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Cross-ecosystem linkages - anthropogenic impacts on subsidy transfer from streams to riparian zones by emergent aquatic insects

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CONTENT

ABSTRACT

Streams and surrounding riparian zones are closely linked and resource fluxes between the systems are important for maintaining aquatic and terrestrial biodiversity. Emergent aquatic insects represent one important food web connection between aquatic and adjacent riparian ecosystems, exporting biomass and nutrients such as the omega-3 polyunsaturated fatty acid (PUFA) eicosapentaenoic acid (EPA), which is scarce in terrestrial systems. However, streams have undergone drastic alterations during the last century and although various measures were implemented, for example in Europe, to improve stream health, many issues remain unresolved. One major problem is the multitude of structures, such as dams and weirs, built along river networks. Barriers disrupt the longitudinal stream continuum, causing bed load deficits, changes in stream morphology and instream assemblages, and potentially affecting important cross-boundary resource transfers between aquatic and terrestrial ecosystems. The general aim of this thesis was to investigate anthropogenic influences linked to instream barriers on lateral food web connectivity to riparian zones. To achieve this, I conducted two field surveys in Switzerland. In the first survey, I examined the effects of sediment traps, a specific type of barrier that retains sediment, on biomass export by emerging aquatic insects. In addition, I considered the influence of sediment traps on the contribution of aquatic subsidies to riparian spider diets using stable isotopes ($\delta^{13}C$, $\delta^{15}N$). Second, I conducted a large field campaign, comparing biomass export by emergent aquatic insects between a near natural and a degraded system with multiple barriers. In the latter project, I also compared differences in nutritional quality (PUFA content) of emergent insects and PUFA transfer to riparian spiders. Here, I used PUFAs, in addition to stable isotope ratios, as dietary tracers.

I found that the impact of sediment traps on the total biomass export by emergent insects and on spider diets was small, although stable isotope ratios alone could not fully disentangle food web linkages. In the second study, the more degraded stream with multiple barriers also showed no reduced total biomass or EPA export compared to the near natural system. However, I found a shift in insect groups contributing to biomass export with less stoneflies that represent a functional connection to ground-dwelling predators in the degraded system. Using PUFAs as additional dietary tracers helped determine food web linkages, although some caution must be taken due to the possibility of bioaccumulation and metabolic changes. My research showed that emergent insects are important vectors of PUFA transfer. Nutritional quality as well as functional traits of different insect groups should be considered when assessing the cascading effects of stream degradation on adjacent terrestrial ecosystems.

ZUSAMMENFASSUNG

Flussläufe und die umgebenden Uferzonen sind eng miteinander vernetzt und der Austausch von Ressourcen zwischen den Systemen ist notwendig, um die aquatische und terrestrische Biodiversität zu erhalten. Emergierende aquatische Insekten stellen eine wichtige Verbindung über das Nahrungsnetz zwischen aquatischen Systemen und angrenzenden Uferzonen dar, indem sie Biomasse und Nährstoffe wie mehrfach ungesättigte omega-3 Fettsäuren (PUFAs), insbesondere Eicosapentaensäure (EPA), das in terrestrischen Systemen rar ist, exportieren. Aber viele Flussläufe wurden im Laufe des letzten Jahrhunderts drastisch verändert, und obwohl zum Beispiel in Europa viele Maßnahmen umgesetzt wurden, um die Gewässerqualität wieder zu verbessern, bleiben zahlreiche Probleme noch immer bestehen. Ein großes Problem Stellen die Vielzahl von Verbauungen, wie zum Beispiel Dämme und Wehre dar, die in vielen Flussläufen errichtet wurden. Verbauungen unterbrechen den natürlichem longitudinalen Verlauf der Flüsse, erzeugen Geschiebedefizite und Veränderungen in der Flussmorphologie sowie in den Lebensgemeinschaften im Fluss. Potenziell wird auch der wichtige Ressourcenaustausch über die Grenze von aquatischen und terrestrischen Systemen hinweg beeinflusst. Das generelle Ziel dieser Arbeit war es menschliche Einflüsse, insbesondere solche die mit Flussverbauungen verknüpft sind, auf die seitliche Vernetzung mit der Uferzone zu untersuchen. Um das zu erreichen habe ich zwei Felduntersuchen in der Schweiz durchgeführt. In der ersten Feldstudie habe ich die Effekte von Geschiebesammlern, einem speziellen Typ der Verbauung die insbesondere Geschiebe zurückhält, auf den Biomasseaustrag über emergierende aquatische Insekten untersucht. Zusätzlich habe ich den Einfluss von Geschiebesammlern auf den Anteil aquatischer Nahrung in der Ernährung von Uferspinnen analysiert. Als Methode habe ich hier das Verhältnis von stabilen Isotopen von Kohlenstoff und Stickstoff verwendet. Im zweiten Projekt habe ich eine große Felduntersuchung durchgeführt, bei dem Biomasseexport zwischen einem natürlichen Flusslauf und einem Flusslauf mit mehreren Verbauungen verglichen wurde. Hier habe ich auch die Nährstoffqualität (PUFA Gehalt) der emergierenden Insekten in Betracht gezogen und den Transfer von PUFAs zu Uferspinnen verglichen. Zusätzlich zu den stabilen Isotopen, wurden hier PUFAs als trophische Marker verwendet.

Ich habe herausgefunden, dass der Einfluss von Geschiebesammlern auf den Biomasseexport durch emergierende aquatische Insekten und die Ernährung von Uferspinnen klein zu sein scheint, obwohl stabile Isotopenverhältnisse von Kohlenstoff und Stickstoff nicht ausreichend waren, um das Nahrungsnetz vollständig zu entschlüsseln. Auch in der zweiten Studie habe ich, in dem weniger natürlichen System mit mehreren Verbauungen, keinen reduzierten Biomasseexport festgestellt. Aber ich habe eine Verschiebung in den Insektengruppen, die zum Biomasseaustrag beitragen, beobachten können. Im Speziellen fand im weniger natürlichen System ein geringerer Biomasseaustrag über Steinfliegen statt, die eine wichtige funktionelle Verbindung zu bodenlebenden Jägern am Ufer darstellen. Die Verwendung von PUFAs als zusätzlicher trophische Marker hat dazu beigetragen

Verbindungen im Nahrungsnetz besser nachzuvollziehen. Trotz allem dürfen PUFAs nur mit Bedacht als trophische Marker eingesetzt werden, da die Möglichkeit von Bioakkumulation und metabolischen Umwandlungen besteht. Meine Forschung hat gezeigt, das emergierende aquatische Insekten wichtige Vektoren für den Transfer von PUFAs sind und sowohl die Nährstoffqualität als auch funktionelle Eigenschaften verschiedener Insektengruppen einbezogen werden müssen, um Effekte von Flussverbauungen auf angrenzende terrestrische Systeme abschätzen zu können.

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PREFACE

This thesis is based on original work of the author. I did the analyses and wrote the original texts. All contributions of others including field- and lab work to manuscript drafts are acknowledged at the end of each chapter.

The manuscript of **Chapter 1** is in preparation for submission likely to the journal `Food Webs`.

A version of **Chapter 2** was published in the Frontiers in Ecology and Evolution under the following citation:
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The manuscript was reformatted for Chapter 2 and minor typographic errors were corrected.

A version of **Chapter 3** was submitted to the journal Science of the Total Environment on 3.2.2022 and was in review at the time this thesis was submitted, with the ID: STOTEN-D-22-05028.
The manuscript was submitted as: Carmen Kowarik, Dominik Martin-Creuzburg, Kate L. Mathers, Christine Weber, Christopher T. Robinson: Stream degradation affects aquatic resource subsidies to riparian ground-dwelling spiders.

A version of **Chapter 4** will be published by the Federal Office for the Environment in autumn 2022 as part of: FOEN (Ed.) 2022. Riverscape – sediment dynamics and connectivity. Practice-oriented research in hydraulic engineering and ecology. Federal Office for the Environment (FOEN), Bern. Environmental Studies, Chapter 3: Carmen Kowarik, Christopher T. Robinson: Aquatic–terrestrial resource fluxes.
The manuscript was reformatted for Chapter 4 and Figures represent drafts of the final figures that will be used in the publication.

GENERAL INTRODUCTION

Connectivity

The increasing degree of habitat fragmentation is considered one of the largest threats to biodiversity (Wilcove et al. 1998, Crooks and Sanjayan 2006). Fragmentation is such a severe problem because many systems can only function with a high degree of connectivity, which can be defined as the degree of movement of organisms and processes (Crooks and Sanjayan 2006). Different processes, including gene flow between population, dispersal, migration, or energy transfer through food webs, can represent connections between systems (Ward et al. 1999, Frankham 2006, Garcia et al. 2017). To maintain a high degree of connectivity, it is crucial that free movement of organisms and material is possible. However, in a world with ever increasing anthropogenic infrastructure, ecosystem connectivity is often drastically reduced (Crooks and Sanjayan 2006). In fluvial ecosystems different spatial dimensions of connectivity play a role 1) the longitudinal connectivity (up- and downstream in stream channel) 2) the vertical connectivity between hyporheic zone, the river and the atmosphere and 3) the lateral connectivity of the main channel to floodplains and riparian zones (Ward 1989, Grill et al. 2019). Anthropogenic changes have dramatically reduced connectivity; fluvial networks are, for example, disrupted by an estimated number of 16 Mio barriers and only 36 % of rivers (>1000 km) remain free-flowing (Grill et al. 2019). The loss of connectivity fundamentally alters the movement of water, but also bed load, organic matter, nutrients and organisms, and threatens biodiversity and essential ecosystem services (Dudgeon et al. 2006, Cardinale et al. 2012, Grill et al. 2019). In this thesis, the primary focus is on the effects of disrupted longitudinal connectivity regarding sediment transport on lateral connectivity, and on nutrient transfer from aquatic to terrestrial systems in particular.

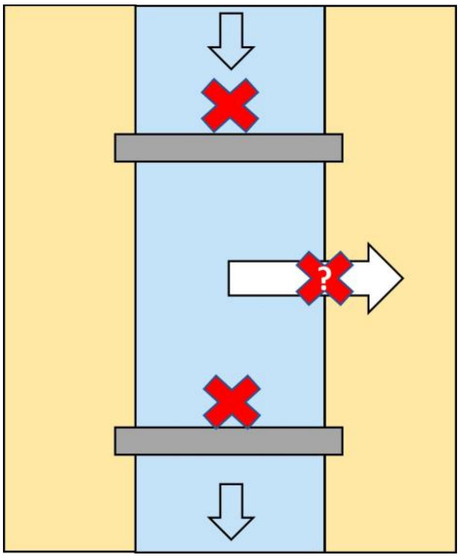

Figure 1: Influence of longitudinal connectivity on lateral connectivity. Artificial structures fragmenting fluvial networks can also disrupt lateral connectivity to riparian zones.

Aquatic - terrestrial linkages

Aquatic and terrestrial systems are often considered separately, but they are connected in a "tangled web" with multiple fluxes in both directions (Baxter et al. 2005). The transfer of resources from one system to the other is called a subsidy. A classic example for terrestrial subsidies to aquatic ecosystems is leaf litter inputs (Vannote et al. 1980, Wallace et al. 1997). Here, however, we focus on the export of aquatic subsidies into terrestrial systems. Export can happen along different pathways, such as fish predation by riparian predators, water birds feeding on aquatic organisms, or the emergence of amphibiotic organisms that spend part of their life cycle in terrestrial systems (Richardson et al. 2010, Gladyshev et al. 2013). Although the biomass of fluxes from aquatic to terrestrial systems is often smaller than in the opposite direction (Bartels et al. 2012), aquatic subsidies represent important food sources for riparian species "living at the edge" between both systems (Pätzold 2004). Riparian areas and floodplains form those transitional zones linking streams and terrestrial ecosystems, and provide unique habitats that support many specialist species with specific resource requirements (Ramey and Richardson 2017, Twining et al. 2018b). Riparian areas and floodplains represent, due to high habitat heterogeneity, a hotspot for biodiversity (Tockner and Stanford 2002) Their ecological importance is evident when considering the following facts from Switzerland: Floodplain areas cover only 0.26 % of the surface area but 10 % of the fauna is restricted to floodplains; 28 % frequently use floodplains and 44 % are occasionally found in floodplains. Meaning, in total about 80 % of regional fauna is associated with floodplains. Riparian and floodplain specialist predators, including spiders, lizards and birds, have been shown to depend on the influx of resource subsidies from aquatic systems via emergent insects (Sabo and Power 2002, Kato et al. 2003, Paetzold et al. 2006, Twining et al. 2018b). For example, riparian tetragnathid spider densities appear dependent on biomass export by aquatic insects (Kato et al. 2003), and breeding success of tree swallows has been linked to available amounts of emergent aquatic insects (Twining et al. 2018b).

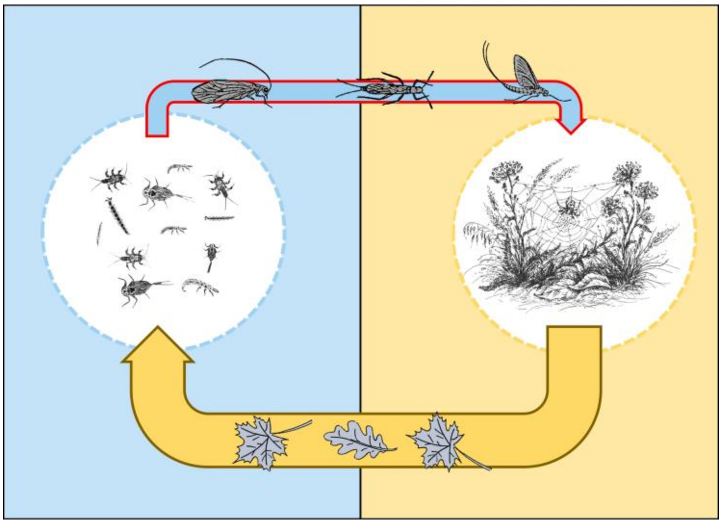

Figure 2: Aquatic-terrestrial linkages. Leaf litter represents an important resource into streams, while emergent aquatic insects represent an important food source for riparian predators such as spiders.

Quality matters

Aquatic subsidies represent additional resources, but are especially important because aquatic and terrestrial resources are not nutritionally reciprocal for consumers (Twining et al. 2019). Besides caloric density and elemental stoichiometry, the content of essential nutrients like essential amino acids and fatty acids should be considered when comparing nutritional quality (Anderson and Pond 2000, Karasov and del Rio 2020). In contrast to terrestrial food sources, aquatic subsidies provide a rich source of omega-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) (Gladyshev et al. 2009, Martin-Creuzburg et al. 2017, Shipley et al. 2022). PUFAs are a group of fatty acids containing multiple double bonds and can, depending on the position of the last double bond, be categorized into omega-3, or omega-6 PUFAs. Some important examples are the omega-6 PUFAs linoleic acid (LIN) and arachidonic acid (ARA), and the omega-3 PUFAs alpha-linoleic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are long-chain (> 20 C) omega-3 PUFAs that are essential for maintaining crucial physiological functions in animals because of their role in membrane physiology, signal transduction and immune function (Mitchell et al. 2003, Stillwell and Wassall 2003, Stanley 2014, Schlotz et al. 2016). Animals vary in their ability to synthesize and bio-convert different PUFAs. LIN and ALA are considered essential for all animals, although some exceptions such as nematodes have been discovered (Watts and Browse 2002, Malcicka et al. 2018). Dietary EPA and DHA uptake has been proven beneficial for different animal groups, including humans (Martin-Creuzburg and von Elert 2009, Twining et al. 2016b, Zhang et al. 2019), because bioconversion of EPA, and other long-chain PUFAs like DHA from shorter chain dietary precursor PUFAs, is thought to be inefficient and costly (Pawlosky et al. 1997, Brenna et al. 2009, Castro et al. 2012). Some predators including cats have been shown to completely rely on dietary intake of long chain PUFAs because they do not have the necessary metabolic pathways for bioconversion (Rivers et al. 1975, Pawlosky et al. 1997, Wang et al. 2020).

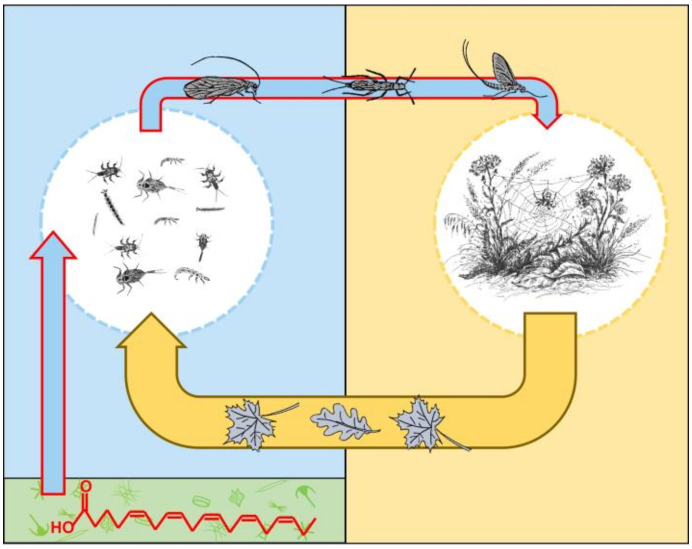

Figure 3: Aquatic systems as an EPA source - Phytoplankton at the base of aquatic food chains produce EPA that is transferred to higher tropic levels. Emergent aquatic insects represent one vector for transfer into terrestrial systems.

The reliance on dietary uptake creates a potential for resource limitation (Twining et al. 2016a) because PUFAs are not equally distributed throughout the landscape due to a vast dichotomy in PUFA content between primary producers (Hixson et al. 2015, Twining et al. 2020). EPA is synthesized by aquatic primary producers and accumulates in aquatic consumers of higher trophic levels, while it is scarce in terrestrial food webs (Twining et al. 2019) because it is not produced by vascular plants (Harwood 1996, Sayanova and Napier 2004). Studies have shown that aquatic organisms, including emergent insects, contain on average 6 times more omega-3 PUFAs than terrestrial insects (Hixson et al. 2015, Mathieu-Resuge et al. 2021). Riparian specialist species have likely adapted to the easy access to EPA provided by aquatic subsidies and may rely on consuming aquatic organisms to cover their EPA demands. For example, for tree swallows, a species that can be considered a riparian specialist, dependence on EPA in the diet was shown (Twining et al. 2018a), while more generalist species like blue tits do not seem to depend on aquatic EPA (Twining et al. 2021). The different supply of nutrients such as PUFAs creates "nutritional landscapes" that can filter the occurrence of species. That concept is illustrated, for example, by the expansion of marine sticklebacks into freshwater systems. Only lineages with a certain metabolic gene, granting them the ability to convert chain PUFAs into DHA, were able to expand their range into freshwaters that are generally deficient in DHA (Ishikawa et al. 2019).

Human impacts – "match-mismatch"

Environmental changes can influence the nutritional landscape and can therefore alter community composition by excluding species maladapted to new conditions. The production of PUFAs in aquatic systems is predicted to be altered due to increasing anthropogenic impacts, with potential cascading effects to organisms of higher trophic levels (including humans) (Hixson and Arts 2016, Colombo et al. 2020). In addition, the amount and timing of aquatic subsidies are susceptible to global change stressors (Larsen et al. 2016, Muehlbauer et al. 2020), further altering nutritional landscapes. Alterations are predicted to cause mismatches between available and required resources; for example, temporal mismatches between availability of aquatic subsidies and breeding time of birds have been reported (Shipley et al. 2022). Such mismatches are especially important to species that are adapted as riparian specialists. Riverine systems seem particularly important in that regard because they represent not only point sources, but have long shorelines spanning the landscape that provide access to PUFAs to a wide range of organisms. Stream fragmentation and morphological changes potentially alter this lateral connection between aquatic and terrestrial systems that provides EPA, where it would otherwise not be easily available.

Aim of this study: The aim of this thesis was to get a better understanding of the impacts of anthropogenic changes, in particular instream barriers, on lateral food web connectivity between streams and riparian zones.

In Chapter 1, I investigated the impact of sediment deficits on lateral food web connectivity using sediment traps build for hazard mitigation in many Swiss high gradient streams. I focused on the biomass export via emergent aquatic insects and the contribution of aquatic food sources to spider diets. I used floating emergence traps to quantify biomass export via emergent insects and stable isotope analysis to investigate the contribution of aquatic and terrestrial prey to spider diets.

In Chapter 2, I focused on the transfer of polyunsaturated fatty acids (PUFAs) from aquatic ecosystems by emergent insects to two different groups of riparian predators. I investigated seasonal patterns and differences between a near-natural and degraded stream system with multiple barriers using two 6th order streams in the Thur catchment in northeastern Switzerland. I used stable isotope analysis, fatty acid analysis and compound specific isotope analysis to investigate the flux of carbon and essential nutrients (PUFAs) to riparian spiders.

In Chapter 3, I investigated the impact of stream degradation on EPA export and transfer from aquatic to riparian zones. I quantified the biomass export by emergent insects along two stream networks in the Thur catchment and compared the emergent insect community. While one stream had near-natural conditions, the other suffered from habitat degradation. I examined if there were differences in the quantity and quality (biomass and PUFA content) of emergent insects between the two river networks in relation to differences in nutrients and stream-bed composition. I not only compared total export, but also considered the accessibility of different emergent insect groups for different types of riparian predators.

In Chapter 4, I developed a factsheet about the importance of lateral food web connectivity in the context of “Riverscape – sediment dynamics and connectivity. Practice-oriented research in hydraulic engineering and ecology”) for practitioners and the general public, highlighting some relevant findings from this thesis.

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CHAPTER 1 - INFLUENCE OF INSTREAM SEDIMENT TRAPS ON RESOURCE SUBSIDIES TO RIPARIAN ZONES

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Abstract

Sediment transport is an important structural driver along unregulated stream networks. However, a multitude of instream barriers have impaired sediment transport dynamics, leading to severe local sediment deficits and changes in stream morphology such as bed incision. These changes can influence aquatic communities, including macroinvertebrates, and potentially alter important lateral food web linkages to riparian zones. Here, we examined the potential effects of barriers, disrupting instream sediment transport, on lateral resource exports to riparian spiders. We estimated resource export of emergent insects at sites upstream and downstream of sediment traps and compared to streams without sediment traps. We found no difference in total biomass export via emergent insects when comparing sites upstream and downstream of sediment traps, but insect groups contributing to export were more similar to each other at downstream sites with sediment deficits. The contribution of aquatic and terrestrial food sources to spider diets could not be fully disentangled due to overlapping stable isotope ratios (C, N), but no differences upstream and downstream of sediment traps were found. We conclude that the influence of instream sediment traps on lateral food web connectivity appears small in these streams, although more research is needed on the cumulative effects of such barriers along stream networks.

Keywords: connectivity, emergent insects, sediment trap, resource subsidies, stable isotopes

1. Introduction

The composition of instream sediments and their transport are key features of fluvial networks (Waters 1995, Whiting et al. 1999, Recking et al. 2016, Grill et al. 2019) and the importance of a natural sediment regime, has been recognized (e.g. Wohl et al. 2015, Poeppl et al. 2020). However, the sediment continuum in many streams is disrupted by a multitude of instream barriers. Globally, only 37 % of rivers (longer than 1000 km) remain free-flowing and only 23 % flow into the ocean without interruptions (Grill et al. 2019). An estimated 16.7 million barriers (with reservoir areas > 100 m^2) fragment fluvial networks and severely limit bed load transport (Lehner et al. 2011). One type of barrier that specifically prevents sediment transport especially the transport of the coarse fraction (bed load) are sediment traps (Waters 1995). Sediment traps are widely used throughout the world for a variety of purposes, including torrent control, water supply enhancement, agricultural land development, and watershed restoration (Lucas-Borja et al. 2021). The number of sediment traps worldwide remains unknown, but in Switzerland for example, over 4000 have been installed on streams (Mathers et al. 2021).

In this study, we focused on sediment traps built for hazard control in high gradient streams that have major sediment transport during high flows (Rudolf-Miklau and Suda 2013, Piton and Recking 2016). Mitigation has been necessary because high gradient streams create a potential for natural hazards, being prone to unpredictable high magnitude flood events that mobilize large amounts of stream-bed material (Rickenmann 1997, Swanson et al. 1998, Rickenmann et al. 2016, Galia et al. 2017). For instance, Hayward (1980) highlighted that 90% of the sediment transport occurred during < 1 % of the flow period, i.e., during high flow events. The central part of a sediment trap typically consists of a slot or slit dam (open check dam) (Zollinger 1984, Armanini and Larcher 2001, Schwindt 2017), retaining transported sediment during floods through mechanical (obstruction by coarse material) or hydraulic control (exceeded discharge capacity) (Piton and Recking 2016). Sediment traps range in size from ca. 3-15 m in height and ca. 2-35 m in width (Lucas-Borja et al. 2021).

Deficits in sediment (hungry water) can alter channel morphology, leading to channel incision and bank erosion, because the transport capacity is higher than the amount of suspended/transported material (Kondolf 1997). Sediment retention by traps typically causes channel degradation downstream, including local morphological changes such as incision, bed armoring, bank destabilization and changes in the grain size distribution (Comiti 2012, Fortugno et al. 2017, Zema et al. 2018). Although multiple studies have highlighted that sediment traps exert a major human impact on alpine fluvial systems (e.g. Abbasi et al. 2019, Lucas-Borja et al. 2021), very few studies have investigated the ecological effects of sediment traps. In those studies, the focus was predominantly on the effectiveness to restore riparian vegetation (Bombino et al. 2014, Zema et al. 2018). However, bed load deficits have been connected to drastic ecological effects, e.g. below dams (e.g. Ligon et al. 1995, Rollet et al. 2014), because they reduce the availability of habitat-forming substrate (Černý et al. 2003). The changes in habitat caused by sediment traps might have similar ecological consequences. For example, factors like grain size distribution and fine sediment inputs have been shown to be hugely influential for instream assemblages (Matthaei et al. 2010, Lorenz and Wolter 2019). In a first assessment of ecological effects of sediment traps on instream communities, Mathers et al. (2021) found that sediment traps modified benthic community structure, potentially reducing the resilience of macroinvertebrate communities to disturbance.

Sediment traps may also affect adjacent riparian communities that rely on aquatic resources such as emergent insects. A considerable proportion of lotic macroinvertebrate assemblages do not remain in the aquatic environment during their entire life cycle, but emerge into terrestrial ecosystems to reproduce and thereby are integrated into terrestrial food webs (Baxter et al. 2005). Many riparian predators, such as birds, bats, lizards, beetles and spiders heavily depend on aquatic resource subsidies (e.g. Sabo and Power 2002, Kato et al. 2003, Paetzold et al. 2006, Twining et al. 2018) as additional food pulses (Nakano and Murakami 2001) and also because of their high food quality (Moyo et al. 2017, Twining et al. 2019). For example, Kato et al. (2003) found the density of tetragnathid spiders, that build webs in the riparian vegetation to capture prey, depended on the availability of emergent insects. In addition, isotope ratios ($\delta^{13}C$, $\delta^{15}N$) have been used in multiple studies to distinguish the contribution of aquatic and terrestrial material to diet of riparian consumers (e.g. Collier et al. 2002, Kato et al. 2004, Paetzold et al. 2005). For example, Collier et al. (2002) observed via stable isotope analysis that ground dwelling spiders obtained on average 55 % and web building spiders 61 % of their carbon from aquatic sources in two streams in New Zealand, indicating that aquatic subsidies are an important food source for both spider types. However, changes in stream morphology and aquatic assemblages might disrupt resource transfer to riparian predators. For example, Jonsson et al. (2013) found less biomass export via emergent insects in regulated rivers than non-regulated. River regulation also affected upland-forest invertebrate communities with at least some effects arising from links between aquatic emergence and terrestrial predators.

In this study, we examined if sediment traps influenced the lateral connectivity of aquatic-derived resources to riparian predators. For this, we estimated the biomass export via emergent aquatic insects and the amount of aquatic prey in the diet of riparian web-building and ground-dwelling spiders upstream and downstream of sediment traps and compared to unimpacted control streams (without a sediment trap). We expected a change in insect groups contributing to export due to degraded habitat conditions, with altered grain size distribution, downstream of sediment traps (ST). We also expected reductions in the total biomass export at sites downstream (DS) of STs compared to upstream (US) sites as well as a lower contribution of aquatic prey to riparian spider diets because of the reduced export. In contrast we expected export and spider diet to be similar at US and DS sites in Control streams. Importantly, investigating sediment trap streams allows the study of sediment deficits without other co-occurring disturbances like residual flows or hydropeaking, that can also affect aquatic community assemblages.

2. Material and Methods

2.1. Study location and design

Study sites were located in central Switzerland, in Canton Obwalden. We sampled six 3rd order streams around lake Sarnen (Figure 1); three streams with sediment traps (ST streams) and three streams without trap (Control streams). All streams were of similar altitude and size. Sediment trap streams were selected from a list of over 4000 sediment traps in Switzerland (Mathers et al. 2021) using GIS software and aerial images. We used the following selection criteria: 1) 3rd order streams, 2) located at elevations between 500 - 700 m, 3) no other alterations such as hydropeaking, flow regulation or low water quality, and 4) mobile bed load in downstream reach. Initial selection was followed by field visits to assess suitability for this study, where most streams showed strong anthropogenic alterations such as artificial channels and no mobile sediment downstream of the sediment trap. Finally, we found a set of streams fulfilling the criteria in relatively close proximity to each other that made parallel sampling of insect emergence possible. Sediment trap streams included Rufibach (Ruf), Bizighoferbach (Biz) and Edisriederbach (Edi) and Control streams were Foribach (For), Altibach (Alt) and Zimmertalbach (Zim) (Figure 1). From the six streams used in this study, four streams (Ruf, Edi, For and Alt) were sampled in parallel for another study investigating the effects of sediment traps on instream communities. For details on trap design and information about the specific sediment traps see (Mathers et al. 2021). We conducted our sampling at two sites in each stream. In sediment trap streams, we used one sampling site two wetted widths upstream and another sampling site the same distance downstream of the trap, whereas in control streams we used, as described in Mathers et al. (2021), one site upstream and one downstream of a slope knickpoint between a steep canyon and a lower gradient alluvial fan (Piton et al. 2017).

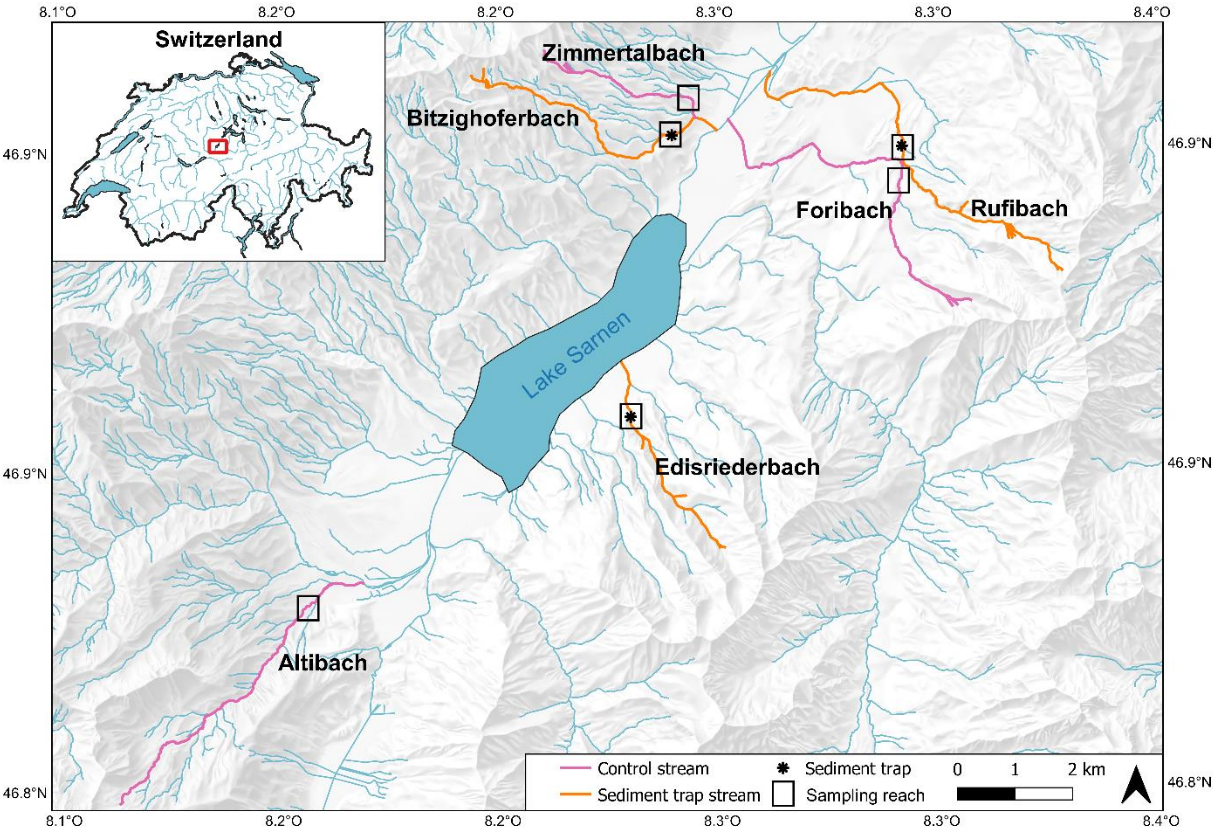

Figure 1: Map of study streams around Lake Sarnen in Canton Obwalden, central Switzerland (Map insert at top left).

2.2. Field Sampling

2.2.1. Physico-chemical parameters and stream-bed composition

A water sample was taken at each site in a triple-rinsed 0.5 l plastic bottle to ensure water quality was similar among sampling sites. Samples were analyzed in the laboratory for pH, alkalinity, nitrate, nitrite, total nitrogen (TN), ortho-phosphate (OP), total phosphorus (TP), dissolved organic carbon (DOC) and total organic carbon (TOC) as detailed in Tockner et al. (1997). See Supplement A for values in each stream. To assess the grain size distribution, 100 particle Wolman counts (Wolman 1954, Bunte and Abt 2001) at each site were undertaken using transects and blindly selecting and measuring (b-axis) a grain after each step using a gravelometer.

2.2.2. Aquatic and terrestrial insect/spider sampling

The ecological sampling campaign took place in June and July 2019. Emergent insects were collected alive using floating emergence traps (0.25 m^2, 100-µm mesh). Emergence traps (n = 3 traps per site) were deployed 2-3 days over 6 sampling periods (n = 3 per month) at each site. In addition, 30s kick-samples were taken using a standard pond net (500 µm mesh) at the two streams not sampled by Mathers et al. (2021). As detailed there, three samples were taken per site covering the range of present habitats, to assess macroinvertebrate assemblages and composition. Terrestrial insects were collected using a sweep net in the area around sampling streams. In addition, ground-dwelling and web-building spiders were collected in riparian areas 0-2 m from the river edge. All ground-dwelling spiders belonged to the family Lycosidae and web-building spiders to the family Linyphiidae. Samples were brought back to the laboratory, left alive overnight to allow gut clearance and then frozen until further analysis.

2.3. Laboratory Procedures

Emergent insect samples were sorted generally to family level into taxonomic groups, freeze-dried and weighed using an ultra-fine balance to determine dry mass export. Samples from both spider groups, emergent aquatic insects from each site, and terrestrial insects were used for stable isotope analysis. Samples were ground up and for each sample 0.7-0.9 mg of freeze-dried ground material was weighed and placed into tin capsules to determine carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios to estimate the contribution of aquatic-derived material to riparian spiders. Individual samples were combusted in a Vario PYRO Cube elemental Analyzer (Elementar Analysensysteme GmbH, Langenselblod, Germany) connected to an IsoPrime isotope ratio mass spectrometer (GV instruments Ltd, Manchester, UK). The δ13C and $\delta^{15}N$ ratios values are presented in permille (‰) after normalization to reference material (NBS 19, L-SVEC, IAEA-N-1 and IAEA-N-2, provided by Biogeochemical Laboratories, Indiana University). Analytical uncertainty was 0.1 ‰ for $\delta^{13}C$ and 0.2 ‰ for $\delta^{15}N$ ratios.

2.4. Data Analysis

All analyses were conducted in R 3.6.2 (R Core Team 2019). Pebble counts were used to characterize stream-bed composition by calculating the percentage of different grain sizes contributing to classes as defined by Blair and McPherson (1999). To check if stream type (Control, ST) explained variance among sites, we used adonis2 (package “vegan”; Oksanen et al. (2019)) to perform a non-parametric multivariate analysis of variance (PERMANOVA) on Bray-Curtis similarity matrices. Grain size classes were used in a non-metric multidimensional scaling (nMDS) with Bray-Cutis dissimilarities to visualize stream and site-specific differences in bed composition. Similarity percentage (SIMPER) analysis was used to determine which grain size classes contributed most to the separation. Based on the pebble counts, we also calculated Shannon´s diversity index (diversity, package “vegan”) using the grain size distribution data.

We compared aquatic invertebrate community composition among streams and between US and DS sites using data from Mathers et al. (2021) and from kick-samples taken at the two additional streams using PERMANOVA. Differences were visualized using nMDS with Bray-Curtis dissimilarities. For differences in community composition see Supplement B and Mathers et al. (2021).

We calculated mean biomass export using data from emergence traps (n = 3 traps per site) to represent the average export from different habitats. The data were normalized to represent export per square meter per day. We used a mixed model (lmer, package "lme4") with date specified as a random effect to investigate stream specific differences in biomass export. The comparison between US and DS sites was run separately for ST and Control streams using mixed models. In addition, we calculated differences in biomass export between US and DS sites for ST and Control streams by subtracting the mean biomass export per month (June and July) per stream at US sites from that at DS sites. Positive values represent higher export at DS sites and negative values represent lower export at DS sites.

We visualized the contribution of different insect families to biomass export at US and DS sites using nMDS and calculated the contribution of stream identity, month and US/DS on explained variation using PERMANOVA. In addition, variance among US sites and variance among DS sites was calculated using PERMANOVA. The nMDSs and PERMANOVAs were run separately for Control and ST streams.

Differences in isotope ratios among predator and prey groups were investigated using type III ANOVA (Anova, package "car"). Multiple pairwise comparisons of estimated marginal means (EMMs) with Tukey corrections (package "emmeans"; (Lenth 2021) were performed as post-hoc tests. In addition, isotope ratios in spiders were compared between US and DS sites using a mixed model with stream set as random effect. The differences between US and DS sites were analyzed separately for ST and Control streams and the two predator groups. The relationship between biomass export via different insect orders and $\delta^{13}C$ ratios in riparian spiders was investigated using mixed models with stream as a random effect. As a proxy for biomass export, the average export via the different insect orders over the whole sampling period at each sampling site was used. The analysis was done separately for the two spider types.

3. Results

3.1. Comparison of stream-bed composition

Stream-bed composition differed between control and ST streams, with stream type explaining 26 % of variation (PERMANOVA: F = 3.52, p = 0.034). In particular DS sites in ST streams were separated from control stream sites in nMDS space (Figure 2A). In ST streams, we also found differences in stream-bed composition between US and DS sites in two of the three study streams (Figure 2B), with cobbles, medium pebbles and sand explaining 50 % of the difference (SIMPER). Only at Edi did the US and the DS sites have a similar substrate composition. In control streams, substrate composition appeared to mainly differ among streams. US and DS sites at For and Zim clustered relatively close together in the nMDS, but at Alt we observed a separation between sites with more cobbles at the US site than the DS site. In all study streams, the diversity of stream-bed material (Shannon´s diversity index) was similar between sites (ST: US = 2.47 ± 0.08; DS = 2.50 ± 0.06; $p > 0.05$; Control: US = 2.44 ± 0.08; DS = 2.45 ± 0.05; $p > 0.05$).

