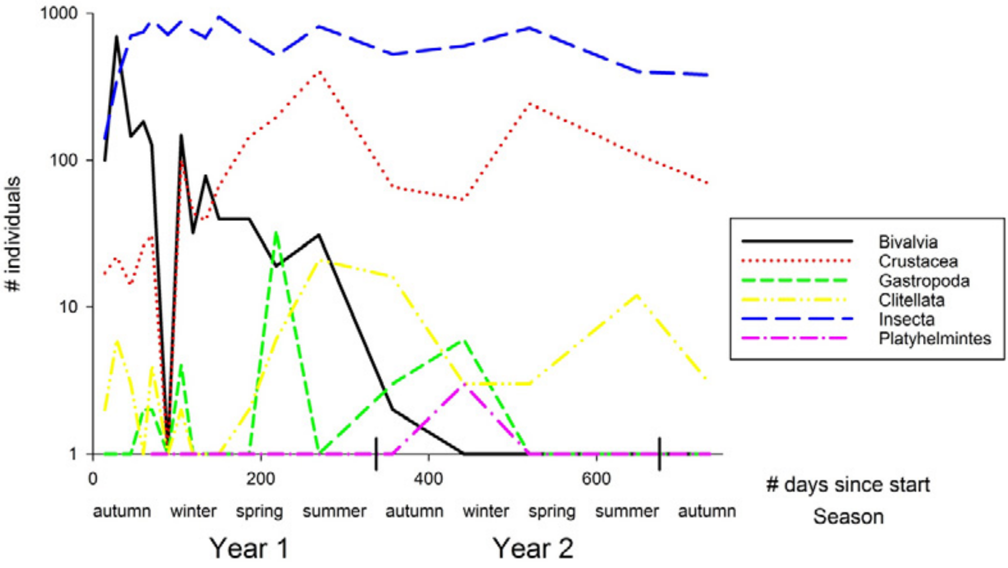


Figure 4.3. Changes in abundance (log (# individuals)) per taxonomical macroinvertebrate class over time (# days since start). Insects form the largest class of colonist from the early onset of succession, maintaining equal levels of large abundance over time.

The four most abundant orders of insects showed annual patterns with a large variation in abundance over time (Fig 4.4). Some taxa showed abundance peaks midwinter (Panel B. Plecoptera; *N. cinerea*) , while other taxa were most abundant in summer (Panel C. Trichoptera; *H. radiatus*) or spring (Panel D. Coleoptera; *O. villosus*). Peaks in larval abundance are often found right after adults have oviposited their eggs. Univoltinistic patterns are found for most taxa, while some (e.g. Micropsectra sp.) show semivoltine or flexivoltine life cycles with more than one peak in abundance per year. Annual patterns were similar in both years.

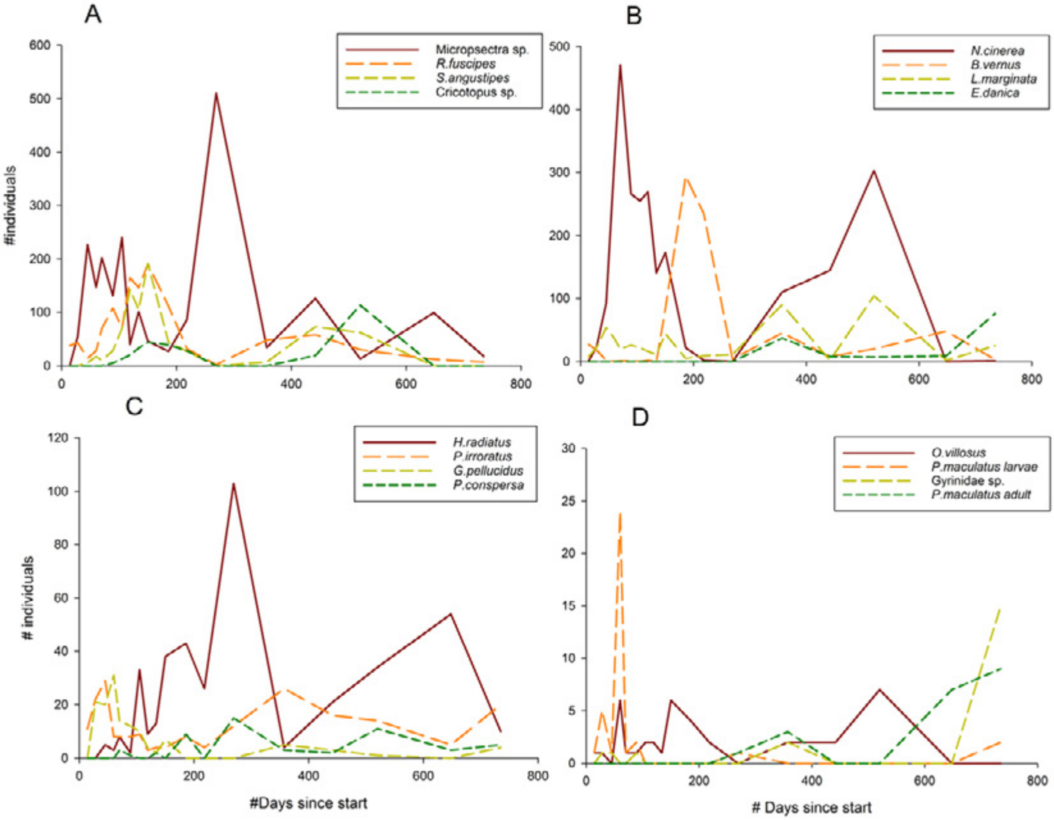


Figure 4.4. Changes in abundance over time (days since start) of four omnipresent taxa of insects, divided by order. A: Diptera, B: Ephemeroptera/Plecoptera, C: Trichoptera, D: Coleoptera.

Functional trait composition

Of the 29 traits considered in this study, divided into six different trait categories, 25 traits were present from the first sample moment onward. The overall majority of these traits (20 out of 29 traits) remained present without significant decreases and increases in relative occurrence over time (Fig 4.5). We do see significant increases in number of shredders, active filter feeders and burrowers (Table 4.2) over time. Significant increases were also found for taxa with univoltine and semivoltine reproductive cycles over time. Semi-rare taxa increased over time while rare and very rare taxa did not show significant changes. Passive filter feeders and aerial passive dispersers decreased over time.

Table 4.2. Additive linear regression model results with season (autumn, winter, spring, summer) and time since start as predictors and relative trait occurrence as dependent variable. Only significant results are shown in this table.

Trait category	Trait class	*p*	$F_{4,13}$	R^2
Feeding group	shredder	0.018	4.849	0.599
Feeding group	active filter feeder	0.004	4.692	0.591
Feeding group	passive filter feeder	0.020	5.655	- 0.635
Locomotion	burrowing	0.008	4.128	0.559
Rarity score	semi-rare	0.020	3.407	0.512
Dispersal mode	aerial passive	0.016	5.852	- 0.643
Dispersal mode	aerial active	0.014	2.996	0.480
Reproductive cycles	semivoltine	0.000	9.265	0.740
Reproductive cycles	univoltine	0.041	4.244	0.566

Predator taxa were most abundant (average of 34% of the total community) throughout the whole study period compared to gatherers, grazers, miners, filter feeders and shredders. Active walking taxa were most abundant (48%) throughout the whole period compared to active swimming, drifting, sessile and burrowing taxa. Taxa with a r-strategy were most abundant (87%) compared to K-strategists. Univoltinistic taxa were most abundant (62%) compared to bivoltine, trivoltine, semivoltine and multivoltine taxa. Taxa with different rarity-scores were all present in equal ratios (18%-22%), apart from the very rare taxa, which are not abundant (6%) at all times (Fig 4.5). Equal ratios were also present for aquatic passive and aerial active taxa, which both account for 32% of the total community.

A NMDS of the taxa (Fig 4.6A) and trait composition (Fig 4.6B, Table 4.3) show that seasonality as well as days since start of experiment are important factors shaping the community composition. For the taxa-ordination, the stress value at the optimal solution is 0.159. The R-squared for the non-metric fit between observed

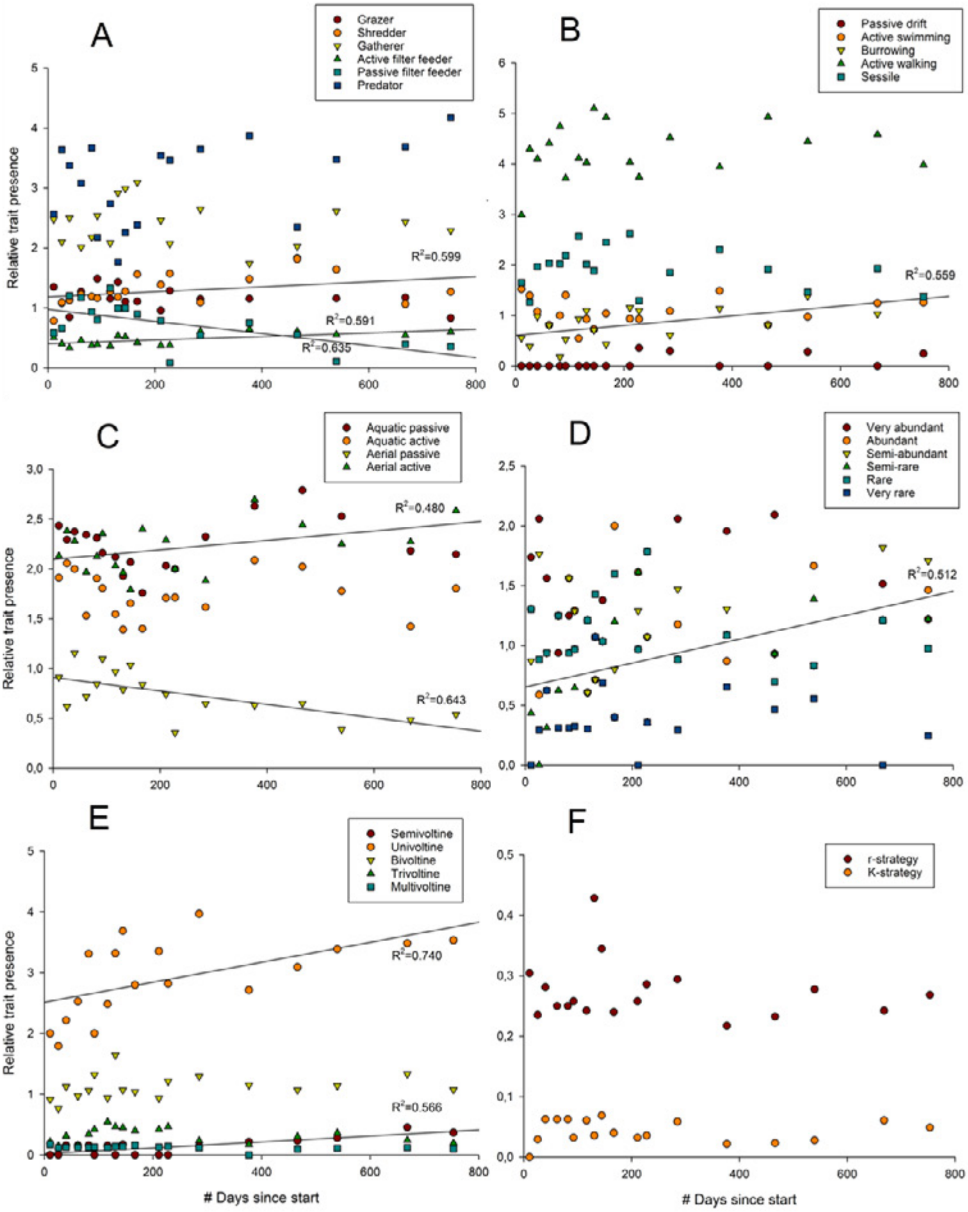


Figure 4.5. Changes in functional trait composition (relative trait presence) over time of six different trait categories. Linear regressions of functional traits over time were calculated to test if specific functional traits were of increasing or decreasing importance during succession. Only taxa presence/ absence data was considered in this case, multiplied with specific trait values and divided by the total number of species present per moment. Regression lines and coefficient are only shown for significant regressions. A: Feeding group, significant regressions: shredder, active filter feeder, passive filter feeder, B: Locomotion type, sig. regr.: burrowing, C: Dispersal mode, sig. regr.: aerial passive, aerial active, D: Rarity, sig. regr: semi-rare, E: Reproductive cycle, sig. regr: semivoltine, univoltine, F: r-K selection strategy, no sig. regr.

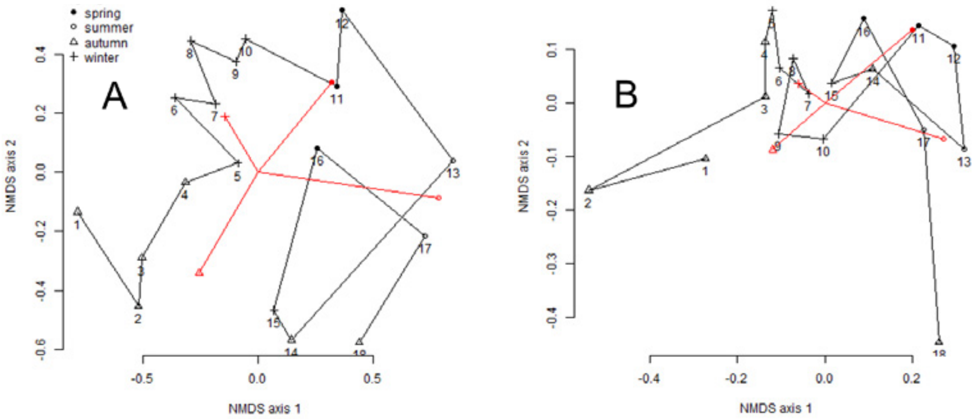


Figure 4.6. Non-metric multidimensional scaling plot (NMDS; a rank-based approach to ordination, representing the pairwise dissimilarity between all cases). For both the species (A) and the trait-based (B) analysis, the data is fitted to two axes, using the Bray-Curtis distance metric.

dissimilarity and ordination distance is 0.975. Season explains the variation of the taxa in the reduced space with an r^2 of 0.598, while days since start explains it with an r^2 of 0.710. When analysing the contribution by season and days since start in a distance-based RDA, both factors also turn out to be significant (permutation-based p-value of 0.001 for season and 0.002 for days), with r^2 values of 0.16, 0.40 and 0.51 for model including respectively days since start, season and days since start+season.

Table 4.3. Relative functional trait composition per season. Only significant different traits are shown. Different letters indicate significant differences between groups. Bold number indicates which season has the highest trait value.

Trait category	Trait class	Autumn	Winter	Spring	Summer
Feeding group	grazer	a (0.79)	ab (0.88)	b **(1.74)**	ab (0.88)
Feeding group	predator	ab (1.14)	a (0.35)	ab (0.83)	b **(1.81)**
Locomotion	passive drifting	a (0.00)	a (0.00)	a (0.00)	b **(0.04)**
Locomotion	active swimming	a (0.98)	a (0.55)	b **(3.16)**	b (2.11)
Locomotion	sessile	ab (1.89)	a **(2.50)**	b (1.41)	ab (2.28)
Selection strategy	K-strategy	ab (0.50)	ab (0.69)	a (0.26)	b **(1.52)**
Rarity score	very common	a (0.83)	a (0.63)	b (2.30)	b **(2.92)**
Rarity score	rare	a **(1.01)**	b (0.32)	ab (0.53)	ab (0.28)
Dispersal mode	aquatic active	a (1.93)	a (1.77)	b **(2.31)**	ab (2.04)
Reproductive cycles	bivoltine	a (1.13)	ab (1.57)	b **(2.13)**	ab (2.08)
Reproductive cycles	flexivoltine	a **(0.27)**	b (0.03)	ab (0.00)	ab (0.07)

For the trait-ordination, the stress value at the optimal solution is 0.11. The R-squared for the non-metric fit between observed dissimilarity and ordination distance is 0.987. Season explains the variation of the traits in the reduced space with an r^2 of 0.418 and days since start with an r^2 of 0.574. A distance based-RDA on the traits shows that both factors are significant (permutation-based p-value of 0.002 for season and 0.004 for days), with r^2 values of 0.27, 0.41 and 0.58 for model including respectively days since start, season and days since start+season.

So, the differences between the observation times in terms of taxa as well as trait diversity can be represented well in a reduced (two-dimensional) space both season and days since start of the experiment explain a considerable part of the variability for taxa as well as traits.

Discussion

This study gives insight into the shifts in community composition on a taxonomic as well as a functional trait-level during the early stages of colonization of a temperate lowland stream. Our results show that new trajectories of temperate streams are characterized by a rapid colonization by multiple species with different traits in large abundances. This observational study was performed in a highly connective system with constant supply of allochtonous organic material. These parameters could be the agent of success that caused a high rate of succession. Circumstances that are thought to increase succession rate are small distances to the regional source pool (Horváth et al., 2016; Sundermann et al., 2011), species with active dispersal mechanisms (Kappes & Haase, 2012; Miller & Labandeira, 2002), specific seasonal effects (Miguélez & Valladares, 2008) and suitable new habitat. We will discuss how these four elements have played a role in our study and how they affect the process of succession in temperate lowland streams.

Distance and dispersal mechanisms

Parkyn and Smith (2011) consider dispersal constraints low if a new habitat has an upstream supply of invertebrates and additional aerial recolonists are no further than 2 km away. Under such conditions the new habitat could resemble a reference community within 10-50 years after disturbance. Sundermann et al. (2011) and Winking et al. (2014) consider dispersal constraints high when there is no direct upstream community and recolonists are more than 5 km away. Those communities are very unlikely to resemble a reference community ever again.