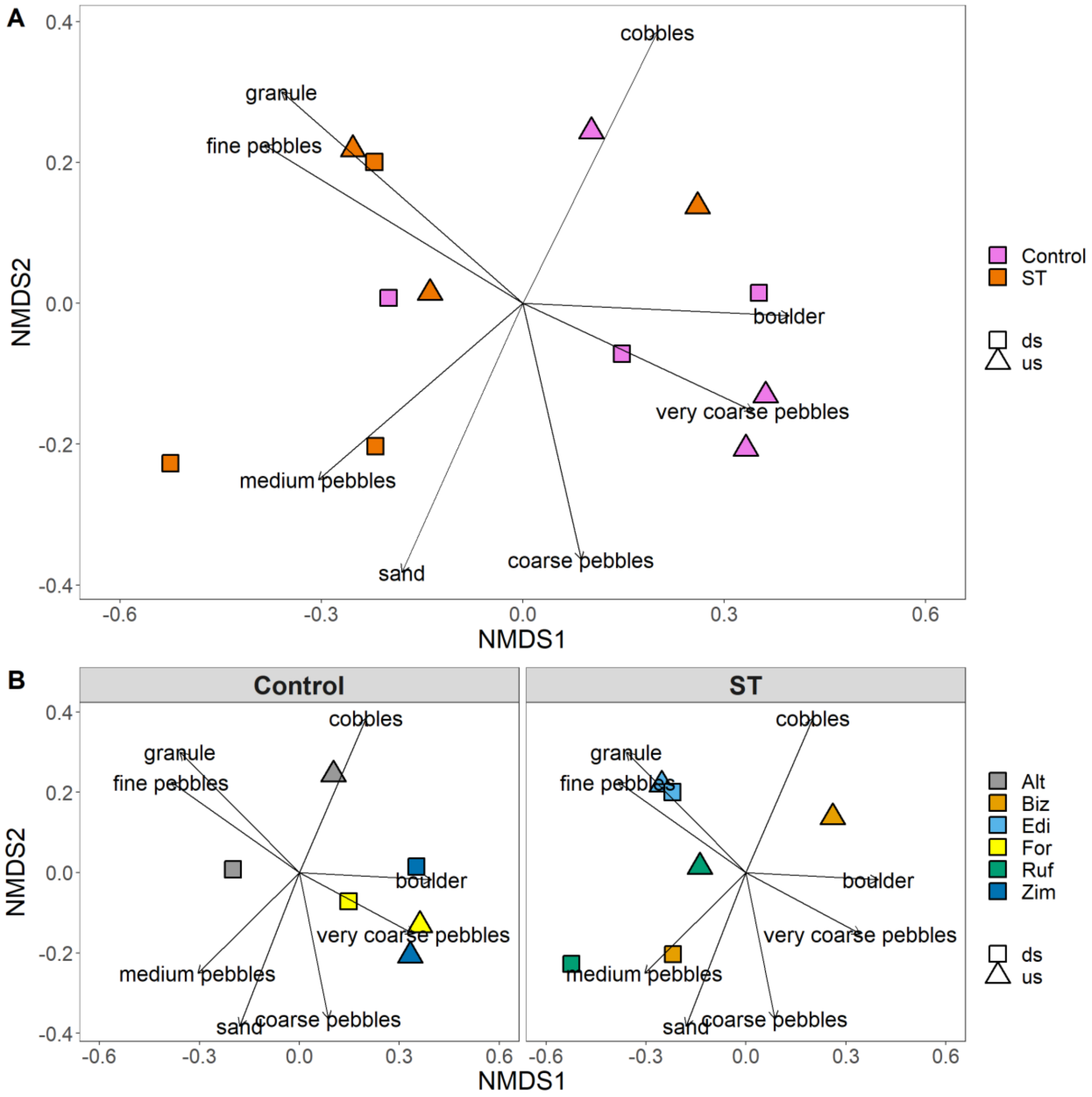

Figure 2: Non-metric multidimensional scaling (nMDS) of stream-bed composition at US and DS sites in control and sediment trap (ST) streams; A) differences between control and ST streams B) differences between US and DS sites in control and ST streams.

3.2. Comparison of biomass export by emergent insects

Total biomass export differed among streams (F = 7.87, $p < 0.001$) with the highest export recorded at Alt (22.6 ± 7.5 mg m^{-2} day^{-1}) and Biz (23.5 ± 17.1 mg m^{-2} day^{-1}) and the lowest export at Edi (5.3 ± 3.2 mg m^{-2} day^{-1}) (Figure 3A). Month had no significant effect on total biomass export (F = 0.22, $p > 0.05$), and there were no differences between ST and control streams ($p > 0.05$). In both sample months, mayflies and caddisflies contributed the most to total biomass export with peak exports of up to 10 mg m^{-2} day^{-1}. However, Diptera also had high biomass export especially at Alt in July with 14.2 ± 5.2 mg m^{-2} day^{-1}. Stonefly export was low during June and July being highest at Ruf in June (1.9 ± 0.4 mg m^{-2} day^{-1}). In control streams, total export (F = 6.78, p = 0.014) and export by caddisflies (F = 4.33, p = 0.047) was higher at US than DS sites (Figure 3B), whereas other insect orders showed no differences between US and DS sites (p > 0.05). There was no difference in total export between US and DS sites at ST streams (F = 0.42, p = 0.523), only stonefly export was higher at US than DS sites (F = 4.94, p = 0.034).

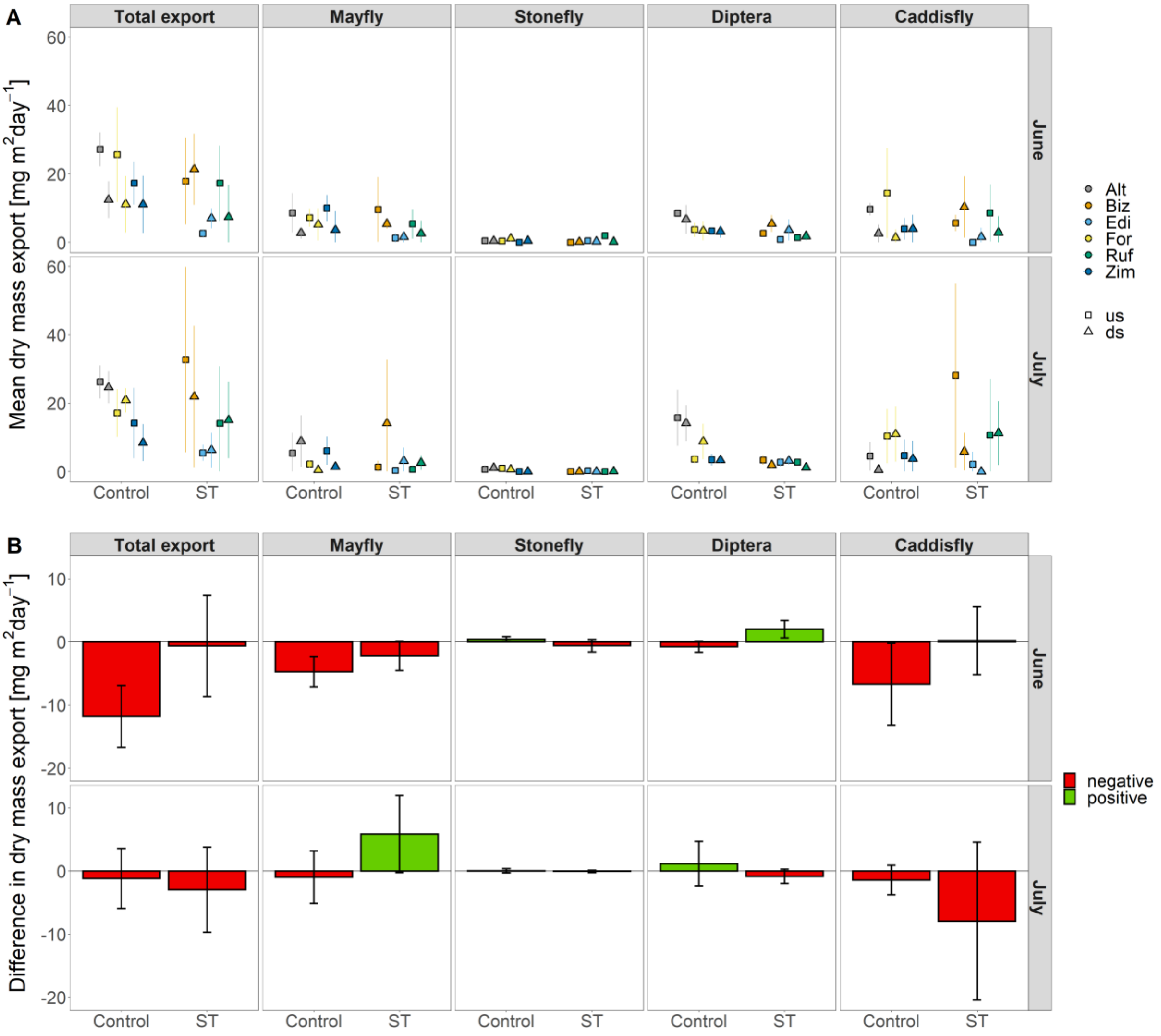

Figure 3: A) Biomass export by different groups of emergent insects in June and July from US and DS sites in sediment trap (ST) and control streams. B) Difference in average biomass export by different insect groups in June and July between US and DS in ST and control streams. Red bars (< 0) represent lower export at DS sites; green bars (> 0) represent higher export at DS sites.

3.3. Contribution of different insect groups to biomass export

The variation in the contribution of different insect groups to biomass export in ST streams was explained 13 % by stream identity (PERMANOVA: F = 2.64, p = 0.003) and 10 % by month (PERMANOVA: F = 4.08, p = 0.001) (Figure 4). The difference between US and DS explained no significant variation in the data (PERMANOVA: F = 0.93, p= 0.456) and we saw no shift in the biomass composition in the nMDS between US and DS in a specific direction. However, we observed shifts in groups contributing to biomass export in two of the three ST streams. At Edi the composition of groups contributing to biomass export US and DS were similar during both months. In contrast, the shift between US and DS was especially large at Ruf in June and Biz in both months. Further, while US emergent insect communities at ST streams were distinct from each other, especially in June (PERMANOVA: June: $R^2 = 0.46$, F= 2.51, p=0.013, July: $R^2 = 0.26$, F = 1.03, p = 0.381), DS communities were similar to each other in both months (PERMANOVA: June: $R^2 = 0.25$, F = 1.01, p=0.445; July: $R^2 = 0.33$, F = 1.49, p = 0.24). In control streams, the variation in the contribution of different insect groups to biomass export was explained 21 % by stream identity (PERMANOVA: F = 4.94, p = 0.001) and 9 % by month (PERMANOVA: F = 5.02, p = 0.001). The difference between US and DS sites explained 4.5 % of the variation (PERMANOVA: F = 2.50, p = 0.013). Here, all DS sites clustered further to the right than respective US sites in the nMDS in both months, although this separation was small e.g., at Alt in July.

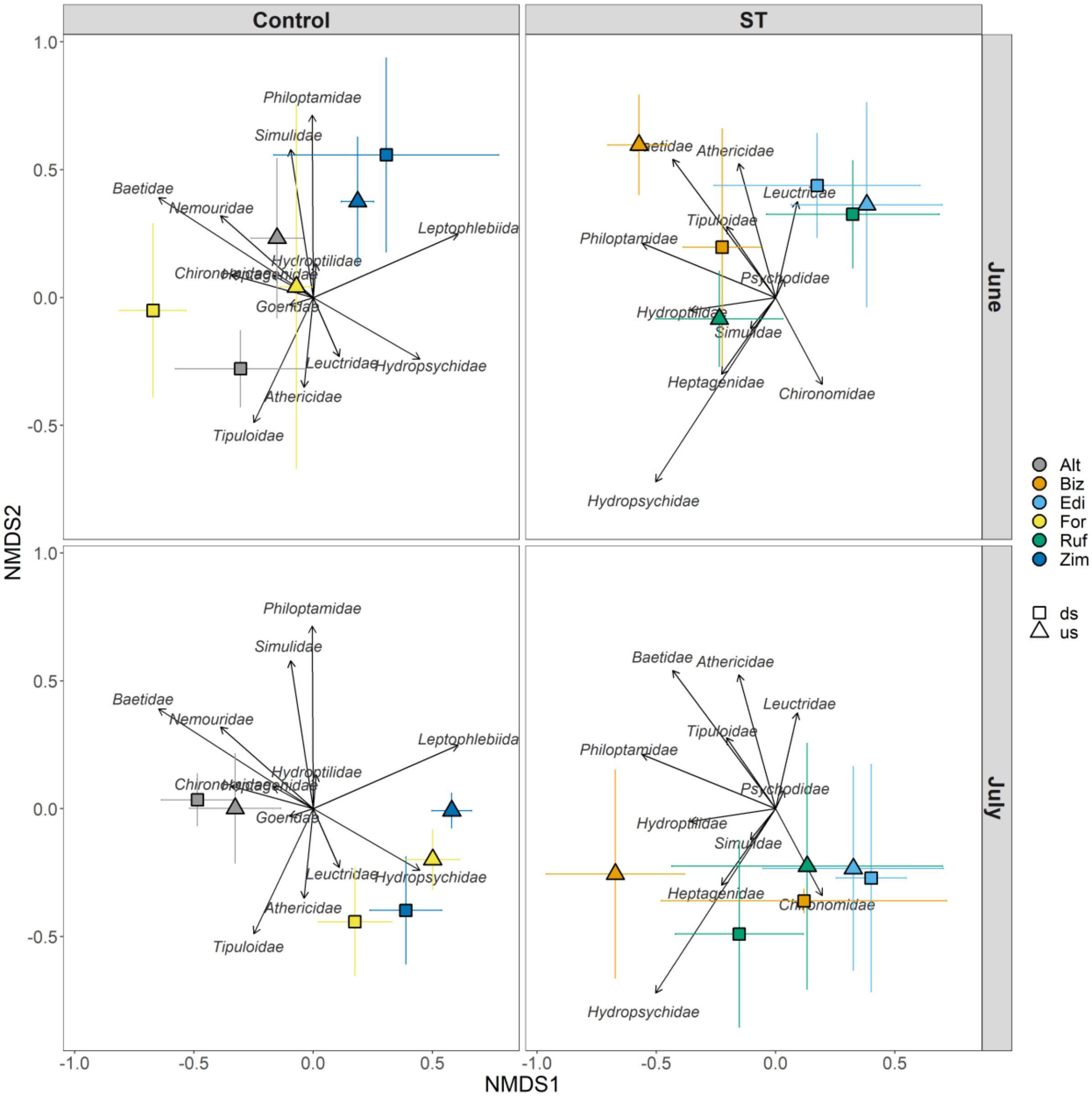

Figure 4: Non-metric multidimensional scaling (nMDS) of dry mass export by different insect groups at US and DS sites in control (stress = 0.23) and ST streams (stress = 0.22) in June and July.

3.4. Stable isotope data

3.4.1. Isotope ratios of predator and prey groups

We found more negative δ^{13}C values in web spiders (-27.34 ± 0.72 ‰) than in ground spiders (-26.8 ± 0.72 ‰) (F = 5.18, p = 0.026). Further, δ^{15}N was more positive in web spiders (5.45 ± 1.68 ‰) than in ground spiders (4.28 ± 2.10 ‰) (F = 8.20, p = 0.006) (Figure 5). Both ratios differed among prey groups (δ^{13}C: F =18.76, p <0.001; δ^{15}N: F = 2.70, p = 0.0338). Aquatic Diptera (δ^{13}C: -27.86 ‰, δ^{15}N: 3.45 ± 2.46 ‰), stoneflies (δ^{13}C: -28.56 ‰, δ^{15}N: 1.07 ± 2.68 ‰) and terrestrial insects (δ^{13}C: -28.36 ‰, δ^{15}N: 3.16 ± 3.23 ‰) had largely overlapping δ^{13}C and δ^{15}N ratios, whereas mayflies had more negative δ^{13}C ratios (-31.76 ±2.59 ‰) than the other groups ($p < 0.001$). Caddisflies showed a wide span of δ^{13}C and δ^{15}N ratios, and δ^{15}N in stoneflies was less enriched than in caddisflies (t.ratio = -2.89, p = 0.036). As there was no clear isotopic distinction between aquatic and terrestrial prey groups, we could not use a mixed model to calculate the contribution of aquatic and terrestrial food sources to spider diets, except for mayflies in contrast to all other groups combined (Supplement C).

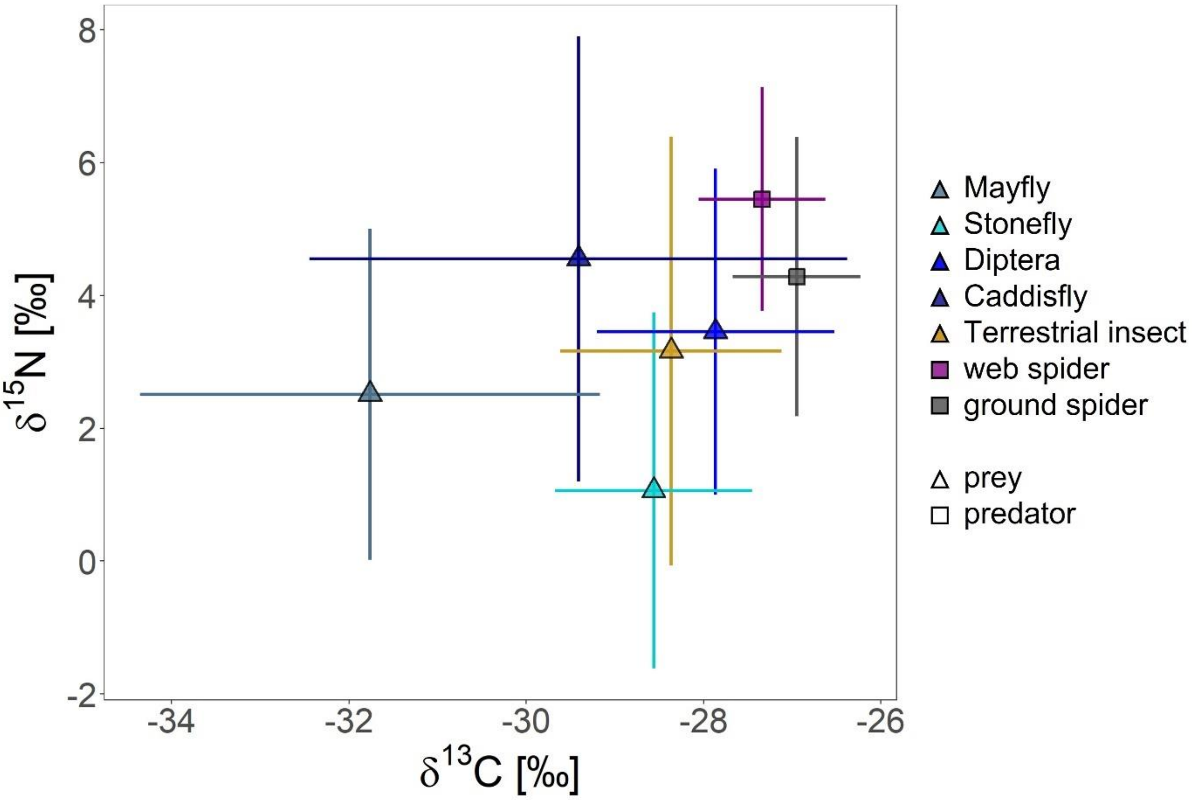

Figure 5: δ^{13}C and δ^{15}N ratios of web-building spiders, ground-dwelling spiders and potential insect prey groups. Symbols represent mean and error bars standard deviation.

3.4.2. Difference in isotope ratios of spiders between US and DS sites

In web spiders, we found no difference in δ^{15}N ratios between US and DS sites in ST (F = 0.23, p = 0.636) or control streams (F = 2.27, p = 0.149) (Figure 6A). In ground spiders, δ^{15}N ratios differed between US and DS in both ST (F = 5.78, p = 0.033) and control streams (F = 6.09, p = 0.035). In both spider types, we found no differences in δ^{13}C ratios between US and DS sites in control or ST streams ($p > 0.05$) (Figure 6B). While the δ^{13}C ratios in web spiders were generally more negative, the differences in δ^{13}C ratios between sites followed the same pattern in both spider types; e.g., being on average equal between US and DS in Edi, and more negative US than DS in Biz.

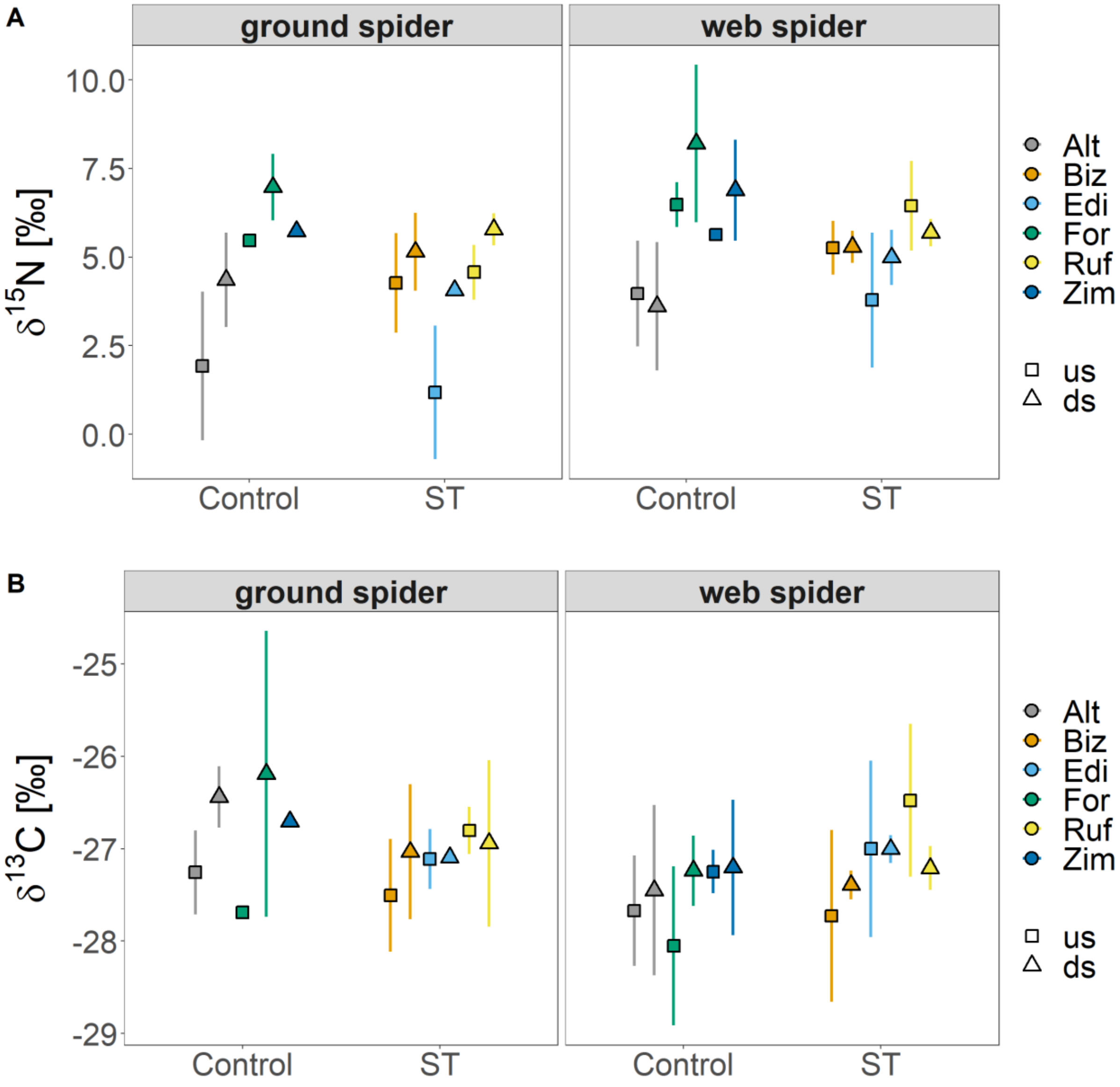

Figure 6: A) Differences in δ^{15}N in riparian ground-dwelling and web-building spiders between US and DS sites in control and sediment trap (ST) streams. B) Differences in δ^{13}C in riparian ground-dwelling and web-building spiders between US and DS sites in control and ST streams.

3.4.3. Relationship between emergent insect biomass export and δ^{13}C in riparian spiders

The average δ^{13}C ratios of web-building spiders (per site) were correlated with the average total biomass export by emergent aquatic insects, with more negative ratios at sites with higher total biomass export (F = 7.48, p = 0.02, R^2m = 0.40) (Figure 7). We also saw a trend of more negative ratios in relation to export in mayflies (F = 3.77, p = 0.08, R^2m = 0.26) and caddisflies (F = 4.10, p = 0.07, R^2m = 0.27). In contrast, we found less negative δ^{13}C ratios in web spiders at sites with more stonefly export (F = 14.43, p = 0.009, R^2m = 0.30). We found no relation between δ^{13}C ratios of web spiders and Diptera export. In ground spiders, we found more negative δ^{13}C values at sites with high caddisfly export (F = 21.77, p = 0.007, R^2m = 0.53). No further relationships between aquatic insect biomass export and δ^{13}C ratios in ground spiders were found.

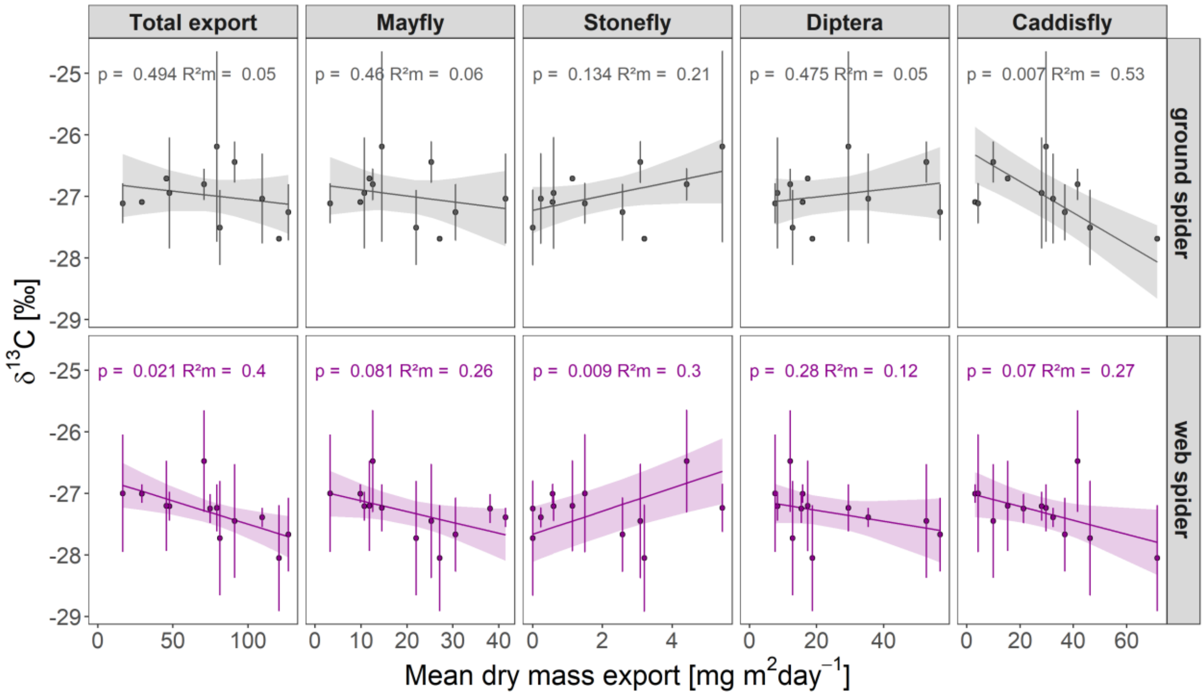

Figure 7: Relationship between average dry mass export by different insect orders and δ^{13}C ratios in riparian web-building and ground-dwelling spiders.

4. Discussion

4.1 Changes in stream-bed composition

The stream-bed composition at DS sites and therefore instream habitat conditions for aquatic macroinvertebrates differed relative to US sites in two of the three ST streams, whereas the composition at US and DS sites at control streams was rather similar, showing more variation among streams (except Alt, more cobbles US than DS). A change in grain-size distribution was also observed in other studies investigating morphological changes downstream of sediment traps (Fortugno et al. 2017, Zema et al. 2018). In contrast to these studies, we observed a higher proportion of fine material (sand) in DS than US sites at two of the ST streams. This fine material was likely eroded from local stream banks by “hungry” water and deposited in the stream; i.e., bank erosion is a common phenomenon downstream of sediment traps (Boix-Fayos et al. 2007). In the third ST stream (Edi), we observed no differences in stream-bed composition probably because the banks were artificially protected against erosion. Ruf and Biz, the two ST streams without bank protection, had downstream sites that were similar to each other regarding stream-bed composition, but the stream bed composition at those two sites was clearly distinct from all other sampling sites.

4.2 Aquatic invertebrate community and biomass export

Aquatic macroinvertebrate assemblages mainly differed among streams, with slight differences between US and DS sites at ST streams that were not present in control streams. Differences in assemblages were described in more detail in Mathers et al. (2021) with main differences found in resilience traits (relative abundance of functional resistance forms like statoblasts or dormancy) between US and DS sites. We found no significant differences in total biomass export between US and DS sites of ST streams. This result is similar to a study on the effect of dam removal (small low head dam) on emergent insect biomass export, which was found to be relatively small (Sullivan et al. 2018). The similar biomass export between US and DS sites in ST streams contrasted with control streams where total export and export by caddisflies was lower at DS than US sites. This change between sites at control streams might represent expected longitudinal changes. For example, Freitag (2004) also reported a decrease in total emergence in the downstream direction. This finding suggests that sediment traps may disrupt the longitudinal continuity in ST streams.

Stonefly export was slightly lower at DS than US sites at ST streams, although it was generally on a low level at all sites. In this context it is important to note that all sampling was conducted in late spring/early summer and therefore the results should be interpreted with this in mind as emergence of many aquatic insects occurs during specific periods of the year (Finn et al. 2022). Further, stoneflies were present in aquatic macroinvertebrate samples (Mathers et al. 2021), but scarcely captured in emergence traps, indicating that sampling occurred outside of the main emergence period of stoneflies. Consequently, the effect of bed load deficits on stonefly emergence could not be fully assessed in this study. Sampling during early spring, when stonefly emergence peaks were reported in other systems in Switzerland (Kowarik et al 2022, in review), would be optimal to assess effects on stonefly emergence. In addition, sediment trap design (e.g., % of material retained) and distance of sampling sites to the sediment trap likely play a role in the effect size. For this study, we chose sediment traps that retained at least 95 % of the transported material to be able to assess the strongest possible effects and sampled relatively close to sediment traps where the greatest implications were found by Mathers et al. (2021).

4.3 Groups contributing to biomass export

We observed shifts in insect families contributing to biomass export between US and DS sites in ST streams, especially in the two streams where stream-bed composition differed between US and DS sites (Ruf and Biz). For Edi, both sites had similar community and stream-bed compositions, linking habitat and emergent insect communities. Edi might be different to the other studied streams due to two main reasons. First, in a visual assessment, the US site at Edi appeared to have undergone some anthropogenic changes including small vertical barriers not present in the other streams (see aerial image of reach upstream of sampling site, Supplement D). Second, Edi had artificial bank protections downstream of the trap, and therefore less fine material could be introduced directly below the sediment trap. The water remained “hungry”, and erosion and effects further downstream were recorded in Edi by Mathers et al. (2021).

We also observed some shifts in the groups contributing to export in control streams, especially at Alt (June) and Zim (July). Observed differences might therefore be normal fluctuations in emergence between reaches. However, the shifts in the control streams between US and DS sites all occurred in the same direction, and this was not the case in ST streams. In ST streams, the direction of the shift in groups contributing to biomass export differed among streams. We did not find, for example, more chironomids and less caddisflies at all DS sites compared to US sites. However, while the insect community contributing to the biomass export in ST streams was diverse at US sites, all DS sites had similar community composition to each other. Further sampling, including more sites would be necessary to support the pattern we found. Unfortunately, additional ST streams were logistically difficult to include in this study, because other ST trap streams present in the local area had completely artificial stream-beds downstream of the ST. This artificial stabilization of the stream bottom is a common measure to prevent erosion by “hungry” water. Irrespective, DS assemblage composition in ST streams were more similar likely because the stream-bed composition was comparable (except Edi) favoring more similar communities (Xuehua et al. 2009). The consequences for riparian communities remain unclear without further assessment, but less distinct communities among streams might also change riparian communities (e.g. spider and bird assemblages) because of a lower diversity in prey groups with unique traits.

4.4 Stable isotope data

We were unable to calculate the contribution of aquatic and terrestrial material to spider diets because $\delta^{13}C$ ratios of stoneflies, aquatic Diptera and terrestrial insects overlapped. These prey groups had overlapping carbon ratios, probably because terrestrial leaf litter was part of their respective diets and contributed to carbon signatures (Rounick et al. 1982, Rasmussen 2010). Mayflies, on the other hand, were represented by different grazing families that feed on autochthonous sources (periphyton) and had $\delta^{13}C$ ratios distinct from terrestrial insects. Because of the contribution of mayflies to the diet, we likely observed a more aquatic signal (more negative $\delta^{13}C$ ratios) in some spiders. For instance, web-spiders had more negative $\delta^{13}C$ than ground-spiders, likely due to the ingestion of greater numbers of mayflies. In other studies, web-spiders also showed stronger aquatic contribution to diets than ground-spiders (e.g. Collier et al. 2002). For web-spiders, we found no significant difference in $\delta^{13}C$ ratios upstream and downstream of sediment traps. For ground-spiders, we found a significant difference between upstream and downstream sites at ST streams, but the difference also occurred in control streams, indicating that differences in $\delta^{13}C$ ratios are normal fluctuations across sites. This result might indicate that diet sources were similar upstream and downstream of STs and compared to control streams and thus sediment traps had little impact on lateral food web connectivity. Similarly, Sullivan et al. (2018) found that removal of a low head dam had minimal impact on the contribution of aquatic carbon sources to the diet of riparian predators. However, with the overlapping isotope ratios, effects could be hidden in the present study. We found a trend with more negative $\delta^{13}C$ ratios in web spiders at sites with higher export by mayflies, that have more negative $\delta^{13}C$ ratios than other aquatic taxa, as well as total aquatic export, indicating that the availability of aquatic insects likely influenced the carbon composition in web-spiders. For ground-spiders, stoneflies, that emerge via crawling, have been suggested as the most important aquatic subsidy (Paetzold et al. 2005), even though the $\delta^{13}C$ ratio in ground-spiders was correlated with caddisfly biomass in this study. The missing link between $\delta^{13}C$ ratios in ground-spiders and stonefly biomass is unsurprising, as $\delta^{13}C$ of stoneflies and terrestrial insects were indistinguishable. The lower $\delta^{15}N$ ratio found in ground-spiders than in web-spiders could however indicate that they fed on more stoneflies, because stoneflies had on average lower $\delta^{15}N$ ratios than other insect groups, albeit only significantly for caddisflies. Overlapping isotope ratios make estimates of diet contribution difficult and $\delta^{13}C$ and $\delta^{15}N$ ratios alone might be inadequate to estimate contribution of aquatic material to ground spider diet, because their most likely food source, stoneflies (especially shredder families like Leuctridae), can be isotopically similar ($\delta^{13}C$) to terrestrial insects. Other trophic markers, such as polyunsaturated fatty acids in addition to isotopes (Twining et al. 2020, Kowarik et al. 2021), might help clarify the importance of aquatic subsidies for ground-dwelling spiders.

5. Conclusion

The sediment traps in this study did not appear to severely impact lateral food web connectivity. For instance, we found no difference in the total biomass export by emergent insects or isotope ratios of riparian spiders upstream and downstream of sediment traps, although spider diets could not be fully disentangled due to overlapping isotope ratios. However, we observed some changes in invertebrate groups contributing to the biomass export, where communities of emergent insects downstream of sediment traps were more similar to each other than at upstream sites. Differences between upstream and downstream sites were stream specific, and other factors, like bank protection and artificial streambeds commonly associated with sediment traps, might have a stronger impact than the sediment traps themselves. Further research that includes different seasons to capture more emergence periods of specific taxa like stoneflies and using additional trophic markers (e.g. fatty acids) are necessary to better clarify the effect of sediment traps on lateral food web connectivity. Sediment traps serve an important function in hazard mitigation and novel designs are continuously being developed (e.g., semi-permeable traps) (Schwindt et al. 2018, Moldenhauer-Roth et al. 2021) to reduce ecological impacts. A wider realization of such novel designs in practice could mitigate remaining problems associated with sediment traps.

6. Author contribution:

Conceptualization: CK, CR, CW, KM; formal analysis: CK, KM; writing - original draft preparation: CK; writing - review and editing: CK, CR, CW, KM; visualization: CK; project administration: CK, CR; funding acquisition: CR, CW; All authors have read and agreed to the version of the manuscript.

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CHAPTER 2 - CROSS-ECOSYSTEM LINKAGES: TRANSFER OF POLYUNSATURATED FATTY ACIDS FROM STREAMS TO RIPARIAN SPIDERS VIA EMERGENT INSECTS

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Abstract

Polyunsaturated fatty acids (PUFAs) are essential resources unequally distributed throughout landscapes. Certain PUFAs, such as eicosapentaenoic acid (EPA), are common in aquatic but scarce in terrestrial ecosystems. In environments with low PUFA availability, meeting nutritional needs requires either adaptations in metabolism to PUFA-poor resources or selective foraging for PUFA-rich resources. Amphibiotic organisms that emerge from aquatic ecosystems represent important resources that can be exploited by predators in adjacent terrestrial habitats. Here, we traced PUFA transfer from streams to terrestrial ecosystems, considering benthic algae as the initial PUFA source, through emergent aquatic insects to riparian spiders. We combined carbon stable isotope and fatty acid analyses to follow food web linkages across the ecosystem boundary and investigated the influence of spider lifestyle (web building vs. ground dwelling), season, and ecosystem degradation on PUFA relations. Our data revealed that riparian spiders consumed considerable amounts of aquatic-derived resources. EPA represented on average 15 % of the total fatty acids in riparian spiders. Season had a strong influence on spider PUFA profiles, with highest EPA contents in spring. Isotope data revealed that web-building spiders contain more aquatic-derived carbon than ground dwelling spiders in spring, although both spider types had similarly high EPA levels. Comparing a natural with an anthropogenically degraded fluvial system revealed higher stearidonic acid (SDA) contents and $\Sigma\omega3/\Sigma\omega6$ ratios in spiders collected along the more natural river in spring but no difference in spider EPA content between systems. PUFA profiles of riparian spiders where distinct from other terrestrial organism and more closely resembled that of emergent aquatic insects (higher $\Sigma\omega3/\Sigma\omega6$ ratio). We show here that the extent to which riparian spiders draw on aquatic PUFA subsidies can vary seasonally and depends on the spider's lifestyle, highlighting the complexity of aquatic-terrestrial linkages.

Keywords: ecosystem degradation, emergent insects, fluvial systems, food web, PUFA, resource subsidies, riparian spiders, stable isotopes

1. Introduction

Aquatic and terrestrial ecosystems are open units that are connected in a tangled web via various resource fluxes oriented in both directions (Baxter et al. 2005). This cross-boundary transfer of resources (allochthonous resource subsidies) is essential towards sustaining both ecosystems. Some relevant examples of resource transfer from aquatic to terrestrial systems are fish predation by riparian predators (or humans), amphibiotic organisms leaving the water during their life cycle, and water birds feeding on aquatic plants (Richardson et al. 2010, Gladyshev et al. 2013). Riparian communities often rely on aquatic-derived subsidies with whole populations at times being fueled by aquatic resources, most notably in resource poor environments (Anderson and Polis 1998, Sabo and Power 2002). Emergent aquatic insects play a crucial role in adjacent riparian food webs, as they serve as prey for various predators, such as birds, bats, lizards (Sabo and Power 2002, Iwata et al. 2003, Lam et al. 2013) spiders or beetles (Paetzold et al. 2005). The abundance of spiders, for instance, has been shown to correlate strongly with the amount of emergent aquatic insects (Kato et al. 2003, Kato et al. 2004, Paetzold et al. 2005, Iwata 2007, Burdon and Harding 2008, Paetzold et al. 2008, Paetzold et al. 2011) and stable isotope analysis revealed that some riparian spiders obtain up to 100 % of their carbon from aquatic-derived organic matter (Sanzone et al. 2003). Stable isotope analysis has been used successfully to disentangle food webs and differentiate between allochthonous and autochthonous carbon sources in consumer tissues (DeNiro and Epstein 1978), although its use is dependent on the degree of isotopic discrimination between basal resources (Collier et al. 2002). Aquatic-derived organic material is generally more depleted in $\delta^{13}C$ than terrestrial organic material (Rounick and Winterbourn 1986) and this difference can be used to estimate dietary contributions. More recently compound-specific stable isotope analysis (CSIA) has been suggested as an additional method to trace energy flows through food webs (Twining et al. 2020b).

The strong effect of aquatic subsidies on riparian communities cannot be explained solely by quantity of carbon transfer, as biomass fluxes from aquatic to terrestrial ecosystems are often lower than fluxes in the opposite direction (Bartels et al. 2012). Possible explanations are still poorly understood. One aspect to consider are differences in insect phenology between aquatic and terrestrial ecosystems. Aquatic insects typically emerge earlier in the year than terrestrial insects thus representing an important dietary supplement for riparian predators when the amount of terrestrial food sources in spring is still low (Nakano and Murakami 2001). In addition, aquatic subsidies are thought to be of higher nutritional quality and energy density than terrestrial subsidies (Moyo et al. 2017).

One important determinant of nutritional quality is the content of polyunsaturated fatty acids (PUFAs), especially the content of ω-3 PUFAs, which is typically much higher in aquatic than in terrestrial resources (Hixson et al. 2015). It is generally assumed that the C18 PUFAs linoleic acid (LIN, 18:2ω-6) and α-linolenic acid (ALA, 18:3ω-3) are essential for all animals and thus have to be taken up with food, albeit the ability of some insects to synthesize LIN has been documented (Blomquist et al.

1991). Dietary uptake of the physiologically important long-chain (LC, ≥ C20) PUFAs, such as eicosapentaenoic acid (EPA, 20:5ω-3), is also important. Even though many animals are able to elongate and desaturate dietary C18 precursors to LC-PUFAs, this internal bioconversion pathways is often inefficient and energy consuming and thus incapable of covering LC-PUFA demands (Pawlosky et al. 1997, Brenna et al. 2009, Castro et al. 2012). In this context, it is generally assumed that consumers largely reflect the PUFA profile of their food (Iverson et al. 2004, Brett et al. 2006, Torres-Ruiz et al. 2010). LC-PUFAs are important because of their central role in membrane physiology and as precursors for signaling molecules acting on reproduction and the immune system (Stillwell and Wassall 2003, Stanley 2014, Schlotz et al. 2016). Better growth and development on naturally LC-PUFA-rich diets and diets that were supplemented with LC-PUFAs has been documented for different species (Müller-Navarra et al. 2000, Martin-Creuzburg et al. 2010, Twining et al. 2016b).

The fundamental dichotomy in the occurrence of PUFAs in aquatic and terrestrial ecosystems, with aquatic systems being generally richer in ω-3 PUFAs, especially long chain ones, derives from differences in PUFA profiles of the respective primary producers (Hixson et al. 2015). Several algal groups produce high amounts of LC-PUFAs, such as EPA, whereas most terrestrial plants, except for some bryophytes, lack the ability to synthesize PUFAs with more than C18 atoms (Harwood 1996, Sayanova and Napier 2004, Uttaro 2006, Hixson et al. 2015). In fact, aquatic ecosystems have been suggested to be the principal source of LC-PUFAs and a net transfer from aquatic to terrestrial systems has been recognized (Moyo et al. 2017). Emergent aquatic insects represent an important vector for LC-PUFA transfer across the ecosystem boundary to terrestrial consumers (Gladyshev et al. 2013, Martin-Creuzburg et al. 2017, Popova et al. 2017, Fritz et al. 2019) and some riparian predators might have specialized on exploiting this high-quality prey. Indeed, low dietary LC-PUFA provision can cause impaired development or lower breeding success of certain species (e.g., tree swallows), even with an abundance of other food sources (Twining et al. 2016a, Twining et al. 2018b). PUFA availability might also be the reason why, e.g., riparian lizards without access to aquatic insects show reduced growth rates (Sabo and Power 2002). Low amounts of aquatic-derived subsidies (i.e., low LC-PUFA input) are also linked with reduced immune function in riparian spiders (Fritz et al. 2017). Human impacts on river morphology (e.g. channelization, construction of barriers) or water quality (e.g. pollution, nutrient levels) can alter cross-ecosystem nutrient fluxes, potentially resulting in resource mismatches and a decoupling of aquatic and terrestrial systems (Laeser et al. 2005, Paetzold et al. 2011, Larsen et al. 2016, Muehlbauer et al. 2020).

Here, we examined the content of five physiologically important PUFAs, i.e., linoleic acid (LIN, 18:2ω-6), α-linolenic acid (ALA, 18:3ω-3), stearidonic acid (SDA, 18:4ω-3), arachidonic acid (ARA, 20:4ω-6), and eicosapentaenoic acid (EPA, 20:5ω-3) in riparian spiders along two rivers with different degrees of anthropogenic disturbance (Figure 1). In addition, we checked samples for the physiologically important docosahexaenoic acid (DHA, 22:5 ω-3), although DHA levels are normally lower in freshwater than in marine systems (Twining et al. 2020a). Two types of riparian spiders were analyzed at different times of the year: ground-dwelling spiders roaming the riparian area for prey and web-building spiders that are relatively stationary in prey capture. We explored potential differences in individual PUFA content between spider types and to what extent the different spider types reflected the PUFA profiles of aquatic emergent insects. We used site-specific differences in PUFA provision to track PUFA transfer from periphyton via emergent aquatic insects to riparian spiders.

Our main hypotheses included: 1) riparian spiders are rich in EPA and their $\Sigma\omega3/\Sigma\omega6$ ratio is comparable to aquatic organisms because they feed on emergent aquatic insects; 2) aquatic-derived PUFA content of spiders is highest in spring when aquatic emergent insects are most abundant; 3) emergent aquatic insect consumption depends on spider type (web-building vs. ground-dwelling), because the two groups have access to different prey types (e.g., ground vs aerial prey) resulting in differences in PUFA content between them; and 4) riparian spiders living along anthropologically disturbed systems ingest low amounts of aquatic material and contain less aquatic-derived PUFAs because human impacts can alter cross ecosystem linkages such that spiders cannot draw on aquatic resources. To test our hypotheses, we used a combination of fatty acid, carbon stable isotope and CSIA analyses. To our knowledge, this is one of the first studies showing a tight link between PUFA profiles of riparian spiders and emergent aquatic insects.

2. Material and Methods

2.1 Site description

Sampling was conducted at eight sites along two streams in the Thur River catchment in northeast Switzerland (Figure 1). Both streams (Necker, Glatt) are sixth-order pre-alpine streams with a flow regime including regular natural flood events. The Necker is a non-regulated system with low nutrient levels and a natural flow-sediment regime. It drains a basin of ca. 126 km^2 with elevation ranging from 1550 to 550 m a.s.l. and has a long-term mean annual discharge of 3.3 m^3/s (hydrograph station Achsäge). Sampling sites (n = 4) ranged in elevation from ca. 700 (upper most site) to 550 m a.s.l. (lower most site). The Glatt is a heavily regulated system with many channel alterations realized over the years, including numerous barriers impeding sediment movement and changing river morphology. In addition, nutrient levels are higher (TN: 4-8 mg/l, T-P: 30-70 µg/l) than in the Necker, due to the strong agricultural land use. The Glatt drains a basin of ca. 90 km^2 with elevation ranging between 950 and 480 m a.s.l. Long-term mean annual discharge is 2.7 m^3/s at the hydrograph station Oberbüren. Here, sampling sites (n = 4) ranged in elevation between 620 to 500 m a.s.l.

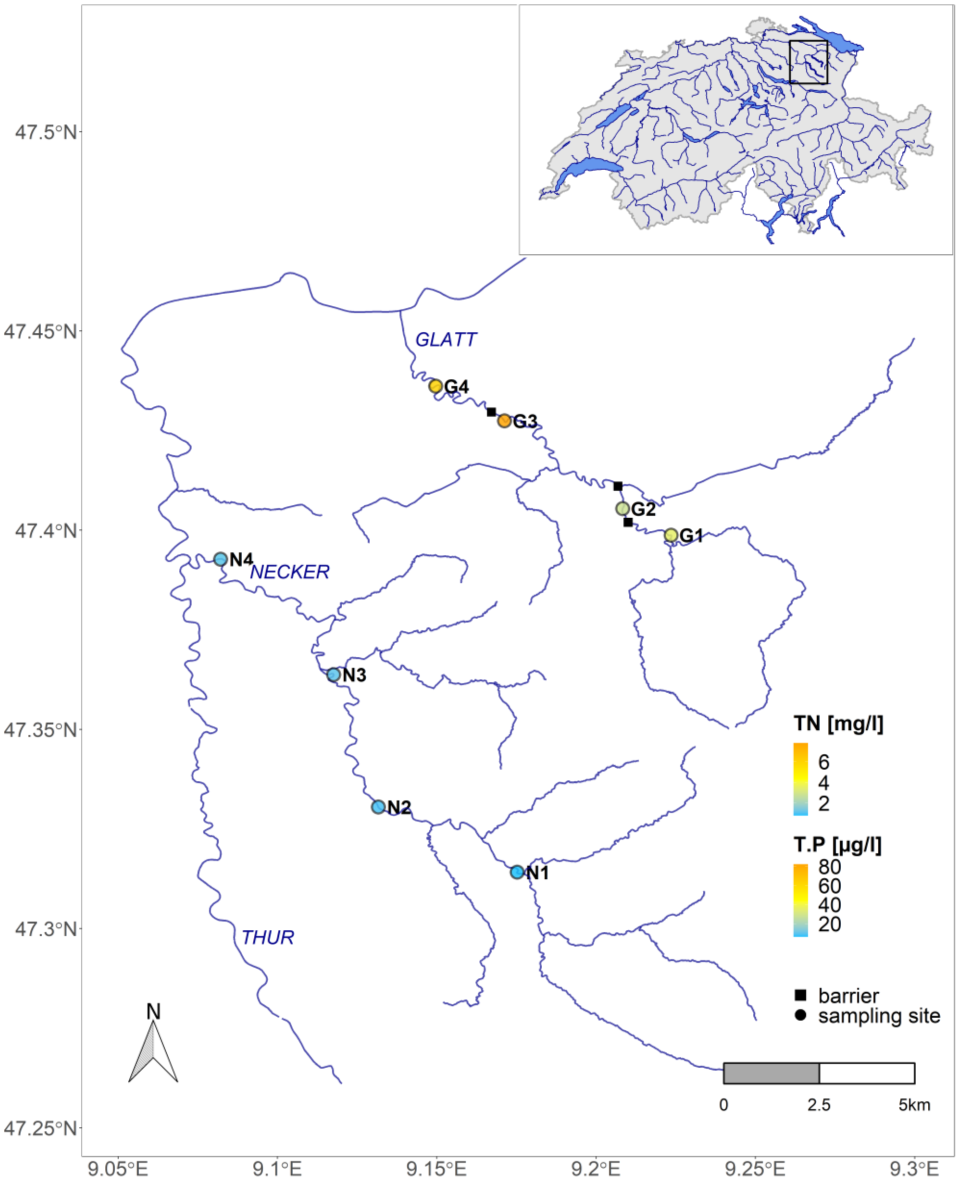

Figure 1: Map of sampling sites on the Necker (N) and Glatt (G) in the Thur catchment, northeast Switzerland (map insert at top right). The color gradient represents nutrient levels (T.P – total Phosphorus, TN – total Nitrogen). Circles represent sampling sites; squares represent major barriers blocking bed movement.

2.2 Field sampling

Samples were taken between April and October 2019. Water samples, periphyton, emergent aquatic insects, and spiders were collected in spring (April/May), summer (June/July/August) and autumn (September/October) to assess seasonal differences. No sampling was conducted during winter as most spiders are not active during the winter month, because they are either hibernating in the soil or die in autumn and the next generation hatches from resting eggs in spring. Benthic algae (as periphyton) were taken to quantify resource availability at each site and season. Three stones were randomly selected from the riverbed and returned to the laboratory. The biofilm on each stone was detached with a metal brush and an aliquot of the periphyton suspension was filtered through a glass fiber filter (Whatman GF/F). This procedure was done twice (2 aliquots), one filter was used for fatty acid extraction (frozen at -20 °C until analysis) and the other for determination of ash free dry mass (AFDM). For AFDM estimation, filters were dried at 60 °C, weighed, combusted for 3 h at 500 °C, and then reweighed.

Emergent aquatic insects were captured alive using floating emergence traps with a base area of 0.25 m^2 and mesh size of 100 µm. Three emergence traps were deployed at each site. Sampling started in early April with emergence traps deployed weekly (2-3 days/week) until mid-May and then monthly (2x 2-3 days/month) from June to October. Adult aquatic insects were collected from emergence traps after 2-3 days on each deployment. Samples were returned to the laboratory, frozen, and then counted and identified to the lowest practicable taxonomic level. Samples were stored short-term (a few weeks) at -20 °C and longer (month) at -80 °C when necessary. Trichoptera were not considered in this study because of too few samples for adequate assessment. As reference material, terrestrial insects were collected once per season using a sweep net, returned to the laboratory and frozen as described above until further analysis.

Riparian spiders were collected within 0-2 m distance from the shoreline once per season at each site. We collected spiders from two different functional feeding guilds (Wise 1993, Nyffeler et al. 1994, Sanzone et al. 2003); i.e., ground-dwelling spiders hunting for their prey on the ground (ground spiders) and spiders that use orb-webs for prey capture (web spiders). All ground spiders belonged to the family Lycosidae (genera *Pardosa* or *Pirata/Piratula*) and all web spiders belonged to the family Tetragnathidae (genus: *Tetragnatha*). Spiders in both groups were collected using a mouth aspirator. In addition, spiders were sampled at different distances from the stream at the Necker (site N4) in June 2020 to assess potential changes in PUFA content with distance from the river. Here, at least three individuals of each group were collected at 0-2 m, 5-10 m, 20-30 m, 40-50 m distance from the stream shore. All collected spiders were returned to the laboratory, kept in containers over night to allow gut clearance, and then frozen for later analysis.

2.3 Laboratory procedures

2.3.1 Fatty acid analysis

We analyzed fatty acids of 72 periphyton samples, 233 emergent insect samples (85 Diptera, 75 Ephemeroptera, 73 Plecoptera), 140 spiders (69 web spiders, 71 ground spiders, in addition 24 spider samples for distance gradient) and, as reference, 9 terrestrial insect samples (Diptera). The PUFAs analyzed included LIN, ALA, SDA, ARA, and EPA. Prior to fatty acid extraction, samples were freeze-dried, and the dry mass was determined with an ultrafine balance. Weighed samples were extracted in dichloromethane/methanol (2:1 v/v) over night and an internal standard (17:0 ME and 23:0 ME) was added for analysis. After 5 min in an ultrasound bath, particles were separated by centrifugation (3500 g, 5 min). The supernatant of each sample was evaporated to dryness under nitrogen, resuspended in 4 ml methanolic HCl for transesterification, and then incubated at 60 °C for 20 min to obtain fatty acid methyl esters (FAMEs). After cooling, hexane was added, and each sample was mixed and the upper phase consisting of hexane was transferred to a new vial. This procedure was repeated two more times, the hexane fractions pooled, and the composite sample evaporated to dryness under nitrogen. Each sample was resuspended in 20 to 200 µl of hexane, depending on initial sample dry mass, and analyzed for FAME content using gas chromatography (7890B gas chromatograph, Agilent Technologies, Waldbronn, Germany); configuration details (Martin-Creuzburg et al. 2010). fatty acids were identified based on their retention time and their mass spectra, and quantified by comparison to internal standards using multipoint calibration curves of known concentration (for details see Martin-Creuzburg et al. (2017)).

2.3.2 Stable isotope analysis

We used the carbon stable isotope ratio (δ^{13}C) to estimate the contribution of aquatic-derived material to riparian spiders and to compare with the results from fatty acid analysis. Samples from both spider groups, emergent aquatic insects from each site and season, and terrestrial insects were used for carbon stable isotope analysis (sampled as described above). For each sample, 0.7-0.9 mg of freeze-dried ground material was weighed and placed into tin capsules. Individual samples were combusted in a Vario PYRO Cube elemental Analyzer (Elementar Analysensysteme GmbH, Langenselblod, Germany) connected to an IsoPrime isotope ratio mass spectrometer (GV instruments Ltd, Manchester, UK) to measure total C content and δ^{13}C. The δ^{13}C values are presented in permille (‰) after normalization to reference material (NBS 19, L-SVEC, IAEA-N-1 and IAEA-N-2, provided by Biogeochemical Laboratories, Indiana University). Analytical uncertainty was 0.1 ‰ for δ^{13}C.

2.3.3 Compound-specific stable isotope analysis

To validate the aquatic origin of LC-PUFAs, we analyzed 12 fatty acid extracts using CSIA. Carbon isotope ratios of combined C18 fatty acids and combined C20 fatty acids were analyzed using a GC-IRMS (Thermo Scientific; Trace Gas 1310 with a DB5MS column connected with GC Isolink to IR-MS: Delta V Advantage). Peak separation on this column was sufficient to differentiate between C18 und C20 fatty acids but did not allow to differentiate between single fatty acids. We used the following equation to account for differences in isotope signals due to addition of a methyl group during the conversion from fatty acids to FAME:

$$\boldsymbol{\delta^{13}C_{FA}} = \frac{(\mathbf{n}+\mathbf{1}) \times \boldsymbol{\delta^{13}C_{FAME}} - \boldsymbol{\delta^{13}C_{MeHCl}}}{\mathbf{n}} \ (\mathbf{1})$$

where, *n* represents the number of C atoms in the fatty acids, $\delta^{13}C_{FAME}$ isotope ratio of fatty acid methyl esters and $\delta^{13}C_{MeHCl}$ isotope ratio of MeHCl used for the derivatization. All samples were measured twice, and the average used for validation. The $\delta^{13}C$ of MeHCl was -23.01‰.

2.4 Data Analysis

2.4.1 PUFA content in riparian spiders and transfer across trophic levels

All analyses were conducted in R 3.6.2 (R Core Team 2019). Five individual PUFAs (LIN, ALA, SDA, ARA, EPA) were examined (DHA was detectable in trace amounts only in a few samples and therefore excluded from the analyses). We investigated individual PUFA content of three trophic levels, i.e., periphyton, emergent aquatic insects and riparian spiders. Individual PUFA content was calculated on a relative basis (% of total FA).

Spider individual PUFA content and $\Sigma\omega3/\Sigma\omega6$ ratio was compared among spider types, seasons and the two fluvial systems using a factorial ANOVA (type III) (factors = season, spider type, river) (Anova, package “car”; Fox and Weisberg (2019)). Main and interaction effects were calculated for all three levels. When interaction effects were significant ($p < 0.05$), post-hoc tests were run separately for each pair of combinations. When interactions were not significant ($p > 0.05$), significant main effects ($P < 0.05$) were tested. In both cases, multiple pairwise post-hoc comparisons of estimated marginal means (EMMs) with tukey correction (package “emmeans”; Lenth (2021)) were conducted. Data were tested for normality and homogeneity of variance assumptions and transformed where required. Percent data were transformed using the arcsine square root transformation (Sokal and Rohlf 1995). The values reported represent untransformed means.

We used the difference in PUFA provision (PUFA content in emerging aquatic insects and periphyton) between sites and seasons (see supplementary material) to explore the transfer of PUFAs across trophic levels. If the same differences occur at all levels, transfer is likely. We calculated average individual PUFA content for periphyton, each of the emergent insect orders, and the two spider types per site and season (as measurement were not paired within site); the values were arcsine square root transformed. We used average values to investigate if there is a positive relationship among trophic levels in regard to individual PUFAs. We used a linear mixed effects model (lmer package “lme4”; Bates et al. (2015)) with individual PUFA content of the lower trophic level as the predictor variable. We included season and river as a random effect to account for the two different systems and multiple sampling at the same sites. The first step was from periphyton to emergent insects, the second from emergent insects to spiders. Conditional R^2 (variance explained by complete model; R^2c) and marginal R^2 (variance explained by fixed effects; R^2m) were calculated (package "MuMIn"; (Barton 2020)) to estimate the variance explained by fixed effects (Nakagawa and Schielzeth 2013). If variance explained was < 0.1, predictive effect was not considered to be ecologically significant. If R^2m was < 0.3 it was considered as a weak effect, for R^2m < 0.5 as a moderate effect and for R^2m > 0.5 as a strong effect (Nakagawa and Cuthill 2007). A significant predictive effect of individual PUFAs of the lower trophic level for the higher trophic level is an indicator of transfer of the respective PUFA

2.4.2 Multivariate Analysis of Similarity

We used the five selected PUFAs for non-metric multidimensional scaling (nMDS) to ordinate PUFA composition among predator and prey in two dimensions using rank-order dissimilarities. All values were arcsine square-root transformed before ordination. Multivariate outliers were removed by the minimum volume ellipsoid-based robust distance (R package "mvoutlier" P. Filzmoser (2018)). To assess similarities in PUFA composition between spider types, and among predators and their food sources, we used analysis of similarity (ANOSIM, R package "vegan"; Oksanen et al. (2019)) with 999 permutations. We used the ANOSIM global R value of the pairwise comparisons as an indicator of similarity. The value can range between -1 to 1, with global R value close to 1 indicating the sample pairs being different, while values close to 0 indicate that the pair is similar. In greater detail, a global R value of < 0.25 indicates that groups are hardly separated, R < 0.5 shows that groups differ but with some overlap, and R > 0.75 implies groups are well separated (Jaschinski et al. 2011). Negative values can occur and indicate greater dissimilarity within a group than among groups (Chapman and Underwood 1999).

2.4.3 Food source reconstruction

The $\delta^{13}C$ of riparian spiders were compared in a similar fashion as described above (factorial ANOVA) to see if spider type, season, and system influenced carbon isotope ratios. We used a two-source mixing model (aquatic/terrestrial insect) to calculate the proportion of carbon obtained from aquatic sources (P_{aq}) in spider tissue. Carbon isotope ratios of aquatic and terrestrial insects were compared to see if food sources can be discriminated from each other. Average $\delta^{13}C$ was calculated per river and season for both prey and predator groups. When food sources where distinct, the proportion of aquatic diet was calculated using the equation (modified from DeNiro and Epstein (1978), Sanzone et al. (2003)):

$$\boldsymbol{P}_{aq} = \frac{\delta^{13}\boldsymbol{C}_{\boldsymbol{pred}} - \delta^{13}\boldsymbol{C}_{\boldsymbol{ter}} - \boldsymbol{TEF}}{\delta^{13}\boldsymbol{C}_{\boldsymbol{aq}} - \delta^{13}\boldsymbol{C}_{\boldsymbol{ter}}} \quad (2)$$

where; $\delta^{13}C_{pred}$ = natural abundance of $\delta^{13}C$ of riparian spiders, $\delta^{13}C_{ter}$= natural abundance of $\delta^{13}C$ of terrestrial insect reference samples, and $\delta^{13}C_{aq}$= natural abundance of $\delta^{13}C$ of emergent aquatic insects. TEF is the average trophic enrichment factor between predator and prey (approximately 1.1 ‰), similar to (DeNiro and Epstein 1978), and similar to experimentally determined values for lycosid spiders (Oelbermann and Scheu 2002).

We then included PUFA profiles in the food source reconstruction, as a combination of PUFA and carbon isotope data can provide more detailed information about the use of different prey groups by spiders at the different sites and seasons. Likely food sources were investigated using a Bayesian model (package “MixSiar”; Stock et al. (2018)) combining the information about the diet from all measured parameters (δ^{13}C and 5 PUFAs). For δ^{13}C, we used the same TEF as described above (approximately 1.1 ‰, (DeNiro and Epstein 1978). Metabolic effects are an important topic for PUFAs when trying to estimate food sources. Although it is generally assumed that predators largely reflect the PUFA signature of their prey, metabolic effects should be taken into account (Galloway and Budge 2020). We used two approaches to verify that LC-PUFAs we found in riparian spiders derived from aquatic food sources, or if spiders were able to elongate shorter chain PUFAs into LC-PUFAs (some recent studies suggest that more species than previously thought are able to do this conversion or that LC-PUFAs might be produced in the soil food web (Kabeya et al. 2018, Menzel et al. 2018a, Garrido et al. 2019, Kabeya et al. 2020): 1) a distance gradient and 2) an approach using CSIA. For the distance gradient, we used the samples collected from different shoreline distances at Necker site N4 from June 2020 (see above). We compared samples from the different distances using a one-way ANOVA with post-hoc pairwise comparisons of estimated marginal means when significant. To capture trends (decrease or increase with distance from shore), we also used a general additive model (GAM, package “mgcv“, Wood (2017)). For the second approach, we analyzed carbon isotope ratio for combined C18 fatty acids and combined C20 fatty acids of a small subsample of aquatic insects, terrestrial insects, web spiders and ground spiders (3 per group). Terrestrial insects only contained trace amounts of C20 fatty acids, so we used the carbon isotope ratio of C18 fatty acids as a baseline. This is a valid approximation, because carbon isotope ratios of C18 and C20 fatty acids in aquatic insects were very similar to each other (C18: -39.3 ‰; C20: -39.6 ‰).

We used the information from the distance gradient and CSIA to exclude PUFAs that might have been internally metabolized from the Bayesian model. Due to a lack of feeding experiments, a trophic enrichment of zero was used for the remaining PUFAs. To check if further assumptions of the mixing model were fulfilled, we checked if values of predators (-TEF) lay within range of prey values using NMDS analysis. We used the Bayesian model to find dietary contribution of the different prey groups in the different systems and seasons. Average values per river and season were used for prey groups and the model was run separately for the two spider types with uninformative priors. River was set as a random effect and MCMC chain length was set to 300 000. Gelman-Rubin and Geweke diagnostics were applied to test model convergence.

3. Results

3.1 PUFA content in riparian spiders

PUFAs comprised on average 39.0 ± 8.2 % of total fatty acids in web spiders and 41.8 ± 8.4 % in ground spiders. Riparian spiders were especially rich in ω-3 PUFAs (Table 1). EPA made up the largest proportion with 14.3 ± 7.0 % of total fatty acids in web spiders and 15.6 ± 7.4 % in ground spiders (Figure 2, Table 1). This was slightly lower than EPA content in aquatic insects (18.8 ± 6.3 %), but higher than in the measured terrestrial insects (1.1 ± 1.2 %). Levels of ALA and SDA were also close to those in emergent aquatic insects (Table 1; for PUFA content in spiders per dry mass see Supplement Table 1). The ratio of Σω3/Σω6 of riparian spiders was lower than in most emergent aquatic insects (3.9 ± 4.6), but on average close to 1 (web spiders: 1.34, ground spiders: 0.97), while measured terrestrial insects had Σω3/Σω6 ratios of 0.4 ± 0.2.

Table 1: Carbon isotope ratios and individual PUFAs (% of total fatty acids) for the different trophic levels by stream and season (mean ± standard deviation). N = Necker, G = Glatt.

		season	river	$\delta^{13}C$ mean±SD	$\delta^{13}C$ n	LIN mean±SD	ALA mean±SD	SDA mean±SD	ARA mean±SD	EPA mean±SD	Σω3/Σω6 mean±SD	fatty acid n
periphyton		spring	G			5±2.7	5.2±2.4	2.7±1.7	0.8±0.9	13.7±6	4.8±2.7	10
			N			8.2±6.6	4.9±1.3	10.8±3.3	0±0	4.1±1.1	3.2±1.3	9
		summer	G			7.5±2.9	4±3.5	0.2±0.6	0.2±0.7	2.3±2.8	0.9±0.8	12
			N			5.9±1.3	4.5±1.1	1.4±1.5	1±1.3	3.5±2	1.4±0.5	12
		autumn	G			6±2.5	4.8±3.2	1.1±1.2	1.2±1.2	3.2±2	1.3±0.5	12
			N			12.4±4.6	3.6±0.9	1.6±1.3	0.1±0.3	2.2±1.5	0.8±0.9	12
Emergent aquatic insect	**Diptera**	spring	G	-30.4±2.4	2	13.3±4.8	6.1±2.3	2.6±2.9	0.8±1.1	22.4±5.4	2.6±1.3	17
			N	-33.8±1.5	2	11.6±8	6.6±3.2	5.9±3.7	0.6±1	17.4±6.6	3.6±2.3	18
		summer	G	-29.0±1.5	9	21.5±5.4	6.4±4.3	0.9±1.4	5.5±1.7	19.3±7.1	1.1±0.5	14
			N	-29.1±0.6	4	17.8±4.8	7.8±1.9	0.9±1.3	4.7±2.6	23.5±7.4	1.6±0.8	11
		autumn	G	-29.7±2.3	7	23±8.6	8.3±6.4	0.8±0.8	3.7±2.1	21.2±5.6	1.5±1.2	13
			N	-28.8±0.4	5	22.8±5.4	7.8±1.9	1.2±1.1	3.3±2	19.2±8.6	1.2±0.5	12
	Ephemeroptera	spring	G	-33.0±1.5	4	3.5±1.5	5.3±2.8	2.9±1.6	0.8±1.1	14.5±5.2	6.5±3.7	11
			N	-32.6±0.3	2	3.6±2.7	4.8±2	6.1±2.9	1.5±3.4	13.9±7.6	8±4.3	10
		summer	G	-33.3±2.0	7	4±1.3	11.2±6.7	2.8±1.3	1.5±1	17±5.8	6.6±3.8	17
			N	-33.8±1.5	7	3.5±1.2	8±3.8	4±2.5	1.1±0.6	19.3±5.7	7.2±1.9	17
		autumn	G	-34.2±0.5	5	3.7±1.4	11±5.8	2.1±0.9	0.9±0.5	21.5±6.8	7.8±2.7	11
			N	-34.1±0.7	3	13±32.6	4.8±3.7	2.9±1.4	0.6±0.6	19.8±9.3	10.6±5.7	9
	Plecoptera	spring	G	-35.5±1.5	2	13.9±10.8	14.9±9.7	1.1±1.5	2.9±2.5	17.2±9.7	2.8±1.9	9
			N	-31.3±0.4	4	8±4.1	12.1±2.4	7.5±4.4	2±0.8	18.3±3.2	4.5±2	20
		summer	G	-28.3±1.0	3	15.1±3.3	8.6±1.7	0.7±0.7	7.6±2.3	18.5±2.7	1.3±0.3	10
			N	-28.8±0.3	3	14.8±5.2	8.7±2.1	1±0.9	6.9±2.1	19.9±5.5	1.5±0.6	12
		autumn	G	-28.3±0.9	4	14.6±3.3	7±2	0.6±0.5	6.7±1.3	16.3±3.5	1.2±0.3	13
			N	-27.7±0.9	4	17±4.8	10.4±3.6	0.9±1	5.4±1.6	17.4±5	1.3±0.4	10
Terrestrial insect		spring		-27.9±1.0	4	14.6±3.4	7±4.9	0±0	0.3±0.1	0.5±0.2	0.5±0.2	3
		summer		-28.4±0.4	4	13±4.3	5±0.6	0±0	1.1±1.1	2.2±1.9	0.5±0.2	3
		autumn		-29.1±0.3	4	8.8±1	1.7±0.2	0±0	0.5±0.3	0.7±0.2	0.3±0	3
Riparian predator	**ground spider**	spring	G	-27.8±0.5	13	17±3	3±1.2	0.1±0.5	7.3±2.3	20.3±4.2	1±0.2	12
			N	-27.4±0.5	12	15±4.2	3±1	0.4±0.9	7.2±3	20.7±7.7	1.1±0.4	11
		summer	G	-28.1±0.6	15	15.9±4.4	2.8±2.2	0.2±0.4	7.1±3.2	16.7±5.6	0.9±0.3	12
			N	-28.0±0.8	12	16.7±6.5	5.1±2.3	0.4±0.8	5.7±2.1	12.8±7.8	1±0.7	12
		autumn	G	-28.5±0.6	8	16.5±3.8	4.6±1.7	0.5±0.7	3.9±2.2	10.3±5.5	0.8±0.4	12
			N	-27.7±0.4	12	14.4±4.7	5.1±2.3	0.4±0.7	4.8±3	13.2±7.5	1±0.5	12
	web spider	spring	G	-28.7±1.2	12	16.2±4.3	3.2±1.2	1.5±1.8	2±1.4	19.7±4.2	1.5±0.6	12
			N	-28.7±0.5	7	11.5±6.4	5.7±2.1	6.5±3.9	1.7±1.2	19.9±3.5	3.2±2.7	9
		summer	G	-28.7±1.3	11	14.7±4.4	4.6±1.4	0.3±0.5	4.8±2.1	17.2±4.4	1.2±0.5	12
			N	-28.0±0.8	11	15.1±5.5	5.2±1.1	0.5±0.8	3.2±1.8	15.5±6.4	1.4±0.8	12
		autumn	G	-28.3±1.0	10	21.7±5.6	4.7±2.6	0.3±0.6	3±1.7	8.3±4.8	0.6±0.3	12
			N	-28.0±0.8	10	17±4	2.9±1.2	0.4±1.1	1.7±0.9	6.5±4.6	0.6±0.6	12

Season, spider type, fluvial system and the interaction of the factors had different effects on individual PUFAs (Table 2). We found the most pronounced seasonal difference in EPA content with highest values in spring in both spider types. An opposite pattern, with significantly highest values in autumn, was visible for LIN content. For the other PUFAs, seasonal patterns were less strong and differed between spider types and fluvial systems (Figure 2, Table 1). The strongest difference between spider types we found in ARA content, which was significantly higher in ground spiders than in web spiders. In contrast the SDA content was significantly higher in web spiders than in ground spiders, but only in spring. The differences in LIN and ALA between spider types were specific to season and fluvial system. For EPA, we found no significant difference between spider types. Between the two systems the only overall significant difference was a slightly higher LIN content in riparian spiders at the Glatt. In spring, we found a higher SDA content and Σω3/Σω6 ratio in web spiders than ground spiders at the Glatt than at the Necker (Table 2).

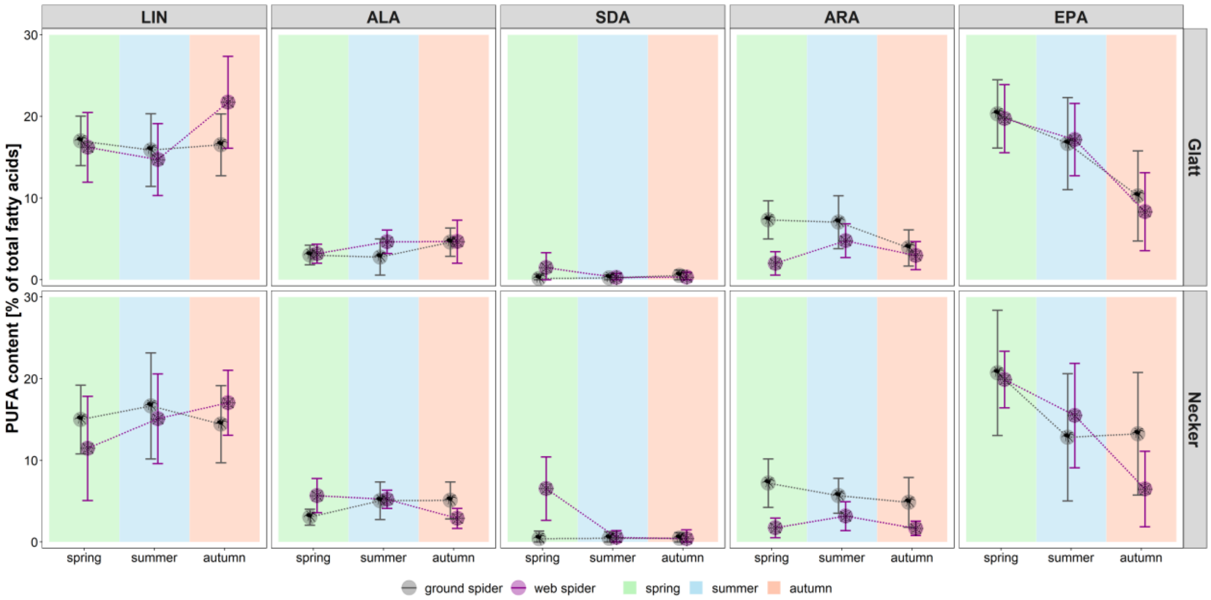

Figure 2: Individual PUFA content (as % of total fatty acids) in web spiders (n = 69) and ground spiders (n = 71) separated by stream and season. Points represent mean content, and error bars represent standard deviation, background colors represent the three seasons (spring, summer, autumn).

Table 2: Factorial ANOVA of PUFA content in riparian spiders (factors: spider type = type, season, fluvial system = river) and significant post-hoc comparisons (web = web spider, ground = ground spider, N = Necker, G = Glatt).

	Factor	Df	F value	Pr(>F)		contrast	season	type	river	t.ratio	p
LIN	type	1	0.54	0.465							
	season	1	7.48	0.007	**	spring-autumn				-2.6	0.0270
	river	2	3.17	0.045	*	G - N				2.7	0.0071
	type:season	1	1.65	0.202							
	type:river	2	2.52	0.084							
	season:river	2	2.64	0.075							
	type:season:river	2	0.44	0.648							
ALA	type	1	4.65	0.033	*						
	season	2	4.23	0.017	*						
	river	1	5.85	0.017	*						
	type:season	2	3.92	0.022	*						
	type:river	1	1.23	0.269							
	season:river	2	4.45	0.014	*						
	type:season:river	2	4.48	0.013	*	summer - autumn		ground	G	-3.4	0.003
						spring - autumn		web	N	2.9	0.018
						summer - autumn		web	N	2.8	0.018
						ground - web	summer		G	-3.5	0.0006
						ground - web	spring		N	-2.6	0.010
						ground- web	autumn		N	2.5	0.012
						G - N	spring	web		-2.7	0.007
						G - N	summer	ground		-3.5	0.0005
SDA	type	1	1.50	0.223							
	season	2	0.70	0.5							
	river	1	7.90	0.006	**						
	type:season	2	3.61	0.03	*						
	type:river	1	5.70	0.018	*						
	season:river	2	6.52	0.002	**						
	type:season:river	2	4.02	0.02	*	spring - summer		web	N	7.1	2.7e-10
						spring - autumn		web	N	7.7	9.6e-12
						ground - web	spring		G	-2.9	0.005
						ground - web	spring		N	-7.5	1.03e-11
						G - N	spring	web		-5.5	1.7e-07
ARA	type	1	28.81	3.62e-7	***						
	season	2	7.20	0.001	**						
	river	1	3.04	0.083							
	type:season	2	6.76	0.002	**	ground - web	spring			8.2	2.5e-13
						ground - web	summer			3.4	8.1e-04
						ground - web	autumn			3.6	4.6e-04
						spring-autumn		ground		3.9	0.0005
						summer-autumn		ground		2.8	0.015
						spring-summer		web		-3.9	0.0005
						summer-autumn		web		3.0	0.0010
	type:river	1	1.98	0.162							
	season:river	2	1.06	0.35							
	type:season:river	2	0.80	0.453							
EPA	type	1	0.26	0.608							
	season	2	19.19	5.15e-8	***	spring-summer				3.4	2.2e-03
						spring-autumn				8.1	1.2e-12
						summer-autumn				4.8	1.4e-05
	river	1	1.04	0.309							
	type:season	2	0.36	0.695							
	type:river	1	0.08	0.776							
	season:river	2	1.45	0.239							
	type:season:river	2	1.60	0.205							
Σω3/Σω6 ratio	type	1	1.53	0.218							
	season	2	6.53	0.002	**						
	river	1	6.03	0.015	*						
	type:season	2	2.12	0.124							
	type:river	1	0.98	0.325							
	season:river	2	3.00	0.053							
	type:season:river	2	3.40	0.036	*	spring - autumn		web	G	3.8	0.0006
						summer - autumn		web	G	3.0	0.009
						spring - summer		web	N	4.6	2.7e-05
						spring - autumn		web	N	7.8	5.6e-12
						summer - autumn		web	N	3.4	0.002
						ground - web	spring		N	-5.1	1.4e-06
						ground - web	autumn		N	2.4	0.017
						G - N	spring	web		-4.0	9.3e-05

3.2 PUFA transfer across trophic levels

Individual PUFA content in periphyton and emergent aquatic insects showed site and season specific differences (see Supplement Figure 1). Therefore, a different PUFA pool was available for riparian spiders at different sites and seasons. EPA generally made up the largest proportion of PUFAs in both periphyton and emergent aquatic insects.