Recent studies have shown that succession can be a slow process if the connectivity between streams is poor (Haase et al., 2013; Milner et al., 2008; Parkyn & Smith, 2011).

In our case, the re-connected trajectories were directly, and imperatively, connected to the upstream and downstream existing stream channel and therefore dispersal constraints were considered low. Furthermore, hydrological connectivity and riparian vegetation was present at all times, which enabled rapid colonization of aerial and aquatic dispersers from the start. Even though there is still a debate on which pathway makes up the majority of dispersal, it has been found that short-distance dispersal mainly occurs due to aquatic drift (Parkyn & Smith, 2011; Sundermann et al., 2011; Tonkin et al., 2014) while long-distance dispersal by adult insect flight is often the primary mechanism for recolonization of restored trajectories in separate catchments (Schultheis et al., 2002; Hughes, 2007). However, many species are incapable of actively dispersing themselves and rely on animal vectors, wind, or water flow to provide passive transport between sites (Bilton et al., 2001). In terrestrial ecosystems, wind-mediated dispersal is the prime mechanism that enables early stage succession (Taylor, 1954). In running waters, flow facilitates colonization from the first moment on (Allan, 1995; Mackay, 1992) and connectivity to source populations will further speed up arrival.

All four dispersal mechanisms (aquatic/aerial active/passive) were present among taxa from the onset of succession. Aerial passive dispersers decreased over time, all other dispersal mechanisms remained present at equal ratios over time. This finding contradicts recent studies where aerial active generalists were found to colonize most rapidly and weakly dispersing generalists immigrated much later (Li et al., 2016; Winking et al., 2014). This contradiction and our observation leads us to hypothesize that categorizing species by dispersal mechanisms alone does not give sufficient information on actual dispersal rate.

A clearer view of how the community developed during the process of succession is obtained by looking at the functional trait composition. We found that while some traits were dominant over others, overall trait composition did not change over time. For terrestrial systems, new habitat is colonized by species that have rapid life cycles and are r-strategists. In later stages, species with competitive traits, such as predatory behavior, are able to settle in the new habitat. Analogous, Gore (1982) showed that collector-gatherers and collector-filterers were the initial colonizers of stream habitat instead of predators. Surprisingly, the traits found to be dominant in this study contradict the colonizer-competitor trade-off concept (Huston & Smith, 1987; Tilman, 1990, 1994) and the findings of Gore (1982). From the onset of succession, predatory species were present in the new habitat. The diversity of predators was even higher than the diversity of filter feeders. In accordance with other studies (Gore, 1982; Li et al., 2016; Tonkin et al., 2014; Leps et al., 2016) our study shows that the trait composition of the newly arriving species community probably depends on the regional species pool in the proximity, rather than develop according to previously theorized sequence of functional traits.

Succession and Seasonal effects

Our results indicate that there is a temporal trend for the duration of the project (visible in the results by distance-based RDA), which might be successional. On top of that there are clear seasonal effects (also shown in distance-based RDA). The results show that most species either immigrate in the first season or exactly one year later in the same season. Both immigration events took place in autumn. This indicates the importance of seasonality during the colonization process in temperate running waters. Seasonality can effect dispersal processes directly, in terms of optimal hydrological connectivity (Gallardo et al., 2014) and species availability due to life-cycle timing (Hynes, 1976; Jacobi & Gary, 1996; López-Rodríguez et al., 2009), and indirectly, in terms of increases and decreases of specific environmental parameters that trigger organisms to disperse (Miguélez & Valladares, 2008). Peak flows after heavy rainfall are common in Dutch lowland streams during the end of summer and autumn. The sudden increase in current velocity can cause species to become dislodged from the substrate and enter a state of drift (Verdonschot et al., 2012). Additionally, an increase in the volume of water due to melting or rainfall can expand potential habitat surface and increase hydrological connectivity. This improves instream dispersal and colonization patterns (Knispel & Castella, 2003). In the late summer and early autumn temperate lowland streams are populated by many juvenile and first larval stages specimens of many species (Higler, 2008; Buffagni et al., 2009; Graf et al., 2008). These young animals are often susceptible to drift, either passively or actively as dispersal trait, and prone to transportation downstream where they can settle.

Throughout the year, also some new species immigrate into the denuded habitat. This can be explained by both ongoing habitat development and the ability of species to inhabit these new habitats due to their functional traits (Townsend et al., 1997; Verberk et al., 2008). Interestingly, species from different insect orders show annual peaks in abundance, but alternate in the timing of these peaks. By partitioning resources and interspecific competition, more species are able to coexist while total abundance levels are stable and succession continues (Townsend, 1989).

The result that species and trait composition is correlated to seasonality indicates that seasonal life history characteristics provide the opportunity to colonize, survive and reproduce and not just time since existence or dispersal capacity alone. This finding is supported by previous studies on stream restoration effects (Leps et al., 2016), and can be explained by the different life history strategies needed to exploit (a)biotic resources (Verberk et al., 2008; Jacobsen et al., 2008). Surprisingly, little attention has been given to seasonal patterns in macroinvertebrate colonization in temperate streams. In our study, the combination of strong hydrological connectivity, high availability of juveniles, and availability of habitats and resources of re-connected trajectories set the requisites for enhanced autumn colonization.

Habitat suitability

Once the macroinvertebrates arrive at the newly restored habitat, they have to be able to settle and reproduce before recruitment can be considered successful. However, the presence of favorable in-stream and riparian habitat alone is not enough for community recovery (Palmer et al., 2010). Distances between leaf patches and sizes of these organic refuges have a profound effect on species survival (Westveer et al., 2017; Lancaster, 2000). Species might not be able to recolonize the stream before multiple new microhabitats have established in the stream (Lancaster & Hildrew, 1993a,b). The rapid increase in biodiversity and functional diversity right after water flow commenced indicates that microhabitat formation was not a limiting factor for the first species to arrive. High hydrological connectivity between the upstream source pool of organic matter and the reconnected trajectory together with the input of allochtonous material from the autumn-shed leaves along this temperate lowland stream could have provided a fast formation of suited habitats.

To conclude, this study shows that temperate lowland stream colonization is marked by a rapid increase in species richness and abundance right after water flow commences, when hydrological connectivity is not a constraint. The community is heterogeneous in terms of functional trait diversity from the early onset, with no clear sequence in colonizer to competitor trait characteristics. The immigration rate of new species is affected by seasonality. To strengthen the interpretation of these observations, data from nearby established streams help to standardize the community composition by season and determine at what time point the assembled communities of new streams become statistically indistinguishable from established streams. This could unravel the proportion of both spatial and temporal effects on successional patterns. Nonetheless, the observed patterns show that colonization and subsequent succession rate can be high in restored stream trajectories, yet effects of the regional species pool might be a limiting factor in community restoration and worth to explore in future studies.

Acknowledgements

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Substrate homogenization impacts survival and fitness in the lowland stream caddisflies *Micropterna sequax* and *Potamophylax rotundipennis*: a mesocosm experiment

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Abstract

Loss of substrate heterogeneity or patchiness is common in lowland streams with disturbed hydrological regimes. On reach scale, peak discharges tend to homogenize the stream bed and decimate the availability of specific microhabitat types. This spatial shift in habitats towards a more homogeneous landscape could have large negative effects on species that perform essential ecosystem processes. An aquatic mesocosm experiment was performed to test the effect of habitat homogenization on survival and fitness of two species of Trichoptera (*Micropterna sequax* and *Potamophylax rotundipennis*). Caddisflies were used as model organisms due to their abundance in lowland streams, their representativeness for the total shredder community and the significant role they play during the process of leaf litter decomposition. Larvae were reared in artificial recirculating channels containing leaf and sand patches in 3 different spatial configurations, differing in homogeneity of substrates, varying from few large patches to many small patches. Emergence rate was used as a measure of survival, and biomass and wing span of the adults were used as fitness correlates. In *M. sequax* survival was lower in the homogeneous treatment in comparison to the heterogeneous configurations, but no effect of patch configuration on fitness were found. In *P. rotundipennis* survival was unaffected by the spatial configuration of the patches, but effects on fitness were found instead: the longest forewings were found in the homogeneous configuration for both males and females. Our results suggest that both species experience intraspecific resource competition arising from the spatial distribution of patches, expressed as an investment in wing development (e.g. dispersal capacity) in *P. rotundipennis* and resulting in lower survival rates in *M. sequax*. Our results indicate the importance of knowledge of trait-based responses and highlight the effects of the configuration of stream bottom substrate for its inhabitants on microscale.

Keywords: habitat heterogeneity, substrate patchiness, habitat preference, Trichoptera, macroinvertebrates, functional traits

Introduction

Attempts to predict responses to natural and anthropogenic disturbances have been and continue to be a pervading theme in ecological research (Fritz & Dodds, 2004; Harrison 1979; Pimm 1984; Vieira et al., 2004). The ability of an ecosystem to endure specific disturbances without losing its structure or functions (ecological resistance) and the ability to absorb or recover while maintaining its functions and services (ecological resilience) gives us an indication of how severely certain changes can disrupt an ecosystem (Holling, 1973). The resistance and resilience of an ecosystem depend on the impact of disturbances on species and the processes that contribute to ecosystem functioning. It has been shown that species are affected by disturbances, while the ability to express certain traits allows them to cope with environmental change (Townsend & Hildrew, 1994; MacGillivray & Grime, 1995). This makes detailed knowledge on species life history (e.g. survival, fecundity and growth) a necessity to understand ecosystem resistance and resilience.

Multidimensional organic substrate types serve, amongst others, as physical refuges from larger predators and physical stress like peak flows (Bond & Downes, 2000; Lancaster & Hildrew, 1993; Lancaster, 2000), while the carbon-based substrates also serve as a food resource for many macroinvertebrates (Reice, 1980; Arunachalam et al.,1991). Previous studies have shown that lowland stream macroinvertebrates have specific substrate and structure preferences, as well as the spatial configuration of these resources (Heino, 2013; Jähnig et al., 2009; Schröder et al., 2013; Tolkamp, 1980; Townsend & Hildrew, 1994). If these resources disappear, so will their associated species (Townsend, 1989). The disappearance of these species could have a considerable impact on stream ecosystem functioning, since many species play a vital role in the stream food web and contribute substantially to a variety of ecosystem processes (McKie et al., 2008; Vannote et al., 1980). Examples of important macroinvertebrate mediated ecosystem processes are bioturbation of sediments (Mermillod-Blondin et al., 2002; Jonsson & Malmqvist, 2003; Nogaro et al., 2009) and the decomposition of organic material (Malmqvist & Oberle, 1995). This makes the loss of substrate heterogeneity, as well as the loss of its associated macroinvertebrates, a process that could negatively affect the whole ecosystem.

We explore this ecological issue by focusing on streambed homogenization resulting from hydrological extremes in lowland streams. High current velocities during peak discharges could wash out a variety of streambed substrates homogenizing the substrate mosaic, increasing the area of sand at the expense of organic patches (Lake, 2000; Resh et al.,1988). Most habitat homogenization studies have been performed in terrestrial systems (Fahrig, 2003). Only few attempts have been made to test the importance of the spatial configuration of resource patches for stream invertebrates, but clear effects of microscale patchiness on densities, emergence success and larval biomass have been reported (Palmer, 1995; Palmer et al., 2000; Silver et al., 2000; Lancaster & Downes, 2014). Loss of habitat mosaics can decrease suitable habitat and quantity of patch interfaces, increasing competition for resources. Furthermore, small patches can become unsuitable to sustain a population, while large distances between patches can be difficult to bridge for non-mobile species (Fahrig, 2003). Palmer (1995) describes how inter-patch movements lead to predation or dislodgment risk and how stressful resource acquisition might explain the observed negative effects of a homogenized environment.

In this study, we aim to expand knowledge on the effect of streambed homogenization on macroinvertebrate survival and fitness. Caddisflies (Trichoptera) were selected as model organisms because of their abundance and species richness in lowland streams (Giller & Malmqvist, 1998), their representativeness as shredder within the macroinvertebrate community and their vital role in decomposition of leaf litter (Malmqvist & Oberle, 1995). An aquatic mesocosm experiment was performed to quantify the effects of patch homogenization (resulting in increased patch sizes, increased distance between patches, decreased edge length) on two caddisfly species with organic patch inhabiting larvae: *Micropterna sequax* (McLachlan, 1875) and *Potamophylax rotundipennis* (Brauer, 1857). They are characteristic of northwestern European low-gradient streams and occur in streams with sand and particulate organic material as dominant substrates. Both species belong to the family Limnephilidae, subfamily Limnephilinae, and are both shredders (Waringer & Graf, 2011).

We address the following research questions: (1) is there a relationship between caddisfly survival (emergence success) and habitat homogenization, (2) does habitat homogenization affect caddisfly fitness (wing span and biomass) (3) do trait differences between species result in different responses to habitat homogenization?

Regarding the latter, both species have similar habitat preferences (i.e. patches of coarse particulate organic material), but *P. rotundipennis* is a rather mobile species, whilst *M. sequax* displays more sedentary behavior and tends to live at the organic-mineral interface (Verdonschot et al., 2012; Verdonschot et al., 2014; Tolkamp, 1982). We hypothesize that species displaying a lack of mobility and a high preference to interface microhabitats will show a low resistance to habitat homogenization.

In this case, we expect the behavior and microhabitat preference of *M. sequax* to result in a lower emergence success and a lower fitness in a homogeneous spatial configuration with less patch interfaces than the more mobile *P. rotundipennis*. We use emergence success as a measure for survival since both species are known to be univoltine (Higler & Solem, 1986; Graf et al., 2008) and no life larvae were recovered post-experiment, indicating that all non-emerged individuals had died. Wing span and biomass were used as fitness correlates because of their effect on respectively dispersal capacity (Hoffsten, 2004) and fecundity (Honěk, 1993).

In previous studies, correlations between environmental changes and biotic responses were tested to explain an organism merely as a product of its adaptations, determined by the combination of functional traits it possesses (Tachet et al., 2000, Verberk et al., 2008). Yet, ongoing research continues to prove that each organism expresses its traits in ways that are very plastic and dependent of various factors (Statzner & Dolédec 2011; Verdonschot et al., 2012). We elucidate this issue by performing this study with two species and their response on environmental change.

Material and methods

Experimental design and mesocosm set-up

In total, 6 indoor stream mesocosms were used, each consisting of 4 recirculating channels (24 channels in total). Each channel was divided into 2 compartments, which served as experimental units (48 units in total; Fig. 5.1A) that were supplied with 20 individuals of either *M. sequax* or *P. rotundipennis*. Some channels carried both species in separate compartments, yet the mutual effects were negligible as the mesh between compartments (1 mm) prevented any substrate material from passing through and communication by pheromones has only been shown for adult caddisflies (Wood & Resh, 1984). There are no records of chemical communication by caddisfly larvae. Channel bottoms consisted of sand (layer of 5 cm thick, grain size 1-3 mm) and patches of leaves. Leaf patches were composed of abscised oak (Quercus robur) leaves and were arranged in 3 different spatial arrangements, differing in the number of patches, patch sizes and total patch interface length (Fig. 5.1B, Table 5.1). The spatial configuration of the mesocosms mimicked a natural situation in which streambeds have various substrate patterns, before (heterogeneous) and after a peak flow (homogeneous). With 3 different levels of patchiness and 2 different species, we set up 6 treatment combinations.

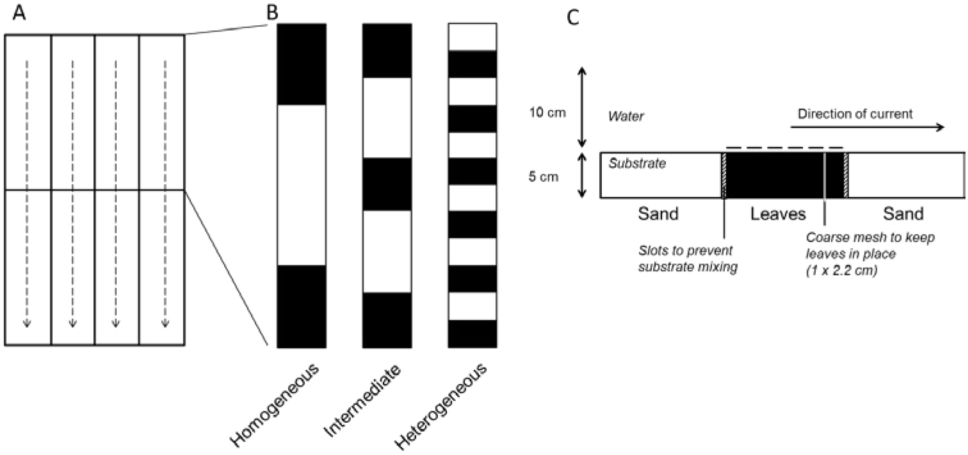


Figure 5.1. A: Outline of a mesocosm with four stream channels. Each channel was divided into two compartments with mesh (1 x 1 mm) to keep water flowing (direction of arrow) but prevent exchange between compartments. Six identical stream mesocosms were used, with a total of 48 compartments/experimental units. B: Top down view of three different patch configurations. One configuration per experimental unit, with decreasing substrate homogeneity. Homogeneous = large patches at large distances, Intermediate = medium sized patches at medium distance, Heterogeneous = many small patches at small distances. C: Side view of bottom substrate of the experimental compartments.

Each combination was replicated 5 times and randomly distributed across 30 experimental units (Fig. 5.2). Given previous experiments in these mesocosms used a set-up of 4 replicates per treatment and similar population pressures (Verdonschot et al., 2012; Verdonschot et al., 2014), we used 5 replicates to account for any Type I errors.

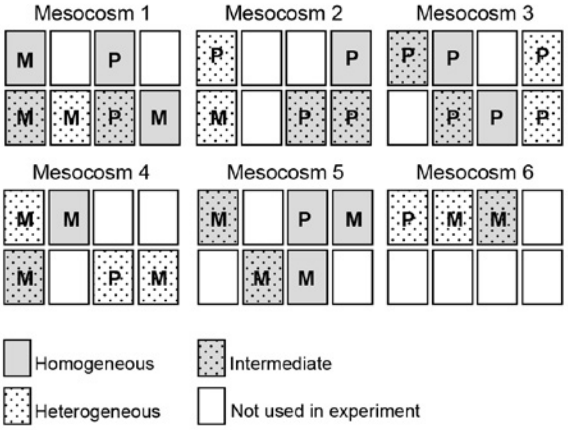


Figure 5.2. Random distribution of experimental units with different species and different treatments. Units with 'M' indicate a population of 20 *M. sequax* individuals. Units with 'P' indicate a population of 20 *P. rotundipennis* individuals.

Table 5.1 Mesocosm set-up with a homogeneous, intermediate and heterogeneous treatment

Homogenization parameter	Patchiness Treatment Level		
	Homogeneous	Intermediate	Heterogeneous
Number of leaf patches	2	3	6
Number of sand patches	1	2	6
Number of patch interfaces	2	4	11
Surface of single leaf patch (cm^2)	243	162	81
Surface of single sand patch (cm^2)	486	243	81
Length of total patch interface (cm)	24	48	132

Total patch size (40.5 x 12 x 5 cm) and biomass of leaves was kept constant in all compartments; the experiment started with 10 g of leaves, divided equally over the patches. To compensate for consumed organic material by the caddisflies, extra leaf material (5 g) was evenly divided amongst all leaf patches in each compartment halfway through the experiment. This was done to prevent food availability becoming a limiting factor during larval development, and mimics a natural situation in which *Q. robur*, a slowly decomposing litter type (Swift et al., 1979), is occasionally distributed across the stream bottom in forested areas throughout the year (Minshall, 1967; Reice, 1974). Furthermore, additional food in the form of 1 cube (2 g) of frozen Tubifex oligochaetes (Dutch Select Food Tubifex; crude protein 6.2%, crude fat 2.6%, crude fiber 0.6%, ash 0.5%) was supplied every two weeks in each compartment to prevent cannibalism or predation in species which supplement their diet with (dead) invertebrates (Wissinger et al., 2004). These extra food sources were added to ensure that all potential components of the caddisflies diet were available in the experiment with litter still being their main food source till pupation enhanced.

To keep patch configuration intact during the experiment slots were placed in the sediment on the borders of the patches of sand and leaves. To prevent downstream transport of the leaves by the current patches were covered by mesh (sized 1 x 2.2 cm; Fig. 5.1C). Both species were able to reach the leaves without constraints as the diameter of their larval cases was on average 0.5 cm, allowing them to move through the coarse mesh.

The water depth in the channels was 10 cm. Current velocity was kept constant at 10 cm/s in all units, similar to the natural velocity preference of both species

(Verdonschot et al. 2012). The mesocosm water was constantly recirculated from a 600 liter reservoir and consisted of preconditioned water. Due to large volumes of water involved, water consisted of a mixture of 550 liter ground water and 50 liter stream water (preconditioning time of two weeks). Stream water and species came from nearby streams. Light regime followed natural seasonal conditions. Water temperature was kept constant at 12°C, air temperature at 16°C. Each compartment was covered with a Perspex plate to prevent emerged caddisflies from escaping. To facilitate collection of adults, emergence traps were installed above each compartment.

Study organisms

For this experiment, fifth instar larvae were hand-collected in the field in February. *P. rotundipennis* was collected at Rode Beek near Roermond (51° 8'34.35"N, 6° 7'19.38"E), while *M. sequax* was collected at Seelbeek near Heveadorp (51°58'24.23"N, 5°48'58.48"E), the Netherlands. All individuals were acclimatized before being transported to the mesocosms.

Subsequently, the fifth-instar larvae completed their larval development and pupation during the experiment. Resource acquisition is an important factor in this final instar life stage, since the larvae spend the largest part of developmental time as a fifth instars (Hickin, 1967). The eggs of *M. sequax* and *P. rotundipennis* hatch in late autumn (Bitsch & Frochot 1962; Higler 2005). Both species rapidly develop through the first four instars before winter when growth stops. After an extended stage as fifth instar, the larvae pupate and start emerging as terrestrial winged adults. *P. rotundipennis* emerges from July until September, while *M. sequax* emerges from May until September (Higler, 2005). The experiment was terminated in November after emergence had come to halt for several weeks.

Data collection and statistical analyses

Adults were collected within 48 hours after emergence and frozen at -18°C until further processing. The number of emerging individuals (emergence success) and the number of males and females (sex-ratio) was counted and length of the left forewing, the total adult dry biomass, and the dry biomass of the abdomen were measured. Forewing length (Distance between base of subcostal vein to wing tip, between radius R4 and R5. Terminology based on the Comstock-Needham system.) was measured by one person using a dissecting microscope with an eyepiece micrometer to the nearest 0.1 mm. Measurements were confined to wing length since strong linear relationships have been found between forewing length and forewing area for many caddisfly species (Kovats et al., 1996). To determine dry mass, the caddisflies were dried at 60°C for 48 hours to a constant weight and weighed on a microbalance to the nearest 0.0001 g. All results regarding emergence success and survival do not include drowned individuals. Additionally, all results regarding fitness do not include the drowned, nor the deformed individuals. Drowned individuals were regarded as dead, while deformed individuals were regarded as alive and counted for emergence rate, yet their deformities were so severe that it potentially compromised their reproductive success and were therefore disregarded during fitness analysis. Since male and female caddisflies differ in wing span and weight, they were analyzed separately.

All individuals were considered pseudo replicates within their experimental unit. Therefore, survival and fitness means and standard deviations were calculated per unit and subsequently tested for mesocosm effects (mesocosm number as independent factor). No significant effects of mesocosm were found (Table 5.2), nor were there any significant differences in the timing of emergence for the species in

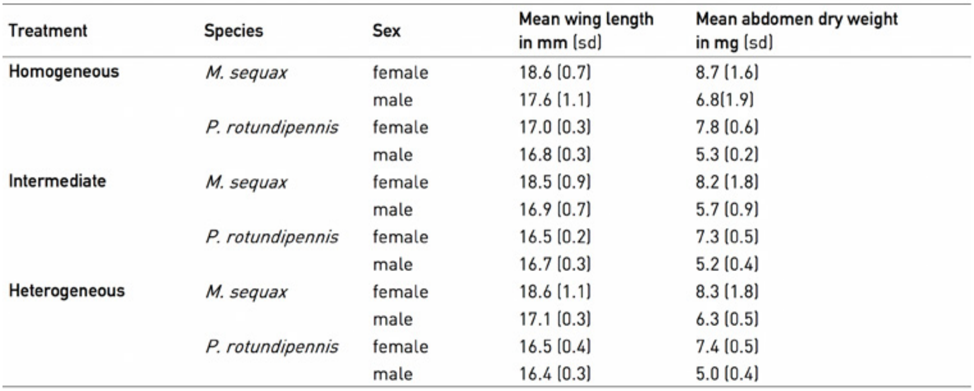
Table 5.2. Overview of statistical results of mesocosm effects and treatments (spatial configuration) effects on survival and fitness correlates.

Species	Sex	Dependent factor	Effect of mesocosm		Effect of treatment (df =2)	
			F	p	F	p
M. sequax	-	Emergence rate	0.532	0.715	4.418	**0.036**
P. rotundipennis	-		2.543	0.116	2.776	0.106
			(df=4)			
M. sequax	female	Wing length	1.595	0.250	0.249	0.791
		Abdomen biomass	0.580	0.684	0.265	0.779
	male	Wing length	0.737	0.590	0.953	*0.459*
		Abdomen biomass	0.584	0.682	1.067	0.427
			(df=5)			
P. rotundipennis	female	Wing length	1.486	0.285	3.477	**0.035**
		Abdomen biomass	0.824	0.563	1.161	0.317
	male	Wing length	0.190	0.959	3.657	**0.029**
		Abdomen biomass	0.419	0.824	2.061	0.132

Note All bold results are statistically significant analyzed with one-way ANOVA’s (p<0.05). One result in Italics comes from non-normally distributed data, analyzed with a Kruskal-Wallis non-parametric ANOVA with Bonferroni-corrected Mann-Whitney U tests (Bonferroni corrected p-value = 0.017). Six mesocosms were used in this experiment, resulting in 5 degrees of freedom while testing the effects of mesocosm. However, random distribution of species across mesocosms caused one mesocosm to not have any experimental units with *M. sequax*, resulting in five different mesocosms and four degrees of freedom to test for effects.

the different treatments. Mean dependent factor values (Table 5.3) were calculated by averaging all replicates per treatment per species. All normally distributed data were analyzed with one-way ANOVA's with patchiness level as fixed factor, followed by Tukey's post hoc procedures. Non-normal data were analysed using Kruskal-Wallis non-parametric ANOVA's, with Bonferroni-corrected Mann-Whitney U-tests to examine differences between treatment pairs (Divided p-value by three, due to three pair-wise comparisons; Table 5.2). Statistical tests were conducted in IBM SPSS Statistics (version 19.0/IBM Corp, Armonk, NY, USA).

Table 5.3. Mean wing length (mm) with standard deviation and mean abdomen dry weight (mg) with standard deviation of all 3 treatments (Homogeneous = large patches at large distances, Intermediate = medium sized patches at medium distance, Heterogeneous = many small patches at small distances) for both species, separated by sex.



Treatment	Species	Sex	Mean wing length in mm (sd)	Mean abdomen dry weight in mg (sd)
Homogeneous	*M. sequax*	female	18.6 (0.7)	8.7 (1.6)
		male	17.6 (1.1)	6.8(1.9)
	P. rotundipennis	female	17.0 (0.3)	7.8 (0.6)
		male	16.8 (0.3)	5.3 (0.2)
Intermediate	*M. sequax*	female	18.5 (0.9)	8.2 (1.8)
		male	16.9 (0.7)	5.7 (0.9)
	P. rotundipennis	female	16.5 (0.2)	7.3 (0.5)
		male	16.7 (0.3)	5.2 (0.4)
Heterogeneous	*M. sequax*	female	18.6 (1.1)	8.3 (1.8)
		male	17.1 (0.3)	6.3 (0.5)
	P. rotundipennis	female	16.5 (0.4)	7.4 (0.5)
		male	16.4 (0.3)	5.0 (0.4)

Note All mean values (standard deviation) are obtained by calculating averages of fitness correlates (wing length & abdomen weight) per experimental unit. Experimental units were distributed randomly across 6 mesocosms and replicated 5 times per treatment. Therefore, n=5 per given value.

Results

The emergence rate of the species differed, with a higher emergence success in *P. rotundipennis* in comparison to *M. sequax*. Mean development time (post-winter larval development till emerged adult) in *M. sequax* was faster in comparison to *P. rotundipennis* (respectively an average of 111 and 132 days). However, variation in development time was larger in *M. sequax*, with individuals emerging over a period of 166 days. The emergence time of *P. rotundipennis* was approximately one month shorter with an average 132 days (Table 5.4).

Treatment effects on adult emergence success

Emergence success in *M. sequax* was significantly lower in the homogeneous treatment than in the other two treatments (Fig. 5.3A). In *P. rotundipennis* no difference in emergence success between treatments was detected (Fig. 5.3B).

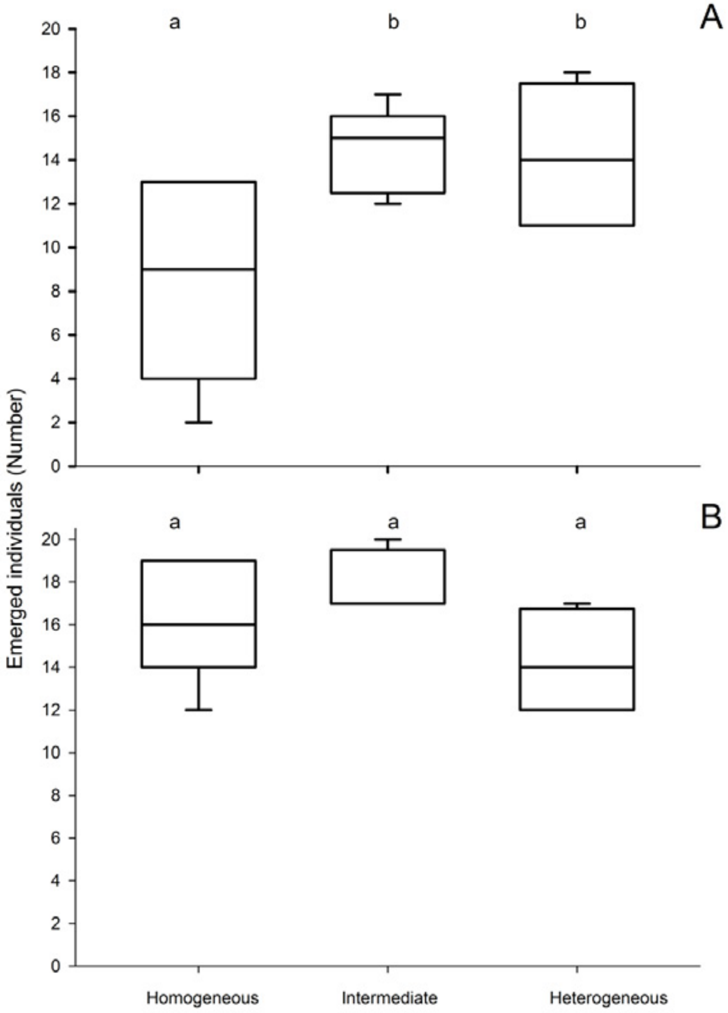


Figure 5.3 Median, 10th, 25th, 75th, 90th percentiles with error bars are displayed as vertical box plots for emerged individuals (#) of **A:** *M. sequax* and **B:** P. rotundipennis per patchiness treatment level. Degree of patchiness: homogeneous, intermediate, heterogeneous. Boxplots with different letters are significantly different. **A:** *M. sequax*; one-way ANOVA and Tukey HSD post hoc comparison, n=193, F= 4.4; p=0.036. **B:** P. rotundipennis; one-way ANOVA and Tukey HSD post hoc comparison, n=288, F= 2.77; p=0.106.

Treatment effects on fitness - wing length

For *M. sequax*, no significant differences in forewing length were found between the treatments (Fig. 5.4A; Tables 5.2 and 5.3). Contrastingly, forewing length of both male and female *P. rotundipennis* differed between treatments (Fig. 5.4B).

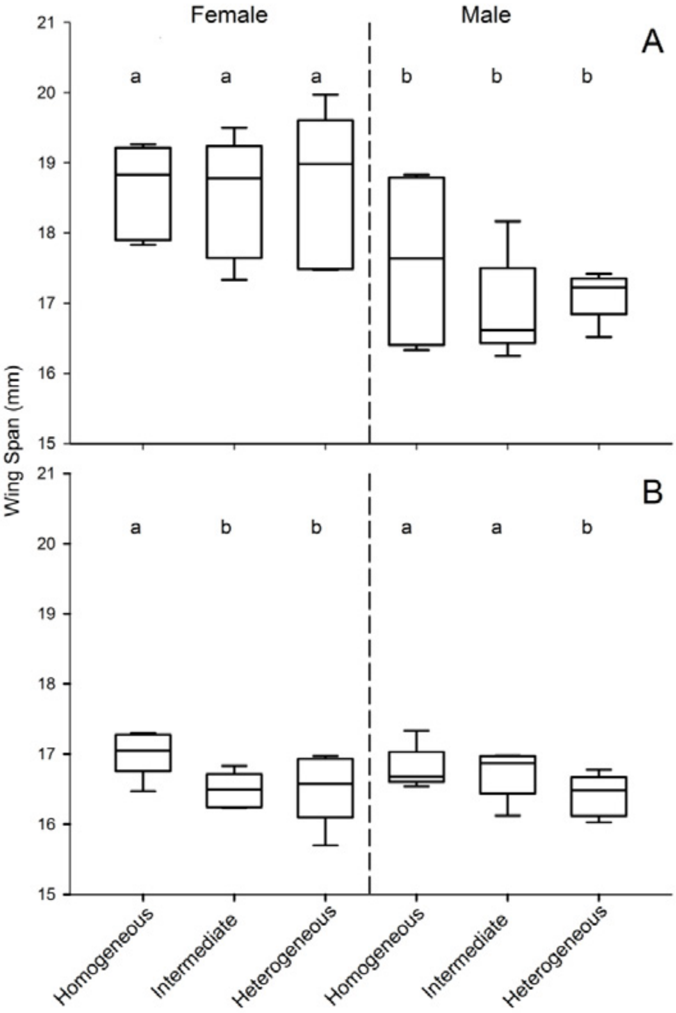


Figure 5.4 Median, 10th, 25th, 75th, 90th percentiles with error bars are displayed as vertical box plots for wing span of left forewing (mm) of *M. sequax* and P. rotundipennis (female and male) per patchiness treatment level. Plots with different letters are significantly different. **A:** *M. sequax* female: one-way ANOVA and Tukey HSD post hoc comparison, male: Kruskal-Wallis non-parametric ANOVA with Bonferroni-corrected Mann-Whitney U-tests. **B:** P. rotundipennis female and male: one-way ANOVA and Tukey HSD post hoc comparison.

Both female and male *P. rotundipennis* developed longer forewings in the homogeneous patch configuration than in the heterogeneous patch configuration. Additionally, males also displayed longer forewings in the intermediate patch configuration compared to the heterogeneous patch configuration (Fig. 5.4B).