The mixed effects model indicated average ALA content in periphyton was a moderate predictor for ALA content in aquatic Diptera ($R^2m = 0.31$, $p < 0.05$) (Figure 3A). Model results showed SDA content in periphyton was a strong predictor for SDA content in aquatic Diptera ($R^2m =0.51$, $p < 0.001$) and Plecoptera ($R^2m = 0.53$, $p < 0.05$). LIN content in Diptera ($R^2m = 0.22$, $p = 0.05$) and ALA content in Ephemeroptera ($R^2m = 0.15$, $p > 0.05$) were weak predictors for the respective PUFAs in riparian web spiders. Average SDA and ARA in emergent aquatic insects of all orders were a predictor for SDA (Diptera: $R^2m = 0.47$, $p = 0.01$; Ephemeroptera: $R^2m = 0.1$, $p = 0.08$); Plecoptera: $R^2m = 0.57$, $p < 0.001$) and ARA (Diptera: R2m = 0.49, $p < 0.05$; Ephemeroptera: $R^2m = 0.19$, $p < 0.05$; Plecoptera: $R^2m = 0.20$, $p > 0.05$) content in web spiders (Figure 3B). For the other PUFAs, the variance explained by the lower trophic level was close to zero ($R^2m < 0.1$, $p > 0.05$). Individual PUFAs of emergent aquatic insect had low predictive power for PUFAs in ground spiders ($R^2m < 0.1$, $p > 0.05$) (Figure 3B). There was only a weak predictive effect of ALA in Diptera ($R^2m = 0.17$, $p < 0.05$) and ARA in Ephemeroptera ($R^2m = 0.10$, $p = 0.055$).

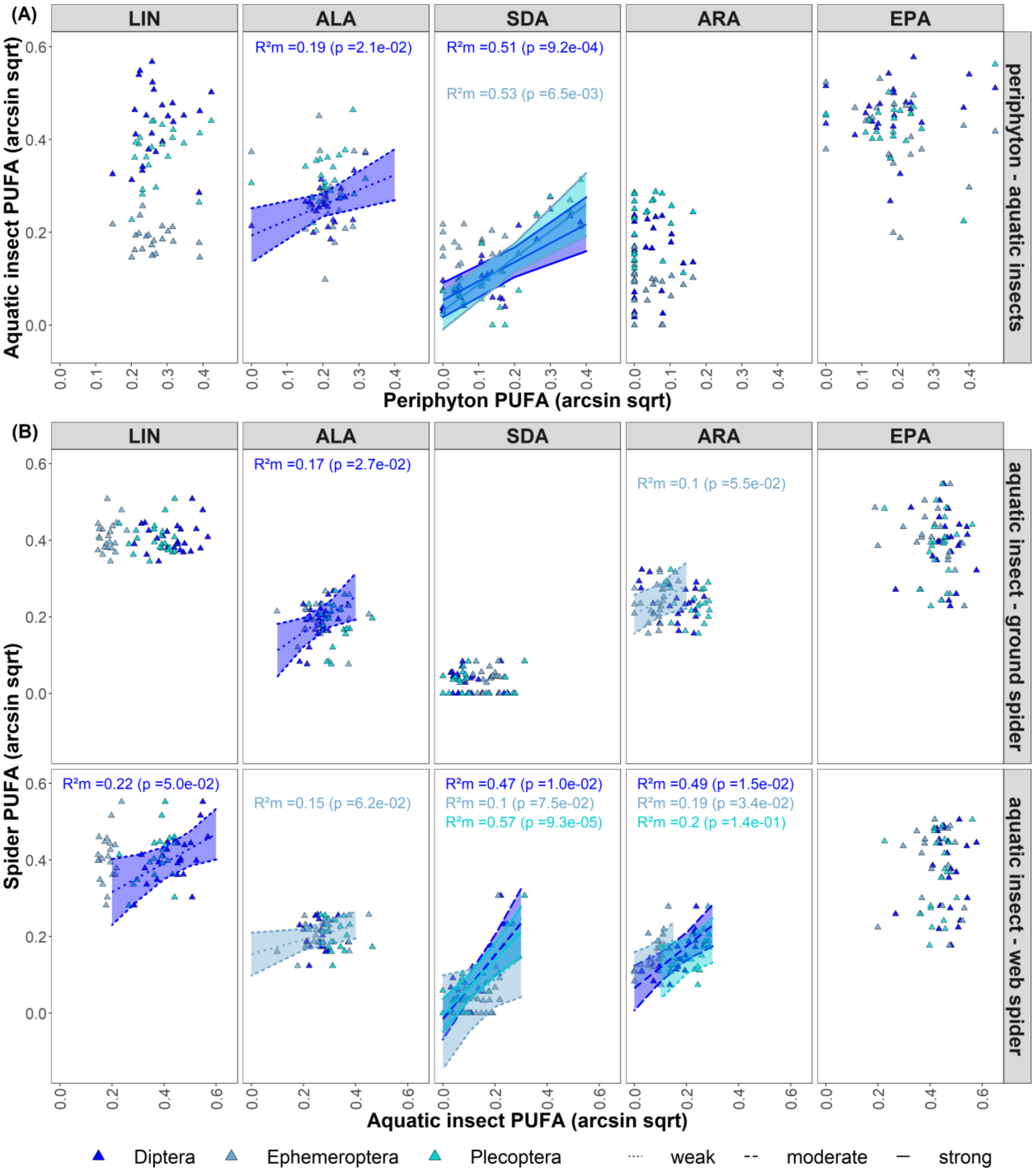

Figure 3: A) Mixed model showing effect of periphyton individual PUFA content on individual PUFA content of aquatic insects, with river and seasons as random effects. B) Mixed model showing effect of aquatic insect individual PUFA content on individual PUFA content of riparian spiders, with river and seasons as random effects. If no trendline is shown, predictive effect of fixed effect is close to zero (R^2m < 0.1), dotted lines represents weak effects (R^2m < 0.3), dashed lines represent moderate effects (R^2m < 0.5) and solid lines represent strong effect (R^2m > 0.5).

3.3 Multivariate analysis of similarity

Multivariate comparisons of similarity revealed seasonal differences in PUFA profiles for both spider types. Web spiders in spring were clearly separated from those in summer and autumn (seasonal effect: ANOSIM: R = 0.31, p = 0.001) (Figure 4A), and the difference was more pronounced at the Necker. Ground spiders also showed some, albeit less pronounced, seasonal differences (ANOSIM: R = 0.15, p = 0.001), (Figure 4A). PUFA profiles of web spiders and ground spiders differed in spring (R = 0.46, p = 0.001), and the difference was more pronounced at the Necker (R = 0.60, p = 0.001) than the Glatt (R = 0.36, p = 0.001). In contrast, the two spider types had similar PUFA profiles in summer (R = 0.04, p = 0.051) and autumn (R = 0.12, p = 0.004) in both rivers (Figure 4B, Table 3). Lastly, web spiders showed some differentiation between streams in spring (R = 0.21, p = 0.015).

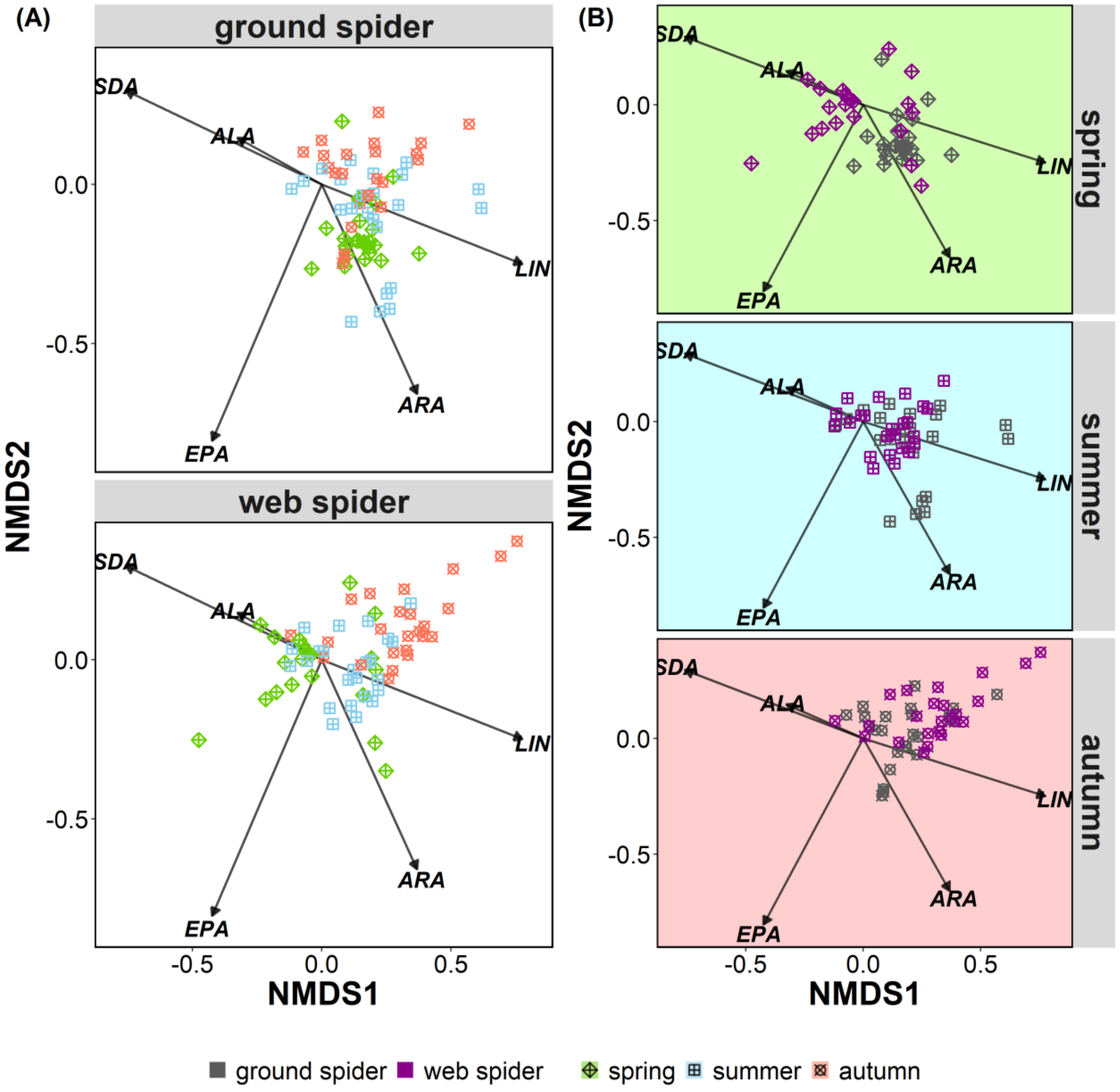

Figure 4: Non-metric multidimensional scaling (nMDS) of arcsine-square-root-transformed PUFA (% of total fatty acids) of riparian spiders. Stress level = 0.15. *LIN* - linolenic acid, *ALA* - alpha-linolenic acid, *SDA* - stearidonic acid, *ARA* - arachidonic acid, *EPA* - eicosapentaenoic acid. Organized by A)

seasonal differences in ground spiders and web spiders, and B) differences between spider types per season.

Table 3: Pairwise Similarity (ANOSIM) of PUFA profiles of prey and predator groups at each of the systems (Necker, Glatt) and overall (both rivers)

	season	**river**	**ground spider**		**Diptera**		**Plecoptera**		**Ephemeroptera**		**terrestrial insect**	
			R	p	R	p	R	p	R	p	R	p
web spider	spring	Glatt	0.36	0.001	0.15	0.024	0.34	0.001	0.70	0.001	1.00	0.001
		Necker	0.60	0.001	-0.03	0.604	0.48	0.001	0.52	0.001	0.95	0.010
		overall	0.46	0.001	0.07	0.085	0.34	0.001	0.56	0.001	0.94	0.001
	summer	Glatt	0.04	0.179	0.09	0.058	0.38	0.001	0.76	0.001	0.90	0.002
		Necker	0.02	0.316	0.18	0.022	0.28	0.002	0.73	0.001	0.60	0.001
		overall	0.04	0.051	0.14	0.001	0.31	0.001	0.74	0.001	0.77	0.001
	autumn	Glatt	0.05	0.153	0.34	0.001	0.51	0.001	0.87	0.001	0.87	0.002
		Necker	0.23	0.004	0.55	0.001	0.64	0.001	0.90	0.001	0.61	0.008
		overall	0.12	0.004	0.46	0.001	0.53	0.001	0.87	0.001	0.72	0.001
ground spider	spring	Glatt			0.64	0.001	0.54	0.001	0.96	0.001	1.000	0.007
		Necker			0.63	0.001	0.95	0.001	0.96	0.001	0.97	0.002
		overall			0.59	0.001	0.75	0.001	0.96	0.001	0.99	0.001
	summer	Glatt			0.14	0.012	0.27	0.001	0.85	0.001	0.75	0.005
		Necker			0.15	0.022	0.20	0.004	0.75	0.001	0.47	0.025
		overall			0.14	0.002	0.25	0.001	0.78	0.001	0.60	0.001
	autumn	Glatt			0.31	0.001	0.22	0.001	0.84	0.001	0.90	0.001
		Necker			0.35	0.001	0.24	0.011	0.92	0.001	0.99	0.003
		overall			0.35	0.001	0.23	0.001	0.86	0.001	0.94	0.001

Web spider PUFA profiles were most similar to those of emergent aquatic insects, especially Diptera (ANOSIM spring: R = 0.07, p =0.085, summer: R = 0.14, p = 0.001) and Plecoptera at both rivers in spring and summer, but were well separated from terrestrial reference samples (ANOSIM spring: R = 0.94, p = 0.001, summer: R = 0.77, P = 0.001) (Figure 5, Table 3). In autumn, web spiders were more separate from all emergent aquatic insect groups (Diptera: ANOSIM R = 0.46 p = 0.001; Plecoptera: ANOSIM R = 0.53, p = 0.001; Ephemeroptera: ANOSIM R = 0.87, p = 0.001) but still distinct from terrestrial insect reference samples (ANOSIM R = 0.72, p = 0.001).

Ground spiders were more separated from emergent aquatic insects in spring than web spiders (Figure 5, Table 3). Ground spiders showed some overlap to aquatic Diptera (ANOSIM R = 0.59, p = 0.001) but were well separated from Plecoptera (ANOSIM R = 0.75, p = 0.001) in spring; the overlap with Plecoptera was larger at the Glatt than Necker (Figure 5, Table 3). In summer, ground spiders were similar to aquatic Diptera (ANOSIM R = 0.14, p = 0.001) and Plecoptera (ANOSIM R = 0.25, p = 0.001). In autumn, ground spiders were most similar to Plecoptera (ANOSIM R = 0.23, p = 0.001) and showed some overlap with Diptera (ANOSIM R = 0.35, p = 0.001). Ground spider PUFA profiles were separated from those of Ephemeroptera and terrestrial reference samples during all seasons (Figure 5, Table3).

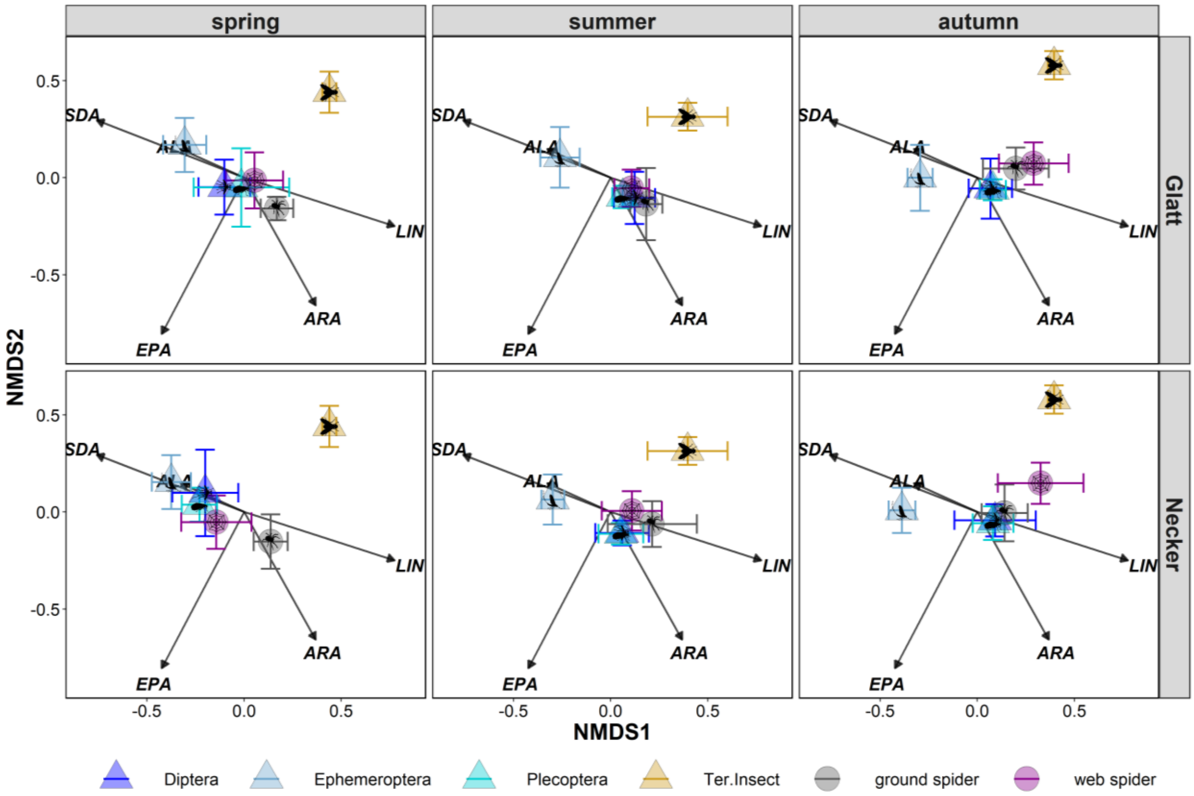

Figure 5: Non-metric multidimensional scaling (nMDS) of arcsine-square-root-transformed PUFA (% of total fatty acids) of prey (aquatic Diptera, Ephemeroptera, Plecoptera and terrestrial insects) and predator groups (web spiders, ground spiders). Stress level = 0.15. Points represent means and error bars represent standard deviation. Results were divided post-hoc by stream and season. *LIN* - linolenic acid, *ALA* - alpha-linolenic acid, *SDA* - stearidonic acid, *ARA* - arachidonic acid, *EPA* - eicosapentaenoic acid.

3.4 Food source reconstruction

3.4.1 Food web analysis using $\delta^{13}C$

Riparian spiders had average $\delta^{13}C$ ratios of -27.9 ± 0.6 ‰ in ground spiders and -28.4 ± 1.0 ‰ in web spiders. We found a significant main effect of river (ANOVA, F = 5.56, p = 0.02) and a close to significant interaction effect of season and spider type (F = 2.90, p = 0.056) on $\delta^{13}C$ ratios. In spring, the $\delta^{13}C$ ratio of web spiders (-28.7 ± 0.9 ‰) was significantly more depleted than ground spiders (-27.6 ± 0.5 ‰) (t.ratio = 5.17, p < 0.001). In summer and autumn, web spiders and ground spiders displayed similar $\delta^{13}C$ isotope ratios (web spider: summer -28.4 ± 1.1 ‰, autumn -28.1 ± 0.9 ‰; ground spider: summer: -28.0 ± 0.6 ‰, autumn: -28.02 ± 0.7 ‰, p > 0.05). There was no significant difference in $\delta^{13}C$ ratio among seasons for either spider type (p > 0.05). However, spiders were more depleted at the Glatt than at the Necker (p < 0.05).

In spring, the $\delta^{13}C$ ratio of web spiders (-28.7 ± 0.9 ‰) was between that of aquatic (-32.5 ± 1.7 ‰) and terrestrial (-27.9 ± 1.0 ‰) insects, indicating that web spiders consumed a mixture of aquatic and terrestrial food sources (Figure 6). In contrast, ground spider $\delta^{13}C$ ratios (-27.6 ± 0.5 ‰) were similar to terrestrial insects, inferring a mostly terrestrial source in the diet. The $\delta^{13}C$ ratio of all groups of emergent aquatic insects were distinct from terrestrial insects in spring (t.ratio = 2.1, $p < 0.001$) but not in summer and autumn (Diptera and Plecoptera overlapped with terrestrial insects); thus the proportion of aquatic-derived carbon in spiders was only calculated in spring. Based on mixing model results (Equation 2), web spiders obtained on average 38 % aquatic-derived carbon at the Glatt and 44 % at the Necker in spring. In contrast, ground spiders obtained on average 19 % of aquatic-derived carbon at the Glatt and 13 % at the Necker. The difference in proportion of carbon obtained from aquatic sources in spring was more pronounced between spider types at the Necker.

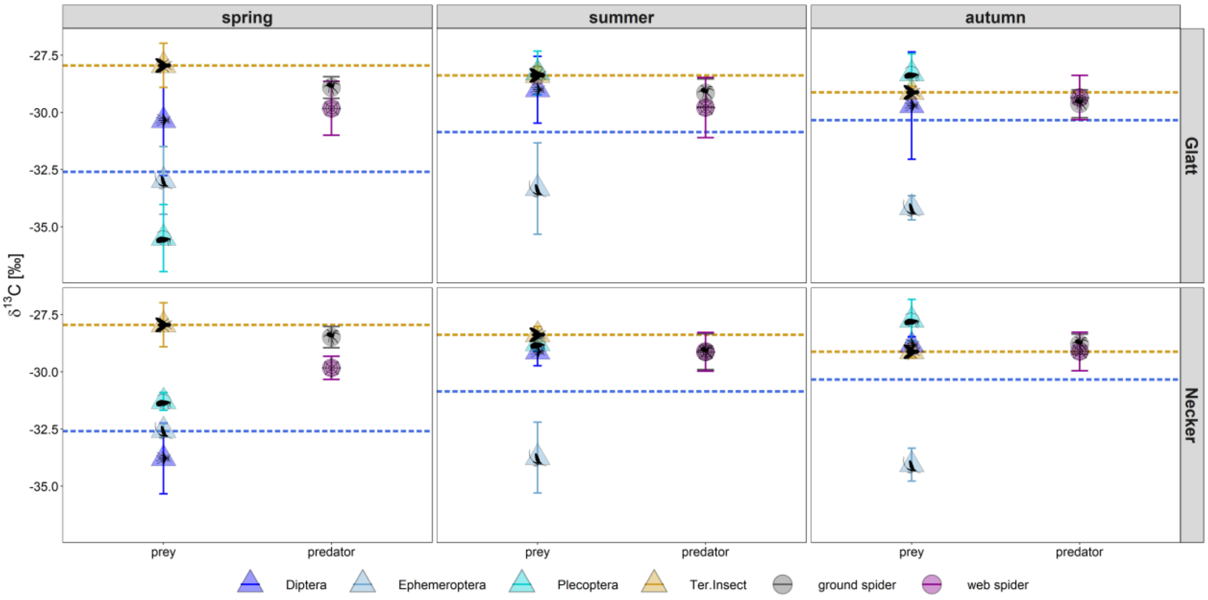

Figure 6: $\delta^{13}C$ ratio of emergent insects, terrestrial insects and riparian spiders. Dashed lines represent average ratios for aquatic insects (blue) and terrestrial insects (orange). Error bars represent standard deviations. For number of samples per group see Table 1. To adjust for fractionation between trophic levels, TEF = 1.1 ‰ was subtracted from predator $\delta^{13}C$. On the left side of the plot are prey values, on the right site are predator values.

3.4.2 Including PUFAs in food source reconstruction

The $\delta^{13}C$ ratio and PUFA profiles of spiders changed with distance from the shore. The $\delta^{13}C$ ratio of spiders had a more aquatic signature close to the shoreline (0-2 m) than 40-50 m away from the shore (Table 4, Figure 7A). The correlation between distance and $\delta^{13}C$ ratio was significant for ground spiders (GAM $p = 0.007$) with a trend to less depleted values further from the shoreline, also the direct comparison between 0-2 m and 40-50 m showed a difference that was close to significance ($p = 0.052$). A multivariate comparison of similarities showed that PUFA profiles of spiders sampled 0-2 m from shore were separated from those spiders sampled further away from shore (ground spiders: ANOSIM R = 0.99, $p = 0.008$; web spiders: ANOSIM R = 0.62, $p = 0.012$). We found higher EPA and SDA content close to shore and decreasing values with distance (Table 4), the difference between 0-2 m and 40-50 m being significant for both spider types for SDA ($p < 0.05$) but not EPA ($p > 0.05$) (Figure 7A). The GAM trend line also showed decreasing content with distance, but the correlation was not significant ($p > 0.05$) (Figure 7A). A decrease in ALA content with distance from shore was evident for ground spiders

(GAM $p < 0.05$), but not web spiders (GAM $p > 0.05$) (Table 4, Figure 7A). Both LIN and ARA increased with distance from shore (LIN: both spider types GAM $p < 0.05$; ARA: ground spiders GAM $p < 0.05$). LIN in both spider types increased from ~10 % (0-2 m) to nearly 25 % of total fatty acids at 40-50 m distance from shore (Figure 7A, Table 4). The $\Sigma\omega3/\Sigma\omega6$ ratio differed between 0-2 m (web spider: 1.3 ± 0.6, ground spider: 1.7 ± 0.4) and 40-50 m (web spider: 0.7 ± 0.1, $p = 0.069$; ground spider: 0.3 ± 0.1, $p= 0.002$), although this difference was significant only for ground spiders.

Table 4: $\delta^{13}C$, individual PUFA content (% of total fatty acids) and $\Sigma\omega3/\Sigma\omega6$ ratio (mean ± standard deviation) in spiders at different distances from shore (site N4).

	distance	**δ¹³C**	**LIN**	**ALA**	**SDA**	**ARA**	**EPA**	**Σω3/Σω6**	**n**
ground spider	0-2m	-28.4±1.0	11.2±2.0	7.2±2.2	2.7±2.8	1.6±0.4	11.2±1.1	1.7±0.4	3
	5-10m	-26.7±0.3	23.7±0.5	4.4±0.8	0.8±0.3	4.4±1.7	10.8±3.9	0.6±2	3
	20-30m	-27.0±0.3	25.8±5.6	1.8±1.7	0.0±0.0	5.7±0.5	9.5±0.8	0.4±1	3
	40-50m	-26.0±0.3	24.3±2.3	2.1±0.3	0.0±0.0	7.8±2.2	8.2±1.4	0.3±03	3
web spider	0-2m	-28.2±1.6	13.8±3.2	4.4±1.8	1.9±1.1	2.5±1.2	13.8±6.0	1.3±0.6	3
	5-10m	-28.5±1.9	20.3±7.4	5.6±1.9	0.4±0.7	3.0±0.7	10.0±3.2	0.8±0.5	3
	20-30m	-27.3±0.4	27.9±2.3	5.3±4.6	0.0±0.0	3.2±1.1	9.5±3.6	0.5±0.1	2
	40-50m	-27.0±0.2	23.1±2.2	7.1±2.5	0.0±0.0	4.1±1.3	10.4±4.5	0.6±0.5	3

The compound-specific $\delta^{13}C$ ratio of C18 fatty acids was less depleted in terrestrial insects (mean $\delta^{13}C$ = -34.8 ± 0.9 ‰) than in aquatic insects ($\delta^{13}C$ = -39.3 ± 3.5 ‰) (Figure 7B). The $\delta^{13}C$ ratios of C18 fatty acids of aquatic and terrestrial insects were used as baselines for comparison with spider $\delta^{13}C$ ratio. Like the bulk isotope ratio, C18 $\delta^{13}C$ ratio of spiders was between aquatic and terrestrial insects with ground spiders ($\delta^{13}C$ = -35.1 ‰) being close to terrestrial insects. In contrast, spider $\delta^{13}C$ ratios of C20 s were close to the aquatic baseline (web spider: -38.6 ‰, ground spider: -38.1 ‰). The main part of C20 fatty acids consisted of EPA (web spiders: 80-90 %, ground spiders: 50-90 %) and the rest was ARA. The sample containing the most ARA was least depleted (supplement Table 2 & 3). This indicates $\delta^{13}C$ of EPA in spiders was similar to $\delta^{13}C$ ratio of fatty acids in aquatic insects, while ARA might have a more terrestrial $\delta^{13}C$ signature. This is in accordance with the results from the distance gradient were ARA was increasing with distance from shore while EPA was decreasing. We found no indication for internal metabolism of EPA or acquisition through terrestrial sources, EPA was therefore used for the food source reconstruction.

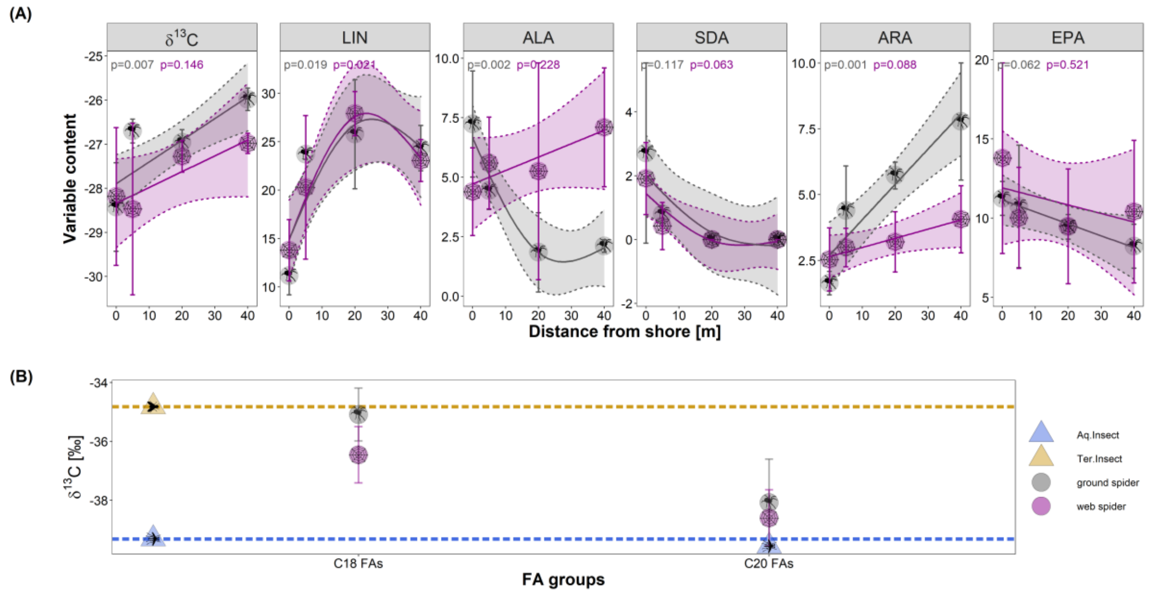

Figure 7: A) The relationship in $\delta^{13}C$ and the different measured PUFAs (as % of total fatty acids) with distance from stream shore at site N4 (Necker) in June (per distance n = 3; except web spider 20 m, n = 2) for web spiders and ground spiders; B) Compound-specific carbon stable isotope ratios ($\delta^{13}C$) of combined C18 fatty acids and combined C20 fatty acids for web spiders (n=3) and ground spiders (n=3). Dashed lines represent average aquatic (blue) and terrestrial (orange) C18 fatty acids carbon isotope ratios. Symbols represent mean carbon isotope value for each group and error bars represent standard deviation.

Based on the results above, we used LIN, ALA, SDA and EPA as tracers for food source reconstruction. ARA was excluded for ground spiders because of potential internal production or an unknown diet source. Model output using combination of the remaining PUFAs and $\delta^{13}C$ indicated a mixed diet, consisting of terrestrial and aquatic insects for both types of riparian spiders (Figure 8). Results are presented as median and 95 % credibility interval.

For web spiders, the model revealed that the diet in spring was dominated by terrestrial insects (Glatt: 45 (31-57) %, Necker: 43 (34-52) %), aquatic Diptera (Glatt: 35 (11-63) %, Necker: 17 (0-26) %) and Plecoptera (Glatt: 16 (0-31) %, Necker: 34 (9-59) %). In summer, the proportion of terrestrial insects and Diptera was lower than in spring at the Glatt (terrestrial insects: 35 (17-54) %, Diptera: 25 (4-51) %) while Plecoptera increased (33 (8-59) %). At the Necker, the proportion of terrestrial insects remained similar as in spring (44 (28-61) %), whereas the contribution of Diptera increased (31 (8-58) %) while Plecoptera decreased (19 (0-43) %). In autumn, terrestrial insects comprised the largest proportion of web spider diet at both rivers (Glatt: 51 (39-62) %, Necker: 69 (59-77) %) followed by Plecoptera (Glatt: 30 (10-51) %, Necker: 9 (0-24) %) and Diptera (Glatt: 18 (0-31) %, Necker: 20 (4-33) %).

For ground spiders, the diet in spring was dominated by terrestrial insects (Glatt: 79 (56-91) %, Necker: 86 (70-94) %). Terrestrial insect contribution was lower at both rivers in summer (Glatt: 49 (11-81) %, Necker: 53 (30-70) %) and autumn (Glatt: 45 (33-57) %, Necker: 47 (35-58) %). In summer, Plecoptera (Glatt: 25 (1-76) %, Necker: 12 (0-43) %) and Diptera (Glatt: 18 (0-37) %, Necker: 28 (3-57) %) made up the largest proportion of aquatic prey. In autumn, Plecoptera were the most important aquatic dietary contribution for ground spiders at both rivers (Glatt: 31 (12-48) %, Necker: 31 (18-46) %). Ephemeroptera were estimated to contribute only small proportions (< 10 %) to spider diets.

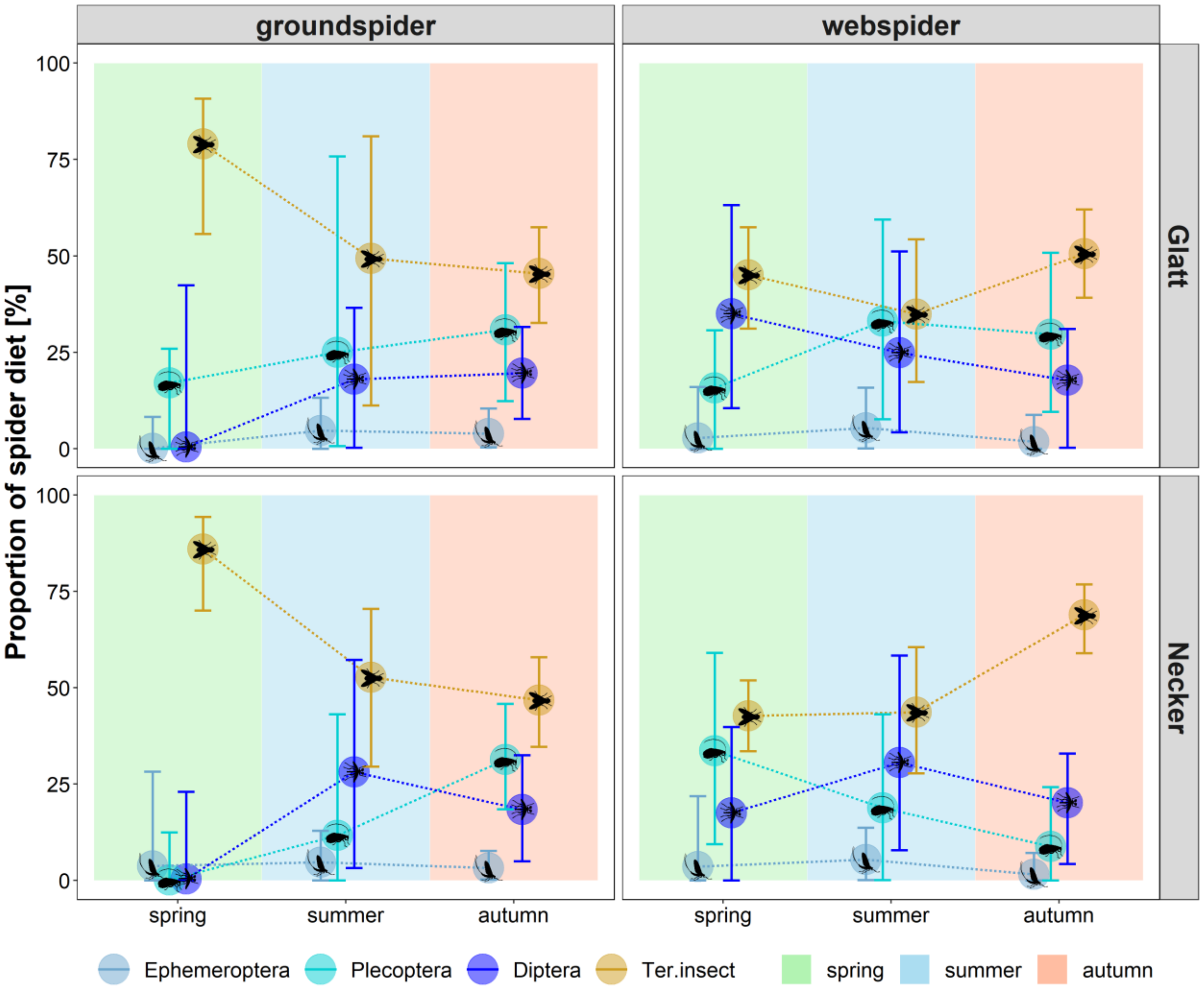

Figure 8: Estimated dietary contribution of aquatic and terrestrial food sources derived from MixSIAR multi-source Bayesian model to diet of web spiders and ground spiders in spring, summer and autumn at the two different rivers. Dietary tracers included $\delta^{13}C$, LIN, ALA, SDA, ARA and EPA for web spiders and $\delta^{13}C$, LIN, ALA, SDA and EPA for ground spiders. Models were calculated separately for each season and spider type. Results are presented as median and 95 % credibility intervals.

4. Discussion

4.1 PUFA transfer

We show here that PUFAs are transferred across ecosystem boundaries, from streams to adjacent terrestrial habitats, via emergent aquatic insects and that these PUFAs are partially incorporated into riparian spider tissues. Riparian spiders had an unusually high EPA content and thus high Σω3/Σω6 ratio for terrestrial consumers. On average, EPA represented 15 % of total fatty acids in riparian spiders (ca. 10 mg/g dry mass). High EPA content in riparian spiders was also reported by other studies (Fritz et al. 2017, Ramberg et al. 2020), although our results show even higher EPA content than e.g. the 2-3 mg/g dry mass found by Ramberg et al. (2020). The Σω3/Σω6 ratio is often employed to distinguish autochthonous versus allochthonous food sources in aquatic food webs and to assess the relative consumption of aquatic versus terrestrial material by consumers. A ratio > 1 is thought to indicate that dietary energy was acquired mainly from autochthonous food sources (Torres-Ruiz et al. 2007, Guo et al. 2016). Most terrestrial organisms are estimated to have an average Σω3/Σω6 ratio of 0.6 (Hixson et al. 2015). In the present study the Σω3/Σω6 ratio was close to 1 in most spiders and even >1 in web spiders in spring and summer, suggesting that aquatic-derived material was a major energy source for riparian spiders.

The high Σω3/Σω6 ratio of riparian spiders implies a trophic transfer of ω3-PUFAs from periphyton via emergent aquatic insects to riparian spiders. We cannot exclude, however, that spiders converted PUFAs internally from dietary precursor PUFAs or used PUFAs from terrestrial sources; e.g., some terrestrial plants produce ALA and SDA especially in seeds (Callaway et al. 1997, Guil-Guerrero 2007) and EPA may be produced in the soil food web (Menzel et al. 2018a). However, the site-specific differences that we observed for individual PUFAs, that were consistent through all trophic levels, support the idea of trophic PUFA transfer across the ecosystem boundary. For fatty acids that are primarily found in aquatic systems (ALA, SDA, ARA, and EPA) (Hixson et al. 2015), we expected to find a correlation in content between periphyton, emergent aquatic insects and riparian spiders. Indeed, we found a positive relationship in SDA content in periphyton, emergent insects and web spiders but not ground spiders, suggesting that SDA was exported from the aquatic system via emergent insects and then incorporated into web spiders. We also saw a positive relationship between ARA content in emergent aquatic insects and web spiders, implying that web spiders obtain their ARA at least partially from emergent insects. While ARA is a ω-6-PUFA associated with aquatic systems (Hixson et al. 2015), it was found in only small amounts in periphyton but higher amounts in aquatic invertebrates, other studies have found similar patterns (Ebm et al. 2020). The ARA source for aquatic invertebrates and ground spiders remains unclear.