Treatment effects on fitness– biomass

No effects of homogenization on abdomen biomass were found for *M. sequax* or *P. rotundipennis* (Fig. 5.5A and 5.5B; Tables 5.2 and 5.3).

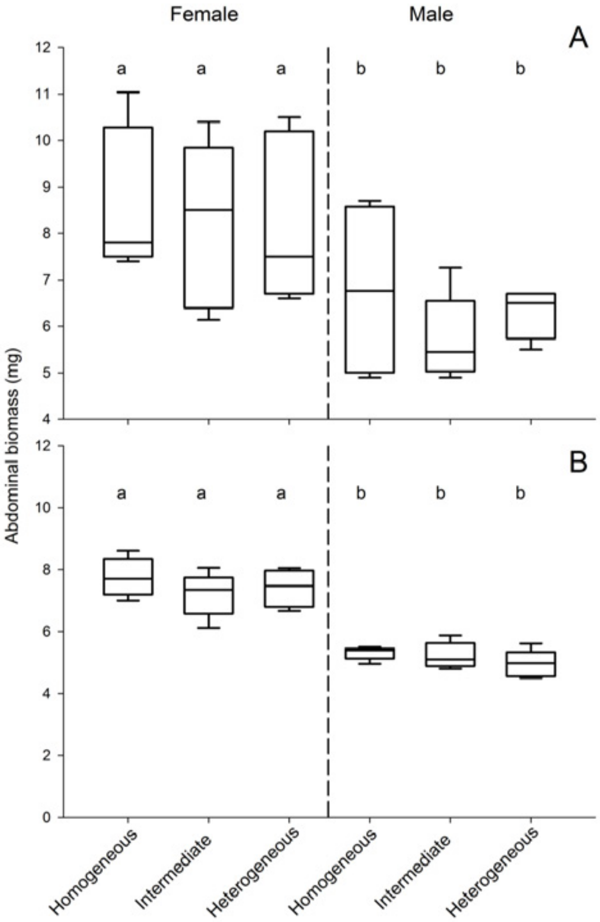


Figure 5.5 Median, 10th, 25th, 75th, 90th percentiles with error bars are displayed as vertical box plots of abdomen dry mass (mg) of *M. sequax* and P. rotundipennis (female and male) per patchiness treatment level. Plots with different letters are significantly different. **A:** *M. sequax* female and male: one-way ANOVA and Tukey HSD post hoc comparison. **B:** P. rotundipennis female and male: one-way ANOVA and Tukey HSD post hoc comparison.

Table 5.4. Overview of species' overall emergence success and development time.

Species	Adult emergence numbers					Development time (days)	
	total (%)	drowned	deformed	male (#)	female (#)	mean	min-max
M. sequax	64.3	0	6 (2%)	74	119	111	55 - 221
P. rotundipennis	96.0	31 (10.3%)	21 (7%)	171	117	132	63 - 195

Note: Percentage of emerged adults is calculated by number of fully developed adults that were found in mesocosm divided by 300 individuals that were introduced into the mesocosm as larvae. Percentages of drowned and deformed adults are calculated by dividing the observed number of drowned/deformed adults by the number of emerged adults. It remains unknown what caused drowning or the development of deformations. Adults were identified as male or female and counted (male/female #).

Discussion

Effect of streambed homogeneity on survival

This study shows that homogenization of the bottom substrate affects the survival and subsequent emergence of caddisflies in a species-specific way. *P. rotundipennis* emergence success is unaffected by substrate homogeneity, whilst *M. sequax* has a lower survival rate in the homogeneous treatment than in the intermediate and heterogeneous treatments. A possible explanation for the observed patterns lies in the preferred microhabitat of both species. *P. rotundipennis* has a rather ubiquitous microhabitat distribution under natural conditions and moves between substrates frequently (Higler, 1975; Schmera, 2002; Urbanič et al., 2005). *M. sequax* inhabits the edge of organic substrate patches (organic-sand interface) and displays sedentary behavior (Verdonschot et al., 2012). Heterogeneous landscapes with many small habitat fragments contain more edges for a given amount of habitat. Increasing intraspecific competition will take place when edge length becomes limited in a homogeneous streambed with lower number of habitat fragments.

Simultaneously, increasing distance between organic substrate patches causes individuals to co-occur at higher densities when they are not dispersing to nearby patches. Its sedentary behavior makes this species vulnerable, for example, if organic patches are washed out by peak flows and individuals of *M. sequax* dismiss the option to disperse to nearby patches. Our findings suggest a threshold in required microhabitat space, since only the homogeneous treatment, with the least amount of edge habitat, showed decreased survival rates. When extrapolated to decreased heterogeneity of habitat patches in a field situation, loss of heterogeneous microhabitat mosaics could negatively influence population size of this species.

Effect of streambed homogeneity on fitness of *M. sequax*

Despite the low emergence success in the homogeneous treatment, no effects on wing length and biomass were found in *M. sequax*. These results suggest that the stress from intraspecific competition does not lead to changes in fitness correlates (Feminella & Resh, 1990) but simply leads to mortality. We did not observe an allocation of resources that compromised either wing length or abdomen biomass. Abdomen biomass is assumed to be strongly correlated with fecundity (Honěk, 1993) since caddisflies will not continue their growth after emergence and nutrients required for egg production must derive from larval feeding (Boggs 2009; Jannot 2009). This is an important factor since fecundity will affect future generation size and has a long-term effect which has consequences for the resilience of aquatic macroinvertebrates both at the individual and population level.

Effect of streambed homogeneity on fitness of *P. rotundipennis*

Adults of *P. rotundipennis* showed an increase in wing length related to habitat homogeneity, which could indicate an investment in wing development during larval stages (Zera & Denno, 1997). The concept of investing in larger wings in unfavorable conditions (Harrison, 1980) could explain the large wing size of *P. rotundipennis* in this microhabitat experiment with large distances between favorable patches. The largest distance between patches in our experiment does not exceed 41 cm, yet we think that distances from this experiment are a good proxy for a natural environment since many caddisfly species exhibit only short distance movement in their aquatic stages (Verdonschot et al., 2012).

The ability to disperse from an unfavorable habitat is an important factor for ecosystem resilience (Öckinger et al., 2010) and positive correlations have been found between flight morphology (thorax mass and wing span) and site-occupancy of caddisflies (Hoffsten, 2004). Our finding indicates that wing development is affected by streambed homogeneity which has a subsequent effect on the terrestrial dispersal capacity of this species.

Trait-based responses to disturbance

Species resistance is often seen as a good prediction method for long-term biological responses. A study by Lake (2003) showed the effects of another disturbance (drought) on aquatic biota. He stated that both resistance and resilience appear to be order-specific. While in this case, we even find species-specific and sex-specific responses to streambed homogenization, probably dependent on trait expression. The importance of carrying out trait-based assessments on disturbance has, for example, been shown by Lancaster (2000). It was found that the size and shape of microhabitat patches appear to be important refuge characteristics after high flow disturbances. Small, soft-edged patches with a high edge/size ratio are more attractive than large patches as refuges for macroinvertebrates that seek shelter (Lancaster, 2000). After a trait-based analysis, it was found that taxa with a high mobility are especially dependent upon small flow refuge during high flow disturbance. Our experiment is complementing this study, by showing that taxa with different levels of habitat ubiquity and mobility display different responses to spatial shifts in microhabitats. Species with stronger habitat preferences appear to respond more negatively to increases in habitat homogenization, which in turn may lead to a lower ecosystem resistance.

The results of this study indicate that the response of organisms to specific disturbances is species-specific and there is little evidence for sex-specific responses. This supports the hypothesis that the level of trait expression should also be taken into account. By viewing an organism merely as a set of specific traits, the degree of response is still uncertain. Trait plasticity and trait combinations could have a severe effect on the organisms' response to certain disturbances. Future research involving more species with similar sets of traits could elucidate this issue. Additionally, a mesocosm set-up with equal edge length and differing patch distances, and vice versa, and simultaneous counting of interpatch movements could give qualitative data on resource acquisition.

Conclusion

We conclude that the studied species are affected by patch homogenization and that these effects are species-specific. Besides direct effects on survival, also potential indirect effects on the fitness of the individuals were observed, acting through forewing length. Ubiquity and mobility appear to play a role in the species response to patchiness. The outcome of this study gives an indication of the resistance of both species to streambed homogenization, a common result of natural and anthropogenic disturbance.

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6

Biotic interactions enhance survival and fitness in the caddisfly *Micropterna sequax* (Trichoptera: Limnephilidae)

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Abstract

Patches of coarse particulate organic matter in lowland streams are inhabited by many different macroinvertebrate species, yet knowledge of interactions among the members of these assemblages is scarce. In a mesocosm experiment we aimed to determine the effect of interspecific interactions on species survival and fitness of two caddisfly species. It was hypothesized that, as a result of positive interactions, mixed species populations yielded a higher survival and fitness than single species populations. Larvae of two caddisfly species, *Micropterna sequax* and *Potamophylax rotundipennis*, were reared in single species and mixed species populations. Emergence rate was recorded and adult fitness was measured in terms of wingspan and biomass. We found that in mixed populations emergence rate, wing length and biomass of *M. sequax* was higher than in single species populations. *P. rotundipennis* was only significantly, yet negatively, affected in terms of biomass of the male individuals. This study showed that occurring together with other species holds advantages for *M. sequax*, and emphasizes the importance of species diversity in streams. Furthermore, the observed positive effects on survival and fecundity might influence population sizes of the interacting species, in turn affecting macroinvertebrate-mediated ecosystem processes such as leaf litter decomposition.

Keywords: macroinvertebrates, interspecific facilitation, niche complementarity, ecosystem functioning, biodiversity

Acknowledgements

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Introduction

High flow induced within-stream habitat fragmentation results in isolated patches of preferred substrate for many macroinvertebrate species (Tolkamp, 1980; Lake, 2000; Jähnig et al., 2009; Schröder et al., 2013). In lowland streams these patches consist of coarse particulate organic material (leaves, leaf fragments, twigs) embedded in a matrix of sand, and are an important resource in terms of, amongst others, food and shelter (Egglishaw, 1964; Lancaster & Hildrew, 1993). As consumers of the material aggregated in the patches, shredders play an important role in the decomposition process (Anderson et al., 1978; Malmqvist & Oberle, 1995; Mermillod-Blondin et al., 2002). With many different species inhabiting these patches, intra- and interspecific interactions are expected to be frequent. Nonetheless, knowledge on biotic interactions among macroinvertebrates performing the same functional role is scarce.

Biotic interactions can be positive (mutualism and commensalism; Milbrink, 1993; Tokeshi, 1993) and/or negative (competition, parasitism, amensalism; Burkholder, 1952; Connel, 1983; Didham et al., 1996) for one or both species. The positive effect of an interaction, also known as facilitation, is seen as a key mechanism in which species diversity positively affects ecosystem processes and functioning (Mulder et al., 2001; Stachowicz & Byrnes, 2006; Bulleri et al., 2016). A study by Cardinale et al. (2002) showed that multiple species from the same functional feeding guild (aquatic suspension-feeders) enhanced each other's feeding success by decelerating the flow from upstream to downstream neighbours. This example shows that it is well possible that changes in species assemblages alter the likelihood of positive species interactions. While Cardinale et al.'s study is valuable for a better understanding of biotic interactions and resource partitioning, it remains unknown how biotic interactions affect species survival and development, and with that the long-term effect on future populations.

Species belonging to the same functional guild within a community could hypothetically become functionally redundant, with several species occupying the same ecological niche and consequently competing for resources (Walker, 1992; Duffy et al., 2001; Dolédec & Bonada, 2013). However, studies across terrestrial, marine and freshwater ecosystems suggest that functional complementarity is more often the case, with species having similar functions while niches amongst species do not overlap (Fargione et al., 2007; Rudolf et al., 2014; Kelly et al., 2016). Functional or niche complementarity results in greater resource uptake efficiency and faster ecosystem process rates (Loreau, 2000; Loreau & Hector, 2001; Fox, 2005; Leibold et al., 2016). Considering the organic patches as rather isolated streambed microhabitats, inhabited by a set of species with similar feeding strategies, it is probable that these species have adapted to cope with frequent biotic interactions, and may even benefit from it. In other words, the high organic patch species diversity observed in lowland streams might be the result of niche complementarity.

In this study, we investigated the effects of interspecific interactions in leaf-shredding caddisfly (Trichoptera) larvae. Caddisflies were selected as model organisms because of their abundance and species richness in lowland streams (Giller & Malmqvist, 1998), their vital role as shredder within the macroinvertebrate community in decomposition of leaf litter (Malmqvist & Oberle, 1995). *Micropterna sequax* (McLachlan, 1875) and *Potamophylax rotundipennis* (Brauer, 1857) belong to the shredder functional feeding guild and are characteristic of and abundant in northwestern European low-gradient streams with a streambed dominated by sand and patches of organic material. Both species belong to the family Limnephilidae, subfamily Limnephilinae. Studying two almost taxonomically and functionally similar species will stress the importance of biotic interactions when differences are found.

Biologically, *M. sequax* differs from *P. rotundipennis* in terms of a longer period of emergence and a larger wing span (Higler, 2008; Graf et al., 2015). Ecologically, *M. sequax* displays more sedentary behavior when positioned at its preferred substratum than *P. rotundipennis*, which is more ubiquitous (Verdonschot et al., 2012). It is known that both species occur on similar habitat patches spatially and temporally, but differ slightly in their preferred microhabitat within the patch (Westveer et al., 2017). This behaviour could be a consequence of interspecific interaction, which caused us to use these two species for this experiment.

We tested the effects of their interaction on adult fitness correlates related to emergence, growth and fecundity, by studying single species and mixed populations in leaf patches differing in the degree of patchiness. In a mesocosm experiment we tested: 1.) if habitat isolation increases interspecific interactions among species, and 2.) how these interactions affected emergence and fitness in the individual species. We hypothesized that mixed species populations yielded a higher emergence rate and fitness as a result of adaptations to living in dense multi-species aggregations and that this effect was more evident under increased interaction pressure resulting from patch isolation.

Material and methods

Collection of specimens

The fifth-instar larvae were hand-collected from natural streams in the first half of March. *P. rotundipennis* was collected at Rode Beek near Roermond, while *M. sequax* was collected at Seelbeek near Heveadorp, the Netherlands. While both species have similar distribution patterns throughout the Netherlands, each species occurred in largest abundance at different locations within the watershed. This provided the opportunity to efficiently collect many individuals from the same instar at the same time. All individuals were acclimatized before being released in the mesocosms. Fifth-instar larvae were used because the larvae spend the largest part of developmental time in this life stage (Hickin, 1967). Resources are acquired during this stage for the remainder of their lifespan (Boggs, 2009; Jannot, 2009). Adults may take up some additional food after emergence but individuals will not continue their growth and nutrients required for adult body parts (e.g. wings, abdomen, eggs and sperm) must derive from feeding during larval stage (Boggs, 1981).

Stream mesocosm setup

Tests were carried out between March and November 2015. In the experiment we used 6 indoor stream mesocosms, each consisting of 4 artificial recirculating channels. Each channel was divided into 2 compartments, from now on referred to as 'experimental units' (Figure 6.1a). Each experimental unit contained 20 individuals of either *Micropterna sequax* or *Potamophylax rotundipennis* (single species population) or 10 individuals of both species (mixed species population). This set-up allowed us to incorporate both species, while population pressure was kept similar across all experimental units. Larvae were similar-sized (fifth instar, n=28), with a mean head-width of 1.737 mm for *M. sequax* and 1.834 mm for *P. rotundipennis*.

Each experimental unit contained a 5-cm-thick stream bottom consisting of sand (grain size 1-3 mm) and patches of leaves (abscised oak; *Quercus robur*). Water depth was 10 cm. Leaf patches were offered in three different spatial arrangements, differing in the number of patches and individual patch size (Fig 6.1b). Total patch size and biomass of leaves was kept constant in all experimental units. Per unit 10 g of leaf material was distributed equally across all leaf patches. To keep patch configuration intact during the experiment and especially to prevent mixing of the substrates, PVC slots were placed between the patches of sand and leaves. To prevent downstream transport of the leaves by the current, patches were covered by a 1 x 2.2 cm mesh (Fig 6.1c). Each unit was covered with a perspex plate to prevent the escape of emerged caddisflies.

With 3 population composition types and 3 spatial leaf patch configurations, this resulted in 9 different treatments. Each treatment was replicated 5 times and randomly distributed across 45 experimental units.

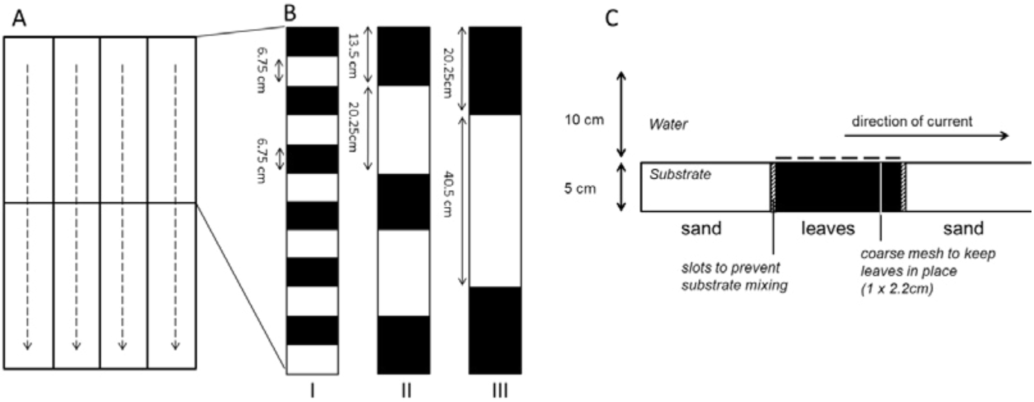


Figure 6.1. A: Outline of a mesocosm with four stream channels. Each channel was divided into two compartments with mesh (1 x 1 mm) to keep water flowing (direction of arrow) but prevent exchange between compartments. Six identical stream mesocosms were used, with a total of 48 compartments/ experimental units. **B:** Top down view of three different patch configurations. One configuration per experimental unit, with increasing patch isolation and subsequent biotic interaction frequency potential. I = low frequency interaction, II=intermediate frequency interaction, III=high frequency interaction.
C: Side view of bottom substrate of the experimental compartments.

Current velocity was kept constant at 10 cm/s in all units and fell within the preferred flow preference range described for both species (Dolédec et al., 2007; de Brouwer et al., 2017). The water inside the units was constantly aerated and recirculated from a 600 L reservoir and consisted of preconditioned water (preconditioning time was 2 weeks, water consisted of a mixture of 550L of tap water and 50L of filtered water from a nearby stream). Light regime followed natural seasonal conditions. Water temperature was kept constant at 12°C, air temperature at 16°C.

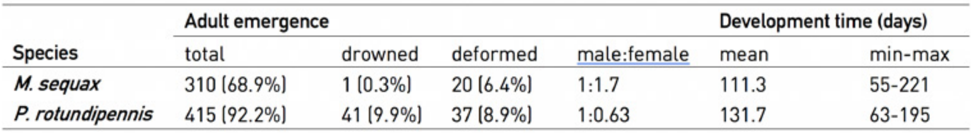
To prevent food availability to become a limiting factor, 5 g of extra leaf material was added per unit halfway through the experiment. This resource addition mimics a natural situation in which *Q. robur* leaves, a very slow decomposing litter type (Swift et al., 1979), is occasionally distributed across the stream bottom in forested areas throughout the year (Minshall, 1967). Furthermore, additional food in the form of 1 cube (2 g) of frozen Tubifex oligochaetes (Dutch Select Food Tubifex; crude protein 6,2%, crude fat 2,6%, crude fiber 0,6%, ash 0,5%) was supplied every two weeks in each compartment to prevent cannibalism or predation in species which supplement their diet with (dead) invertebrates (Wissinger et al., 2004). These extra food sources were added to ensure that all potential components of the caddisflies diet were available and therefore not limit survival and growth during the experiment.

Data collection and statistical analyses

After emergence, adults were collected within 48 hours and frozen at -18°C until further processing. Number of emerged males and females (sex-ratio) was identified and counted (Malicky, 2004). Left forewing length was measured using a microscope with an eyepiece micrometer to the nearest 0.1 mm. To determine adult abdomen dry mass, the caddisflies were dried to a constant weight at 60°C for 48 hours and weighted on a microbalance to the nearest 0.0001 g (minimum measured weight = 0.0020 g). Wing span and biomass were used as fitness correlates because of their effect on respectively dispersal capacity (Hoffsten, 2004) and fecundity (Honěk, 1993).

All results regarding fitness do not include drowned, nor deformed individuals (Table 6.1). Drowned individuals were regarded as dead, while deformed individuals were regarded as alive and counted for emergence rate, yet their deformities were so severe that it potentially compromised their reproductive success. Therefore,

Table 6.1. Overview of species' overall development time and emergence rate.



	Adult emergence				Development time (days)	
Species	total	drowned	deformed	male:female	mean	min-max
M. sequax	310 (68.9%)	1 (0.3%)	20 (6.4%)	1:1.7	111.3	55-221
P. rotundipennis	415 (92.2%)	41 (9.9%)	37 (8.9%)	1:0.63	131.7	63-195

Note Percentage of total emerged adults is calculated by number of fully developed adults that were found in the experimental units divided by 450 individuals that were introduced into the experiment as larvae (Corbet, 1969). Percentages of drowned and deformed adults are calculated by dividing the observed number of drowned/deformed adults by the amount of emerged adults. It is unknown what caused drowning or the development of deformations.

these individuals were disregarded in the fitness analysis. Since male and female caddisflies differ in wing span and body weight, they were analyzed separately.

The number of emerged individuals per experimental unit was expressed as ratios, where 1 equals emergence of 20 individuals in the single species populations and 10 individuals in the mixed species populations. All individuals were considered pseudo replicates within their experimental unit. Therefore, mean values and standard deviations for emergence rate and fitness correlates were calculated per experimental unit (n=5) and subsequently tested for mesocosm effects (mesocosm number as independent factor). No significant effects of mesocosm were found (Independent t-test, n=6, P=0.715 (*M. sequax*), P=0.116 (*P. rotundipennis*)). All data was tested for normal distribution (Shapiro-Wilk). All normally distributed data was analyzed with one-way ANOVA's to test for the effect of population type (single or mixed species) on fitness, and two-way ANOVA's with spatial configuration (I, II, III) and population type as fixed factors, followed by Tukey's HSD post hoc procedures. Non-normal data was analysed using Kruskal-Wallis non-parametric ANOVA's. Statistical tests were conducted in IBM SPSS Statistics (version 22.0 /IBM Corp, Armonk, NY, USA).

Results

The emergence rate of the species differed, with a higher emergence rate in *P. rotundipennis* in comparison to *M. sequax*. The mean development time (post-winter larval development till emerged adult) of *M. sequax* was shorter than that of *P. rotundipennis* (respectively 111.3 and 131.7 days). However, variation in development time was larger in *M. sequax*, with individuals emerging over a period of 166 days (Table 6.1). Treatments with mixed species populations had interspecific interaction potential throughout the vast majority (87%) of the experiment. On average, both species were present together in the experimental unit for 146.2 days before emergence, after which an average of 18.7 days remained with only one species in the unit. Thus, in the final stages of the experiment some mixed population units had low interaction potential, however, this stage was spent while pupating and therefore no biotic interactions would have been expected anyway.

Effects of patch configuration on biotic interactions

All responses, in terms of emergence rate, effects of biotic interactions and of spatial configuration, appeared to be species-specific. *M. sequax* showed a higher emergence rate in mixed populations in all spatial configurations (Figure 6.2a, two-way ANOVA $F_{(1,\ 2)}$=12.05, P=0.002). Additionally, *M. sequax* showed a significant decrease in emergence rate in the habitat with a high patch isolation ($F_{(1,\ 2)}$=9.087, P=0.001). However, a two-way ANOVA showed that combining both factors (interaction population type and spatial configuration) did not cause an additional effect ($F_{(2,\ 24)}$=0.707, P=0.503), indicating *M. sequax* did not respond differently to patch configuration when reared with *P. rotundipennis*. This means that patch

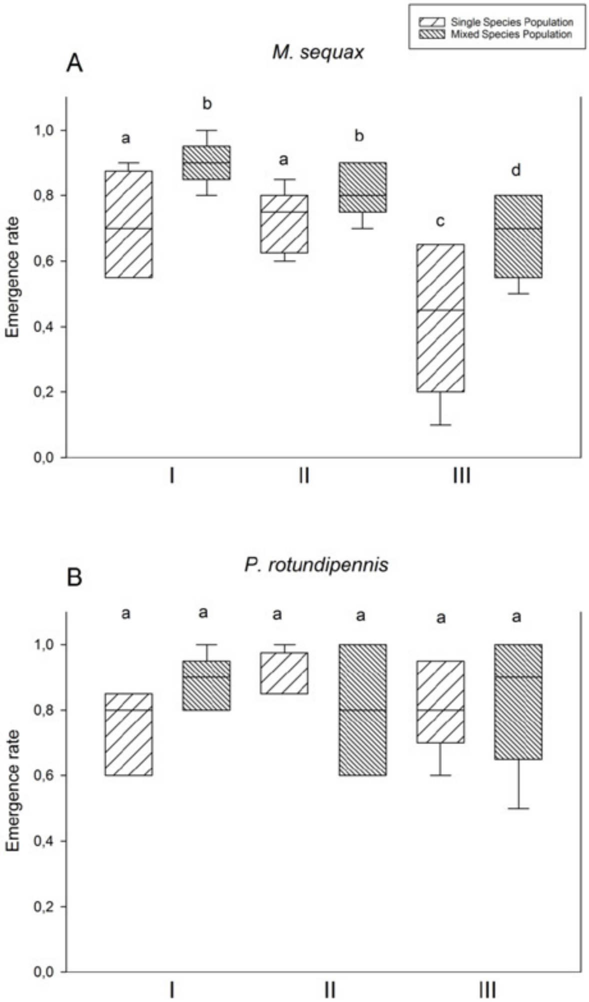


Figure 6.2 Boxplots of the emergence rates of **A:** *M. sequax* and **B:** *P. rotundipennis* with median, 25-75th percentiles in box, 10-90th percentiles as error bars and dots indicating outliers, in single and mixed populations across three spatial configurations range from low patch isolation and low interaction potential (I) to high patch isolation and high interaction potential (III). Different letters indicate significant differences between groups. (Two-way ANOVA, Tukey post hoc test p<0.05).

configuration influences emergence, but the uniform direction of change from single to mixed populations indicate the effect of an interspecific interaction.

P. rotundipennis was unaffected by both biotic interactions with *M. sequax* (two-way ANOVA: $F_{(1, 2)}$=0.136, P=0.716) and spatial configuration ($F_{(1, 2)}$=0.181, P=0.835) in terms of survival rate (Fig 6.2b). This species showed overall high emergence rates, regardless of the population in which the larvae developed or the level of patch isolation and subsequent interaction frequency potential. Once more, no interaction effect was found between the two independent factors ($F_{(2,24)}$=1.630, P=0.217).

Due to the non-significant interaction effect of spatial configuration and population type, all spatial configurations were pooled and data was grouped into either single species population or mixed species population for analysis of biotic interaction effects on emergence and fitness rates.

Effects of biotic interactions on survival and fitness

In *M. sequax* more individuals emerged from the experimental units with a mixed species population (Figure 6.3; Independent samples t-test, t(28)=-2.78, P=0.010). *P. rotundipennis* showed overall high emergence rates, while no significant difference was found between single species- and mixed populations (Independent samples t-test, t(28)=-0.3, P=0.731) (Fig 6.3).

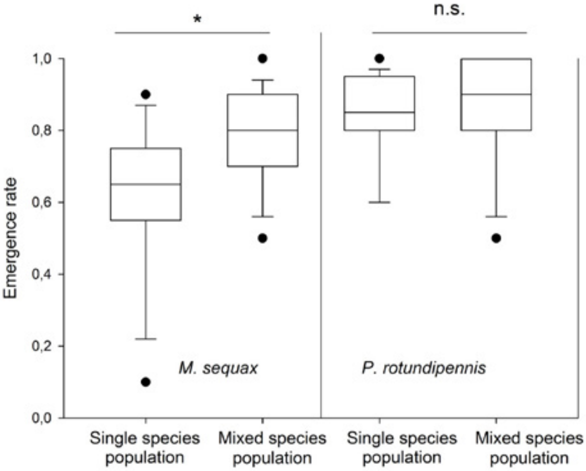


Figure 6.3 Boxplots of the emergence rates of single and mixed *M. sequax* and *P. rotundipennis* populations with median, 25-75th percentiles in box, 10-90th percentiles as error bars and dots indicating outliers (Independent t-tests P <0.05). Asterisk indicates significant difference.

M. sequax females developed larger forewings in mixed populations ($F_{(1,\ 182)}$=14.4, P<0.001) and had higher abdomen dry weights (Kruskal Wallis X^2(1) = 20.28, P=0.000, Figure 6.4, panel A and B). Similar results were found for *M. sequax* males (Fig 6.4, panel C and D) with larger forewings ($F_{(1,\ 103)}$=25.5, P<0.001) and abdomen dry weight ($F_{(1,\ 103)}$=34.5, P<0.001).

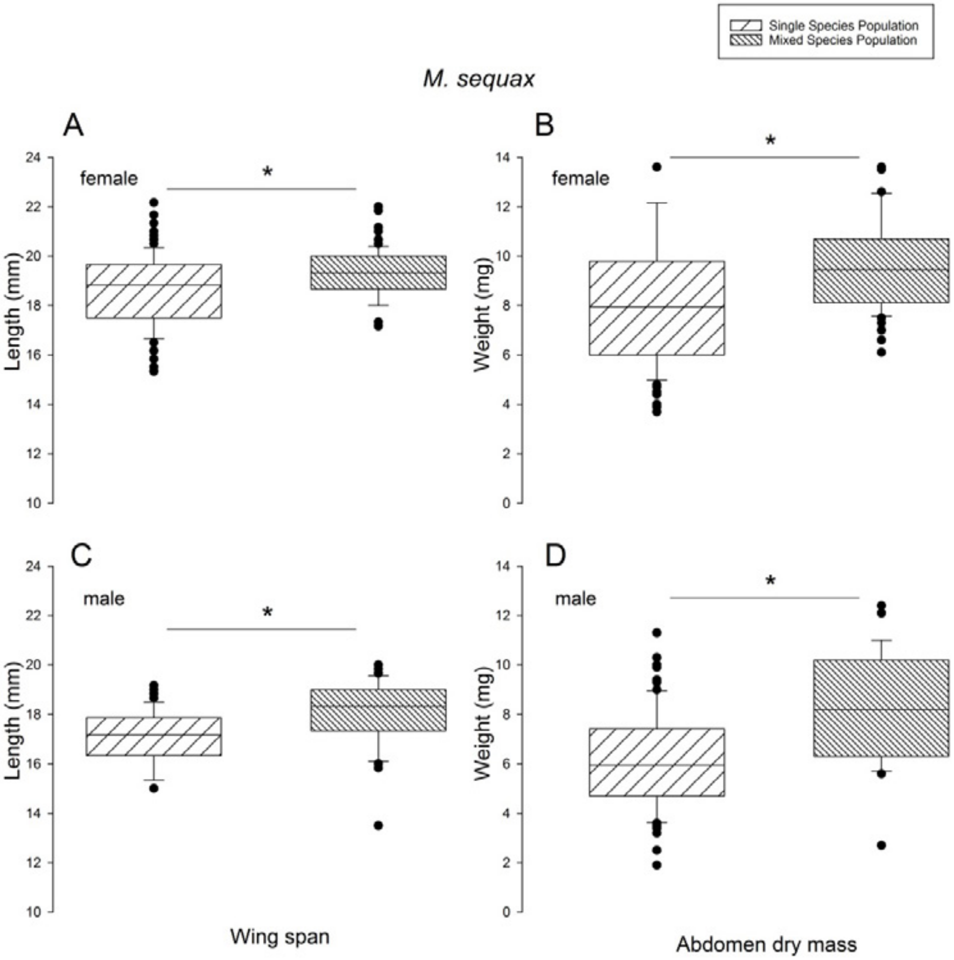


Figure 6.4 Boxplots of the fitness correlates of *M. sequax* in single species and mixed populations **A:** forewing length (mm) of females, **B:** abdomen dry mass (mg) of females (n= 113 for single species population, n= 71 for mixed population), **C:** forewing length (mm) of males , **D:** abdomen dry mass (mg) of males (n=73 for single species population, n= 32 for mixed species population) with median, 25-75th percentiles in box, 10-90th percentiles as error bars and dots indicating outliers. Asterisk indicates significant differences (Tukey post hoc tests P <0.05)

P. rotundipennis females did not show any significant responses to biotic interactions during developmental stages in terms of wing length ($F_{(1,\ 135)}$=1.01, P=0.3) and abdomen dry weight ($F_{(1,\ 135)}$= 1.43, P=0.2) (Figure 6.5, panel A and B). *P. rotundipennis* males showed no significant response for wing length ($F_{(1,\ 197)}$=3.15, P=0.078), but did show significantly lower abdomen weight (Kruskal-Wallis $X^2(1)$ = 11.4, P=0.001) in the mixed populations (Figure 6.5, panel C and D).

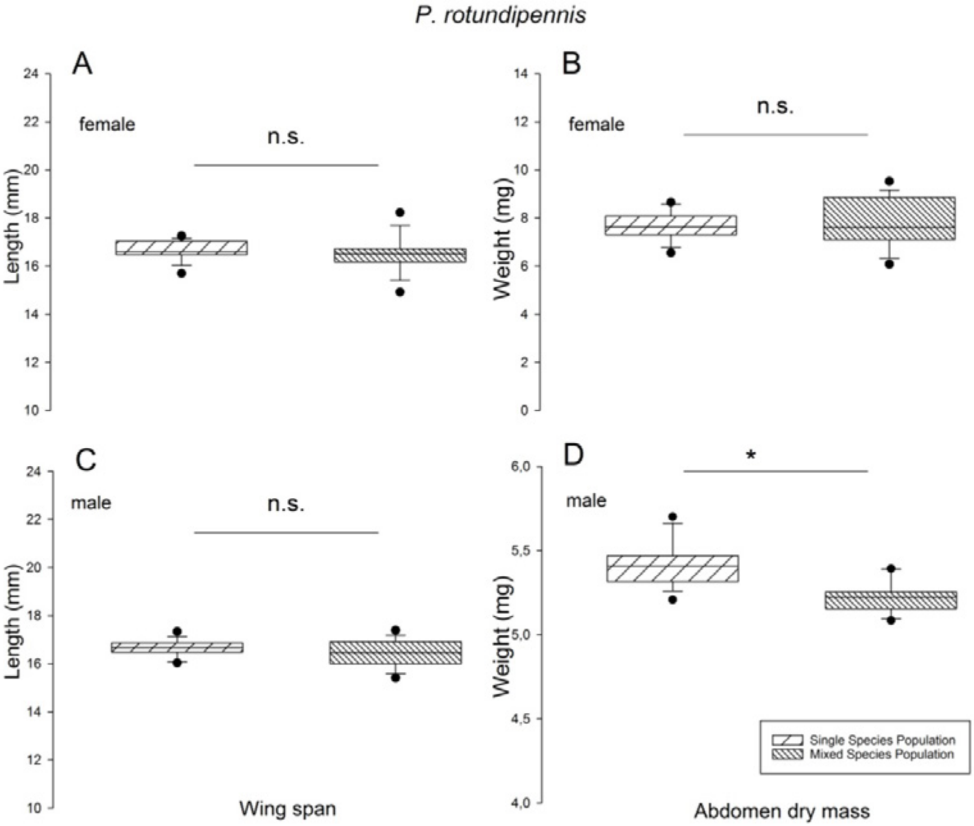


Figure 6.5 Boxplots of the fitness correlates of *P. rotundipennis* in single species and mixed populations **A:** forewing length (mm) of females , **B:** abdomen dry mass (mg) of females (n=101 for single species population, n=37 for mixed species population), **C:** forewing length (mm) of males , **D:** abdomen dry mass (mg) of males (n=135 for single species population, n=64 for mixed species population). Asterisk indicates significant differences (Tukey post hoc tests P <0.05).