We expected EPA content to be correlated among trophic levels, but no correlation was evident. Possible reasons for a lack of EPA association among trophic levels were 1) few strong site-specific differences in emergent insect EPA, 2) EPA accumulation in spider tissues, 3) the biomass of emergent insects and therefore PUFA export might be more important than actual PUFA content in single emergent insects, 4) internal metabolism, and 5) usage of an unknown terrestrial source (e.g., soil organisms). Here, we could exclude large quantities of internal EPA production or major terrestrial EPA sources (4 & 5) based on the distance gradient and compound-specific isotope analyses. The distance gradient showed a decrease in EPA content, albeit non-significant, with distance from shore. Further, in riparian spiders analyzed compound-specific, the $\delta^{13}C$ ratio of C20 fatty acids, where EPA comprised the greatest proportion, was similar to that of aquatic sources. These results support the idea of an aquatic EPA origin because $\delta^{13}C$ ratios of nutritionally valuable PUFAs have been shown to be relatively constant through trophic transfer with only minor fractionation (Burian et al. 2020). Results would have been clearer with a separation between single fatty acids, but this approach could not be used in this study due to limited technical resources. Even though we were not able to completely exclude internal conversion or terrestrial EPA sources, we still found strong evidence for a predominantly aquatic origin of EPA.

The correlation between ALA content in emergent aquatic insects and spiders was relatively weak, and thus did not confirm the aquatic origin of ALA. As terrestrial insects also contain ALA (Hixson et al. 2015), the ALA in spiders is likely a mixture from different sources. Lastly, we did not expect aquatic insects to be the main source of LIN for spiders, as LIN is present in large quantities in many terrestrial organisms, including the terrestrial Diptera that we used as reference material. Indeed, we saw only a weak correlation between LIN content in aquatic Diptera and web spiders. LIN content especially in ground spiders increased with distance from shore, supporting the hypothesis that terrestrial insects are the main LIN source for riparian spiders. However, aquatic insects contain some LIN, so spiders that feed close to 100 % on aquatic subsidies can still fulfill their LIN requirements. Lastly, our results indicate that season and spider type had strong influence on PUFA content and usage of aquatic subsidies in riparian spiders.

4.2 Seasonal patterns

We expected to find the highest proportion of aquatic-derived resources and aquatic PUFAs, especially EPA, in spring when aquatic emergent insects are abundant and terrestrial insects are still relatively scarce (Nakano and Murakami 2001, Uesugi and Murakami 2007). While several isotope studies suggest riparian spiders use most aquatic-derived material in spring (Kato et al. 2004, Paetzold et al. 2005), we saw no significant change in $\delta^{13}C$ ratio in spiders during the year. However, we observed a decrease in EPA from spring to summer to autumn, also indicating that aquatic subsidies are most widely used in spring. The proportion of aquatic vs. terrestrial material in spider diets using $\delta^{13}C$ could be estimated only in spring, where we saw a significant difference in $\delta^{13}C$ between aquatic and terrestrial insects. In summer and autumn, isotope signatures of Plecoptera and aquatic Diptera overlapped with the signal of terrestrial insects. The shift in the carbon isotope ratio of Plecoptera and aquatic Diptera reflected more terrestrial-derived resources being consumed in summer and autumn than spring. As food sources could not be discriminated from each other, the usage of $\delta^{13}C$ as method to reconstruct food sources was not possible. The $\delta^{13}C$ ratio of spiders in summer and autumn showed that ingested carbon originated mostly from terrestrial sources, although likely in parts being cycled through the aquatic system and thereby the biomass being enriched in PUFAs.

The mixing model results, including PUFAs as dietary tracers, revealed higher amounts of aquatic-derived material in web spider diets in spring and summer than in autumn. For web spiders, PUFA profiles showed a changing trend from high EPA and SDA contents in spring to more LIN in autumn. Seasonal differences in ground spiders were less evident, although still containing highest EPA in spring. For ground spiders, a more consistent use of small amounts of aquatic subsidies across seasons seems likely. The mixing model including PUFAs showed a higher proportion of aquatic material in ground spider diet in summer and autumn than in spring and in the multivariate analysis ground spiders were most similar to aquatic insects in summer and autumn. Summarizing, aquatic subsidies provide an important dietary supplement to especially riparian web building spiders in early spring when other food sources are low in abundance, and might play a role in reproductive success as shown for other fauna such as birds (Twining et al. 2018b). Importantly, we also found that EPA quantities in riparian spiders remained relatively high during summer and autumn, indicating an ongoing use of aquatic subsidies throughout the active period of the year.

4.3 Comparison of spider types

We saw a stronger aquatic-derived PUFA and stable isotope signature in web spiders than in ground spiders, especially in spring, as also reported in other studies using stable isotopes to estimate proportion of aquatic material in spider diet (e.g., Collier et al. (2002). The two spider types showed the most pronounced differences (PUFA profiles and $\delta^{13}C$) in spring. For instance, stable isotope mixing model revealed that web spiders consumed on average approximately 40 % and ground spiders 20 % aquatic-derived carbon in spring. Other studies found up to 100 % aquatic-derived material being consumed by spiders, especially web spiders (Sanzone et al. 2003). However, previous studies have found huge site-specific differences (Collier et al. 2002). As we only used terrestrial Diptera as a reference source, we likely underestimated the amount of aquatic-derived material consumed by spiders. We saw that spiders sampled 40-50 m from shore were less depleted in carbon than the terrestrial Diptera samples, indicating that other less depleted terrestrial carbon sources were available. However, although amount of aquatic material in the diet was likely underestimated, the relative difference of about 20 % between the two spider types remained, suggesting that web spiders incorporated more aquatic-derived carbon than ground spiders in spring. Importantly, this finding indicates stationary web spiders, in contrast to roaming ground spiders, use this aquatic subsidy flux in spring when more aquatic than terrestrial insects are available. Ground spiders are more mobile and can find sufficient alternate prey in riparian areas, and appear to use fewer aquatic subsidies, when only considering isotope data.

However, ground spiders contained considerable amounts of EPA. The soil food web (nematodes/collembola) might be an alternative EPA source (Menzel et al. 2018a), but the compound-specific data indicated that the EPA found in ground spiders derived from aquatic sources. As EPA has been shown to be of physiological importance for ground spiders, especially the immune system (Fritz et al. 2017), it seems likely that riparian ground spiders, while accumulating the bulk of carbon from terrestrial sources, fulfill their EPA requirements by supplementing their diet with emergent aquatic insects. In fact, ground spiders might specifically accumulate/retain aquatic EPA such as other organisms that have been shown to have a higher transfer efficiency for PUFAs compared to bulk carbon (Gladyshev et al. 2011). In accordance to that, the mixing model including PUFAs estimated a higher proportion of aquatic material in both spider types' diets than the model using only $\delta^{13}C$. While Ramberg et al. (2020) found a significantly higher EPA content in lycosid than tetragnathid spiders, we found no significant difference in EPA content between the spider types. In contrast, SDA content (and $\Sigma\omega3/\Sigma\omega6$ ratio) was higher in web spiders than ground spiders in spring, albeit only significantly at the Necker. This result indicates that while ground spiders were generally low in SDA, web spiders incorporate this PUFA if available. Indeed, SDA content in web spiders was correlated with SDA content in emergent aquatic insects. A major difference between spider types that occurred consistently during all seasons was higher ARA content in ground spiders than web spiders (also see Ramberg et al. (2020). While the ARA content in web spiders was correlated with ARA content in emergent aquatic insects, ground spiders exhibited constantly higher values. It seems likely that certain spider groups (e.g., Lycosidae) have the metabolic machinery for metabolizing ARA or that they derive it from soil organisms; especially as the ARA content increased with distance from shore. This might also explain the differences between spider types, as ground-dwelling spiders have easier access to the soil food web and thus to potentially PUFA-rich prey, such as collembola. More research is needed regarding origin of ARA in riparian food webs. Apart from ARA, both spider types had similar PUFA profiles and $\delta^{13}C$ ratios in summer and autumn, indicating similar diets, in contrast to spring.

We found multiple differences to what degree PUFA profiles of the spiders resemble those of aquatic insects. First, the time of year that PUFA profiles of spiders were most similar to aquatic insects differed between spider types. Web spiders were closely linked to emergent insects in spring and summer, while ground spiders showed most similarities in summer and autumn. Second, web spiders were most similar to aquatic Diptera, while ground spiders especially in autumn were most similar to Plecoptera. The reason might be that Plecoptera represent an important food source especially for ground-dwelling predators due to their emergence mode (Paetzold et al. 2005, Muehlbauer et al. 2020). Both spider types were distinct from terrestrial reference samples, mainly in their higher content in SDA and EPA, and ARA for ground spiders. As only terrestrial Diptera, being highly abundant, were measured as a reference for terrestrial material, a terrestrial separation in profiles from riparian spiders would likely be less clear if different terrestrial insects were included in the analysis. Here, we suggest additional terrestrial groups be included in future studies to better clarify profiles between aquatic and terrestrial sources.

Interestingly, spiders collected for the distance gradient directly at the shore (0-2m) were more distinct from spiders of the same lifestyle/taxonomic group (genus level) further away from shore (10-50 m) than between lifestyles. Chari et al. (2020) also found a strong decrease of PUFAs associated with the aquatic system with distance from shore. This indicates that access to aquatic insects might be stronger driver of PUFA composition than lifestyle, as aquatic insect biomass steeply declines in the first 50 meters from shoreline (Muehlbauer et al. 2014).

4.4 Fluvial network specific differences

The study was completed along a natural (Necker) and a heavily altered (Glatt) fluvial network. We expected to see lower proportion of aquatic diet (less depleted $\delta^{13}C$ ratios) and less EPA in riparian spiders at the Glatt, because of a decoupling between the system due to the anthropogenic changes. Somewhat surprisingly, $\delta^{13}C$ of spiders was on average more depleted at the Glatt than Necker, indicating higher proportion of aquatic material in the diet at the Glatt. There was no significant difference in EPA in spiders between systems. Other factors, like riparian vegetation structure, have been shown to influence PUFA profiles in riparian spiders (Ramberg et al. 2020), suggesting that site-specific differences along each system might be important as well. However, we saw higher SDA content in web spiders in spring in the more natural Necker. Further, SDA was found in aquatic insects and web spiders at the same sites SDA was present in periphyton, thus differences between systems occurred already at the base of the food web (i.e., primary producers). Different algae assemblages, involving taxa producing SDA in the Necker, might therefore explain the system differences. The nutrient status, higher in the Glatt than the Necker, likely caused differences in algal assemblages (Stelzer and Lamberti 2001). Colombo et al. (2006) also found an association between cold temperatures and high SDA content in some types of algae, which might be important because of the higher water temperatures in the Glatt (mean annual temperature 2019: Glatt 10.7 °C (Hydrograph station Oberbüren); Necker 9.2 °C (Hydrograph station Mogelsberg)).

We also saw a more pronounced difference between the two spider types at the natural Necker in comparison to the anthropogenic Glatt. While we saw no general reduction of the food web connectivity between the two systems, we observed some specific differences especially a lower transfer of SDA and a lower $\Sigma\omega3/\Sigma\omega6$ ratio in spring at the Glatt than Necker. Although the Glatt has elevated nutrient levels and a sediment deficit, its flow regime is mostly natural and many reaches along the river are not channelized, thus partially explaining the lower than expected differences in aquatic-derived subsidies between systems. Rivers showing even stronger regulatory alterations or pollution might show larger effects not only on spider populations (see Paetzold et al. (2011), but also in PUFA transfer.

5. Conclusion

Both web-building and ground-dwelling riparian spiders contained unusually high amounts of EPA for terrestrial consumers. While isotope data suggested that ground spiders derived only a small part of their carbon from aquatic sources, fatty acid analysis showed that both spider types used aquatic subsidies as an EPA source (dietary supplement). This was especially true for web spiders in spring. These results highlight that by including additional tracers, like PUFAs, in food web studies, we can track energy flow and exchange of important nutrients that might otherwise be overlooked. The difference in $\delta^{13}C$ and PUFAs between spider lifestyles was pronounced in spring but small during the other seasons. Lastly, the difference between the anthropogenic and natural system was smaller than expected. We found mainly a higher SDA content and $\Sigma\omega3/\Sigma\omega6$ ratio in web spiders in spring in the more natural system, but no difference in spider EPA content between systems. The high similarity in PUFA profiles, especially between aquatic Diptera and Plecoptera and riparian spiders, highlights the importance of emergent aquatic insects as vectors of PUFA transfer across ecosystem boundaries to riparian predators.

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7. Author contributions

Conceptualization: CR, CK, DMC; formal analysis: CK; writing - original draft preparation: CK, CR, DMC; writing - review and editing: CK, CR, DMC; visualization: CK; project administration: CK, CR; funding acquisition: CR; All authors have read and agreed to the published version of the manuscript.

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CHAPTER 3 - STREAM DEGRADATION AFFECTS AQUATIC RESOURCE SUBSIDIES TO RIPARIAN GROUND-DWELLING SPIDERS

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Abstract

Freshwater systems have undergone drastic alterations during the last century, potentially affecting cross-boundary resource transfers between aquatic and terrestrial ecosystems. One important connection is the export of biomass by emergent aquatic insects containing omega-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA), that is scarce in terrestrial systems. Because of taxon-specific differences in PUFA content and functional traits, the contribution of different insect groups should be considered, in addition to total biomass export. In this context, one important trait is the emergence mode. Stoneflies, in contrast to other aquatic insects, crawl to land to emerge instead of flying directly from the water surface, making them accessible to ground-dwelling predators. Because stoneflies are especially susceptible to environmental change, stream degradation might cause a mismatch of available and required nutrients, particularly for ground-dwelling predators. In this study, we estimated emergent biomass and EPA export along two streams with different levels of habitat degradation. The EPA content in aquatic insects did not differ with different degrees of habitat degradation and total biomass export in spring was with 7.9±9.6 mg m^{-2} day^{-1} in the degraded and 7.3±8.5 mg m^{-2} day^{-1} in the natural system, also unaffected. However, habitat degradation substantially altered the contribution of crawling emergence to the total export in spring, with no biomass export by stoneflies at the most degraded sites. The EPA content in ground-dwelling spiders was correlated with emergent stonefly biomass, making up only 16.0±6.2 % of total fatty acids at sites with no stonefly emergence, but 27.3±3.0 % at sites with highest stonefly emergence. Because immune function in ground-dwelling spiders has been connected to EPA levels, reduced crawling emergence might impact spider fitness. Functional traits, like emergence mode as well as nutritional quality, should be considered when assessing the effects of stream degradation on adjacent terrestrial ecosystems.

Keywords: aquatic subsidies, emergent insects, functional traits, habitat degradation, PUFA, resource quality

1. Introduction

Many streams and associated floodplains have undergone severe changes over the last century (Tockner and Stanford 2002, Verhoeven 2014). In Europe and across the globe, humans have significantly modified almost all large rivers, resulting in drastically changed morphology and increased nutrient levels (Petts et al. 1989, Malmqvist and Rundle 2002, Tockner et al. 2009). Although various measures were implemented under the European Water Framework Directive to improve stream health, many issues remain unresolved (Hering et al. 2010, Voulvoulis et al. 2017). One major problem is the multitude of structures, such as dams and weirs, built along river networks. In Europe, for example, at least 1.2 million barriers fragment fluvial networks (Belletti et al. 2020) and disrupt bed load transport, causing major bed load deficits and changes in stream morphology (Golz 1994, Kondolf 1997). Bed load deficits or so called "hungry waters" lead to bank erosion, which can cause the development of steep banks and increased fine sediment input into streams (Kondolf 1997). Fine sediment, where deposited in large quantities, can have strong negative impacts on various instream assemblages, ranging from periphyton to macroinvertebrates and fish (see reviews by Wood and Armitage 1997, Kemp et al. 2011, Jones et al. 2012). One negative impact of fine sediment is the clogging of interstitial pore spaces in stream beds, which typically leads to homogenization of instream habitats and reduction of accessibility for many stream organisms (e.g., Ryan 1991, Richards and Bacon 1994, Peralta-Maraver et al. 2019).

It is well established that both high nutrient loads and fine sediment input, singly or in combination, act as stressors on aquatic organisms (Matthaei et al. 2010, Wagenhoff et al. 2011). However, instream habitat degradation also affects adjacent terrestrial ecosystems connected in a tangled web via multiple resource pathways. For example, aquatic insects that develop in aquatic ecosystems before they emerge as adults transfer biomass to terrestrial ecosystems (Baxter et al. 2005). Human impacts, including changes in nutrient levels and stream morphology, alter aquatic insect assemblages and can disrupt lateral connectivity to riparian areas, thereby modifying cross-boundary transfers of resource subsidies (Muehlbauer et al. 2020). Riparian areas, the transitional zones linking streams and terrestrial ecosystems, are unique habitats that support many specialist species with specific resource requirements (Ramey and Richardson 2017, Twining et al. 2018b). For instance, many riparian predators, including spiders, lizards, and birds, depend on the influx of resource subsidies from aquatic systems via emergent insects (Sabo and Power 2002, Kato et al. 2003, Paetzold et al. 2006, Twining et al. 2018b).

Aquatic resource subsidies are important because aquatic and terrestrial resources are not nutritionally reciprocal for consumers (Twining et al. 2019). Aquatic subsidies are a rich source of omega-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA), because aquatic organisms, including emergent insects, contain on average 6 times more omega-3 PUFAs than terrestrial insects (Hixson et al. 2015, Mathieu-Resuge et al. 2021). EPA is synthesized by aquatic primary producers and accumulates in many aquatic consumers of higher trophic levels, while it is scarce in terrestrial food webs (Twining et al. 2019). EPA is essential for maintaining crucial physiological functions in animals; e.g., it is an important regulator of eicosanoid biosynthesis and therefore immune response regulation (Schlotz et al. 2016). EPA intake has been proven beneficial for different animal groups, including aquatic invertebrates (Martin-Creuzburg et al. 2018) and tree swallows, that can be considered riparian specialists (Twining et al. 2016). Biosynthesis of EPA and other long-chain PUFAs like docosahexaenoic acid (DHA) from shorter chain dietary precursor PUFAs, which are available in terrestrial systems, is often thought to be inefficient and costly (Pawlosky et al. 1997, Brenna et al. 2009, Castro et al. 2012). Therefore, riparian predators may rely on consumption of aquatic organisms to cover their EPA demands, having not developed or lost the necessary metabolic pathways to produce EPA in adequate amounts (Twining et al. 2018a). However, it should be noted that more generalist species like blue tits (common bird) do not seem to depend on aquatic-derived EPA (Twining et al. 2021).

Human degradation of streams can affect aquatic-derived EPA availability for riparian predators through an interaction by a number of factors. First, human impacts such as enhanced nutrient loads can reduce EPA content at the base of aquatic food chains; e.g., by altering periphyton assemblages to those containing less EPA (Cashman et al. 2013). Lower EPA production at the base of the food chain is transferred to higher trophic levels (consumers), causing aquatic organisms including emergent insects to contain less EPA (Müller-Navarra et al. 2004, Taipale et al. 2016, Scharnweber et al. 2019). Second, the abundance of some stream invertebrates also can be reduced in degraded streams through negative impacts of high nutrient or fine sediment loads; e.g., aquatic invertebrates that emerge from the stream during their lifecycle seem to be particularly susceptible to environmental stressors (Manning and Sullivan 2021). Consequently, the reduced biomass export via emerging insects will reduce EPA export to riparian zones. Third, EPA availability for riparian predators can be influenced by shifts in community composition of emergent insects possessing differing functional traits; e.g., mode of emergence. For instance, chironomids (Diptera) are typically abundant in degraded streams with high amounts of fine sediment, while other, more sensitive taxa, e.g. stoneflies (Plecoptera), may decline, (Wood and Armitage 1997, McCaffrey 2021). While most insects, such as chironomids, emerge directly from the water column and fly away, stoneflies crawl to the shore to emerge (Merritt and Cummins 1996). This emergence behavior makes stoneflies easy prey for ground-dwelling predators, whereas other insects that emerge by flight are presumably less accessible. Indeed, stoneflies have been hypothesized to be a major aquatic food source for riparian ground-dwelling spiders (Paetzold et al. 2005). Consequently, lower stonefly biomass could reduce access to aquatic subsidies for ground-dwelling riparian predators such as wolf spiders. A negative effect of lower access to EPA seems likely because EPA content in ground-dwelling spiders has been linked to immune function (Fritz et al. 2017), although the impact on fitness remains unclear. While organisms can cope with limitations of certain nutrients by using specific metabolic pathways or foraging techniques, adaptation might be too slow to keep up with rapid human-caused environmental changes (Van Der Jeugd et al. 2009, Shipley et al. 2022). This asynchrony can lead to a mismatch between availability and the requirement of essential nutrients.

Emergent aquatic insects clearly transfer important resources to riparian areas, but the question remains if degraded streams provide subsidies in adequate quantity and quality to terrestrial systems. Here, we quantified the biomass export via emergent insects along two stream networks in Switzerland and compared the emerging insect assemblage. While one stream had near-natural conditions, the other suffered from morphological alterations. Further, instream conditions along each stream differed, with higher nutrient loads and fine sediment content at some sites, particularly in the degraded system. We examined if there were differences in the quantity (biomass) and quality (PUFA content) of emergent insects between and along the two stream networks in relation to differences in nutrients and stream-bed condition. Considering accessibility of different emergent insect groups for riparian predators, we compared export via different emergence pathways and investigated the connection between the biomass of "crawling" emergence and EPA content in a ground-dwelling predator (wolf spider). We hypothesized that: 1) the biomass export from the more degraded system (particularly at sites with greater degradation) will be lower than from the near-natural system; 2) emergent insect composition at more degraded sites will differ from near-natural sites with more insensitive (e.g., chironomids) and less sensitive groups (e.g., stoneflies), thereby reducing "crawling" emergence in particular; 3) EPA content will be lower in emergent insects from the degraded than the near-natural system; and 4) EPA content in riparian ground-dwelling spiders is connected to the biomass of emerging stoneflies due to their crawling emergence mode

2. Material and methods

2.1. Site Description

We sampled along two adjacent 6th order streams (Necker and Glatt) in the Thur catchment in northeast Switzerland (Figure 1). The Necker is a system with a natural flow and sediment regime that mostly runs through rural and forested areas. The Necker has a long-term annual mean discharge of 3.3 $m^3 s^{-1}$ (hydrograph station Achsäge) and a basin draining 126 km^2 that ranges in elevation from 550 to 1550 m asl. Elevation of sampling sites (n = 6) ranged from 550 to 740 m asl. The Glatt has undergone many anthropogenic changes, including construction of multiple barriers that prevent bed load transport, leading to a strong bed load deficit (50-80 %) and major morphological impairment in downstream sections. The Glatt mostly flows through an agricultural landscape with mixed forested and urban areas. Mean annual discharge is 2.7 m^3/s (hydrograph station Oberbühren) and the drainage area of the Glatt basin is 90 km^2, ranging in elevation from 480 to 950 m asl. Elevation of Glatt sampling sites (n = 6) ranged between 500 and 620 m asl.

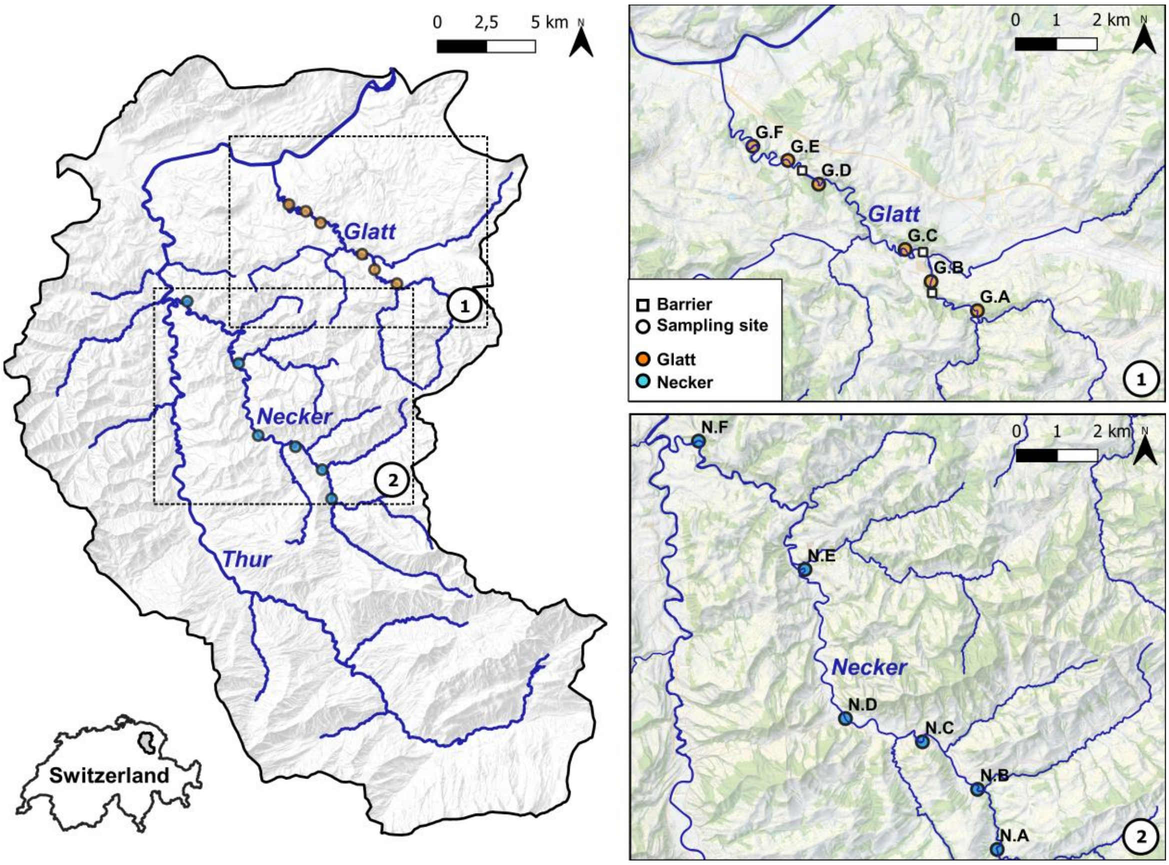

Fig. 1 – Map of the Thur catchment (northeast Switzerland) with sampling sites on the Necker (N) and Glatt (G). Circles represent sampling sites; squares represent major barriers blocking bed load transport.

2.2 Field Sampling

2.2.1. Physico-chemical parameters

At each site, we recorded electrical conductivity (µS cm^{-1} at 20 °C) (WTW LF340, Wielheim, Germany) and collected a water sample in a 0.5-liter plastic bottle (rinsed 3 times, no headspace). Samples were brought back to the laboratory for chemistry analysis of pH, alkalinity, nitrate, nitrite, total nitrogen (TN), ortho-phosphate (OP), total phosphorus (TP), dissolved organic carbon (DOC), total organic carbon (TOC) and total inorganic carbon (TIC) as detailed in Tockner et al. (1997).

2.2.2 Stream bed characteristics

Stream bed characteristics were assessed using three different methods at each site: (1) Wolman pebble counts, (2) visual estimates of the degree of clogging, and (3) quantitative assessment of the surface fine sediment content. We conducted Wolman substrate counts (200 particles) to assess the grain size distribution of the river bed for each site (Wolman 1954, Bunte and Abt 2001). We blindly selected a grain each step along longitudinal transects and measured the b-axis via a gravelometer. At each site, the proportion of fine sediment (defined here as particles < 2 mm) and degree of clogging was assessed using a visual estimate following the protocol of Schälchli et al. (2002). The surface substrates of exposed gravel bars were removed by hand at three points to enable access to the interstitial pore space below. The fine sediment content and clogging of the interstitial space was subsequently assessed through comparison with reference photographs in the field, classifying them into 5 categories as described in Mathers et al. (2021a): 1 – coarse grained, little sand, no cohesive deposits and coarse open pore space; 2 – mix of size classes with some sand present, no cohesive deposits and fine open pore space; 3 – slightly solidified substrate, fine pore space infilled with cohesive particles accounting for approximately 25 % of surface area, majority of other pore space infilled with sand; 4 – strongly solidified substrate, fine pore space infilled with cohesive particles accounting for approximately 50 % of surface area, remaining pore space infilled with sand; and 5 – strongly solidified substrate, fine pore space infilled with cohesive particles accounting for approximately 100 % of surface area. An average of the assessment (category 1-5) of the three points was used as an estimate for the reach. In addition, we used a quantitative resuspension technique, as described by Duerdoth et al. (2015), to quantify fine sediment deposits. In short, an open-ended cylinder was inserted into the riverbed at four locations at the site (two erosional and two depositional) and the water within the cylinder vigorously agitated for ca. 60 seconds to suspend surface fine sediment into the water column before a 50 ml water sample was taken. Samples were routinely processed in the laboratory for mineral content and converted to mass per m^2 using water depths in the stilling well (Mathers et al. 2021b). For each site, the geometric mean of the four samples was taken as the surface fine sediment content measure.

2.2.3 Sampling of emergent aquatic insects

Emergent aquatic insects were captured alive using floating emergence traps with a base area of 0.25 m^2 and mesh size of 100 µm between April and October 2019 to assess emergent aquatic insect biomass and community composition. Emergence traps were deployed weekly (2-3 days per week) from beginning of April to mid-May and afterwards monthly (2 x 2-3 days per month) until end of October. Overall, emergence traps were deployed for 17 sampling periods: 7 periods in spring (April/May), 6 in summer (June/July/August), and 4 in autumn (September/October). No sampling was conducted during winter (November to March) as water temperatures were low and minimal emergence was expected. Three traps were deployed per site, each covering a different habitat type (fast flowing, slow flowing and shallow habitats near the shoreline) to be able to estimate overall biomass export by insects with differing habitat preferences and emergence modes. Collected insects were returned to the laboratory, identified to the family level and frozen (-20 °C or -80 °C depending on storage time) until further analysis.

2.2.4 Spider sampling

Riparian ground-dwelling spiders were collected in spring (April/May) 0-2 m from the shoreline using a mouth aspirator. A minimum of 3 individuals were collected per site, returned to the laboratory, and kept alive in containers overnight to allow gut clearance. Afterwards, they were frozen until further analysis. All collected individuals belonged to the family of wolf spiders (Lycosidae) from the genera *Pardosa* or *Pirata/Piratula*.

2.3. Laboratory procedures

2.3.1. Fatty acid analysis

We analyzed fatty acids of 233 emergent insect samples (85 Diptera, 75 Ephemeroptera, 73 Plecoptera) and 24 ground-dwelling spiders collected from 8 sites (4 per stream) (see Kowarik et al. 2021). We found only a few single individuals of Trichoptera, which were therefore not included in the analysis. In addition, we analyzed 12 ground-dwelling spider samples collected in spring from a further 4 sites used in this study. Emergent insect and spider samples were freeze-dried and weighed using an ultrafine balance to estimate biomass export. We extracted weighed samples in dichloromethane/methanol (2:1 v/v) over night and added internal standard for analysis. We left samples for 5 min in an ultrasound bath and removed particles by centrifugation (3500 g, 5 min). We evaporated samples under nitrogen to dryness and resuspended in 4 ml methanolic HCl. We incubated samples solved in methanolic HCl for 20 min at 60 °C to obtain fatty acid methyl esters (FAMEs). Hexane was added after samples had cooled down, each sample was thoroughly mixed, and the upper hexane phase was transferred to a new vial. We reiterated this process two more times and pooled the hexane fractions into a composite sample, which was evaporated under nitrogen to dryness. We resuspended each sample in 20 to 200 µl hexane, depending on initial sample dry mass. We analyzed samples for FAME content using gas chromatography (7890B gas chromatograph, Agilent Technologies, Waldbronn, Germany);

see configuration details in Martin-Creuzburg et al. (2010). We identified fatty acids based on their retention time and mass spectra, and quantified amounts by comparison to internal standards using multipoint calibration curves of known concentration (for details see Martin-Creuzburg et al. (2017)).

2.3 Data analysis

2.3.1 Environmental variables

All analyses were conducted in R 3.6.2 (R Core Team 2019). To characterize substrate conditions at each site, we used the Wolman pebble counts to calculate grain size percentiles and the statistical parameters of median, percentiles, sorting, skewness and kurtosis (Bunte and Abt 2001). In addition, we estimated substrate diversity by calculating Shannon´s diversity index ("diverse", package "vegan"; Oksanen et al. (2019)) , an ecologically meaningful spatial heterogeneity index, based on the grain size distribution (Mathers et al. 2021a). The index has been used in different fields as a measure of habitat heterogeneity (Yarnell et al. 2006, Turley et al. 2017). Differences in stream-bed characteristics and physico-chemical parameters between the two rivers were tested using one-way ANOVA after log transformation of data to ensure normal distribution. Correlations among variables were checked (supplement A, Fig.1) and one variable for each cluster of correlated variables was used for principal component analysis (PCA) (package "FactoMineR"; (Lê et al. 2008)). Two separate PCAs were conducted from the variables, one describing stream bed characteristics and the other physico-chemical variables. We used adonis2 (package "vegan", (Oksanen et al. 2019)) to perform a non-parametric multivariate analysis of variance (PERMANOVA) on Bray-Curtis similarity matrices to check if stream identity (Necker, Glatt) explained variance among sites. Separation among groups (stream identity) was considered significant at $p < 0.05$. Larger pseudo F-ratios (F) indicate a more pronounced separation among groups. PERMANOVA is a robust method without explicit assumptions about data distribution, as it takes only ranks of dissimilarities into account (Anderson 2001).

2.3.2 Biomass export via emergent aquatic insects

Biomass export was calculated by normalizing measurements from each site and sampling date to dry mass export per standard stream square meter per day. To achieve this, the mean dry mass captured over one sampling period in the three traps (covering different habitats) at each site was calculated and divided by trap area and number of deployment days. Those values were then used to calculate average and standard deviation of dry mass export per month (from all sampling dates in the respective month) and per season. Insect biomass export was split into "crawling" (stonefly) and "flying" (other groups) emergence. We tested if there was a significant difference in total, crawling and flying dry mass export between rivers using a mixed model with square root transformed biomass data and date fitted as a random effect. Tests were run separately for each season. For spring, where aquatic subsidies are believed to be most important (Nakano and Murakami 2001), we did a multivariate comparison of insect families contributing to biomass export using PERMANOVA. We tested how much of the variance was explained by stream identity. In addition, we tested if environmental principal components (chem_PC1, chem_PC2, sed_PC1, sed_PC2) were significant explanatory variables ($p < 0.05$) using PERMANOVA. To investigate which insect families contributed most to observed dissimilarities, we used a similarity percentages routine (SIMPER). We visualized the differences in groups contributing to biomass export using a non-metric multidimensional scaling (nMDS) (package "vegan").

2.3.3 PUFA content in emergent aquatic insects

We examined the content of five individual PUFAs, three omega-3 PUFAs (alpha-linolenic acid - ALA, stearidonic acid - SDA and eicosapentaenoic acid – EPA) and two omega-6 PUFAs (linolenic acid - LIN, arachidonic acid – ARA) in emergent aquatic insects. DHA was only detectable in trace amounts in a few samples and excluded from analysis. Although insects were identified to family level, PUFA contents were compared at the order level similar to other studies considering PUFA export by emergent insects (Martin-Creuzburg et al. 2017, Shipley et al. 2022). We further compared PUFA content (% and mg) among seasons and between the two streams using factorial ANOVA (type III) (Anova, package "car"; (Fox and Weisberg 2019)). We ran post-hoc tests separately for each pair of combinations when interaction effects were significant ($p < 0.05$). We tested the significant main effects when interaction effects were not significant ($p > 0.05$). As post-hoc tests, we performed multiple pairwise comparisons of estimated marginal means (EMMs) with Tukey corrections (package "emmeans"; (Lenth 2021)). We tested data for assumptions for homogeneity of variance and normality, and transformed data where required. For percent data, we used the arcsine square-root transformation (Sokal and Rohlf 1995). We report all values as untransformed means. For spring, we ran a multivariate comparison of PUFA content (% of total fatty acids) using PERMANOVA to assess the proportion of variance explained by insect order, season and stream. In addition, we tested if environmental PCs explained the variance. To investigate which PUFAs contributed most to observed dissimilarities, we used a similarity percentages routine (SIMPER). We visualized patterns in PUFA composition using PCA.

2.3.4 EPA export via emergent aquatic insects and transfer to riparian spiders

We related emergence data to biomass specific average EPA content (mg per dry mass) observed in different insect orders per site and season to calculate daily EPA export. Average EPA content was multiplied with exported dry mass of the respective insect order per standard square meter at the respective site. All sampling dates of each season were treated as replicates to calculate EPA export (mg m^{-2} day^{-1}) for the respective season (mean ± sd). EPA export via "crawling" and "flying" emergence was calculated separately. For an estimation of total EPA export over the complete sampling period, average daily export for each month was calculated and multiplied with number of days to obtain total export for each month. The sum EPA export over all months was used as a rough estimate of total

EPA export over the sampling season, which should be close to export per year as winter months have low export (Nakano and Murakami 2001).

To investigate if EPA content in ground-dwelling spiders was connected to biomass export via crawling emergence, the average exported crawling biomass for each site and EPA content in spiders in spring were correlated. Arsine square-root transformed values of EPA content (% of total fatty acids) were used, while average biomass export was square-root transformed. We used a mixed model, first separately for each stream and then with sites from both streams combined, with stream as a fixed effect and site as a random effect. Analysis was done for spring only, as aquatic subsidies are believed to be most important for riparian predators in spring (Nakano and Murakami 2001).

3. Results

3.1 Differences in environmental variables between streams

3.1.1 Physico-chemical parameters

Nutrient levels (N and P) differed significantly between streams. At the Necker, nutrients were low (TN: 1.2 ± 0.7 mg l^{-1}, TP: 12 ± 7 mg l^{-1}) (mean ± standard deviation), slightly increasing downstream (supplement A, Fig. 2A). Conductivity was 341 ± 24 µS cm^{-1} and relatively low. In contrast, the Glatt had significantly higher TN (3.7 ± 1.6 mg l^{-1}) and TP (61 ± 24 µg l^{-1}) levels, and significantly higher conductivity (582 ± 74 µS cm^{-1}) than the Necker ($p < 0.001$). Carbon constituents and pH did not significantly differ between the two streams but some sites, especially G.A, had a higher pH and the Glatt displayed variable carbon levels. In the Glatt, there was an increase in TN ($p < 0.01$) and TP ($p < 0.001$) between the 3 upstream and 3 downstream sites (supplement A, Fig. 2A). All N and P constituents were highly correlated. In a PCA using TP, TN, DOC and pH as parameters, the two streams had distinct clusters, and stream identity explained 77 % of the variance (PERMANOVA: $F = 33.9$, $p = 0.004$) (Fig. 2A). Chem_PC1 represented a gradient of increasing nutrient (N, P) levels. The division between the upstream and downstream sites on the Glatt was also visible in the multivariate comparison.

3.1.2 Stream-bed characteristics

None of the bed load metrics such as Shannon´s grain diversity and median grain size differed significantly between systems ($p > 0.05$), but surface fine sediment and clogging was generally higher at the Glatt than the Necker ($p < 0.05$) (supplement A, Fig. 2B). For some parameters, especially those connected to fine sediment input, we found strong differences between streams but also among sites, especially along the Glatt; i.e., lower degree of clogging at sites G.A and G.C than the other sites. In the stream-bed PCA, these two sites clustered with the Necker sites along sed_PC2, which was mainly explained by clogging (Fig. 2B). Stream identity did not explain variance among sites (PERMANOVA: $F = 1.5$, $p = 0.28$).