Discussion

Effects of patch configuration on biotic interactions

Patch configuration did not affect the outcome of the biotic interactions among *M. sequax* and *P. rotundipennis*. A positive effect of occurring in mixed populations in the leaf patches was observed in each of the three spatial configurations offered to the larvae. The observed negative effects on *M. sequax* in the least patchy most isolated configuration where comparable in both single and mixed populations, and might be related to the species' microhabitat preference and/or behaviour: being an inhabitant of the edge of organic substrate patches (organic-sand interface) and displaying relatively sedentary behaviour, this configuration might have been less suitable for this species (Higler, 1975; Schmera, 2002; Urbanič et al., 2005; Verdonschot et al., 2012), resulting in a decreased emergence rate which could not be compensated fully by the positive effects of the presence of *P. rotundipennis*.

Positive effects of biotic interactions

Our study showed that *M. sequax* benefitted from the presence of *P. rotundipennis* in the leaf patches. On the contrary, P. rotundipennis did not appear to obtain direct benefits from inhabiting these patches together with *M. sequax*. Positive effects of this biotic interaction include a larger wingspan and a higher abdomen biomass in *M. sequax*. With wing size being an important determinant for dispersal capacity in caddisflies (Hoffsten, 2004), and abdomen biomass being directly correlated to adult fecundity (Honěk, 1993), it is well possible that in our mixed species populations the fitness of M.sequax was increased by the presence of *P. rotundipennis* in the patches. Additionally, in this study a complementarity effect (Loreau & Hector, 2001; Petchey, 2003) is demonstrated, in which positive interactions could lead to increased productivity, in this case biomass.

One of the mechanisms through which one of these species might enhance the other's realized niche, could be their foraging strategy. It is possible that both species prefer different food particle size-ranges, in this case leaf fragments colonized by microorganisms. This concept has also been described as 'selective feeding' by Cummins & Klug (1979). Selective feeding is defined as the ingestion of only certain particles from a range of those that are equally available to the feeding insect. This feeding strategy involves no mechanical interference and therefore excludes some of the materials that need a morphological-behavioural specialization of the insect (Cummins, 1973). Thus, insects do not have to invest in specialized ways to make food available for ingestion, but selectively feed on appropriate food particles due to food partitioning with a similar species. In this case, *P. rotundipennis* potentially cuts leaf fragments into its preferred particle size, after which *M. sequax* forages on the smaller-sized remains.

An alternative explanation could be that *M. sequax* feeds on the faeces of *P. rotundipennis*. A study by Milbrink (1993) showed a mutualistic interaction in freshwater oligochaete communities, where two species of oligochaetes feed on the bacteria that colonize each other's faecal pallets. Even though both species appear to be very similar in their functional traits (Higler, 2005; Graf et al., 2015) they showed species-specific responses. It is known that *M. sequax* is a specialized species when it comes to substrate preference (Rumbos et al., 2010) but unfortunately there is no literature on preferred food particle size.

It has been hypothesized that a shift in feeding ecology from omnivorous shredding to epilithic grazing, and corresponding mandible adaptations to do so, has caused the diversification within the Limnephilidae (Pauls et al., 2008; Waringer et al., 2013). Within this family, evolutionary adaptations have led to spoon-shaped mandibles, toothed mandible edges and mandibles lacking any type of serration. Both *M. sequax* and *P. rotundipennis* have mandibles with teeth which they use for shredding as well as grazing, yet there can be subtle differences in the morphology of the mandibles that lead to a difference in food processing. Future research on food preference and mandible morphology could elucidate this issue.

Neutral and negative effects of biotic interactions

The presence of *M. sequax* did not affect the measured fitness correlates in *P. rotundipennis* females and the wing length in *P. rotundipennis* males. In line with this finding, Cardinale et al. (2002) showed that one out of three species of suspension-feeding Trichoptera did not show significant differences in resource uptake in a mixed population, while the other two species did. This demonstrates the one-way facilitating direction, termed 'commensalism', that an interspecific interaction can have (Connell, 1983). It has been found that Chironomidae often display commensalistic interactions to increase their feeding opportunity, mobility, protection from disturbance and reduce predation risk (Tokeshi, 1993; Callisto et al., 2006), but this entails interactions with other orders of aquatic invertebrates. Within-class commensalism has not been found or described for many aquatic invertebrates, yet could have a large explanatory value when it comes to niche expansion and range shifts (Bulleri et al., 2016).

A negative effect of being reared in mixed populations was found for the abdomen biomass of *P. rotundipennis* males. Biotic interactions in which one species benefits at the expense of the other are referred to as antagonistic interactions. Negative effects on fitness correlates could be explained with developmental trade-offs arising under specific conditions (Stevens et al., 1999; Stevens et al., 2000). For example, while larvae develop into pupae, strategic allocation of resources could favour wing span, and thereby dispersal capacity, over fecundity. However, the lower abdomen biomass did not cause an increased wing span for the males of *P. rotundipennis*, it merely developed equal wing lengths compared to individuals from the single species population. Allocating resources at the expense of fecundity could also be a response to the difference in intraspecific population density. In the mixed species populations both species were present with 10 individuals, while in the single species populations 20 individuals of one of the species was present. Lower sperm competition risk in populations with fewer females of their own species (Gage, 1995; McNamara et al., 2010) could lead to a decreased investment of resources in fecundity. Without measuring the precise allocation of energy, we cannot conclude that a developmental trade-off is apparent in this case, neither is it clear why only *P. rotundipennis* males were affected.

Conclusion

In our study we demonstrated that biotic interactions take place between caddisfly species inhabiting patches of coarse organic material, resulting in positive effects on *M. sequax* and neutral to negative effects on fitness correlates in *P. rotundipennis*. Changes in fitness of individual members of macroinvertebrate assemblages resulting from interspecific interactions could affect future population sizes and species distributions, and with that are an important determinant of stream macroinvertebrate community composition. By linking the enhanced survival and fitness of members of the community to the ecosystem processes performed by these species, the presence of positive interactions is likely to influence ecosystem processes. In this case, decomposition rates in patches of organic material, an essential process in temperate lowland streams. We show that biotic interactions have an impact on population level and thereby, when viewed at a larger scale, might influence stream ecosystem functioning. It is likely that more biotic interactions comparable to what we found occur in lowland stream ecosystems, which should be a theme in future research.

7

Synthesis

The aim of this thesis was to unravel the ecological mechanisms behind dispersal of aquatic invertebrates in restored lowland streams. Reviewing the studies published so far showed that habitat connectivity (Doerr et al., 2014; Durães et al., 2016; Sarremejane et al., 2017), dispersal capacity (De Bie et al., 2012; Kappes & Haase, 2012; Stevens et al., 2013; Cañedo-Argüelles et al., 2015), biotic interactions (Schuwirth, Dietzel & Reichert, 2016), seasonality (Robinson, Tockner & Burgherr, 2004; Murrell, Ives & Juliano, 2014; Bogan et al., 2017; Tonkin et al., 2017b), and habitat suitability (Winemiller et al., 2010; Heino, 2013) are all important parameters contributing to successful recolonization of restored habitat, yet each parameter operates differently at different spatial and temporal scales (Brosse, Arbuckle & Townsend, 2003; Grönroos et al., 2013; Heino et al., 2015; Tonkin et al., 2017a). To investigate habitat suitability and dispersal processes of aquatic invertebrates at various scales, we performed several observational and experimental studies. It has been hypothesized before that the lack of invertebrate community response to reach-scale restorations is caused by a mismatch in the spatial scales of the environmental stressors and restoration projects (Bond & Lake, 2003; Feld et al., 2011; Lake, Bond & Reich, 2007; Roni, Hanson & Beechie, 2008; Stoll et al., 2016). The results of our studies led to an increased understanding of how recolonization of streams is limited by several dispersal and environmental constraints, but more importantly, how the effect-size of these parameters shifts as the scale at which dispersal occurs decreases (Figure 7.1).

In this last chapter of my thesis I will focus on the improved understanding of how the combined effect of these parameters influences dispersal and colonization from a large scale (dispersal between catchments) to a small scale (movements between within stream habitat patches), in order to improve future stream restoration practices.

The role of dispersal filtering from large to small spatial scales

In order to colonize new habitat, species first have to be able to disperse to it. By studying this process at various spatial scales, we noticed a gradual shift in the importance of dispersal capacity and habitat connectivity from large to smaller spatial scales.

At the catchment-scale, aerial dispersal between catchments is seen as an important process in the recolonization of new stream habitat (Mackay, 1992).

Especially isolated headwater streams require long-distance dispersal by aerial active invertebrates (Sarremejane et al., 2017). However, long-distance dispersal has rarely been studied, due to its elusive nature and the presumed unpredictability of such events (Gillespie et al., 2012). Wind-mediated long-distance dispersal has been recorded for Diptera (Verdonschot & Besse-Lototskaya, 2014) as well as other aquatic invertebrates, such as Ephemeroptera, Plecoptera and Trichoptera (Kovats et al.,1996). Unfortunately, the drivers behind overland dispersal remain unclear, as well as the maximum dispersal distance of many species. Aerial active dispersers, benefitted with morphological characteristics such as long wings (Hoffsten, 2004) and small body size (De Bie et al., 2012), seem fit for long distance dispersal. Landscape barriers, such as a lack of vegetation, limit habitat connectivity and thereby dispersal (Macneale, Peckarsky & Likens, 2005).

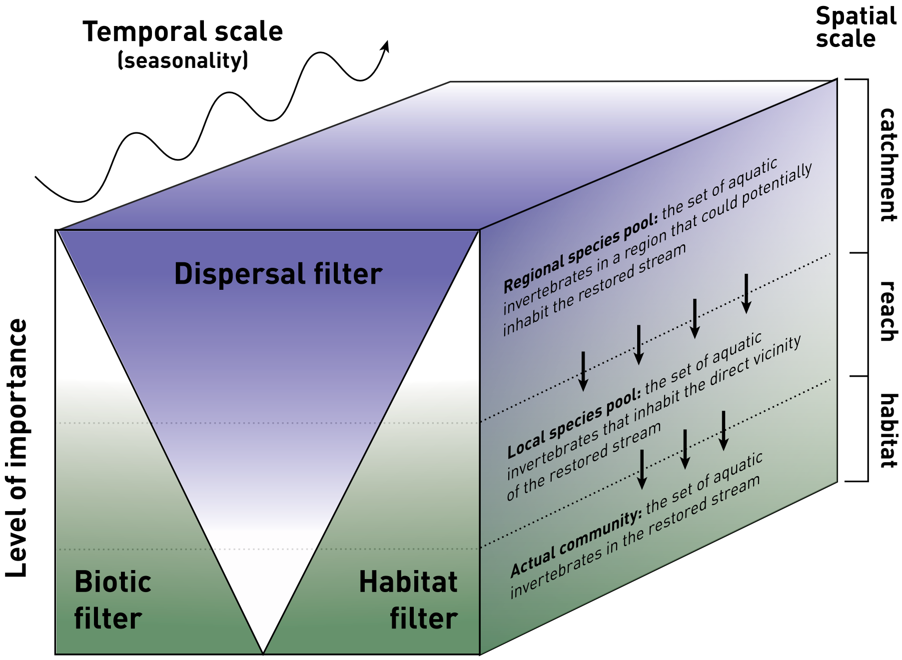


Figure 7.1. Theoretical framework (adapted from Lake, Bond & Reich, 2007, and elaborated with findings presented in current thesis), depicting the gradual processes that determine invertebrate community assembly in a restored lowland stream at various spatial and temporal scales. We call this the 'Community Restoration Continuum'.

In accordance with other studies, we found that successful long-distance overland dispersal events are rare and appear to be stochastic (Kovats et al., 1996; Petersen et al., 1999, 2004; Verdonschot & Besse-Lototskaya, 2014) (Chapter 2). Dispersal by aerial dispersers, between catchments, is probably limited by poor terrestrial habitat connectivity and long-distance flight capacity. At this large scale, biotic interactions or stream habitat suitability are of lesser importance, since the dispersal event is usually only a short period in the lifetime of the invertebrate, occurring right before ovipositioning (Corbet, 1964).

Limitations in overland dispersal are a serious issue because adjacent catchments are likely to be too far apart for most aerial dispersers to bridge. This will ultimately lead to depleted communities over time. Not only will this affect the stream ecosystem, the terrestrial system will also get depleted when populations decline, as adult insects form an important link between aquatic and terrestrial systems as food source for birds (Lancaster & Briers, 2007). There have been some recent studies on the effects of land-use on dispersal range (Petersen et al., 2004; Carlson et al., 2016; Tonkin et al., 2017a), which could provide a start for future studies on how to build the most effective terrestrial corridors to enhance overland dispersal. Additionally, mechanisms behind vector-mediated long-distance dispersal should be explored to elucidate the recolonization of aerial passive dispersers. While passive dispersal via animal vectors has been observed directly and indirectly, the survival of passive dispersers under different regimes and by different vectors is poorly understood (Bilton, Freeland & Okamura, 2001).

At within catchment scale, the community is assembled by dispersal capacity and habitat connectivity through slightly different mechanisms. We showed that dispersal within the same catchment, does not necessarily require an aerial mode of dispersal (Chapter 3). Active and passive drift cause invertebrates to disperse by water flow throughout a catchment, as was already shown by Townsend & Hildrew (1976) and Müller (1982). Smaller scale dispersal, within one catchment, could still be limited by habitat fragmentation, e.g. when stretches are prone to drought (Lake, 2003; Boulton, 2003) or other major stressors. However, our results comply with the finding that species have adapted to use abiotic parameters, such as water flow, to disperse (Downes & Lancaster, 2010; Lancaster & Downes, 2017).

In terms of dispersal capacity, our study showed some surprising results, in the sense that fully aquatic species (i.e., gastropods and molluscs) also arrived quickly in new stream reaches while these are thought to be weak dispersers (Kappes & Haase, 2012). It has been popular to study specific traits in regard to dispersal capacity (De Bie et al., 2012; Winking et al., 2014, 2016), yet a clear succession of 'strong' and 'weak' dispersers was not apparent in our results, which leads us to think that dispersal capacity based on traits is less applicable to streams in comparison to isolated ponds and lake ecosystems (Cáceres & Soluk, 2002; Scheffer et al., 2006; Miguel-Chinchilla et al., 2014). However, thinking beyond morphological traits, there is limited information on buoyancy, or specific drifting or floating behavior of many invertebrates, while these factors can have great impact on dispersal distance by water flow (Verdonschot et al., 2014). Furthermore, species can exhibit upstream movements, while remaining underwater, by walking, swimming or crawling (Elliott, 2003). These movements are thought to be triggered by population densities, but so far, no clear consensus has been found in either the cause, the net difference in number of upstream or downstream movements or the traits that are required for aquatic upstream dispersal (Humphries, 2002). This could be explored in order to gain knowledge on dispersal range and direction within one catchment.

At the stream reach scale, we further noticed that it was a combination of dispersal and environmental filters playing a role in determining the actual community. Population abundance, distance to nearest source population and distribution of source populations (metacommunities) throughout the catchment were important factors that determined which species could actually colonize restored habitat (Chapter 3). A dense population of individuals at one habitat patch leads to dispersal events and overrules potential lower habitat suitability or limited dispersal capacity.

To summarize the effect of dispersal limitations, we found that dispersal capacity is mainly limiting when it comes to overland dispersal, when active movement or vector-mediated movement is essential. At smaller scales, there are effects of dispersal capacity and habitat connectivity, but these effects are mitigated by water flow as species have adapted to use drift as a main mechanism for dispersal. The distribution of dense source populations have an overriding effect on habitat suitability and trigger individuals to disperse within stream networks.

The role of environmental filtering from small to large spatial scales

After reaching a new habitat, the environmental conditions have to fit the needs of the macroinvertebrate in order to actually settle, survive and reproduce there. By studying this process at various spatial scales, we noticed a gradual shift in the importance of habitat suitability and biotic interactions from small to larger spatial scales.

In our study, the smallest spatial scale was the within-stream habitat patch. These patches are inhabited by many different species for food and refuge (Lancaster & Hildrew, 1993; Lancaster, 2000) and interactions are common. While most studies focus on competition and predation (Schuwirth, Dietzel & Reichert, 2016) when it comes to interspecific interactions, we found facilitative interactions (Chapter 6). This indicates that species have adapted to live in close contact with each other and enhance living conditions by decreasing intraspecific competition or increasing resource availability due to selective feeding (Cummins & Klug, 1979; Milbrink, 1993). The occurrence of biotic interactions is important at the habitat-scale, yet less important at catchment-scale due to the different nature of these processes. Resource acquisition, finding refuge and other activities that are required for survival and reproduction take place at the habitat-scale and for most of an individual's lifetime.