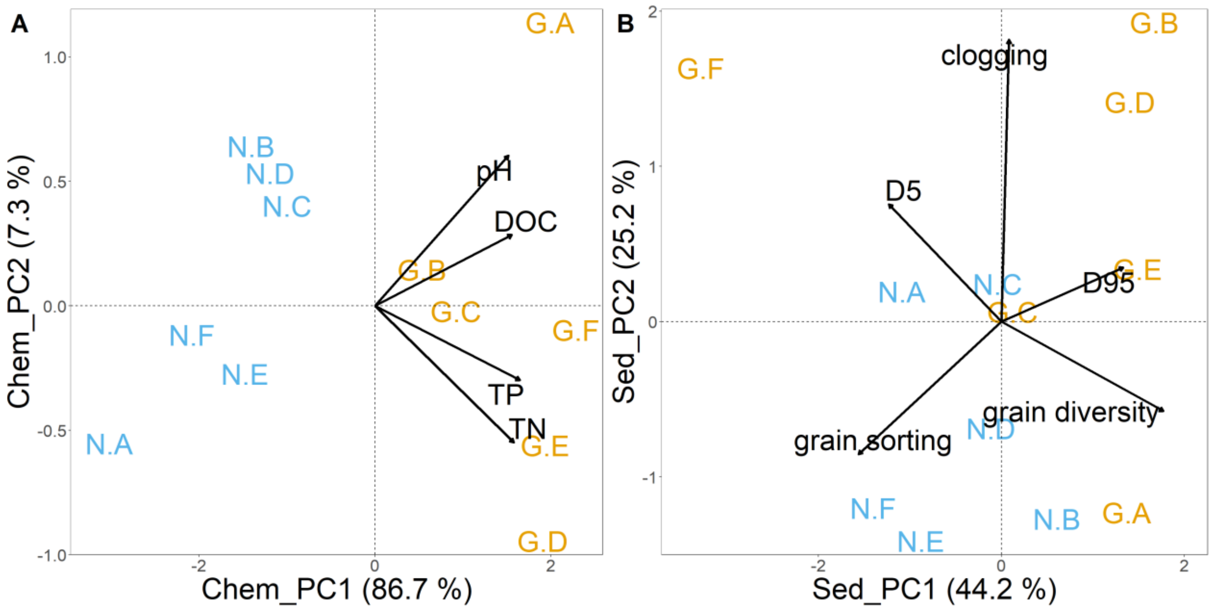

Fig. 2 – A) Principal component analysis of physico-chemical parameters for the different sampling sites along the Glatt (orange) and Necker (blue). DOC – dissolved organic carbon, TP – total phosphorus, TP – total nitrogen. B) Principal component analysis of stream-bed characteristics. Clogging – degree of stream bed clogging by fine sediment, D5 – grain size percentile D5, D95 – grain size percentile D95, grain diversity – Shannon grain diversity.

3.2 Biomass export via emergent insects

3.2.1 Patterns of biomass export

Biomass export via emergent insects showed strong site and season specific variation, ranging from 1.0 to 80 mg m^{-2} day^{-1}. Highest biomass export occurred in summer with highest peaks in July for almost all sites in both streams (Fig. 3A). Total biomass export did not differ significantly between streams in spring (Glatt: 7.9 ± 9.6 mg m^{-2} day^{-1}; Necker: 7.3 ± 8.5 mg m^{-2} day^{-1} ; $p > 0.05$) or summer (Glatt: 17.7 ± 15.2 mg m^{-2} day^{-1}; Necker: 14.2 ± 18.2 mg m^{-2} day^{-1} ; $p > 0.05$) , but there was a statistically higher total export from the Glatt than the Necker in autumn (Glatt: 7.9 ± 5.7 mg m^{-2} day^{-1}; Necker: 4.6 ± 5.7 mg m^{-2} day^{-1}; $p < 0.001$) (Fig. 3B).

The high biomass export in summer was mainly driven by flying emergence, whereas crawling emergence peaked in spring and autumn. Biomass export via crawling emergence was generally lower than via flying emergence, and only at some sites in spring (N.A, N.B) and autumn (G.A, N.F) were levels similar between emergence modes. Flying emergence export was highest at the Glatt during all seasons ($p < 0.05$). We found significantly higher crawling emergence export at the Necker (3.0 ± 6.8 mg m^{-2} day^{-1}) than the Glatt (0.6 ± 1.3 mg m^{-2} day^{-1}; $p < 0.01$) in spring, especially at sites N.A (6.7 ± 6.4 mg m^{-2} day^{-1}) and N.B (9.5 ± 12.6 mg m^{-2} day^{-1}). At the Glatt, sites G.A (1.1 ± 0.7 mg m^{-2} day^{-1}) and G.C (1.8 ± 2.5 mg m^{-2} day^{-1}) had highest crawling emergence export in spring, while crawling emergence export was close to zero (< 0.3 mg m^{-2} day^{-1}) at the other sites. We found no significant patterns present in both streams when comparing crawling, flying and total biomass export along environmental gradients (chem_PC1, chem_PC2, sed_PC1, sed_PC2) within each stream (supplement B).

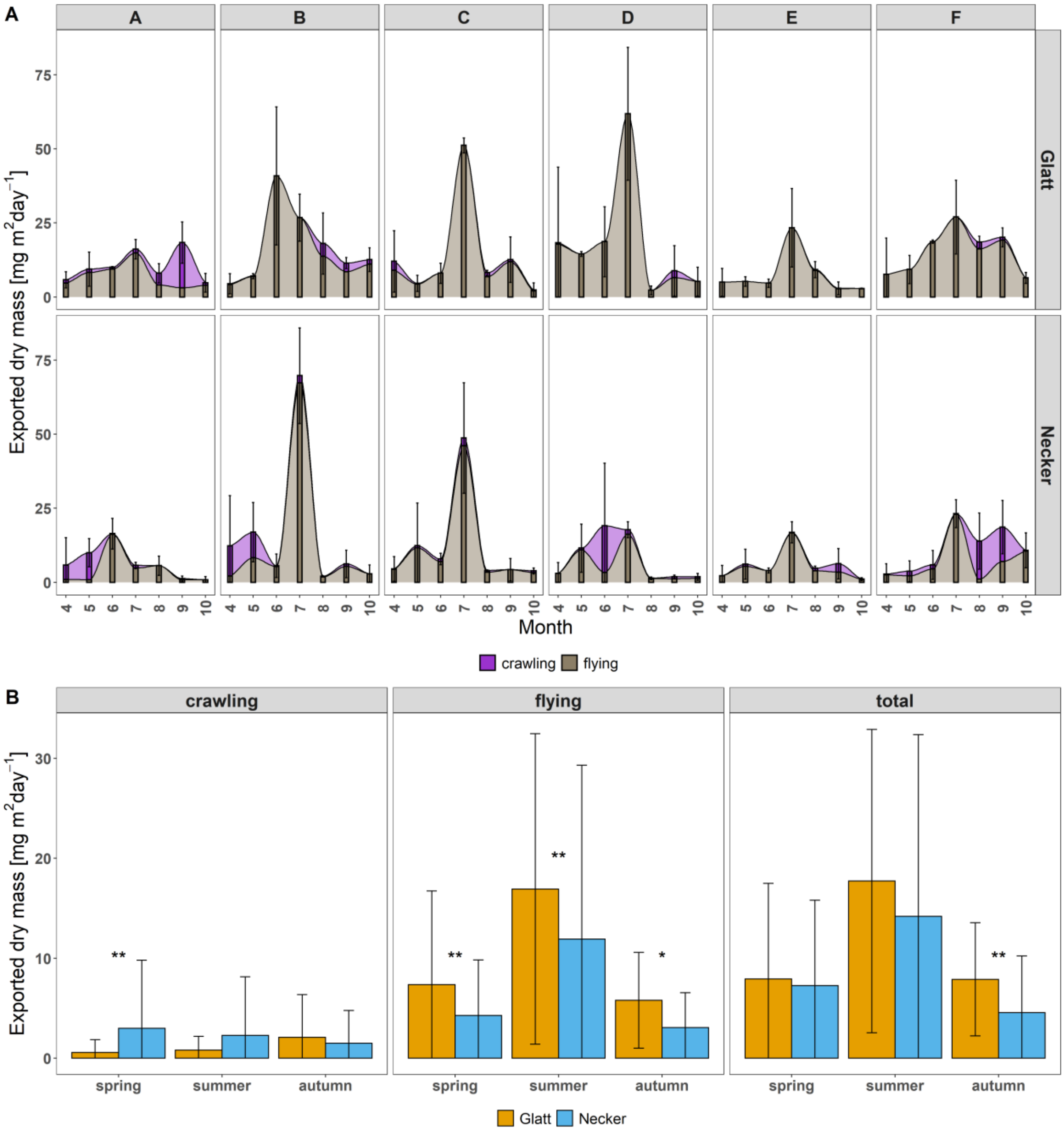

Fig. 3 – A) Dry mass export by emerging aquatic insects through two different emergence modes “flying” (grey) and “crawling” (purple) for the Glatt (upper row) and Necker (lower row). Bars represent monthly means and error bars are standard deviation. Curves represent estimation of dry mass export based on a local smoother function. B) Comparison of biomass export via emergent aquatic insects in spring, summer and autumn in the two streams. Bars represent mean export per season, error bars are standard deviation, and asterisks show significant differences.

3.2.2 Community composition in spring

In spring, 4 % of the variation in emergent aquatic insect composition was explained by stream identity (PERMANOVA: F = 4.0, p = 0.003). The three main taxa contributing to the separation were Chironomidae, Baetidae and Taeniopterygidae (SIMPER: 78 %). Comparing sites, G.A with the most natural stream-bed characteristics in the Glatt, clustered with the Necker sites in the nMDS (Fig. 4). Site G.A also clustered with the Necker sites in the PCA comparing stream-bed characteristics among sites. When we considered environmental PC scores as factors explaining community composition, all terms together explained 10 % of the variance (PERMANOVA: F = 2.2, p = 0.002), but no single term had a significant effect (PERMANOVA, $p > 0.05$). However, the gradient of clogging by fine sediment (sed_PC2) matched the distribution of sites in the nMDS in which crawling taxa (purple in plot) at low fine sediment sites and flying taxa (grey in plot) at higher fine sediment sites drove community separation (Fig. 4).

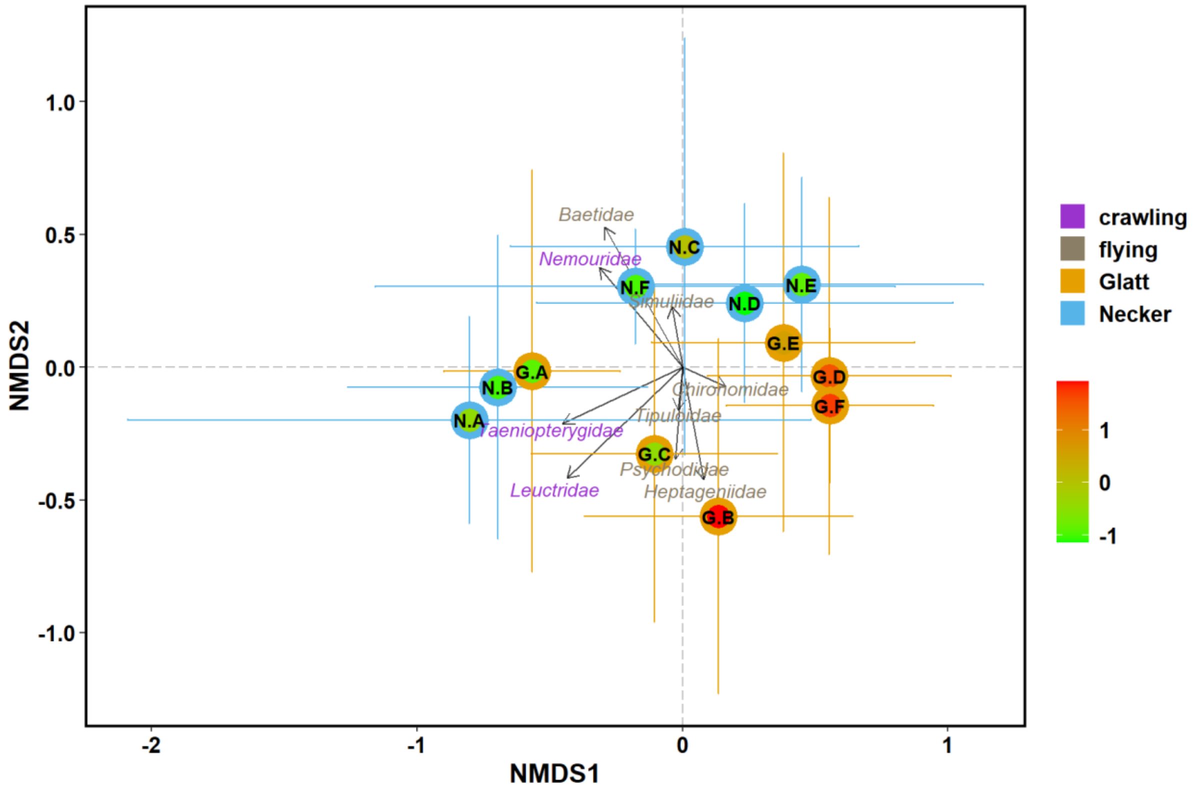

Fig. 4 – Non-metric multidimensional scaling (nMDS) of dry mass export for different insect families at the two study streams in spring (stress level = 0.21). Color of symbol border is stream identity, and color of symbol indicates degree of clogging by fine sediment (sed_PC2 score) at the respective site. Text color represents emergence mode of respective insect group. Points represent mean and error bars are standard deviation.

3.3 PUFA content in emergent insects

3.3.1 Individual PUFAs

3.3.1.1 Differences among orders

In all emergent aquatic insect orders investigated here, EPA made up the largest proportion of all PUFAs (18.8 ± 6.3 % of total fatty acids), with no significant differences found among insect orders ($p > 0.05$) (Fig. 5A). EPA content calculated per dry mass was significantly higher in Ephemeroptera than in other insect orders ($p < 0.001$), while patterns for the other PUFAs were similar when calculated as percentage or per dry mass (for per dry mass see supplement C). ALA was the second most abundant omega-3 PUFA. ALA content was higher in Plecoptera (13.0 ±5.7 %) than in the other orders (Ephemeroptera: 5.1 ± 2.4 %, $p < 0.001$; Diptera: 6.4 ± 2.8 %, $p < 0.001$) in spring, whereas no difference was found among orders during the other seasons ($p > 0.05$). SDA was only present in relatively low quantities (< 10 % of total fatty acids) and content did not differ among insect orders in spring ($p > 0.05$) but was significantly higher in Ephemeroptera than Plecoptera and Diptera in summer and autumn ($p < 0.001$). LIN was the most abundant omega-6 PUFA and LIN content was significantly higher in Diptera (17.7 ± 7.8 %) and Plecoptera (13.2 ± 6.2 %) than Ephemeroptera (3.5 ± 1.6 % $p < 0.001$) in all seasons. ARA was only present in relatively low quantities (< 10 % of total fatty acids) and, except in spring, ARA content was also significantly higher in Diptera (summer: 5.1 ± 2.1 %; autumn: 3.5 ± 2.1 %) and Plecoptera (summer: 7.2 ± 2.2 %; autumn: 6.1 ± 1.6 %) than Ephemeroptera (summer: 1.3 ± 0.8 %; autumn: 0.8 ± 0.6 %, $p < 0.001$). Therefore, with both higher LIN and ARA content Diptera and Plecoptera contained, in general, more omega-6 PUFAs than Ephemeroptera. For details about seasonal differences in PUFA content see supplement D.

3.3.1.2 Stream specific differences

SDA content in all insect groups in spring was significantly higher at the Necker (Diptera: 5.9 ± 3.7 %, $p < 0.001$; Plecoptera: 7.5 ± 4.4 %, $p < 0.001$; Ephemeroptera: 6.1 ± 2.9 %, $p < 0.05$) than the Glatt (Diptera: 2.6 ± 2.9 %, Plecoptera: 1.1 ± 1.5 %, Ephemeroptera: 2.9 ± 1.6 %). We found no other significant differences in PUFA content of emergent insects between the two streams (Fig. 5A).

3.3.2 Multivariate comparison

Insect order explained 27 % (PERMANOVA: $F = 53.9$, $p = 0.001$) of the differences in PUFA profiles followed by season at 10 % (PERMONOVA: $F = 19.4$, $p = 0.001$). Stream identity only explained 1.5 % (PERMAONVA: $F = 6.0$, $p = 0.001$) of the variation in PUFA profiles. The interaction terms of "order:season" and "season:river" were also significant. The PCA revealed a separation between grazing Ephemeroptera (Baetidae, Heptageindae) and other emerging insects (Diptera: Chironomidae, Plecoptera), especially in summer and autumn (Fig. 5B) (separation among insect orders: PERMANOVA: spring: $F = 13.8$, $p = 0.001$, $R2 = 0.25$; summer: $F = 38.5$, $p = 0.001$, $R2 = 0.50$; autumn:

F = 19.7, p = 0.001, R2 = 0.38). While season explained only 11 % of the differences in Ephemeroptera (PERMANOVA: F = 4.2, p = 0.001), season explained 26 % of the differences in Diptera (PERMANOVA: F = 14.5, p = 0.001) and 28 % in Plecoptera (PERMANOVA: F = 13.8, p = 0.001) (supplement D, Fig.1)

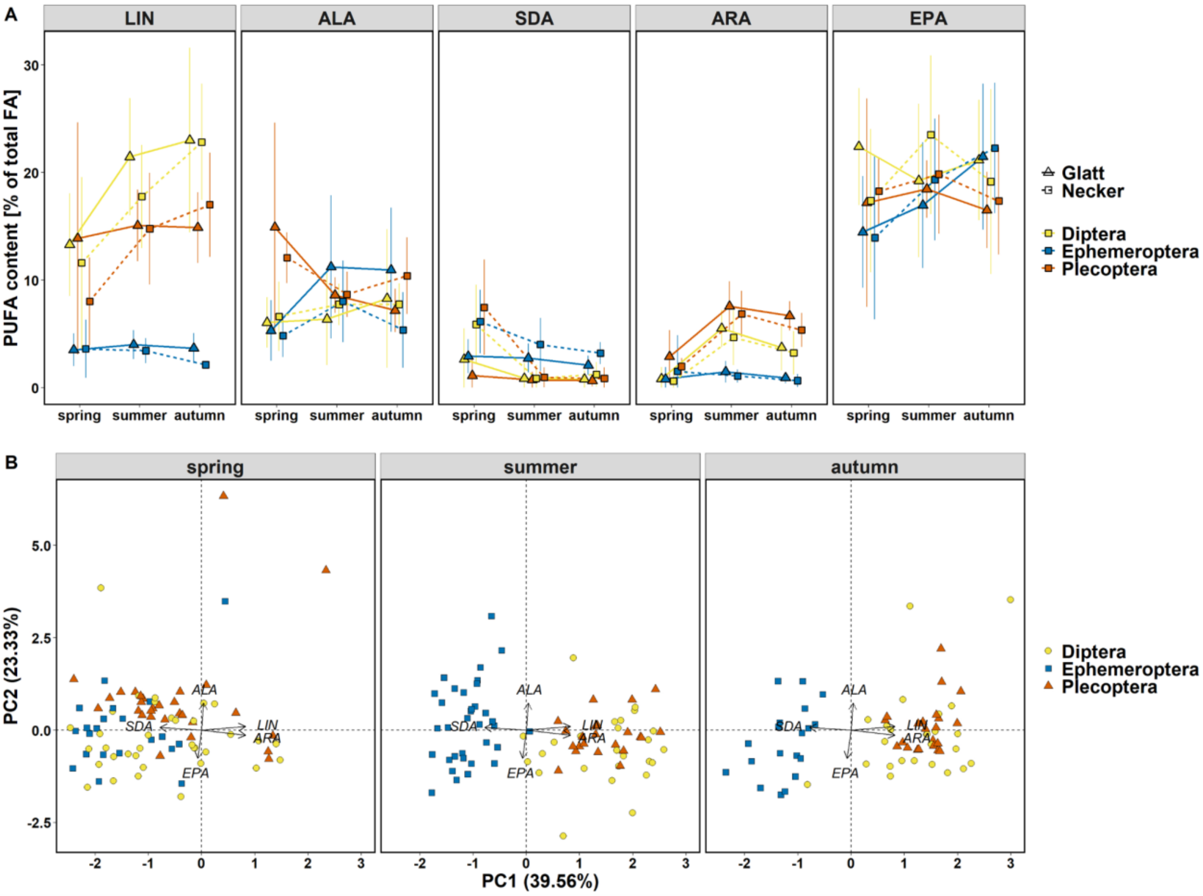

Fig. 5 – A) Individual PUFA content (as % of total fatty acids) in Diptera, Ephemeroptera and Plecoptera by stream (Glatt, Necker) and season. Points represent mean content and error bars are standard deviation. B) Principal component analysis of PUFA profiles of emergent aquatic insects, highlighting differences among orders. *LIN* - linolenic acid, *ALA* - alpha-linolenic acid, *SDA* - stearidonic acid, *ARA* - arachidonic acid, *EPA* - eicosapentaenoic acid.

We found no significant differences in PUFA content between streams in summer and autumn, but in spring 9 % of variation in PUFA content in Diptera (PERMANOVA: F = 3.6, p = 0.005) and 18 % in Plecoptera (PERMANOVA: F = 6.0, p = 0.002) was explained by stream. LIN and EPA contributed mainly to the differences between streams (SIMPER: Diptera: 48 %; Plecoptera: 63 %). However, site G.A again clustered with the Necker sites in spring for Diptera (Fig. 6). Environmental PCs did not explain the variance in PUFA profiles.

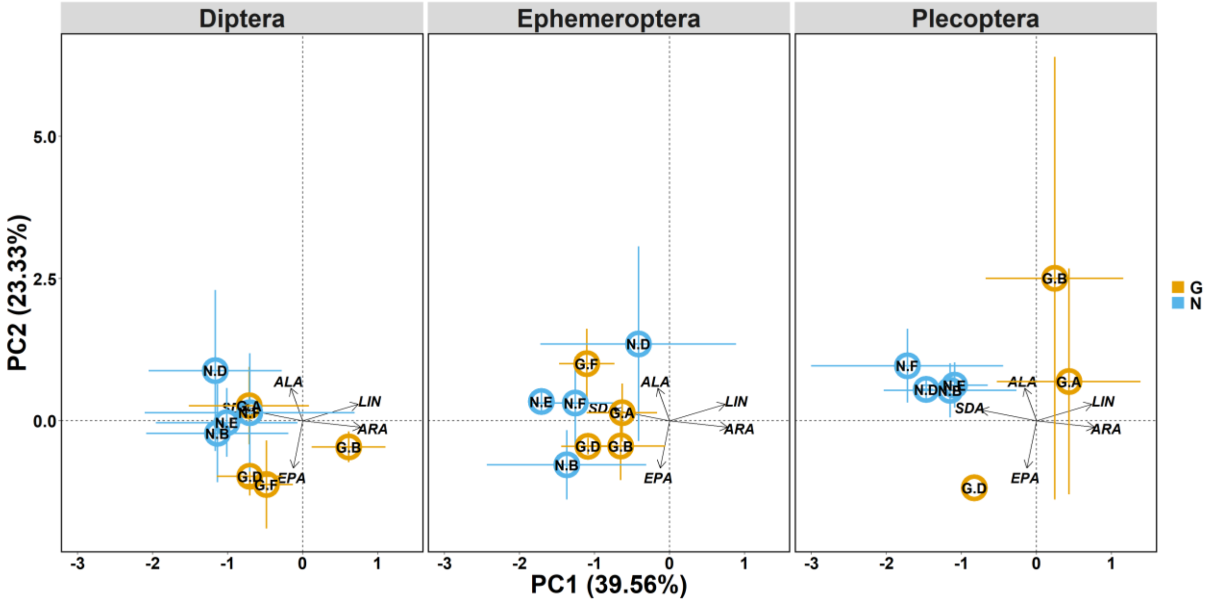

Fig. 6 - Principal component analysis of PUFA profiles of emergent aquatic insects, highlighting differences among sites in spring. Symbol color indicates stream identity (Glatt and Necker). Points represent means and error bars are standard deviation. *LIN* – linolenic acid, *ALA* – alpha-linolenic acid, *SDA* – stearidonic acid, *ARA* – arachidonic acid, *EPA* – eicosapentaenoic acid.

3.4 Estimation of EPA export via emergent insects

Total EPA export followed a similar temporal pattern as biomass export, as EPA content in emergent insects did not significantly differ among seasons (Supplement E). In spring, estimated average export was 0.13 ± 0.18 mg m^{-2} day^{-1} at the Glatt and 0.09 ± 0.12 mg m^{-2} day^{-1} at the Necker. Highest EPA export occurred in summer with up to ~1 mg m^{-2} day^{-1} on some sampling dates. Sites G.D (0.44 ± 0.55 mg m^{-2} day^{-1}) and N.B (0.40 ± 0.53 mg m^{-2} day^{-1}) were sites with the highest average EPA export (Supplement D). On average, 0.27 ± 0.30 mg m^{-2} day^{-1} EPA was exported from the Glatt and 0.23 ± 0.30 mg m^{-2} day^{-1} from the Necker in summer. In autumn, average EPA export was 0.14 ± 0.09 mg m^{-2} day^{-1} at the Glatt and 0.07 ± 0.09 mg m^{-2} day^{-1} at the Necker. EPA export via crawling emergence was very low at the Glatt and especially in spring (no crawling export at sites G.D, G.E, G.F). In contrast, crawling emergence export was high at some sites at the Necker, especially N.B (0.13 ±0.01 mg m^{-2} day^{-1}). Total EPA export was estimated to be 41 ± 15 mg m^{-2} y^{-1} at the Glatt and 32 ± 15 mg m^{-2} y^{-1} at the Necker (study period: April - October).

3.5 EPA transfer to ground dwelling spiders

Dry mass export via crawling emergence was one of the main differences among sites in spring, with highest exports at sites N.A and N.B (Fig. 7A). We also found differences in EPA content in ground-dwelling spiders among sites in spring (Fig. 7B). There was a relationship between biomass exported via crawling emergence (stoneflies) and EPA content in ground-dwelling spiders with EPA making up only 16.0 ± 6.2 % of total fatty acids in spiders at sites with no stonefly emergence, but 27.3 ± 3.0 % at sites with highest stonefly emergence in spring. The correlation between EPA content in ground-dwelling spiders and stonefly biomass was significant at the Necker ($F = 8.24$, $p = 0.012$) but not at the Glatt ($F = 2.41$, $p = 0.14$). Combining data from both streams we found a significant effect of stonefly biomass on EPA content in ground-dwelling spiders ($p = 0.002$, $F = 11.87$), while stream had no significant effect ($F = 0.93$, $p = 0.34$) (Fig. 7C).

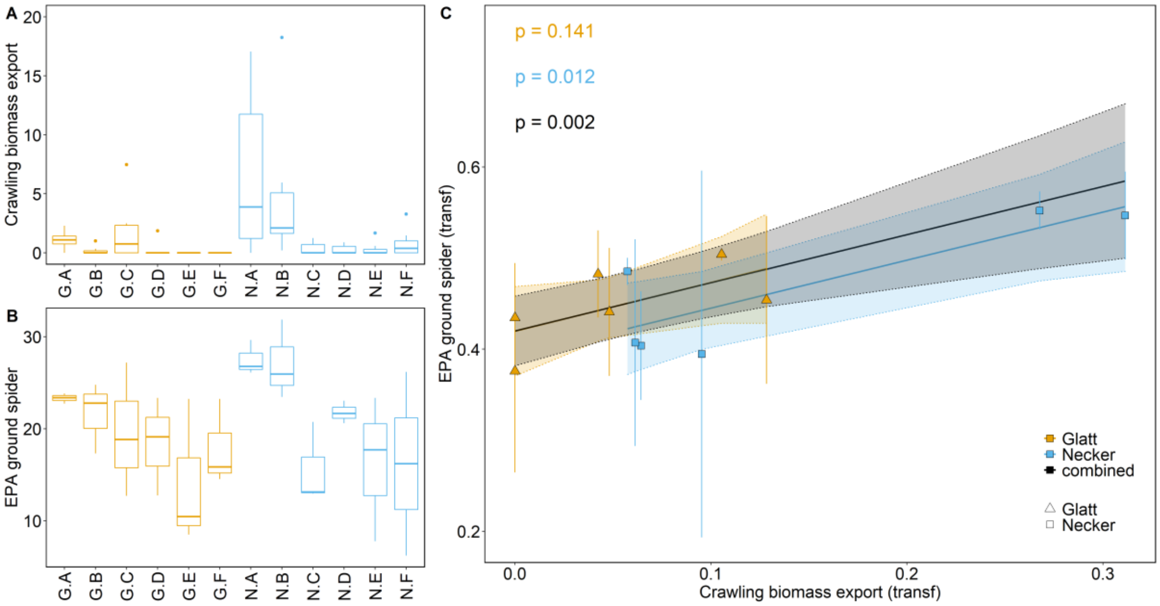

Fig. 7 - A) Plecoptera dry mass export (mg m^2 day^{-1}) per site at the Glatt (orange) and Necker (blue) in spring. B) EPA content (% of total fatty acids) in ground-dwelling spiders per site in spring. C) Association between stonefly biomass and EPA content in ground-dwelling spiders at the two study streams (Glatt and Necker).

4. Discussion

4.1 Stream differences in physico-chemical and stream-bed characteristics

The two study streams differed in nutrient levels, most likely due to clear differences in land use (Omernik 1976). Sites along each stream also displayed differences in environmental conditions. For instance, we saw a slight increase in nutrients in the Necker further downstream, albeit nutrients were at low levels, whereas we found a significant difference between the three upper sites and lower sites at the Glatt. Here, a wastewater treatment plant between the sites G.C and G.D likely explains the increase in nutrient levels at the three downstream sites. In addition, although stream-bed characteristics was more similar than expected, we found a higher surface fine sediment content and degree of clogging in the Glatt than Necker, especially at sites below barriers. For instance, site G.A, which was upstream of all major barriers, had low levels of clogging and fine sediment. Site G.C also had relatively low degree of clogging by fine sediment, despite being below a barrier. Here, and in contrast to other sites, stream banks between the barrier and the sampling site were not easily erodible and therefore little fine sediment entered the system. At other sites in the Glatt, especially G.B, strong bank erosion was visible and likely caused the high degree of clogging we observed.

4.2 Quantitative differences in biomass export of aquatic insects

Biomass export via emergent aquatic insects was strongly dependent on season and site-specific characteristics, with strong variations in emergence likely linked to organism life cycles and weather-related fluctuations in emergence. For example, Ivković et al. (2013) showed that temperature and local weather conditions (e.g. cloudiness) can have a strong influence on emergence. While we expected a major peak of aquatic emergence in spring (Corbet 1964, Nakano and Murakami 2001), peak export was in July at most sites and corresponded with highest water temperatures (supplement F). It is likely that we missed some of the temporal dynamics of emergence due to the non-continuous emergent aquatic insect sampling, as mass emergence can happen during relatively narrow time frames (Sweeney and Vannote 1982, Anderson et al. 2019). For example, Anderson et al. (2019) found some emergence events lasted for only 4-8 days. However, continuous sampling was not possible due to limited resources and recurrent high flow events, during which emergent insects trap deployment was not possible. Total biomass export at both streams was at similar levels in spring and summer, and even higher at the Glatt than Necker in autumn. Complex interactions among different environmental stressors and insect families might explain the observed biomass export patterns; so-called subsidy-stress relationships. For instance, increased nutrient availability, up to a threshold, boost insect population growth via bottom-up effects, while high fine sediment loads have been shown to counteract this effect (Townsend et al. 2008, Matthaei et al. 2010). Total insect biomass export can be affected both in a positive and negative direction depending on the strength and interaction of different stressors.

Importantly, community composition changes and sensitive invertebrate groups, especially EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa, typically are replaced by more generalist species with high fine sediment and nutrient loads (Wood and Armitage 1997, Miltner et al. 1998, Niyogi et al. 2007). In particular, many stonefly taxa are sensitive to high levels of nutrients, excessive fine sediment deposits and habitat degradation (Relyea et al. 2000, Lock and Goethals 2014). Because of the unique emergence mode of stoneflies, lower stonefly emergence equals lower crawling emergence, potentially reducing subsidy transfer to ground-dwelling predators. Our results corroborate this as we observed a reduced biomass export by stoneflies at the Glatt in spring. In summer, stonefly emergence was low in both streams, therefore observed differences were small. In autumn, we observed a stonefly emergence peak at a few Glatt sites, consisting of Leuctridae (supplement G), a stonefly family that seems to be less sensitive to environmental stress (Extence et al. 2013); thus we found no difference in crawling emergence in autumn. Sediment sensitive stonefly families (Nemouridae, Taeniopterygidae) occurred mainly in spring at the Necker and to some extent at the less impaired Glatt site (G.A). It needs to be further investigated how efficient taxa using crawling emergence were caught in comparison to flying taxa. The majority of individuals using crawling emergence were caught in the traps placed close to shore. The differences found between amounts of flying and crawling emergence might be biased by different trapping efficiencies. However, the differences in crawling emergence found between sites should not be affected by a potential sampling bias because the same method was used at all sites.

We also observed within stream differences in emergent insect composition associated with environmental conditions. The most upstream site at the Glatt (G.A), which had, with low levels of fine sediment, stream-bed characteristics comparable to those at the Necker, demonstrated a similar composition of emergent insects as Necker sites, while other Glatt sites with altered stream-bed characteristics also differed in insect groups contributing to the biomass export. Combined environmental PCs were significant predictor variables for variance in composition, but no single environmental PC had a significant effect, likely because high variability in biomass export among sampling dates made comparison of patterns among sites difficult. Although no single environmental PC was found to be a significant predictor variable, the gradient of clogging by fine sediment (sed_PC2) matched the distribution of sites with taxa that emerge via crawling. Those sites with high degree of clogging by fine sediment were associated primarily with taxa tolerant to fine sediment such as Chironomidae that emerge via flight, while a low degree of clogging by fine sediment was associated with those that use crawling emergence, especially the two stonefly families Nemouridae and Taeniopterygidae.

4.3 Qualitative differences in biomass export of aquatic insects

PUFA profiles differed among insect groups, with highest levels of EPA content per biomass in Ephemeroptera (mayflies) as reported previously (Martin-Creuzburg et al. 2017, Twining et al. 2021). With their high EPA content and large size, mayflies are valuable prey for riparian predators, especially those that predominantly catch flying prey (e.g., birds, web-building spiders). In contrast, mayflies may be more difficult to access for ground-dwelling predators. In this study system, mayflies were estimated to make up only a small proportion (< 10 %) of the diet of ground-dwelling spiders (Kowarik et al. 2021). EPA content per biomass in stoneflies was lower, but stoneflies are more accessible to ground-dwelling spiders and we found that stoneflies were especially abundant in early spring when aquatic subsidies are particularly important for riparian predators (Nakano and Murakami 2001). Mayflies, on the other hand, were abundant later in the season, in particular during June and July, when other terrestrial prey items were available to spiders as well. While PUFA profiles were similar during all seasons in mayflies, we observed a shift in PUFA profiles in Diptera and Plecoptera. In Plecoptera, this shift could be largely explained by seasonal shifts in the presence of families. Taeniopterygidae and Nemouridae were only found in spring while Leuctridae were present in all seasons. However, we did also observe a small shift in Leuctridae PUFA profiles between spring and summer (supplement H), likely due to differences at the base of food chain among seasons.

Apart from seasonal differences, the position of sites in the stream network, such as altitude, might also drive differences in community composition as well as PUFA profiles (Grubaugh et al. 1996, Guo et al. 2021). In this study, longitudinal changes did not explain compositional differences among sites, as sites G.A and G.B were near each other with minimal altitudinal difference, but we saw an abrupt shift between these two sites in assemblage composition and PUFA profiles, and not gradually downstream. Here, it is likely that stream-bed characteristics (especially clogging) was an important factor because nutrient levels were comparable but the degree of clogging by fine sediment changed drastically between sites due to a large barrier disrupting the sediment continuum. Downstream of the barrier, we saw enhanced clogging/fine sediment content and altered community structure with stoneflies contributing less to biomass export at the site below the barrier.

We expected a difference in EPA content between streams due to changes at the base of the food chain (periphyton). For example, Scharnweber et al. (2019) found lower EPA in aquatic insects (Chironomidae) with increasing phosphorus concentrations in a mesocosm study. In our study, periphyton at the base of the aquatic food chain contained comparable levels of EPA in both streams (higher at some Glatt sites in spring) (supplement I). Total EPA content in emergent insects was similar at the Glatt and Necker as well. However, the lack of the differences in EPA may be associated with the levels observed in our study sites. Scharnweber et al. (2019) tested much higher phosphorus concentrations (20 -1000 µg/l) than the highest values found at our sites (max P levels < 100µg l^{-1}). As such, we found no reduction in total EPA export in the range of environmental conditions we observed. It may be that stronger human degradation might lead to differences in EPA transfer into riparian systems. While EPA content was relatively similar in emergent insects in both rivers, there was a stream separation of PUFA profiles of emergent insects, especially for Diptera. Only the least degraded Glatt site (G.A) clustered with the more pristine Necker sites. Some of this variation in PUFA composition (especially SDA) between sites occurred at the base of the food chain (supplement J), suggesting that different PUFA composition in periphyton can influence the quality of PUFA export. It remains unclear why this similarity in PUFA profiles to periphyton was not visible in the grazing Ephemeroptera, but internal processes like bioconversion and selective retention may play a role (Kühmayer et al. 2020, Steinberg 2022).

4.4 Emergent aquatic insects as vectors of EPA export

Emergent insects exported biomass and EPA from both the natural and more degraded stream network to adjacent riparian areas. On average, estimated total EPA export per square meter was 30-40 mg over the sampling season (April to November). While aquatic insect emergence also takes place during winter (ice free), this happens only at low levels (Nakano and Murakami 2001), therefore our estimate is comparable to yearly exports. Our values are on the low range of other studies that calculated EPA + DHA export from ~40 up to 4500 mg m^{-2} y^{-1} for different biomes (Gladyshev et al. 2019), but were similar to export from a small German lake (~33 mg m^{-2} y^{-1}) (Martin-Creuzburg et al. 2017). In contrast to other studies, export of DHA via emergent insects was close to zero and therefore not quantified here; most likely because copepods that appear able to synthesize DHA (Nanton and Castell 1998, Nielsen et al. 2019) were not an important part of the food web. In Martin-Creuzburg et al. (2017), predacious *Chaoborus* that are known to prefer copepods as food sources (Swift and Fedorenko 1975, Pastorok 1980) contributed to DHA export, but they were not found in the our study.

Although total EPA export was comparable between streams, there was still a large anthropogenic impact associated with stream degradation. Specifically, less biomass and EPA export came from stonefly emergence in the degraded stream, thus likely being less accessible to ground-dwelling predators like riparian wolf spiders. While there was no connection between EPA content in aquatic insects and ground spiders, and no difference in EPA content in ground-dwelling spiders between the two streams (Kowarik et al. 2021), differences in EPA content in ground-dwelling spiders among sites were correlated with emergent stonefly biomass. Indeed, sites with low or no emergent stonefly biomass also had ground-dwelling spiders with lower EPA content in spring, indicating that ground-dwelling spiders were to some degree dependent on aquatic insects with a crawling emergence to build up EPA deposits. Most stoneflies are sensitive to habitat degradation, thus the transfer of EPA to ground-dwelling riparian predators might be strongly affected by instream habitat degradation. EPA content in ground spiders has been linked to immune function (Fritz et al. 2017). The question remains how important aquatic EPA is for riparian predators. While Hixson et al. (2015) highlight aquatic systems as the principal EPA source, the soil food web has been suggested as an alternative source of EPA (Menzel et al. 2018). However, EPA content decreases with distance from the stream edge (Chari et al. 2020), linking EPA to aquatic systems. Internal conversion from dietary precursors is another possible source of long-chain PUFAs. Recent studies suggest that some web-building spiders can convert dietary precursor PUFAs to long chain PUFAs such as EPA (Mathieu-Resuge et al. 2021, Twining et al. 2021). Further research is required to assess the ability of different predators to cover their EPA demands from metabolic processes, however taking EPA up with the diet likely improves fitness and is less costly.

The loss of stoneflies as a result of instream degradation endangers the existence of an export pathway leading to ground-dwelling predators. While we could not disentangle the effects of high nutrients and fine sediment, the stark contrast between sites G.A and G.B, which were similar in nutrients, suggests fine sediment as an important factor driving lower levels of crawling emergence. Further research is needed to assess the consequences of human impact in aquatic systems on stonefly populations and the cascading effects to riparian ecosystems.

5. Conclusion

Emergent aquatic insects from both systems transported EPA acquired during their larval stages to riparian areas. In the range of environmental conditions studied here, the EPA content in emergent aquatic insects and total biomass and EPA export by emergent aquatic insects was unaffected. Our results indicate however, that when assessing the effects of stream degradation, not just total insect biomass export but the potential modifications to assemblage composition and how those translate to specific mechanisms of PUFA export is important. We highlight, that functional traits, like mode of emergence, unique to specific taxa, must be considered because they relate to certain pathways of resource transfer between aquatic and terrestrial ecosystems. In our study, a distinct shift in insect groups contributing to the biomass export at degraded sites in spring (loss of stoneflies) impacted the functional connectivity to ground-dwelling predators. Although the total export was at similar levels, the EPA export via crawling emergence, accessible to ground-dwelling predators, was reduced. Lower EPA content in ground-dwelling spiders at sites with low stonefly emergence suggests at least a partial dependence of riparian ground-dwelling spiders on this crawling emergence pathway to acquire EPA in adequate quantities. Importantly, stonefly decline is a general result of degradation that can weaken aquatic-terrestrial linkages with potential effects for other ground-dwelling predators such as floodplain beetles, amphibians and lizards.

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7. Author contributions

Conceptualization: CK, CR, CW, DMC, KM; formal analysis: CK, KM; writing - original draft preparation: CK, CR; writing - review and editing: CK, CR, CW, DMC, KM; visualization: CK; project administration: CK, CR; funding acquisition: CR, CW; All authors have read and agreed to the submitted version of the manuscript.

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CHAPTER 4 - FACTSHEET: AQUATIC–TERRESTRIAL RESOURCE FLUXES

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Abstract

This chapter focuses on how rivers and their surrounding landscapes are closely linked, and how resource fluxes between these systems are important for maintaining aquatic and terrestrial biodiversity. It includes a discussion of the export of biomass and specific nutrients, so-called omega-3 PUFAs, as a crucial ecosystem service provided by healthy aquatic systems. Management and restoration projects should take into account this lateral connectivity to improve the success of restoration measures.

1. Importance of cross-boundary fluxes from aquatic to terrestrial systems

Rivers and the adjacent floodplains and riparian areas are interactive, open units connected along multiple pathways (Baxter et al. 2005). Here, we take a closer look into cross-boundary resource fluxes that involve, in this context, the exchange of organic resources (biomass and nutrients) between adjacent aquatic and terrestrial ecosystems (Fig. 1). Resource fluxes occur in both directions, e.g. via leaf litter input into streams and the emergence of aquatic insects into terrestrial systems, creating what Baxter et al. (2005) call a ‘tangled web’. Such cross-boundary fluxes can play crucial roles in sustaining recipient systems.

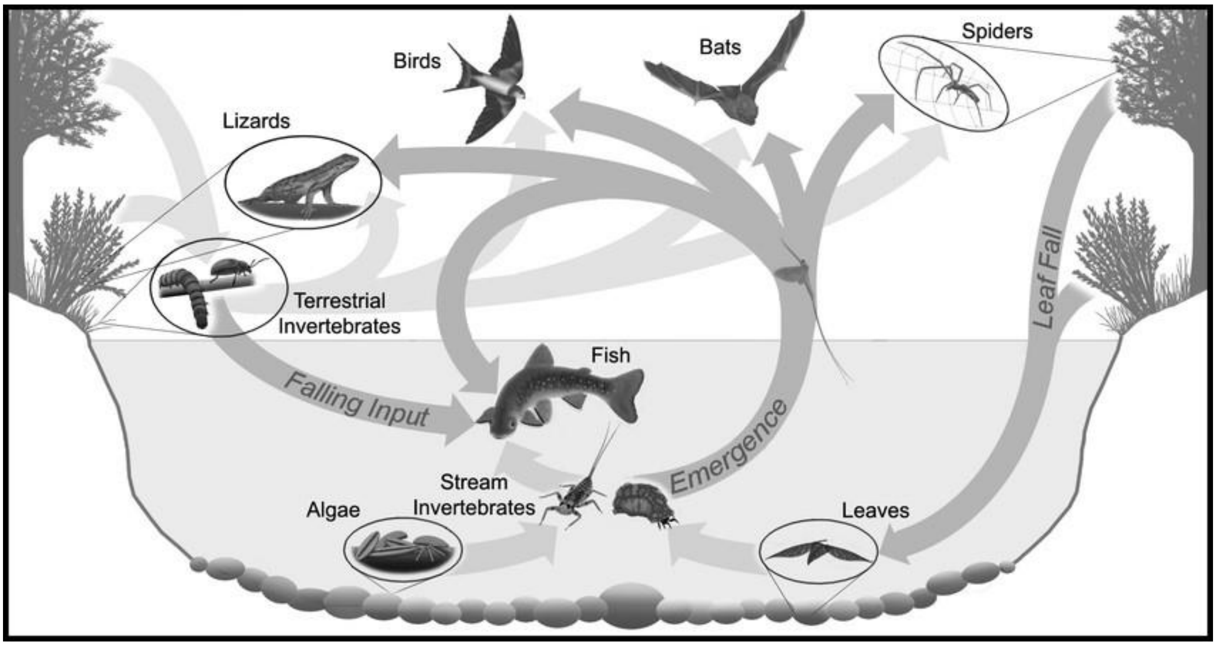
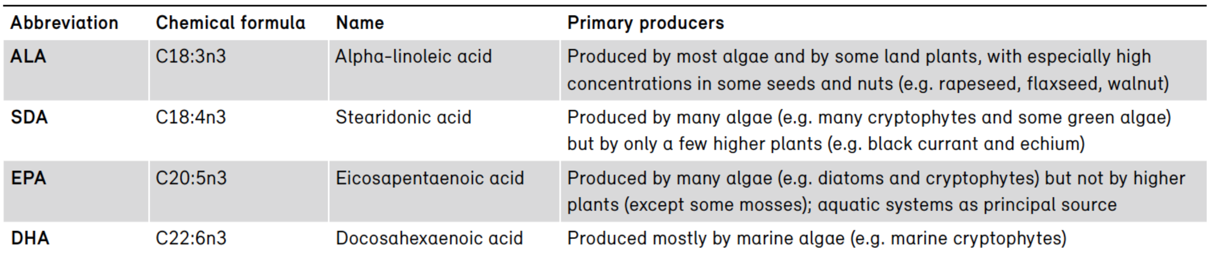


Figure 1: Schematic of cross-boundary resource fluxes between a stream and the surrounding landscape. Source: Baxter et al. (2005)

The present chapter focuses on resource subsidies from aquatic to adjacent terrestrial ecosystems. Aquatic-derived resources provide an additional food source for riparian predators such as spiders, e.g. in the form of emerging aquatic insects. Many aquatic insects have life histories in which the larval stage is aquatic, and the adult reproductive stage is terrestrial. The timing of aquatic subsidies reflects the life histories of local assemblages and leads to seasonal resource pulses. Aquatic insect emergence, especially in spring, provides an important supplement for riparian predators at a time when terrestrial resources are low in abundance. Various studies have shown that riparian predators, such as spiders and birds, are seasonally dependent on aquatic resource subsidies (Iwata et al. 2003, Paetzold et al. 2005, Burdon and Harding 2008).

Aquatic-derived resources not only represent an additional food source, but also contain an important nutrient in low supply in terrestrial ecosystems, the well-known omega-3 fatty acid EPA (Table 1). We find high concentrations of EPA in fish, making them a beneficial food source also for humans, and in other aquatic organisms like insects. In fact, aquatic ecosystems are considered a principal source of EPA (Hixson et al. 2015). EPA belongs to the group of polyunsaturated fatty acids (PUFAs; Table 1) that contain multiple double bonds, which only specific organism groups can produce. While several algal groups, e.g. diatoms, produce large amounts of EPA and it therefore accumulates in aquatic food chains, terrestrial plants, except for some mosses, completely lack this ability (Harwood 1996, Uttaro 2006, Hixson et al. 2015); this makes EPA-rich organisms (aquatic insects) a resource in high demand in terrestrial ecosystems. Preliminary estimates indicate that the quantity of PUFAs exported from aquatic systems can be substantial (Fig. 2), providing an important cross-boundary ecosystem service (Gladyshev et al. 2013).

Table 1 - Important omega-3 polyunsaturated fatty acids (PUFAs).



Abbreviation	Chemical formula	Name	Primary producers
ALA	C18:3n3	Alpha-linoleic acid	Produced by most algae and by some land plants, with especially high concentrations in some seeds and nuts (e.g. rapeseed, flaxseed, walnut)
SDA	C18:4n3	Stearidonic acid	Produced by many algae (e.g. many cryptophytes and some green algae) but by only a few higher plants (e.g. black currant and echium)
EPA	C20:5n3	Eicosapentaenoic acid	Produced by many algae (e.g. diatoms and cryptophytes) but not by higher plants (except some mosses); aquatic systems as principal source
DHA	C22:6n3	Docosahexaenoic acid	Produced mostly by marine algae (e.g. marine cryptophytes)

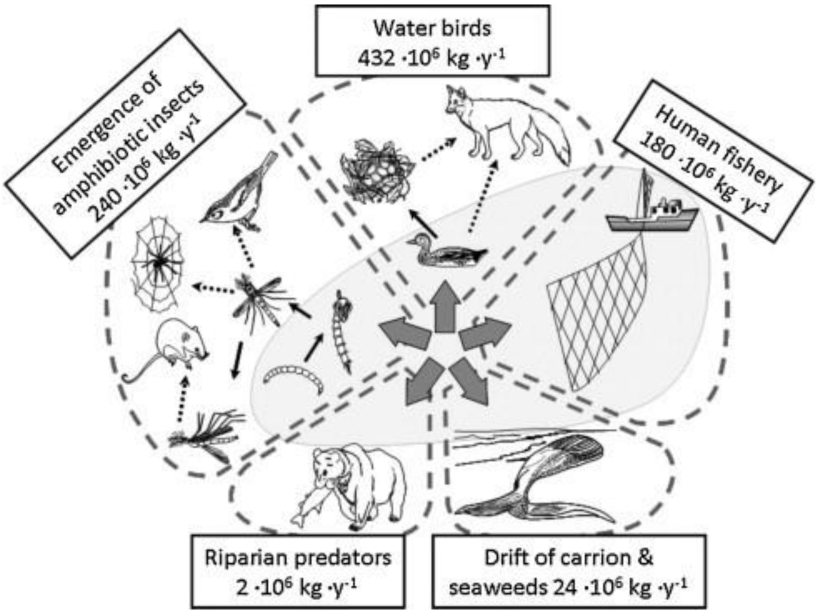


Figure 2: Estimated annual (y) export of EPA + DHA (see Table 2) via different pathways, illustrating the magnitude and importance of this ecosystem service provided by aquatic systems. Source: Gladyshev et al. (2009)

But why are PUFAs so important? In animals, including humans, PUFAs are involved in many physiological processes. They are, for example, essential parts of our cell membranes, have important functions in our immune system, and play a role in signal transduction in the body (Stillwell and Wassall 2003, Stanley 2014, Schlotz et al. 2016). In short, PUFAs are essential for survival und need to be taken up with the diet. Although some organisms can convert other PUFAs to EPA, this process is generally inefficient, and EPA uptake via the diet is thus quite important. In support of this, studies on riparian predators have demonstrated, for example, a positive effect of aquatic-derived EPA fluxes on the development and breeding success of riparian birds, such as tree swallows (Twining et al. 2016, 2018), and on the immune system of riparian spiders (Fritz et al. 2017).

Humans have altered most aquatic ecosystems and especially rivers and streams, in both morphology and water chemistry, thereby causing the ‘dark side of subsidies’ via the cross-boundary transfer of micropollutants and heavy metals (Kraus 2019). Healthy freshwaters clearly sustain the positive side of cross-boundary resource fluxes to adjacent terrestrial systems as an ecosystem service. The extent to which human activities impact aquatic resource subsidies, in terms of both quantity and quality, remains unknown. Some 25% of Swiss running waterways are in a poor eco-morphological state. Specifically, over 100,000 artificial barriers blocking sediment movement occur on Swiss rivers, critically degrading streambed conditions for biota (FOEN), and river shoreline length has been substantially reduced by straightening and shoreline fortifications. Emerging aquatic insect and insectivore bird abundance are positively correlated with shoreline length (Iwata et al. 2003), meaning that less natural river networks with a shorter shoreline may be associated with reduced PUFA transfer. By modifying both rivers and adjacent riparian zones, human activities and infrastructures clearly influence the distribution and amount of cross-boundary resource exchange and flux (Laeser et al. 2005, Paetzold et al. 2011).

Despite the important ecological role of cross-boundary resource subsidies within the context of multi-dimensional riverscapes, they have been largely neglected in practical management. In future projects, restoration measures should therefore account for the lateral connectivity along rivers to incorporate cross-boundary resource fluxes.

2. Aquatic–terrestrial resource subsidies - data from Switzerland

Here we present results about resource subsidies from aquatic to terrestrial systems along two contrasting rivers in Canton St Gallen (Fig. 3a). The Necker (N) is a mostly unregulated river with a natural flow and sediment regime, whereas the adjacent Glatt river (G) is highly regulated, with multiple barriers that alter the flow and sediment regime. Land use also differs between the two catchments, with the Glatt having poorer water quality (higher nitrogen and phosphorus levels) than the Necker. We selected six sites along each river to assess aquatic resource subsidies to adjacent terrestrial ecosystems. We focused on emergent aquatic insects and the export of aquatic-derived PUFAs to two riparian predators (ground-dwelling and web-building spiders). Ground-dwelling spiders (ground spiders) are roaming predators in riparian areas, whereas web-building spiders (web spiders) are stationary predators, catching prey in their webs. Here, we address various aspects of resource subsidies along the two rivers.

2.1 How does regulation influence environmental gradients along river networks?

We evaluated various sediment characteristics, such as grain size distribution and internal colmation. We observed an increase in fine sediment and colmation at sites below structures (barriers) blocking bed movement. Along the Glatt river, the most upstream site (G.A) still had a natural sediment signature, but this changed rapidly downstream of the first structure (G.B). This change in habitat properties is shown in a principal component analysis (PCA) plot (Fig. 3b), where sites that are depicted close to each other have similar bed characteristics and arrows represent different reasons for a separation. G.A clusters with the more natural sites of the Necker river because it has less fine material, while G.B and the other Glatt sites are farther away because it has a higher degree of colmation.

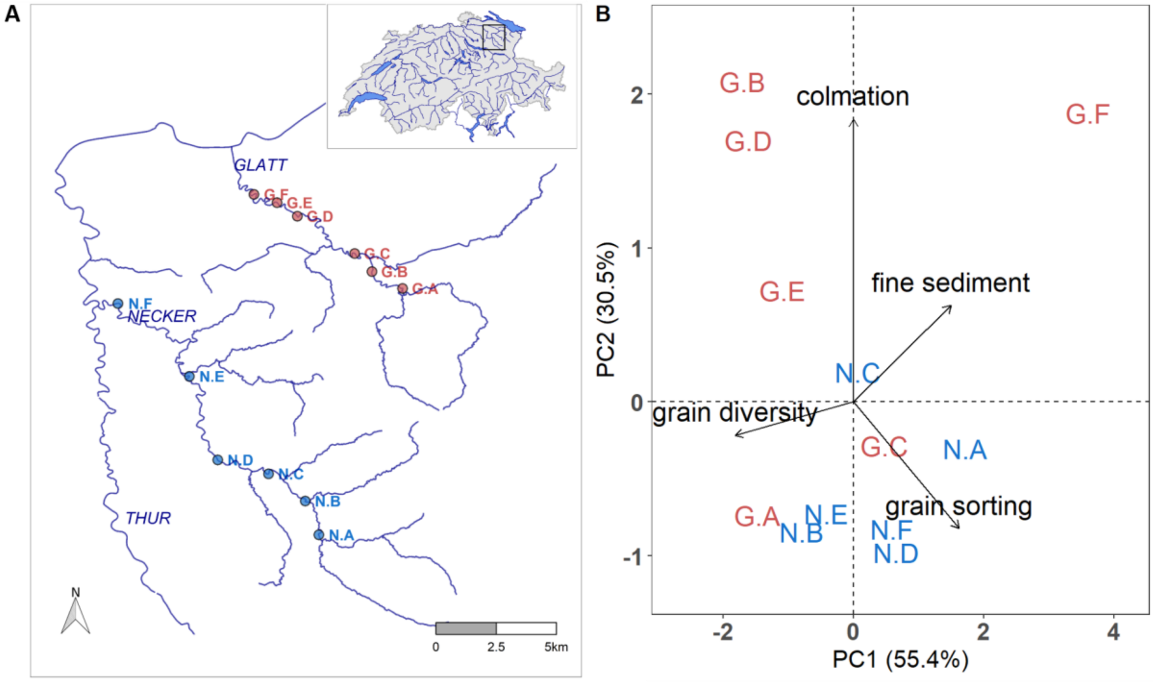
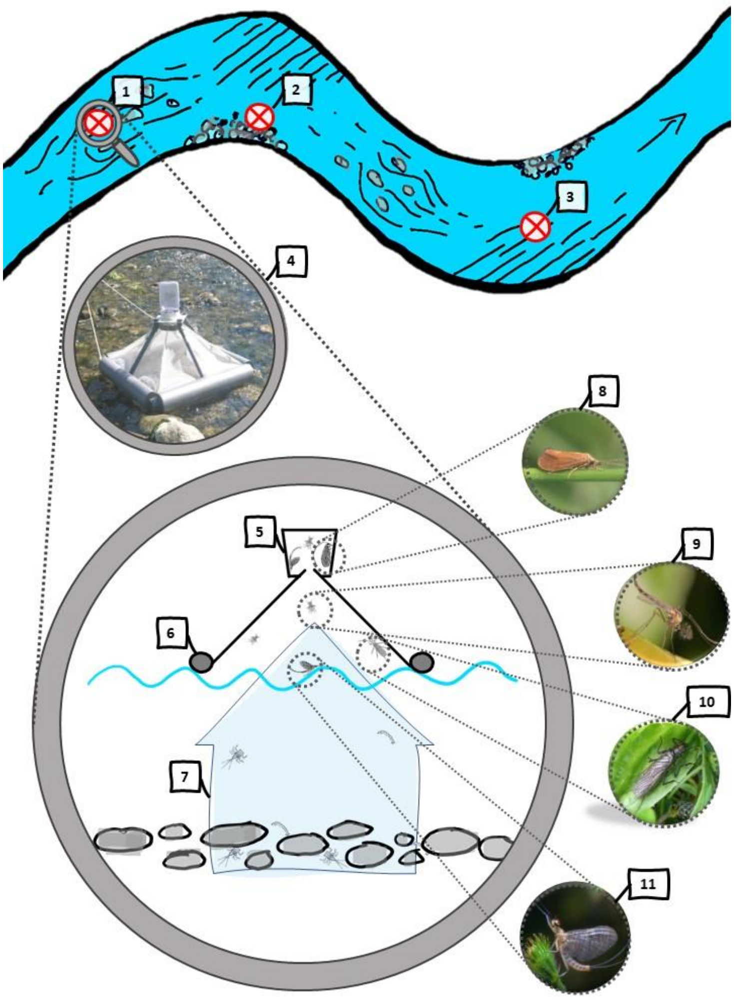


Figure 3: A) Map of sampling sites along the rivers Glatt (G) and Necker (N). B) Principal component analysis (PCA) plot showing the difference in habitat properties between the two rivers. The axes represent dimensions 1 and 2 of the PCA, and the percentage of variance explained by each dimension is given. Sediment variables of colmation, grain (size) diversity, fine sediment (amount) and grain (size) sorting are represented as arrows.

2.2 How does stream degradation influence aquatic subsidies?

Flow regulation often causes habitat degradation in rivers, which typically translates to changes in the communities and abundances of macroinvertebrates in regulated waters relative to free-flowing watercourses. Consequently, the quality and quantity of resource subsidies transferred to adjacent riparian areas also differ. We compared insect biomass export along a bed degradation gradient in the Glatt and Necker rivers, using colmation as a proxy for bed degradation (see Fig.4 for methods and Fig. 5 for results).



Biomass export was estimated using three floating emergence traps per river reach to cover different habitat types: riffle 1 edge 2 and pool 3
.

The emergence traps 4 were equipped with a bottle for insect collection 5 , styrofoam floaters 6 and covered with a net (mesh size 100 µm). The traps float on the river surface and cover an area of 0.25 m^2 each, all aquatic insects emerging from this area 7 are trapped and collected.

Emerging aquatic insects from four orders were collected:

8 Trichoptera (caddisflies)

9 Diptera (midges)

10 Plecoptera (stoneflies)

11 Ephemeroptera (mayflies).

Figure 4: Method for estimating biomass export in the form of emergent aquatic insects.

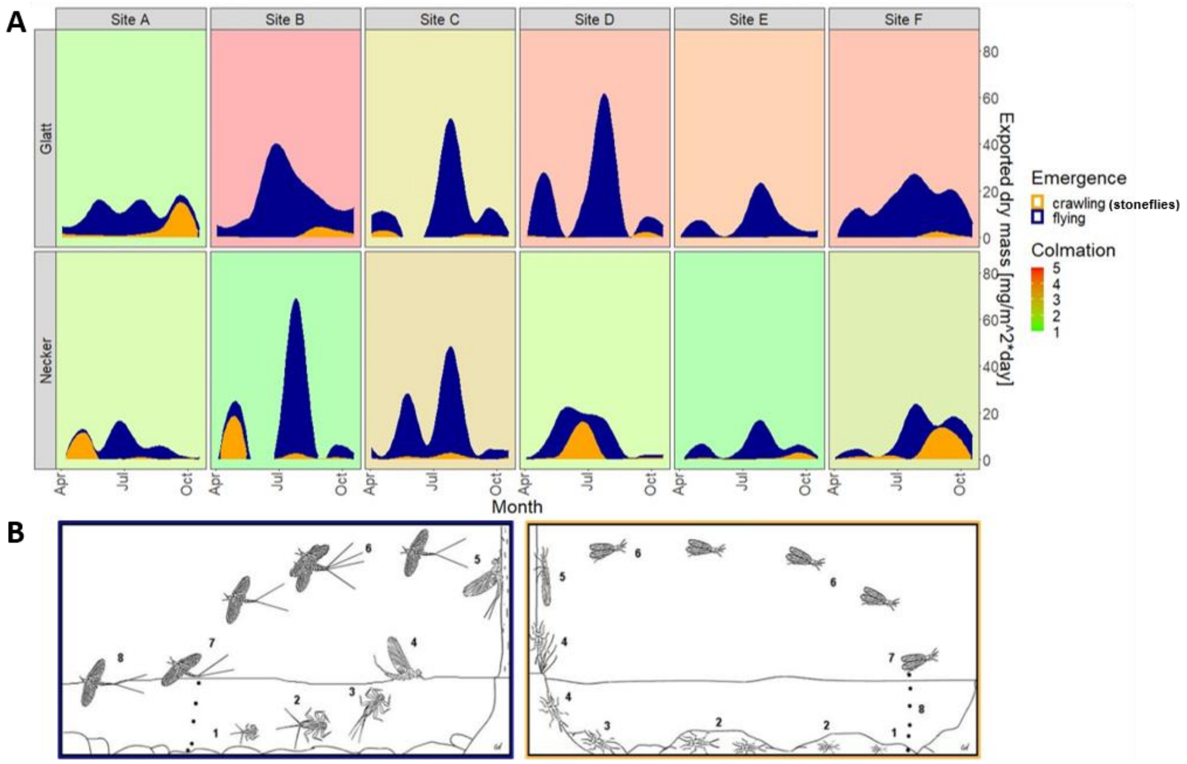


Figure 5: A) Estimation of biomass export in the form of emergent crawling (e.g. stonefly) and flying (e.g. caddisfly, mayfly) aquatic insects along the Glatt river (top row) and the Necker river (bottom row). The sites (A–F) correspond to those shown on the map in Figure 3. B) Illustration of the different emergence modes: flying versus crawling.
Source: B) adapted from http://www.delawareriverguide.net/insects/mayflycyc.html; http://www.delawareriverguide.net/insects/stoneflycyc.html.

No general decline in biomass export was observed with increasing colmation, but there was a change in community composition, with fewer emerging stoneflies in the Glatt than in the Necker river. While a peak in stonefly emergence in autumn, consisting of rather common stonefly species (*Leuctra* spp.), was visible to some extent at most sites along the Glatt river, the important peak in stonefly emergence in early spring was essentially missing along the Glatt river, with a low level of emergence occurring only at sites G.A and G.C (Fig. 5a). This early spring peak consisted of stonefly families that are more sensitive to environmental disturbances, such as an increased fine sediment load (Extence et al. 2013). This lack of stoneflies can have a large impact, as stoneflies express a different emergence behaviour than other aquatic insects, such as mayflies and caddisflies, which emerge in flight directly from the water column. In contrast, stoneflies crawl to shore before they emerge (Fig. 5b), thus representing an important cross-boundary pathway to ground-dwelling predators that is lost in streams without stoneflies (Fig. 6).

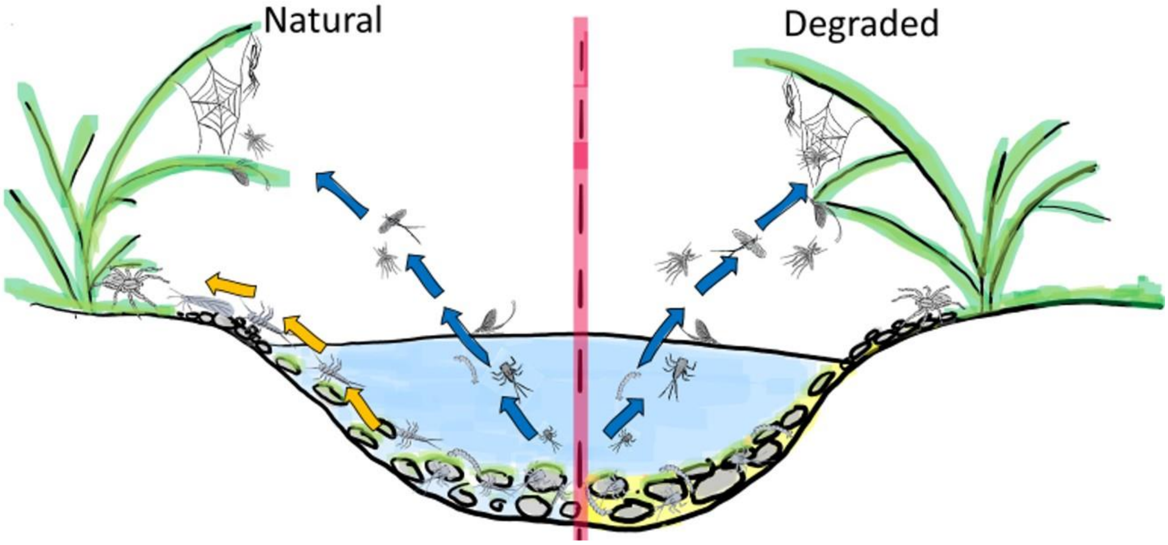


Figure 6: A potential consequence of stream degradation for the cross-ecosystem transfer of resource subsidies from aquatic ecosystems to riparian landscapes. The loss of stoneflies in degraded streams results in the loss of a resource pathway (yellow linkage) to adjacent riparian systems.

2.3 Do emergent insects transfer PUFAs and is there a difference between systems?

EPA and other PUFAs (i.e. ALA + SDA) predominantly found in aquatic environments were present in considerable concentrations in emergent insects (EPA: 15–25% of total fatty acids) and in riparian spiders along both the Glatt and the Necker river (Fig. 7). Web spiders and ground spiders had a similar ALA concentration (~4% of total fatty acids), and both had a very high EPA concentration (~15%) relative to other terrestrial organisms. SDA was higher in web spiders (1.4%) than in ground spiders (0.3%), indicating that predator type played a role in resource transfer.

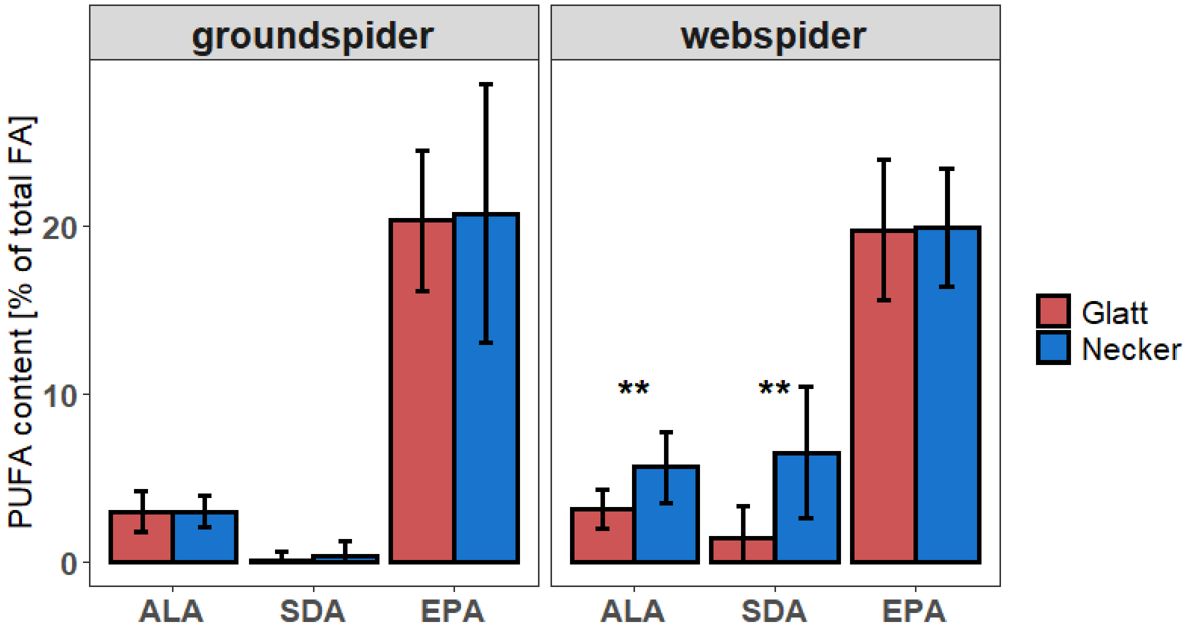


Figure 7: Mean (± SD) polyunsaturated fatty acid (PUFA: ALA, SDA and EPA; see Table 1) concentration, expressed as a percentage of the total fatty acid (FA) concentration in riparian ground

spiders and web spiders in the Glatt and Necker rivers. Asterisks represent significant differences between the two river systems at P <0.01.

We compared PUFA concentrations in riparian spiders between the two systems in spring. In ground spiders, we detected no significant differences. Web spiders, on the other hand, contained more SDA and ALA along the Necker than along the Glatt river, although there was no significant difference in EPA concentration (Fig. 7). We also measured PUFA concentrations in emergent insects and periphyton scraped from rock surfaces and found similar patterns, especially for SDA. It appears that the difference between the systems already occurred at the base of the food chain, potentially because of different environmental conditions. We conclude that SDA production and transfer in particular were very limited along the Glatt river, while the nutritionally important EPA was transferred in comparable quantities.

A closer look at the EPA concentration in riparian spiders reveals some interesting patterns. First, the EPA concentration of riparian spiders was dependent on the distance from shore. At site N.F, where spiders were sampled at different distances from the shore, EPA concentration declined with increasing distance from the shore, with values already lower around 40–50 m from the channel, especially in ground spiders (Fig. 8a). Although differences were not significant due to relatively small sampling size, this pattern is in line with previous findings (Chari et al. 2020) and demonstrates that access to aquatic insects is important for EPA transfer and accumulation. Second, looking at seasonal changes, the EPA concentration in both spider types was highest in spring (Fig. 8b). This finding suggest that emergent aquatic insects are especially important for PUFA transfer into riparian zones in spring.

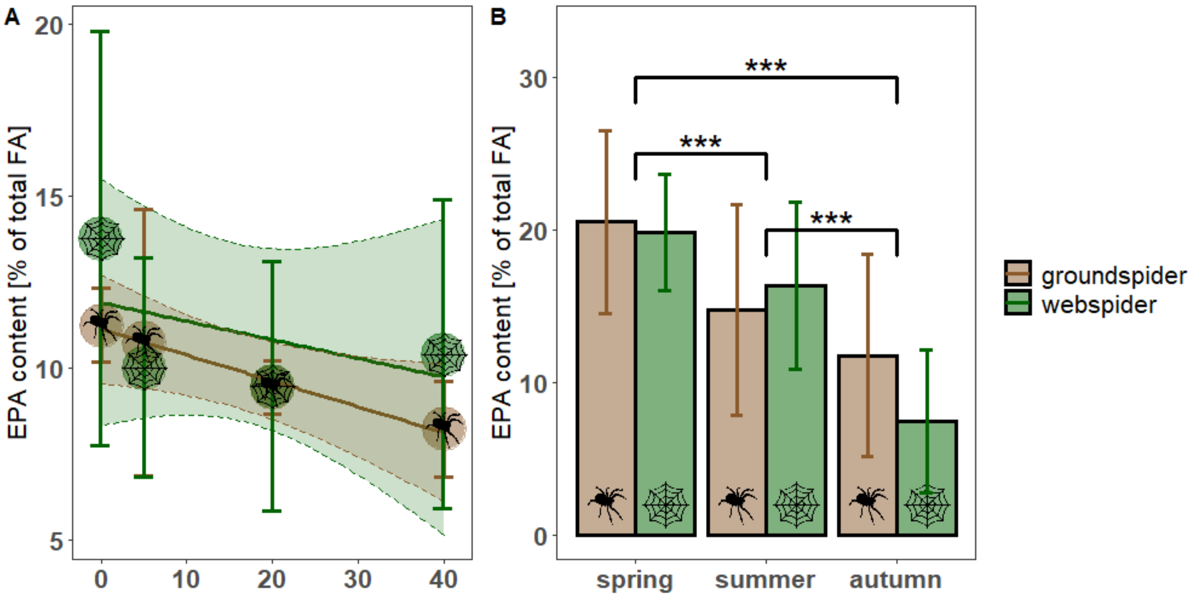


Figure 8: A) Mean ± SD EPA concentration in riparian ground and web spiders (site N.F) at different distances from the river shore. The shaded areas represent 95% confidence intervals. B) Seasonal differences in mean (± SD) EPA concentration in the two spider types, showing the importance of spring emergence. Asterisks represent significant differences between seasons (*** P <0.001).

We found no significant difference in the total EPA export/transfer between the natural Necker and the more degraded Glatt river. However, the difference in macroinvertebrate composition between streams, with reduced stonefly emergence in the Glatt (see Section 2.2), altered EPA availability for different kinds of riparian predators. While web spiders were largely unaffected, the EPA concentration in riparian ground spiders was lower in degraded sites with reduced stonefly emergence in spring (Fig. 9). As mentioned above, stoneflies have a specific ‘emergence mode’ involving crawling to shore. This behaviour makes them easy prey for ground-dwelling predators, while other insects that emerge by flight are much harder to catch. As the EPA concentration in ground spiders is linked with immune function (Fritz et al. 2017), less access to EPA, in this case resulting from reduced stonefly emergence, may have negative consequences on predator survival. Importantly, stonefly decline is a general problem in degraded streams; it weakens aquatic–terrestrial linkages, not only for riparian spiders but potentially also for other ground-dwelling riparian predators, such as lizards and beetles.

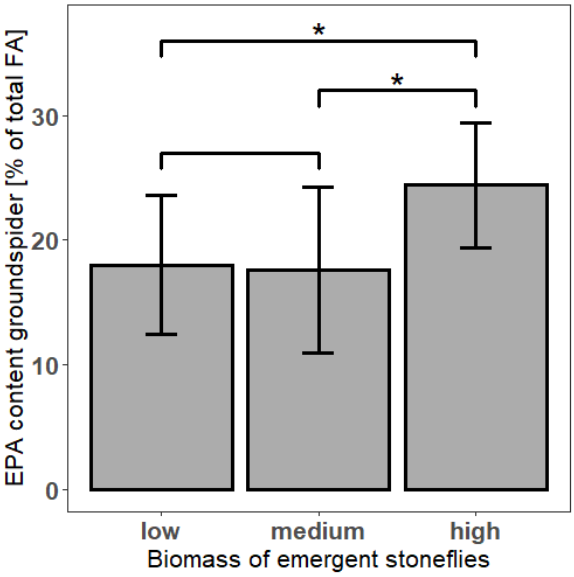


Figure 9: EPA concentration of riparian ground spiders in spring in relation to emergent stonefly biomass. Categories of stonefly biomass: low = dry mass <0.25 mg m^{-2} day^{-1}, medium ≤1 mg m^{-2} day^{-1}, high = dry mass >1 mg m^{-2} day^{-1}. Asterisks represent significant differences at $P < 0.05$.

3. Management implications

We show that both emergent aquatic insects and riparian spiders contain considerable concentrations of EPA and are thus a central link that promotes EPA transfer into terrestrial systems. Waterbodies, which provide aquatic subsidies, and riparian zones, which form the main habitat of riparian spiders, need to be in good ecological condition to sustain healthy populations. In riparian zones in particular, web spider density depends on riparian vegetation such as shrubs and trees (Laeser et al. 2005), and the PUFA concentration in spiders is higher if a riparian buffer zone is present (Ramberg et al. 2020). Conservation of the riverine zone, including a healthy watercourse, is therefore crucial for the maintenance of cross-boundary resource fluxes.

Research on Cross-boundary linkages provides a chance to inform and engage different stakeholders in riparian management projects, as suggested by Muehlbauer et al. (2019). Discussions of restoration projects should take a more holistic perspective, considering terrestrial and aquatic ecosystems in combination. For example, a bird conservation project might have low value if nearby waterbodies are in poor condition and cannot provide needed aquatic resource subsidies such as PUFAs. In this case, PUFA export should be considered a crucial ecosystem service. In this context, it is especially important to stop the general decline in stoneflies, which form a distinct export pathway easily accessible to ground-dwelling riparian predators. Stoneflies cannot live in streams with a poor ecological state, and thus this pathway and resource flux across ecosystem boundaries is lost in degraded riverscapes.

4. Acknowledgment

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SYNTHESIS

Why should we care about maintaining aquatic invertebrate diversity? The importance is often hard to get across, and while there are many possible approaches, my answer is clear: Connectivity. Even small changes in one area can translate to large alterations at another point in the network. While connectivity is a key concept in ecology, some current effects seen in our everyday lives in a globalized world might best explain issues associated with large networks. During the last two years, I have sometimes stood before half-empty shelves in our supermarket and certain products were just not available because of disruptions in the global network due to the covid-19 pandemic. The network is so complex that I was often even unaware what could have caused the disruption. I realized how fragile this connectivity is and how small changes at one point, such as a local lockdown, can tip the balance.

Similar problems occur in ecological connectivity, here not a pandemic, but anthropogenic alterations are often the cause, leading to changes in resource availability. Many of those connections are easy to miss or seem unimportant, but they can still be crucial. When, for example, thinking about streams or other aquatic systems, they are mainly perceived as a source of water, but aquatic systems also provide other ecosystem services that are often forgotten. For instance, nutrients are exported from aquatic systems by the emergence of amphibiotic organisms, such as insects, that spend only the first part of their life cycle in the aquatic environment. In terrestrial systems, those insects represent important prey items for different predators such as riparian spiders. One could argue that predators could find other terrestrial food sources if aquatic subsidies are not available. However, aquatic insects are special because they contain a lot of omega-3 PUFAs, especially EPA, accumulated during their larval stages. Terrestrial insects are not nutritionally reciprocal (Twining et al. 2019), they contain only 3- 25 % of the omega-3 LC-PUFAs of aquatic insects of the same biomass, therefore at least four terrestrial insects would be needed to gather the same amount of EPA (Mathieu-Resuge et al. 2021, Shipley et al. 2022).

Emergent aquatic insects and riparian spiders therefore represent a central link promoting the transfer of EPA to terrestrial systems. They form only the first steps of the food chain, and other animals such as lizards and birds then further distribute EPA throughout terrestrial ecosystems. Predicted changes in PUFA content at the base of aquatic food webs, with a reduced omega-3 PUFAs production (Hixson and Arts 2016, Colombo et al. 2020), can also reach us humans. We might not use aquatic insects as a food source (yet), but we still get a large proportion of our EPA and DHA from aquatic sources, especially from fish. An adequate provision might be an issue in the future, and already alternate sources like transgenic plants producing EPA and other PUFAs are being investigated (Ruiz-Lopez et al. 2013). However, most animals are dependent on natural sources and environmental changes altering nutritional landscapes are an issue.