The patchy environment of lowland streams has led to very specific interactions between species. Organic substrata get distributed across the stream bottom according to the local hydrograph, varying in space and time (Reice, 1980; Arunachalam, 1991). Species have adapted to these dynamic conditions and should therefore be flexible in order to obtain resources. This dynamicity also requires interspecific interaction adaptations, since the patches are inhabited by many different species with the same struggle for life. Furthermore, our findings suggest that these biotic interactions can have a profound effect on increasing or decreasing the organisms' fecundity and thereby influence future population size. Therefore, future studies would do well focusing on these small-scale processes.

At the stream reach scale, between habitat patches, we found that the configuration of the patches matters for survival and fitness of the inhabiting invertebrates (Chapter 5). Substrata can differ in size and configuration, and it has been found that the spatial configuration of resource patches for stream invertebrates has clear effects on densities, emergence success and larval biomass have been reported (Lancaster & Downes, 2014; Palmer 1995; Palmer et al., 2000; Silver et al., 2000). A heterogeneous streambed supports different niches and therefore a more diverse community. Habitat heterogeneity is determined by substrate variation, complexity and stability.

It should be noted that even at this scale, it remains important for the invertebrates to be able to move between patches to acquire sufficient resources and refuge (Palmer, 1995; Winemiller et al., 2010). The movements between patches take place by walking, crawling, swimming or entering active or passive drift when conditions get unfavorable. Therefore, the distance between habitat patches should not exceed certain thresholds and the connectivity between habitats get more important at stream reach scale (Brown, Wahl & Swan, 2017).

Temporal scale effects

The results from our studies have shown that dispersal processes and environmental effects are reflected differently at different spatial scales. However, time is also important to take into account when describing community assembly due to seasonal effects and life history events. It should be noted that our studies did not exceed 3 years of monitoring, which is still a limited time frame when considering colonization and community assembly and development.

We found that the invertebrate community in new habitat, such as a restored stream reach, is not only determined by habitat suitability and biotic interactions, but also depends on season (Chapter 4). We found that the arrival of most species and individuals in our streams happens in autumn, when many juveniles hatch and peak flows occur simultaneously. This 'spawning' event has a massive effect on redistribution of species throughout the catchment and happens regardless of dispersal capacity and habitat heterogeneity.

Air and water temperature, water depth, flow velocity, amount of particulate organic material in the stream and other (a)biotic parameters are strongly related to season and can strengthen dispersal. While species-specific response effects in certain seasons have been well studied (Hynes, 1976; Jacobi and Cary, 1996; López-Rodriguez et al., 2009; Miguélez & Valladares, 2008), it is less well understood how seasonality shapes lowland stream communities.

However, seasonality has been used to explain general phenomena such as life history adaptations (McNamara & Houston, 2008). Ovipositioning, emerging, pupating and diapausing are all processes that have been linked to seasonal parameters. These life history events play a profound role in dispersal timing and behavior (Stevens et al., 2013). It is therefore important to realize that dispersal can be expected to occur more frequently in specific seasons dependent on climate zone (i.e. more aerial dispersal after adult emergence in summer or more passive drift of juveniles in autumn in our temperate zone), and can also be negatively impacted when specific dispersal barriers are present during specific life stages (i.e. no riparian vegetation in summer will limit adult movement (Petersen et al., 2004b)).

It seems that seasonality gives 'a window of opportunity' for some species to disperse to and colonize new habitat, because of a combination of favorable (a) biotic conditions and their adapted life history. With many aquatic invertebrates being univoltine, a yearly cycle of dispersal gives an indication of the rate at which recolonization of new habitat could occur.

Conclusions

The main finding of this thesis is that recolonization of restored stream reaches is determined by both dispersal and environmental constraints operating at different levels of significance at various scales. From our experiments and observations we conclude that 1) dispersal capacity and habitat connectivity are the most valuable predictors when dispersal is regarded at catchment-scale, but become less valuable predictors when regarded at the reach-scale (dispersal of biota within one catchment) because populations in the vicinity will act as sources for colonists if individuals are abundant. 2) After species arrive (active or passively) to new habitat, the environmental filter will determine their livelihood more profoundly. Habitat heterogeneity and biotic interactions have significant effects on species survival and fitness and therefore can affect future population size.

Overall, the significance of the dispersal filter on community assembly decreases when shifting from large scale catchment processes to small scale habitat processes. Meanwhile, environmental filters become increasingly more important at smaller scales. We have combined our findings and previous literature into a 'Community Restoration Continuum' (Figure 7.1). Previously, community assembly was described as a process where the regional species pool goes through several dispersal and environmental filters before the actual community is established. We found that this process can be viewed more as a continuum, where both the dispersal and environmental constraints are dynamic and gradually change based on the scale at which the community is viewed. More precise, the dispersal filter is mostly limiting community development when viewed at larger scales, for example when aquatic invertebrates need to disperse overland or across large distances within the catchment. The dispersal filter plays a minor role at the habitat-scale, where invertebrates have adapted to overcome small inter-patch distances. At that small scale, environmental conditions are limiting species survival and fitness, and thereby community development. The effect of habitat heterogeneity and biotic interactions is very important at the habitat scale, with decreasing level of importance at larger scales since potential barriers at different locations within the catchment (lack of connectivity) will already decrease the chance of community development. Seasonality provides a window of opportunity for species to disperse, as favorable seasonal parameters and life history strategies merge. Communities in restored streams will develop into more functionally and biodiverse communities over time.

This thesis, combined with previous literature, gives an improved and more detailed insight into the various obstacles that an organism needs to pass to be able to get from the regional species pool to the actual community in a restored stream. To give an example of how this concept will transpire in reality, here is the chain of events that an individual macroinvertebrate will come across during its lifetime: the process of dispersal starts with a window of opportunity provided by seasonal parameters and life history strategy. This gives the organism the ability to drift downstream during juvenile stages or fly up- or downstream as recently emerged winged adult. The only specific morphological trait that profoundly increased dispersal distance in our study was the possession of wings, required for inland dispersal. Next is the potential constraint provided by hydrological or terrestrial habitat connectivity, which can be limited by drought, manmade barriers or limited vegetation corridors. Once this obstacle has been overcome (or if conditions are right), the organism will arrive in new habitat such as a restored stream reach. The next constraint to overcome in order to actually establish in the local community is to find a suitable habitat. The suited level of habitat heterogeneity needs to be present for the organism to be able to survive and reproduce. Finally, if the organism has been able to disperse and colonize the new habitat, positive or negative interactions such as facilitation, predation or competition will determine its livelihood and that of future generations. This chain of obstacles, overcome by having the right traits and meeting the right conditions, shows how community assembly can be limited by many dispersal and environmental filters.

These findings can enhance future restoration projects, by assessing the status of several parameters at different scales and drawing subsequent conclusions on which factors are limiting community development.

Stream restoration implications

Our findings aid a more complete understanding of community assembly in lowland streams and recolonization of restored stream reaches, and meanwhile direct towards specific implications to make ecological restoration more successful. I propose that stream restoration design should take the following factors into account:

1. I suggest that colonization of restored streams should not rely on recolonization by invertebrates from adjacent catchments unless terrestrial connectivity is enhanced to increase the range of dispersing winged invertebrates or vector-mediated transport. However, this is very challenging in a fragmented landscape such as the Netherlands. Replanting riparian vegetation aids the terrestrial movement of winged adult invertebrates (Harrison & Harris, 2002), which is also a pathway for recolonization, but does not allow for new species that have not been present in the catchment before, to enter the community. If dispersal conditions are too unfavorable due to several (environmental) barriers, an alternative restoration measure could be to assist recolonization by reintroducing a species or an appropriate assembly of species (Neves, 2004; Rupprecht, 2009; Strayer & Dudgeon, 2010). These species should fit the habitat in terms of ecological functioning.

2. I suggest to locate where the nearest source pools of invertebrates are. Therefore, effort should be put in mapping the existing (meta)communities within the designated catchment. In this manner, locations for restoration can be chosen based distributed patterns and distances to nearest source pool, and hence most successful chance of recolonization. Quantifying how much connectivity is required for optimal dispersal, would be very informative for restoration purposes. Previous studies have focused on preferable distances to the source pool (Sundermann, Stoll & Haase, 2011; Tonkin et al., 2014), some stating that source populations further than 5 km will never be able serve as a viable source for recolonization (Winking et al., 2014) and therefore ecological restoration. However, some factors make these distances differ between studies such as habitat quality and connectivity (Stoll et al., 2016).

3. I suggest to foster and conserve the stream reaches that already have high levels of biodiversity and population abundances. This will cause populations to grow larger than carrying capacity which triggers dispersal (Shmida & Wilson, 1985; Elbersen- van der Straten & Higler, 2002) and thereby increase the chance of reaching and colonizing denuded areas further up- or downstream and cause rapid ecological recovery. Based on our findings, I suggest that metacommunity structure should be the basis for observed recolonization patterns. Viewing a catchment as a patchwork of populations interconnected by dispersal, provides information on the ecological response of an ecosystem after disturbance. In addition to this, 'stepping stone'-restoration efforts could be effective if metacommunities and population abundances are mapped before restoration design (Lancaster & Briers, 2007; Doerr et al., 2014).

4. Assessing the most obvious dispersal limitations within a catchment (human-made barriers, limited connectivity due to unsuitable habitats or reaches, unviable source populations), can already predict the outcome of the developing community in a denuded area. However, when the community does not recover despite increased chances of dispersal, small scale restoration practices should be applied, such as increasing habitat heterogeneity (Frainer et al., 2017; Thompson et al., 2017). This will cause the environmental conditions to improve locally and enhance livelihood of the inhabiting invertebrates. Increasing habitat heterogeneity is a very popular restoration technique, for example by enhancing structural complexity with meanders, boulders, wood, or other structures, but does not always lead to increased biodiversity (Palmer, Menninger & Bernhardt, 2010a). Our findings suggest that habitat heterogeneity is a key factor in ecological restoration, but only after potential dispersal limitations have been resolved.

5. While conservation often aims at protecting specific target-species and sometimes functional groups, I emphasize that even within the same functional group, species are not redundant or replaceable. Species interact with other species directly and through abiotic factors, often forming complex networks of various types of ecological interaction (Olff et al., 2009; Pilosof et al., 2017). While predator-prey interactions often prioritize conservation targets, facilitative interactions can also take place between seemingly similar species (Bruno, Stachowicz & Bertness, 2003). Therefore, I state that biodiversity and functional diversity should still be a main focus within restoration ecology and subsequent small-scale habitat adjustments can enhance this by creating more niches.

6. I suggest that the timing of stream restoration practices should be synchronized with the life history strategy of aquatic invertebrates in order to not limit their chances of dispersal and colonization. For example, efforts should be finished before populations are most vulnerable and unable to respond to disturbance (e.g. when species are pupating). Events of dispersal and emergence can be predicted (Stevens et al., 2013; Tonkin et al., 2017b), which can enhance the effectiveness of specific restoration practices.

The results from our stream restoration survey, as mentioned in chapter 1, show that several water authorities already discuss or implement some of these measures to some extent. Yet, the level of urgency or theory behind several of these measures is largely unclear. As an ecologist, I think there is still a lot of work to be done in bridging science and practice. Hopefully, this thesis will contribute to future discussion and implementation of effective stream restoration measures, timing and targets in Dutch lowland streams. I believe that Dutch water authorities can be protagonists for worldwide improvements in the restoration of freshwater stream ecosystems. Only if we invest in understanding ecological processes and mechanisms, restore key parameters, promote dispersal and give nature time to recover, we will be able to reverse anthropogenic damage and see the local flora and fauna flourish again. A healthy, balanced and living aquatic environment is not only important for future ecosystem services, I believe it is also important for future generations on a spiritual level, or as David Attenborough says it: *"It seems to me that the natural world is the greatest source of excitement; the greatest source of visual beauty; the greatest source of intellectual interest. It is the greatest source of so much in life that makes life worth living."*

Literature

Literature

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Summary

Summary

Freshwater streams have always been of interest to humankind, as they provide resources (e.g. fish and potable water) and serve functions (e.g. irrigation of agricultural crops and drainage of waste water). Anthropogenic alterations were made to enhance these functions, but in the end, this led to an overall deterioration of water quality and available aquatic habitats for stream biota. Subsequently, populations of aquatic flora and fauna got diminished. The European Union recognized the damage to these ecosystems and the Water Framework Directive was installed to prevent further deterioration of European waters. Since then, many restoration projects were carried out, and a lot of effort has been put into recreating suitable aquatic habitats in the deteriorated streams. Unfortunately, these efforts did not always pay off, as populations of aquatic macroinvertebrates do not recover as quickly as anticipated. Until now, it remains mostly unclear why these restored streams are not recolonized by a diverse group of macroinvertebrates.

Source populations are vital for the recolonization of restored stream reaches and imperatively the connection to source populations forms the first hurdle for successful stream restoration. To optimize restoration schemes it is therefore important to know how aquatic macroinvertebrates can disperse from a source pool to a new habitat. A subsequent step in successful stream restoration is for the newly arrived macroinvertebrates to be able to colonize the habitat, survive and reproduce. Which conditions are favorable for their settlement? In this thesis we explore the ecological mechanisms behind the dispersal and subsequent colonization of aquatic macroinvertebrates in restored lowland streams. By doing so, we want to unravel why macroinvertebrates do not always settle in recently restored streams (Chapter 1).

The recolonization of headwaters and isolated stream reaches by aquatic macroinvertebrates usually depends on overland dispersal by active or passive aerial means. This triggered us to study long-distance dispersal (Chapter 2). Our results show that overland dispersal decreases as distance increases. The colonizing species arrived randomly over the course of three years and were all aerial active flyers. We see a distinction between species that actively disperse within their home-range and other species that passively disperse over long-distances, in a stochastic manner. The limited and random colonization by aquatic macroinvertebrates indicate that communities in new stream areas will not develop

rapidly if they only rely on colonists reaching the habitat via overland and long-distance dispersal.

Mid-order stream reaches are generally well connected to tributaries and/or the main channel with several populations of organisms inhabiting the catchment. We unraveled the relative importance of dispersal drivers in new mid-order lowland stream communities by performing a field study during first phase colonization over a period of 2.5 years (Chapter 3). Our results show that successful dispersal depends on the distance between the new habitat and the regional species pool, as well as the abundance of the population within that pool, but not on any specific characteristics related to the dispersal capacity of the individual species. The outcome of this study indicates that limiting distances between source population and restoration sites, as well as reinforcing existing source populations in terms of abundance and diversity will increase the chance of recolonization of new habitat in the restored areas.

When macroinvertebrates reach the newly restored habitat, they have to deal with local environmental conditions. We questioned how invertebrate succession patterns reconcile with general colonization concepts and provided insight into the colonization process in newly created lowland stream trajectories (Chapter 4). Our results show a rapid influx of species, with a wide range of functional traits, during the first season after water flow commenced. Based on more than two years of regular monitoring, we found that immigration rates were highest in autumn, marking the effects of seasonality on invertebrate dispersal. These observations indicate that first phase colonization in freshwater streams can be a very rapid process that results in a high biodiversity and a large variety of species functional characteristics from the early onset of succession.

To establish successfully in the newly restored habitat, habitat suitability plays a major role for survival and reproduction. Loss of substrate heterogeneity or patchiness is common in lowland streams with disturbed hydrological regimes. An aquatic mesocosm experiment was performed to test the effect of habitat homogenization on survival and fitness of two species of Trichoptera (Micropterna sequax and Potamophylax rotundipennis) (Chapter 5). Our results suggest that both species experience intraspecific resource competition arising from the spatial distribution of patches, expressed as an investment in wing development (e.g.

dispersal capacity) in P. rotundipennis and resulting in lower survival rates in *M. sequax*. The outcome of this experiment illustrates the importance of knowledge of trait-based responses, and highlight the effects of the configuration of stream bottom substrate for its inhabitants on microscale.

Next to habitat suitability, also biotic interactions affect species livelihood. Patches of coarse particulate organic matter in lowland streams are inhabited by many different macroinvertebrate species, yet knowledge of interactions among the members of these assemblages is scarce. In a mesocosm experiment we aimed to determine the effect of interspecific interactions on species survival and fitness of the same two caddisfly species (Chapter 6). We found that in mixed populations emergence rate, wing length and biomass of *M. sequax* was higher than in single species populations. P. rotundipennis was only significantly, yet negatively, affected in terms of biomass of the male individuals. This study showed that occurring together with other species holds advantages for *M. sequax*, and emphasizes the importance of species diversity in streams.

Combining the observations from the different studies presented in this thesis, our overall finding is that the obstacle of specific dispersal limitations decreases when shifting from large scale catchment processes to small scale habitat processes (Chapter 7). Meanwhile, environmental limitations become increasingly more important at smaller scales. In the last chapter of this thesis, I have combined our findings and observations from previous literature into a framework that I named the 'Community Restoration Continuum'. Previously, community assembly was described as a process where the regional species pool goes through several dispersal and environmental filters before the actual community is established. We found that in lowland streams, this process could be viewed more as a continuum, where both the dispersal and environmental constraints are dynamic and gradually merge into one another depending on the scale at which the community is viewed. The dispersal filter plays a minor role at the habitat-scale, where invertebrates have adapted to overcome small inter-patch distances. At that small scale, environmental conditions are limiting species survival and fitness, and thereby community development. The effect of habitat heterogeneity and biotic interactions is very important at the habitat scale, with decreasing level of importance at larger scales. Next to these spatial scale dependent processes, seasonality provides a window of opportunity for species to disperse, as favorable seasonal parameters and life history events merge.

Our findings aid a more complete understanding of community assembly in lowland streams and recolonization of restored stream reaches, and meanwhile direct towards specific implications to make ecological restoration more successful. I suggest that 1) colonization of restored streams should not rely on recolonization by invertebrates from adjacent catchments. Instead, a restoration measure could be to assist recolonization by reintroducing an appropriate community of species. 2) Effort should be put in mapping the existing (meta)populations within the designated catchment. In this way, locations for restoration can be chosen based on distributed patterns and distances to nearest source pool, and hence the chance of successful recolonization is increased. 3) By fostering the stream reaches that already have high levels of biodiversity and population abundances, populations will grow larger than carrying capacity which triggers dispersal. 4) Assessing the most obvious dispersal limitations within a catchment, can already predict the outcome of the development community in a restored area. However, when the community does not recover despite increased chances of dispersal, small-scale restoration practices should be applied such as increasing habitat heterogeneity. 5) Biodiversity and functional diversity should still be a main focus within restoration ecology as even within the same functional group, species are not redundant or replaceable. 6) The timing of stream restoration practices should be synchronized with the life history strategy of aquatic invertebrates in order to not limit their chances of dispersal and successful colonization.

Samenvatting

Samenvatting

Rivieren en beken zijn erg belangrijk; ze voorzien ons van goederen (zoals vis en drinkwater) en dienen allerlei functies (zoals irrigatie van landbouwgrond en afvoer van afvalwater). Door rivieren en beken te kanaliseren werden deze wateren op korte termijn functioneler, maar de aquatische flora en fauna nam drastisch af. Dit probleem werd erkend door de Europese Unie en er werd vastgesteld dat verdere degradatie van het ecosysteem moet worden voorkomen, zodat het in de toekomst ook nog functioneel en gezond is. Sindsdien wordt er in Nederland hard gewerkt aan beekherstel.

Helaas zijn de resultaten van dergelijke beekherstelprojecten vaak niet succesvol en keert er maar een kleine gemeenschap aan aquatische (macro)fauna terug in het herstelde gebied (hoofdstuk 1). Dit is een probleem want macrofauna is essentiële voedselbron voor vissen en vogels en ze spelen een sleutelrol in verschillende ecologische processen in de beek. We weten dat de macrofauna uit een bronpopulatie naar het nieuwe, herstelde gebied moet komen, maar met welke strategie (via lucht of water, actief of passief) en over welke afstand vindt dit plaats? En zodra de macrofauna aankomt op een nieuwe plaats, welke condities zijn dan bepalend voor het koloniseren, overleven en voortplanten van alle verschillende soorten? In dit proefschrift zijn de ecologische mechanismen achter deze processen onderzocht en beschreven.

De herkolonisatie van bovenlopen en andere geïsoleerde wateren gebeurt voornamelijk door actieve of passieve verspreiding over het land (hoofdstuk 2). Door een veldexperiment uit te voeren waarbij drie jaar lang is gekeken welke macrofauna via de lucht een nieuwe beek kon vinden, blijkt dat alleen de actief vliegende macrofauna nabijgelegen beken weet te koloniseren, en dat deze slaagkans sterk afneemt naarmate de afstand tussen beken toeneemt. Het moment van kolonisatie in de verder gelegen beken was willekeurig en vond maar zelden plaats, waardoor we een verschil lijken te zien tussen de meeste soorten die binnen hun zogenoemde 'home-range' blijven bewegen en een paar soorten die plotseling ver weg raken van hun geboortegrond, wellicht door met een luchtstroom mee te liften.

De middenloop van beken is veelal goed bereikbaar voor macrofauna via de lucht en het water. Een recent hersteld beektraject kan dan ook snel worden gekoloniseerd wanneer het dicht bij de bronpopulaties van verschillende soorten ligt (hoofdstuk 3). De afstand tussen bronpopulatie en nieuw habitat én de grootte van de bronpopulaties zijn bepalend voor de snelheid en samenstelling van de nieuwe levensgemeenschap in de herstelde trajecten. Uit onze langdurige veldstudie blijkt dat de manier waarop de kolonisten zich verspreiden (actief of passief, vliegend, zwemmend of drijvend) niet bepalend is voor de kolonisatiesnelheid in middenlopen. Wat wel kenmerkend is, is dat de meeste kolonisten aankomen in de herfst en dat de samenstelling van de gemeenschap vanaf het eerste moment een grote verscheidenheid aan functionele eigenschappen bevat (hoofdstuk 4). Een herstelde middenloop met nabijgelegen gezonde bronpopulaties is dus direct biodivers en functioneel divers en daardoor veerkrachtig.

Zodra macrofauna een nieuw beektraject bereikt, moet het habitat geschikt zijn voor overleving en voortplanting om van een succesvolle kolonisatie te spreken. Voor veel aquatische macrofauna is een heterogene omgeving, met afwisseling van bladeren, takken, kiezels en zand op de bodem van de beek, onmisbaar (hoofdstuk 5). Door een experiment uit te voeren waarbij we kokerjuffers in verschillende niveaus van habitatheterogeniteit lieten opgroeien, werd duidelijk dat habitat effect heeft op overlevingskans en groei. Hoe groter de aaneengesloten stukken zand tussen de bladpakketjes, hoe minder individuen overleefden. Waarschijnlijk omdat minder individuen de overtocht naar het volgende bladpakket ondernamen en er meer competitie voor voedsel ontstond. In een andere soort kokerjuffer had de afname aan heterogeniteit een positief effect op de groei van de vleugels, duidend op een investering om verder weg te komen van het ongeschikte habitat.

Tenslotte zijn naast habitatgeschiktheid ook biotische interacties bepalend voor de overleving en groei van macrofauna (hoofdstuk 6). Op een beekbodem bevinden de meeste macrofaunasoorten zich bij de organische structuren, zoals blad en hout, vanwege de voedsel- en schuilmogelijkheden. Er is tot op heden weinig kennis van de effecten van de interacties tussen soorten en individuen, terwijl dit continue plaatsvindt. Uit onze resultaten blijkt dat het samenleven van soorten in sommige gevallen een positief effect heeft op de overlevingskans en groei. Dit benadrukt het belang van biodiversiteit in beken.

Voorheen werd het proces waarbij een nieuwe gemeenschap ontstaat in een (hersteld) habitat gezien als een soort hindernisbaan waarbij de macrofauna vanuit de bronpopulatie een aantal 'obstakels' moest overwinnen om bij de finish te komen (bijvoorbeeld dispersieproblematiek of habitatongeschiktheid). Door al onze bevindingen te bundelen (hoofdstuk 7) kom ik uit op een nieuw kader om dit proces te beschrijven, het 'Community Restoration Continuum', waarin ik de 'obstakels' zie als twee verschillende gradiënten (Dispersie en Habitat) die altijd in meer of mindere mate aanwezig zijn, afhankelijk van de schaal waarop het beekherstel plaatsvindt.

Dit kader helpt ons om het kolonisatieproces van macrofauna in laaglandbeken -en met name herstelde beektrajecten- beter te begrijpen. Tegelijkertijd leidt deze kennis tot aanpassingen of invoeringen van specifieke herstelmaatregelen. Ik stel voor dat 1) herkolonisatie van herstelde bovenlopen niet afhankelijk is van trage kolonisten uit nabijgelegen beken. In plaats daarvan zou een herintroductie van passende soorten een uitkomst bieden. 2) Er zou moeite gedaan moeten worden om bestaande bronpopulaties in kaart te brengen rondom het gebied waar herstel plaats gaat vinden. Door een locatie te kiezen dichtbij gezonde bronpopulaties zullen de herstelresultaten veelal beter zijn. 3) Door bepaalde bronpopulaties te versterken zal de populatie groeien en ter plekke dispersie in gang zetten. Dit is voordelig voor de benedenstroomse trajecten. 4) Wanneer de gemeenschap niet herstelt ondanks het creëren van dispersiemogelijkheden, zullen kleinschalige herstelmaatregelen nodig zijn zoals het verbeteren van habitatheterogeniteit. 5) De planning van beekherstel zou gesynchroniseerd moeten worden met de cycli van macrofauna, waarbij de meest invasieve werkzaamheden niet plaatsvinden tijdens de sessiele stadia (bijvoorbeeld tijdens verpopping van aquatische insecten). 6) Biodiversiteit en functionele diversiteit zou nog steeds een hersteldoel moeten zijn binnen de restoratie van beken. Er is nog niet genoeg kennis om te kunnen zeggen dat bepaalde soorten overtollig zijn.

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Aan al mijn niet-UvA vrienden, bedankt voor het verbreden van mijn tunnelvisie en onvermoeibaar begripvol ja-knikken tijdens uitleg over waterbeestjes. Met name

Auke, bedankt dat je zoveel tijd hebt gestopt in de lay-out van dit boekje, ik vind het prachtig.

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Mijn liefste, mijn rots in de branding, ik weet niet hoe ik het zonder jou had kunnen doen. Ik ben benieuwd met welke stroom we ons laten meevoeren in de toekomst, ik heb er nu al zin in.

Tot slot, dank aan alle 54787 waterdieren die hun leven hebben gegeven voor mijn onderzoek. Achteraf gezien had ik graag een mindere invasieve methode gebruikt om de wonderen van de onderwaterwereld te ontdekken.

Author contributions

Chapter 2: H.G. van der Geest[1], T.B.M. Dekkers[2], P.F.M. Verdonschot[1,2]
JJW and PFMV conceived the ideas and designed methodology; JJW and TBMD collected the data; JJW analysed the data; JJW, HGvdG and PFMV led the writing of the manuscript.

Chapter 3: H. G. van der Geest[1], E. E. van Loon[1], P.F.M. Verdonschot[1,2]
JJW and PFMV conceived the ideas and designed methodology; JJW collected the data; JJW and EEvL analysed the data; JJW, HGvdG and PFMV led the writing of the manuscript.

Chapter 4: H.G. van der Geest[1], E. E. van Loon[1], P.F.M. Verdonschot[1,2]
JJW and PFMV conceived the ideas and designed methodology; JJW collected the data; JJW and EEvL analysed the data; JJW, HGvdG, and PFMV led the writing of the manuscript.

Chapter 5: R. C.M. Verdonschot[2], P.F.M. Verdonschot[1,2]
JJW, PFMV and RCMV conceived the ideas and designed methodology; JJW collected the data; JJW analysed the data; JJW, RCMV and PFMV led the writing of the manuscript.

Chapter 6: R. C.M. Verdonschot[2], P.F.M. Verdonschot[1,2]
JJW, PFMV and RCMV conceived the ideas and designed methodology; JJW collected the data; JJW analysed the data; JJW, RCMV and PFMV led the writing of the manuscript.

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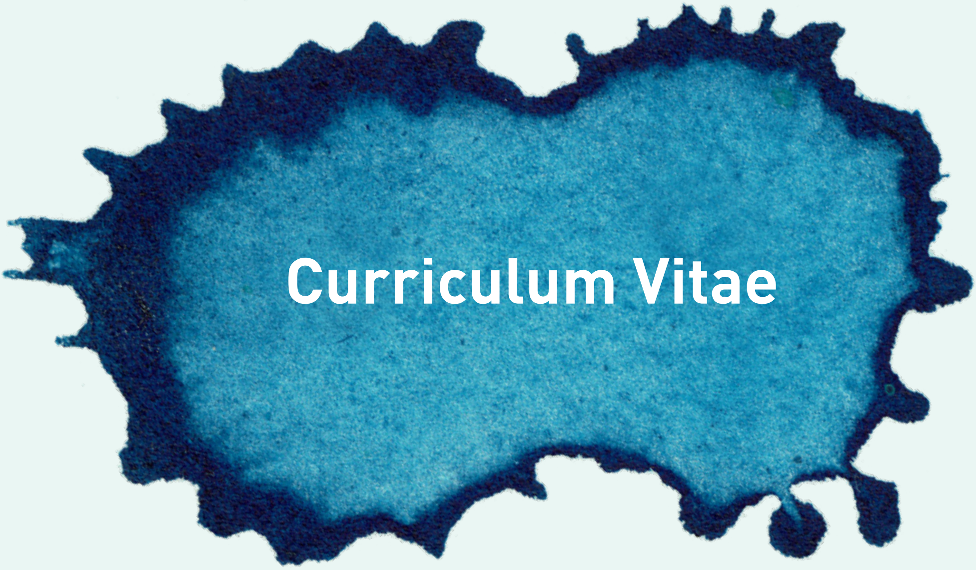


Curriculum Vitae

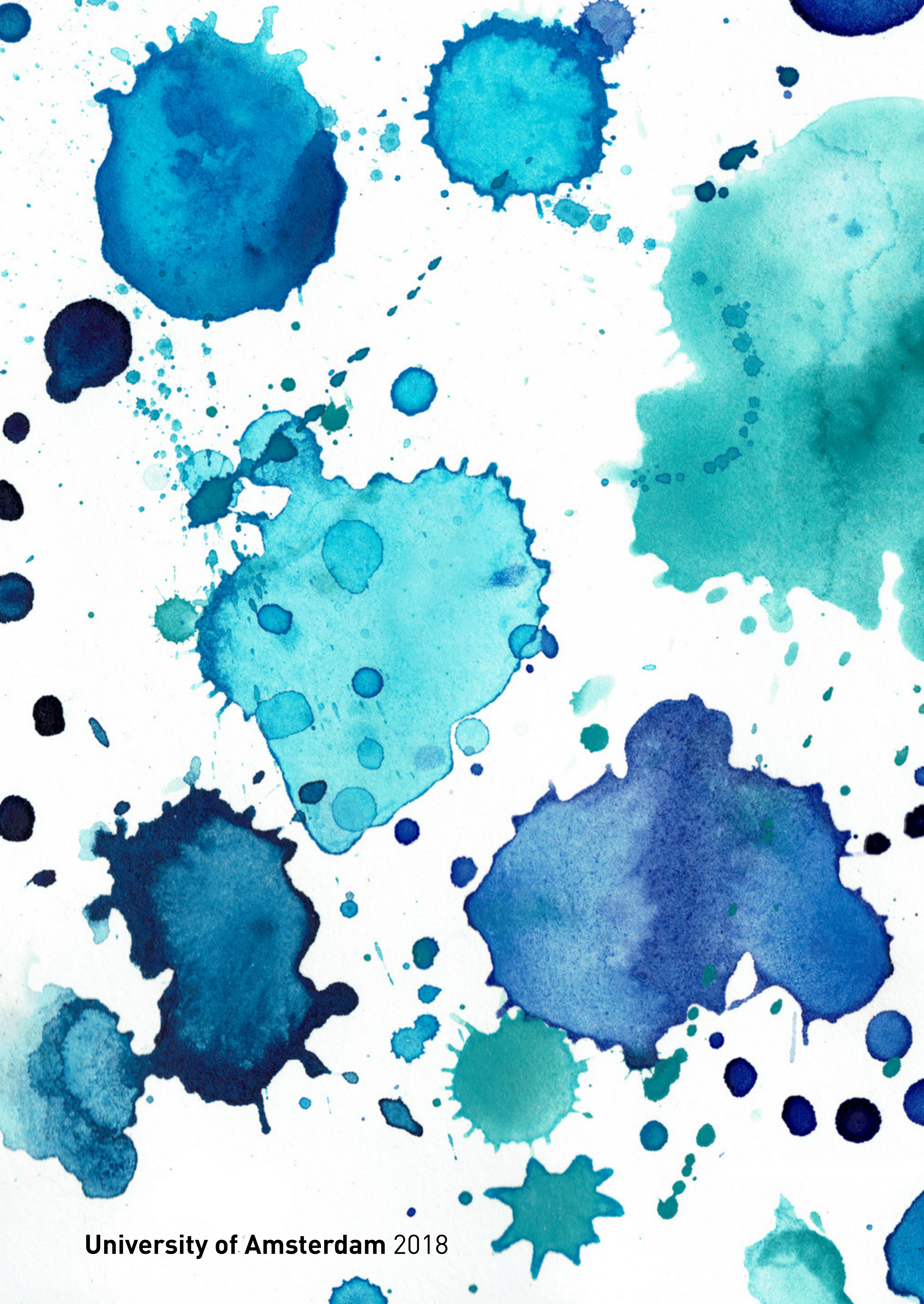
Judith Westveer was born on the 3rd of May 1987 in Amsterdam, the Netherlands. She spent her childhood playing in the garden, listening to stories from her grandparents about the jungle of Indonesia. Judith and her family vacationed in the tropics several times and she was fascinated by flora and fauna of the pristine wilderness that they stumbled upon. This led her to study Ecology and Evolutionary Biology at the University of Groningen from 2006 onward.

In 2009, between her bachelor and master studies, she spent five months in the tropical rainforest of Peru as a resident naturalist in an isolated research station. This is where it became clear to her that not many pristine areas are left on this planet, that the few remaining wildernesses are under constant anthropogenic pressure and that more research and action is needed to restore the already affected natural areas. From that moment onward, she focused her research projects on the conservation and restoration of nature.

After completing her MSc at the Conservation and Community Ecology group at the University of Groningen in 2012, she worked at Artis Zoo, did editorial work for nature documentary 'De Nieuwe Wildernis' and took on a Rijkswaterstaat internship for 'Programma naar een Rijke Waddenzee'. These projects enhanced her educational and communicative skills.

Her scientific education and interdisciplinary side projects paved the way to start a PhD at the University of Amsterdam in 2014, at the IBED department of Freshwater and Marine Ecology, with a topic that combined fundamental biology and applied restoration ecology. This trajectory (February 2014 – March 2018) led to the PhD thesis you now hold in your hand.

Judith is interested in finding ways to make people understand how important nature is, and meanwhile be involved in actively restoring nature. She wants to pursue this challenge, hopefully, by continuing her career within the field of nature conservation.



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