In this thesis, I investigated human impacts, especially those connected to instream barriers and stream degradation, on lateral food web connectivity between streams and riparian zones with a focus on the biomass and PUFA export by emerging aquatic insects and their transfer to riparian spiders. I used two study systems in Switzerland in this thesis. In the first study system (**Chapter 1**), I investigated the local effects of sediment traps on biomass export by emergent aquatic insects and the contribution of aquatic subsidies to riparian spider diets using Sediment Trap and Control streams around Lake Sarnen. In the second study system (**Chapter 2 & 3**), I investigated two 6th order streams in the Thur catchment. While the first stream (Necker) had near natural conditions, the second stream (Glatt) suffered from habitat degradation due to multiple barriers. Here, I also compared biomass export by emergent aquatic insects. In addition, I compared PUFA content in emergent aquatic insects and PUFA transfer to riparian spiders between the more degraded and near-natural stream. In **Chapter 4,** results from **Chapter 2 & 3** were translated into a factsheet about the importance of lateral food web connectivity for practitioners and the general public.

Sediment traps and lateral connectivity

The cascading effects of instream alterations on surrounding terrestrial communities have been shown in different contexts such as stream pollution (Paetzold et al. 2011) or regulation (Jonsson et al. 2013). Even so, removal of a low had dam, for example, caused only minor changes to adjacent systems (Sullivan et al. 2018), and the sediment traps investigated in my first study appeared to have only minor effects on lateral connectivity (although the contribution of aquatic and terrestrial food sources could not be fully assessed due to overlapping isotope ratios). I found no difference in the total biomass export by emergent insects. While I observed some changes in groups contributing to the biomass export, differences between upstream and downstream sites were stream specific and this also occurred in control streams. One conclusion was that further study including different seasons to capture more emergence periods of specific taxa like stoneflies and using additional trophic markers are necessary to better clarify the effect of sediment traps on lateral food web connectivity. Sediment traps serve an important function in hazard mitigation and novel designs are continuously being developed (e.g., semi-permeable traps) (Schwindt et al. 2018, Moldenhauer-Roth et al. 2021) to reduce ecological impacts. A wider realization of such novel designs in practice could mitigate remaining problems associated with sediment traps.

Quantity and Quality

In my second study, the total biomass export by emergent insects was also similar in both stream networks. I then divided biomass export by mode of emergence into “crawling” and “flying” export. Crawling export was reduced in the degraded system compared to the near-natural stream in spring. In addition, nutritional quality was considered. While I did not find clear differences in EPA content in either emergent aquatic insect or in riparian spiders between the degraded and near-natural system, other PUFAs, especially the EPA precursor SDA, showed some stream-specific differences in spring, with less SDA in the more degraded system. This difference was found in all trophic levels from periphyton and emergent aquatic insects up to web-building spiders (less for ground spiders), clearly indicating aquatic insects are not only believed to be vectors of PUFA export, but that PUFAs are actually transferred to riparian spiders. The aquatic input also became apparent in the, for terrestrial organisms, unusually high EPA and content Σω3/Σω6 ratio found in riparian spiders. The Σω3/Σω6 ratio places riparian spiders somewhere between aquatic and terrestrial organisms, which might be typical for organisms "living at the edge" between systems.

Human impacts - "match – mismatch"

Mismatches between available and required nutrients are increasingly becoming a problem, with human alterations unhinging the existing balance and a growing body of research reporting mismatches for example in timing (Regular et al. 2014, Larsen et al. 2016, Visser and Gienapp 2019, Shipley et al. 2022). The dependence on aquatic subsidies makes riparian predators especially susceptible to changes in aquatic systems. Do human alterations linked to instream barriers also cause changes in lateral connectivity and cause mismatches? One aim of this thesis was to find answers to this question. In **Chapter 1**, it appeared that the effect of local structures disrupting sediment transport on lateral connectivity was small. The study system used in **Chapter 2 & 3** was more complex. Here, one of the largest problems was to find a study system that was only disrupted by barriers and lacking the presence of multiple other stressors like hydropeaking or flow abstraction. In addition to barriers, the Glatt system used in **Chapter 2 & 3** also had high nutrient levels. I could not fully disentangle the influence of the different factors, but excessive amounts of fine sediment appeared to play a major role in determining the groups contributing to biomass export. Most degraded sites with a high input of fines were missing the early spring stonefly peak that provides EPA easily accessible to ground-dwelling spiders (Figure 1). This result demonstrates a mismatch, which could be a problem also for other ground-dwelling hunters because stonefly decline is a general problem in degrade streams. For example, no stonefly emergence was reported from agricultural streams by Raitif et al. (2018). In this thesis, I highlight that functional traits and nutritional quality should be considered when thinking about potential mismatches and in assessing the effects of instream alterations that are transferred to terrestrial systems.

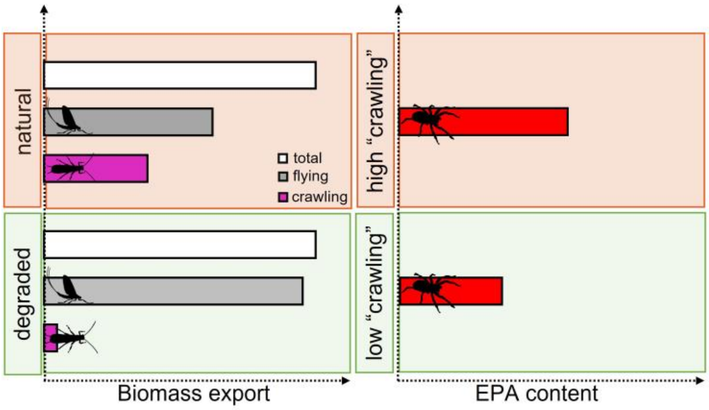

Figure 1: Concept how stream degradation can alter EPA transfer to ground-dwelling riparian predators, even if total biomass export remains stable.

Dietary tracers

In **Chapter 1,** I observed that C and N stable isotope ratios alone can be lacking as dietary tracers because isotope ratios of different food sources are often overlapping. In addition, a maximum of three food sources can be separated with C and N ratios. More dietary tracers are needed to differentiate among more sources and to be able to assess not only carbon sources but also the transfer of essential nutrients such as PUFAs. This was illustrated in **Chapter 2**, where I used both isotopes and PUFAs in combination with compound specific isotope analysis. When only considering carbon isotope ratios, ground spiders appeared to ingest relatively small amounts of aquatic prey, but EPA levels were similar to web spiders and compound specific analysis indicated an aquatic origin of EPA.

This apparent contradiction can be explained. As described in Shipley et al. (2022), it was calculated that even if 75 % of the ingested biomass is from terrestrial sources, 91% of EPA is from aquatic sources because of the low EPA content in terrestrial sources (Figure 2). PUFAs are a valuable addition to the toolbox that can be used to disentangle food webs, but have to be used with care due to the possibility of internal bioconversion and bioaccumulation. PUFAs should best be used with previous feeding experiments or in combination with compound specific isotope analysis of carbon and deuterium, which is a promising new tool that has been recently developed (Twining et al. 2020, Mathieu-Resuge et al. 2021).

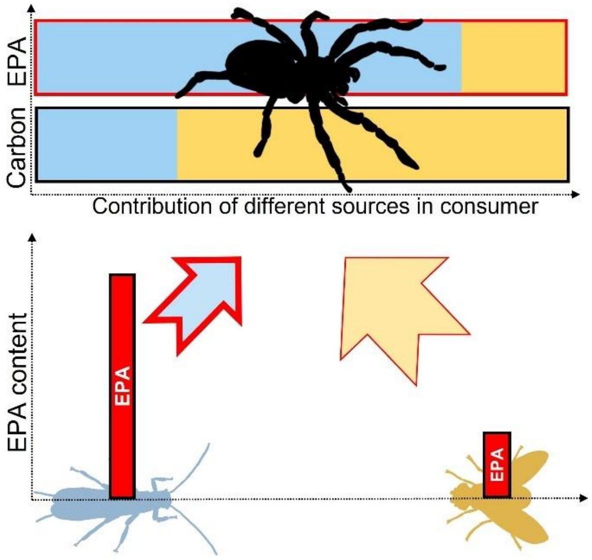

Figure 2: Contribution of aquatic (blue) and terrestrial (brown) sources to riparian ground-dwelling spider Carbon and EPA composition (adapted from Shipley et al. 2022).

Further perspectives

To be able to use PUFAs more widely as dietary tracers and evaluate the importance of aquatic EPA in terrestrial systems, we need to know more about PUFA metabolism by different species. Some first steps in this direction were taken, for example, by Kabeya et al. (2018), listing animals able to synthesize omega-3 PUFAs de novo. However, further research is needed on metabolic traits and the ability of different species for bioconversion using genetic analysis or compound specific isotope analysis of fatty acids and feeding experiments. To assess more dimensions of the cascading effects of instream alterations on surrounding terrestrial systems, a wider range of environmental conditions and morphological changes should be considered. In addition to emergent insects, other vectors like amphibians that export PUFAs (Fritz et al. 2019) should be included in research projects. Further, the effects of different PUFA supply on the fitness of riparian predators, such as shown by Twining et al. (2016), need to be more closely investigated. Further research in this area is important because the connections between aquatic and terrestrial systems appear crucial, but we yet have to fully understand many of the underlying mechanisms.

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APPENDIX

APPENDIX 1: SUPPLEMENTARY MATERIAL CHAPTER 1

Supplement A – Physico-chemical parameters

Table 1: Physico-chemical parameters

stream	**site**	**pH**	**Alkalinity (mmol l^{-1})**	**Nitrate (mg l^{-1})**	**Nitrite (µg l^{-1})**	**OP (mg l^{-1})**	**TP (mg l^{-1})**	**DOC (mg l^{-1})**	**TOC (mg l^{-1})**	**TN (mg l^{-1})**
Alt	DS	8.320	2.731	0.322	<1.0	0.003	0.011	1.724	2.028	<0.5
Alt	US	8.130	2.715	0.335	<1.0	0.002	0.005	1.757	2.456	<0.5
Biz	DS	8.180	3.432	0.456	<1.0	0.018	0.015	2.808	3.252	0.612
Biz	US	8.170	3.481	0.497	<1.0	0.013	0.018	3.528	3.561	0.669
Edi	DS	8.040	2.995	0.463	<1.0	0.001	0.002	1.536	1.647	0.518
Edi	US	8.150	3.085	0.471	<1.0	0.002	0.002	1.459	2.965	0.521
For	DS	8.260	4.242	1.120	1.300	0.031	0.034	2.091	2.450	1.348
For	US	8.090	4.257	1.108	1.200	0.033	0.036	1.983	3.341	1.311
Ruf	DS	7.980	3.221	0.766	<1.0	0.003	0.004	3.438	3.665	0.884
Ruf	US	7.960	3.050	0.740	<1.0	0.002	0.005	1.147	1.642	0.909
Zim	DS	8.080	3.697	0.410	<1.0	0.028	0.030	3.550	3.669	0.594
Zim	US	8.020	3.629	0.259	<1.0	0.037	0.041	4.829	5.003	<0.5

Supplement B - Community composition

Community composition showed large stream specific differences, and 60 % of the variation was explained by stream identity (PERMANOVA: F = 8.94, p = 0.001). Differences between US and DS were not significant in both control (PERMANOVA: F = 1.24, p = 0.265) and ST (PERMANOVA: F = 1.51, p = 0.169) streams. To take a closer look into differences between US and DS sites without interference of stream identity, we did an nMDS and subtracted DS values from US values. The difference between US and DS sites was close to zero in Control streams. In ST streams, we found negative values, especially for nMDS1, indicating a difference in community composition between US and DS sites. SEs were overlapping zero for Edi and Ruf, indicating that they were not significantly different from zero.

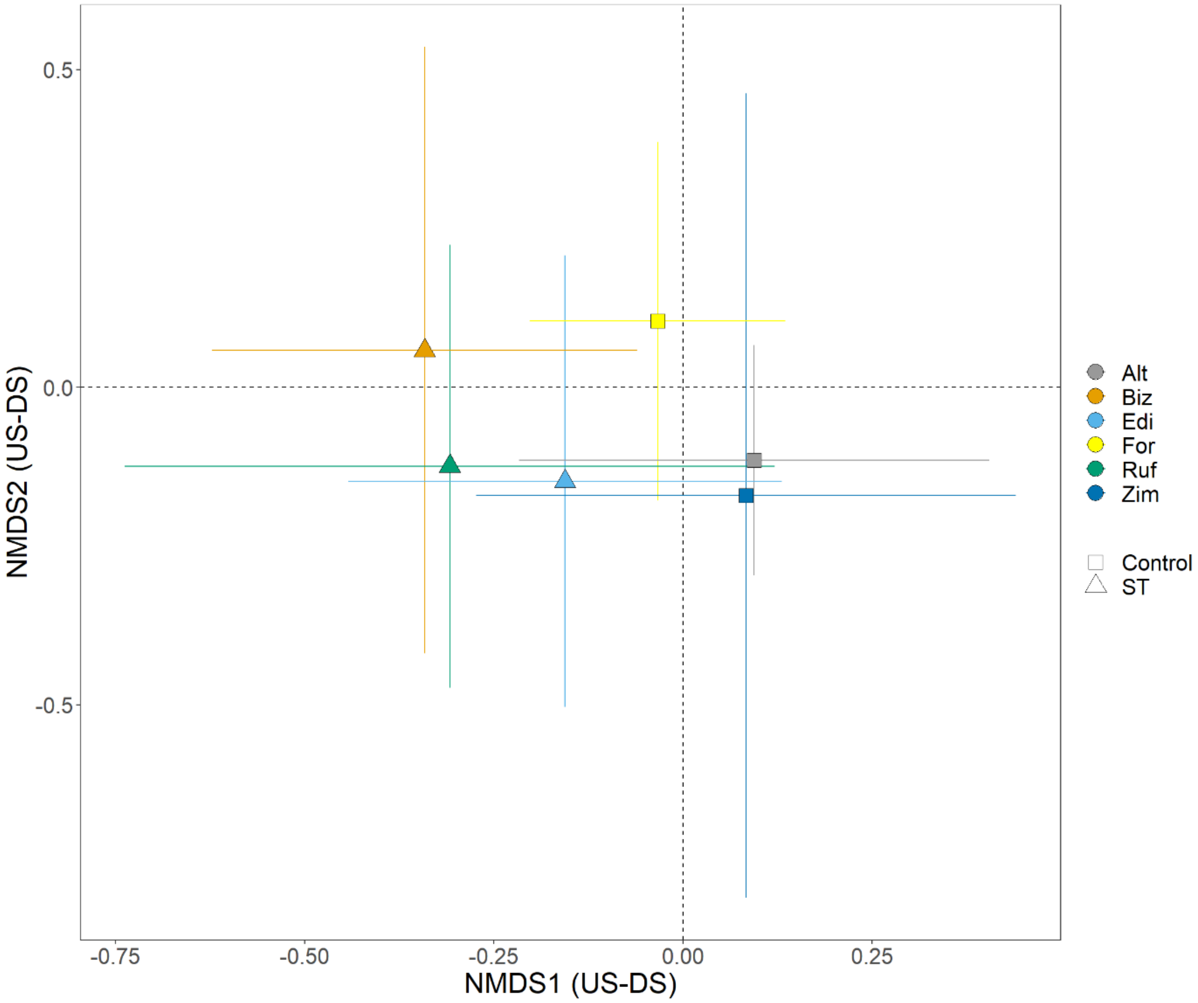


Figure 1: Difference between US and DS sites in nMDS (mean ± SE)

Supplement C – Spider diet

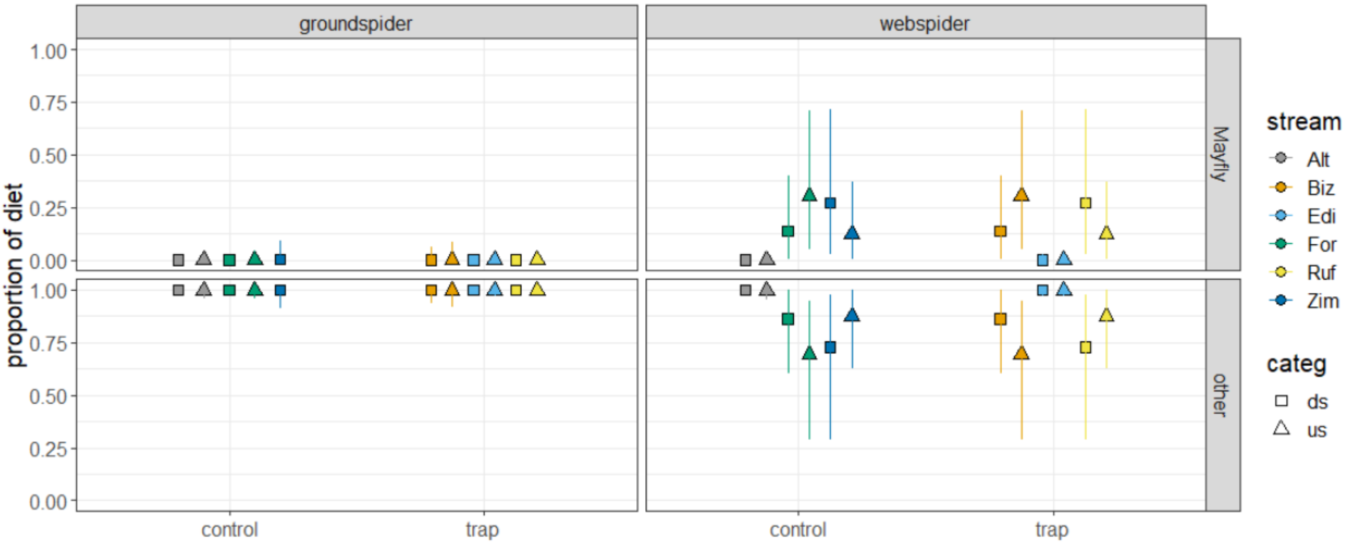


Figure 1: Results from Bayesian mixed model, calculating the contribution of mayflies to diets of ground-dwelling and web-building spiders in control and ST streams. Symbol represents median, error bar represents 50 % credibility interval.

Supplement D - Edisriederbach

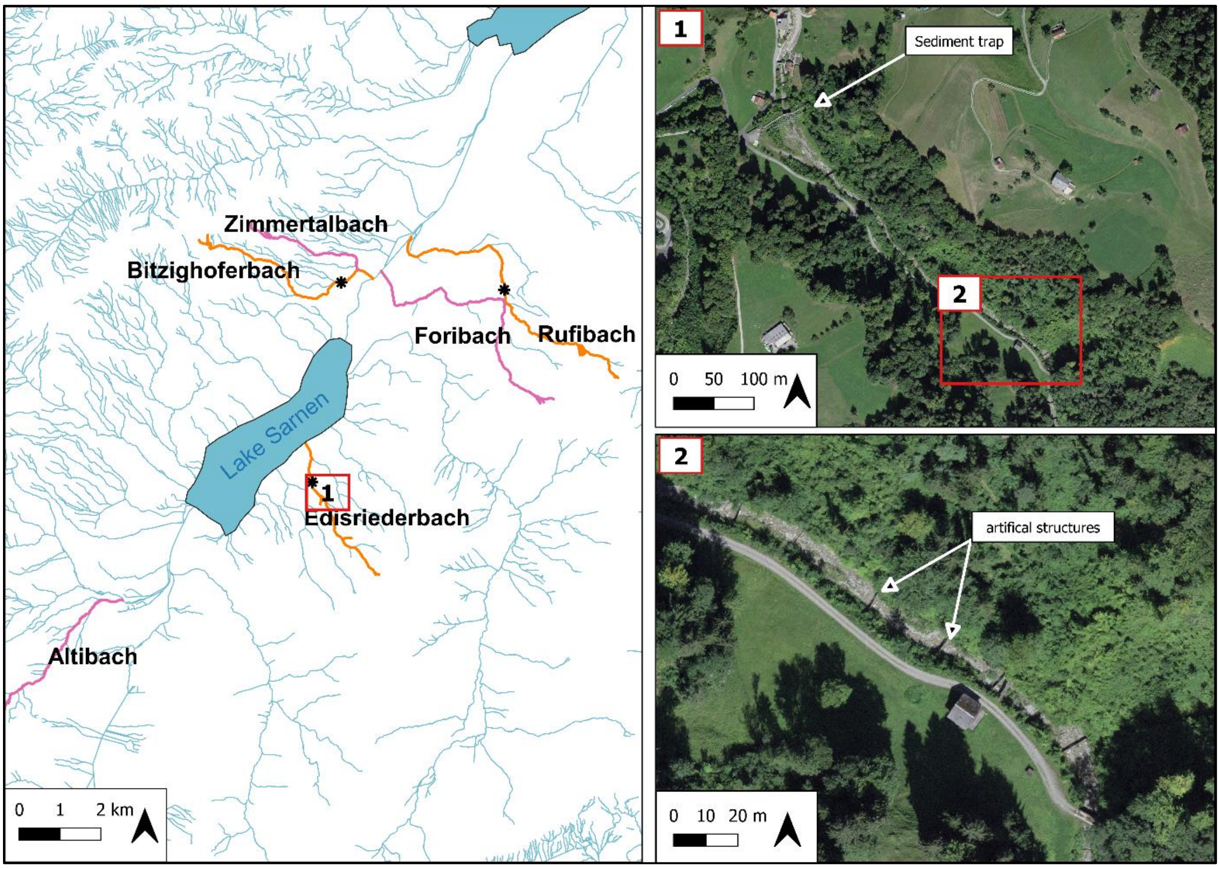
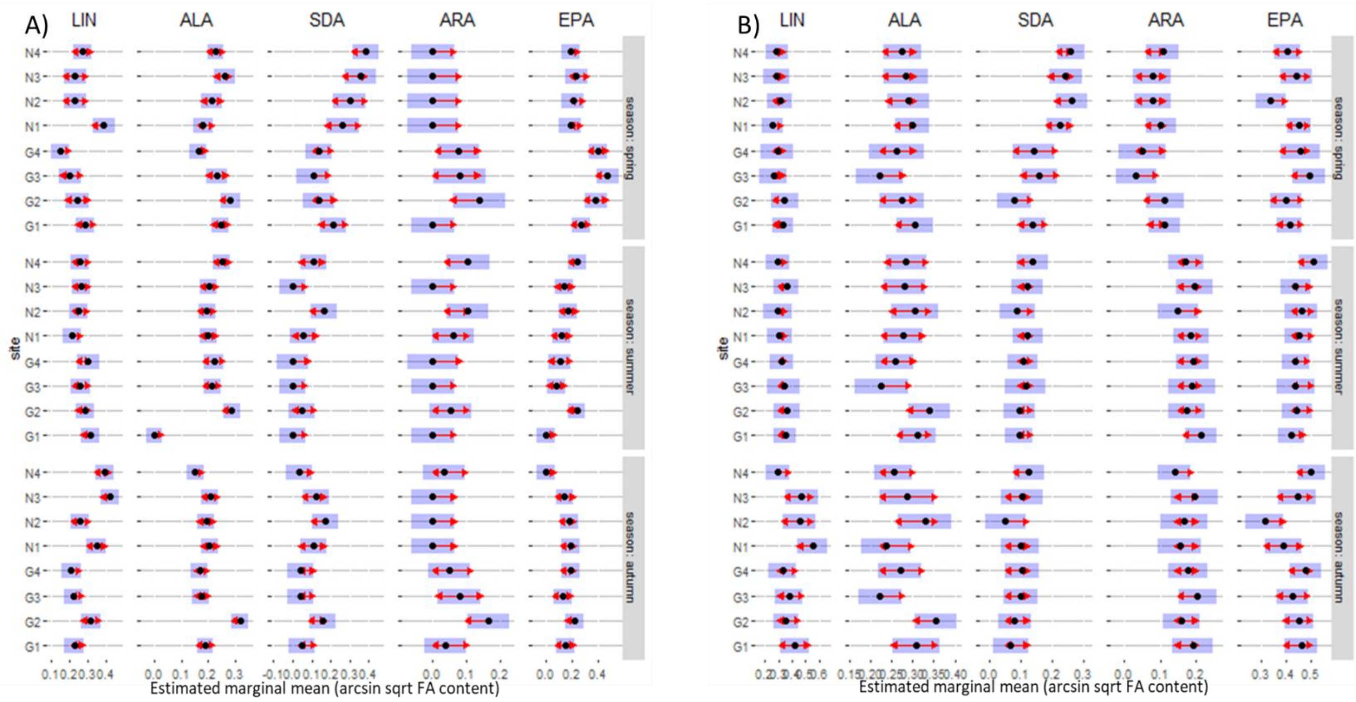


Figure 1: Artificial structures upstream of sampling sites at Edisriederbach (Edi).

APPENDIX 2: SUPPLEMENTARY MATERIAL CHAPTER 2

S -Table 1: Individual PUFA content in riparian spider per dry mass (mg/d dry mass).

river	season	spider type	FA	mean	SD	n
Glatt	spring	groundspider	LIN	8.23	2.81	12
			ALA	1.48	0.84	12
			SDA	0.09	0.32	12
			ARA	3.36	0.89	12
			EPA	9.70	2.70	12
		webspider	LIN	14.01	4.21	12
			ALA	3.13	1.68	12
			SDA	1.57	1.88	12
			ARA	1.45	0.74	12
			EPA	17.87	6.66	12
	summer	groundspider	LIN	10.47	5.96	12
			ALA	2.20	1.99	12
			SDA	0.27	0.42	12
			ARA	3.95	0.97	12
			EPA	9.69	2.28	12
		webspider	LIN	9.20	3.31	12
			ALA	3.04	1.58	12
			SDA	0.24	0.43	12
			ARA	2.79	0.75	12
			EPA	10.37	1.63	12
	autumn	groundspider	LIN	18.42	7.80	12
			ALA	4.74	2.00	12
			SDA	0.60	0.76	12
			ARA	3.67	1.31	12
			EPA	9.80	4.09	12
		webspider	LIN	20.06	9.63	12
			ALA	3.97	2.16	12
			SDA	0.35	0.66	12
			ARA	2.70	1.15	12
			EPA	8.38	4.87	12
Necker	spring	groundspider	LIN	8.99	6.73	11
			ALA	1.91	1.75	11
			SDA	0.46	1.17	11
			ARA	3.61	1.67	11
			EPA	10.42	5.74	11
		webspider	LIN	9.22	3.68	9
			ALA	4.69	1.61	9
			SDA	6.11	4.56	9
			ARA	1.39	0.70	9
			EPA	16.45	4.53	9
	summer	groundspider	LIN	9.75	7.91	12
			ALA	2.88	2.46	12
			SDA	0.23	0.54	12
			ARA	3.10	1.96	12
			EPA	8.20	5.32	12
		webspider	LIN	10.17	5.36	12
			ALA	3.56	1.45	12
			SDA	0.39	0.60	12
			ARA	1.99	0.75	12
			EPA	9.51	3.25	12
	autumn	groundspider	LIN	14.25	7.99	11
			ALA	5.04	3.51	11
			SDA	0.64	1.03	11
			ARA	3.28	0.89	11
			EPA	9.74	3.74	11
		Webspider	LIN	18.36	7.79	12
			ALA	3.22	1.88	12
			SDA	0.49	1.33	12
			ARA	1.84	0.66	12
			EPA	7.33	5.00	12



S-Figure 1: Site specific differences in PUFA content [percent of total fatty acids] in A) periphyton and B) emergent aquatic insects.

S-Table 2: CSIA results for riparian spiders

sample	type	Corrected C18 fatty acids	Corrected C20 fatty acids
N4_2006_SW_e0_1	web spider	-35.7	-38.2
N4_2006_SW_e0_3	web spider	-36.1	-37.9
N4_2006_SW_e40_1	web spider	-37.5	-39.7
N4_2006_SP_e0_1	ground spider	-35.8	-39.6
N4_2006_SP_e0_2	ground spider	-35.4	-38.1
N4_2006_SP_e40_1	ground spider	-34.1	-36.6
Ter.in_1	ter.Insect	-36.0	
Ter.in_2	ter.Insect	-35.3	
Ter.in_3	ter.Insect	-33.9	
Ter.in_4	ter.Insect	-34.2	
N_F1_190824_EM_4	aq.insect	-39.3	-38.6
N_E2_190824_EM_1	aq.insect	-35.8	-37.4
N_F2_190403_EM_1	aq.insect	-42.8	-42.7

S-Table 3: Fatty acid content (percent of total fatty acids) of CSIA spider samples

sample	type	Distance [m]	C14:0	C16:0	C16:1	C18:0	C18:1n7	C18:1n12/9	C18:2n6 (LIN)	C18:3n6	C18:3n3 (ALA)	C18:4n3 (SDA)	C20:4n6 (ARA)	C20:5n3 (EPA)
N4_2006_SP_E0_1	ground	0-2	0.8	17.7	5.5	11.6	10.4	18.7	12.7	0.5	9.7	0.6	1.3	10.3
N4_2006_SP_E0_2	ground	0-2	1.8	18.3	10.0	10.5	17.9	8.2	11.9	0.5	5.4	1.6	1.5	12.4
N4_2006_SP_40_1	ground	> 40	4.2	15.7	1.0	10.7	27.0	1.9	23.4	0.0	2.9	0.0	6.3	6.8
N4_2006_SW_E0_1	web	0-2	4.5	17.5	8.0	8.7	14.4	6.3	11.4	0.6	6.4	3.2	1.9	17.2
N4_2006_SW_E0_3	web	0-2	8.2	18.5	3.7	10.6	12.1	4.2	17.4	0.0	2.8	1.2	3.9	17.4
N4_2006_SW_40_1	web	> 40	5.5	7.3	2.0	15.7	14.6	7.2	18.6	2.1	4.3	1.6	2.2	18.9

S-Table 4: Proportion of different insect groups in spider diet (results from Bayesian models)

predator type	**season**	**prey group**	**river**	**mean**	**sd**	**2.50%**	**25%**	**50%**	**75%**	**97.50%**
Web spider	spring	Diptera	Glatt	36	14	11	26	35	46	63
			Necker	17	11	0	8	17	26	40
		Ephemeroptera	Glatt	4	4	0	1	3	6	16
			Necker	6	6	0	1	4	8	22
		Plecoptera	Glatt	15	9	0	8	16	22	31
			Necker	34	14	9	24	34	45	59
		Ter.insect	Glatt	45	7	31	41	45	49	57
			Necker	43	5	34	40	43	46	52
	summer	Diptera	Glatt	26	11	4	18	25	32	51
			Necker	32	13	8	22	31	40	58
		Ephemeroptera	Glatt	6	4	0	3	5	9	16
			Necker	6	4	0	3	5	8	14
		Plecoptera	Glatt	33	13	8	24	33	41	59
			Necker	19	12	0	9	19	28	43
		Ter.insect	Glatt	35	9	17	29	35	41	54
			Necker	44	8	28	38	44	49	61
	autumn	Diptera	Glatt	17	8	0	13	18	22	31
			Necker	20	7	4	15	20	25	33
		Ephemeroptera	Glatt	2	2	0	1	2	4	9
			Necker	2	2	0	0	2	3	7
		Plecoptera	Glatt	30	10	10	23	30	36	51
			Necker	10	7	0	3	9	15	24
		Ter.insect	Glatt	51	6	39	47	51	55	62
			Necker	69	5	59	66	69	72	77
Ground spider	spring	Diptera	Glatt	6	12	0	0	0	4	42
			Necker	4	6	0	0	0	7	23
		Ephemeroptera	Glatt	1	3	0	0	0	1	8
			Necker	9	10	0	0	4	19	28
		Plecoptera	Glatt	15	8	0	13	17	20	26
			Necker	2	4	0	0	0	3	12
		Ter.insect	Glatt	78	8	56	75	79	83	91
			Necker	85	7	70	79	86	90	94
	summer	Diptera	Glatt	18	9	0	12	18	24	37
			Necker	29	14	3	19	28	37	57
		Ephemeroptera	Glatt	5	4	0	2	5	8	13
			Necker	5	4	0	2	5	8	13
		Plecoptera	Glatt	28	20	1	13	25	40	76
			Necker	14	12	0	4	12	22	43
		Ter.insect	Glatt	49	18	11	36	49	61	81
			Necker	52	10	30	46	53	59	70
	autumn	Diptera	Glatt	20	6	8	16	20	23	32
			Necker	18	7	5	14	18	23	32
		Ephemeroptera	Glatt	4	3	0	2	4	6	10
			Necker	3	2	0	2	3	5	8
		Plecoptera	Glatt	31	9	12	25	31	36	48
			Necker	31	7	18	27	31	36	46
		Ter.insect	Glatt	45	6	33	41	45	50	57
			Necker	47	6	35	43	47	51	58

APPENDIX 3: SUPPLEMENTARY MATERIAL CHAPTER 3

Supplement A – Correlation among environmental variables

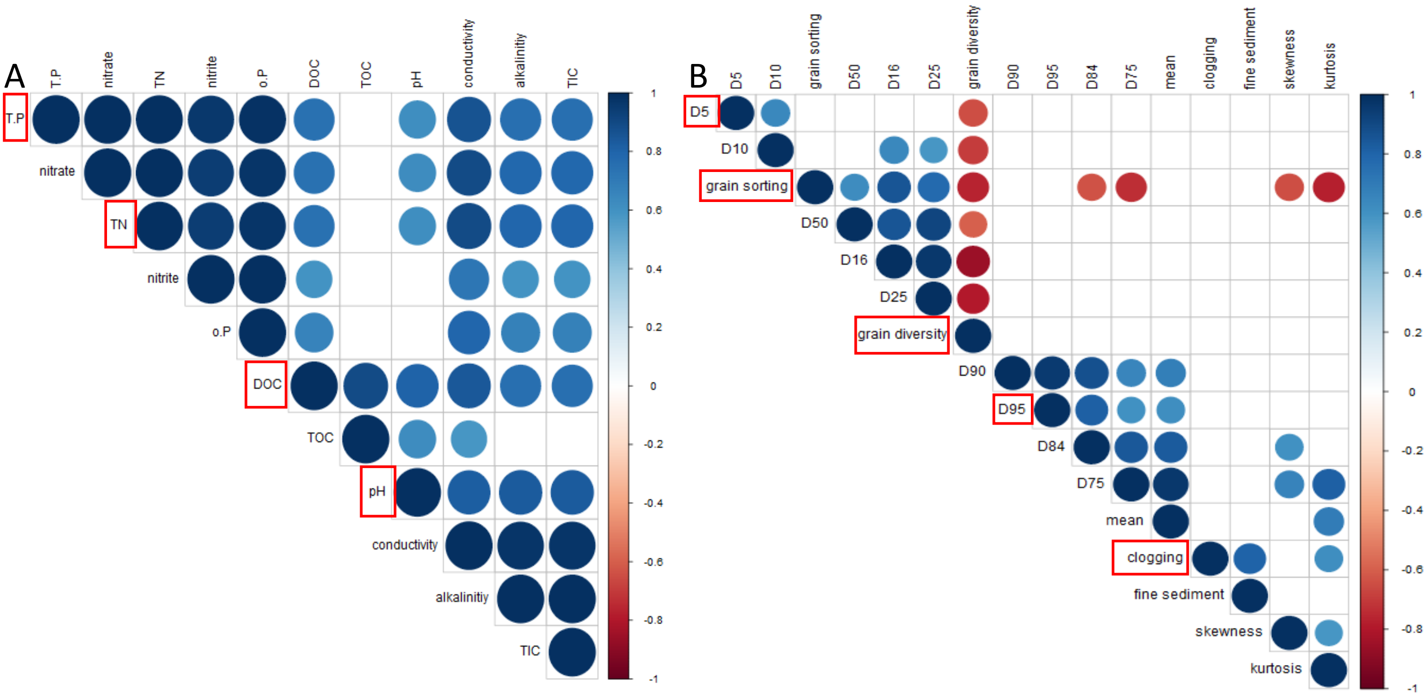


Figure 1: Correlation matrices for A) Physico-chemical parameters and, B) Stream bed characteristics collected from the two studied rivers. Variables marked in red were used for PCA. Colour ramp and bubble size indicates strength and direction (positive or negative) of correlation coefficient.

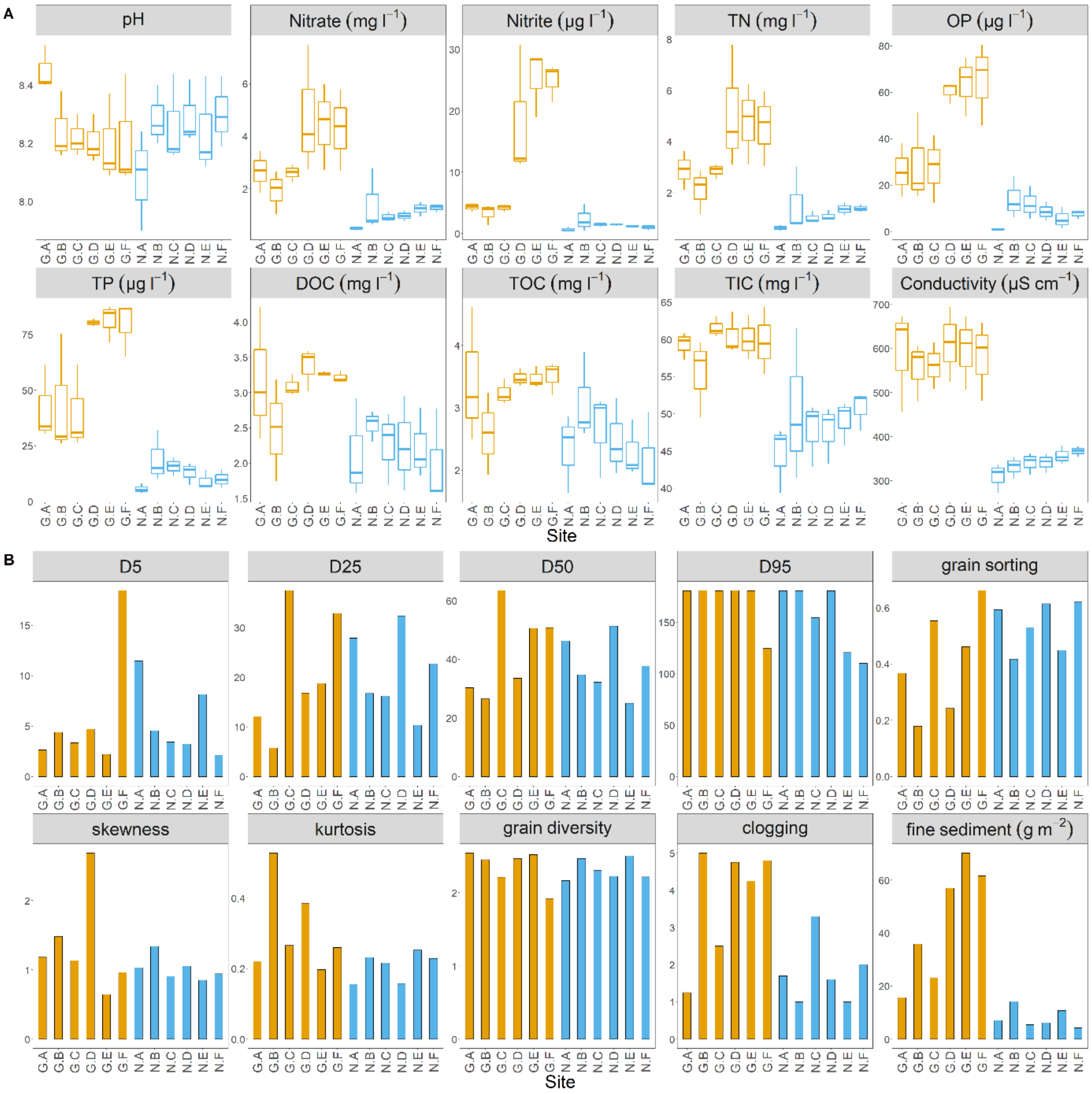


Figure 2: A) Physico-chemical parameters at the different sampling sites along the Glatt (orange) and Necker (blue). B) Stream-bed characteristics at different sampling sites along the Glatt and Necker.

Supplement B – Biomass export via different emergence modes and environmental PC scores

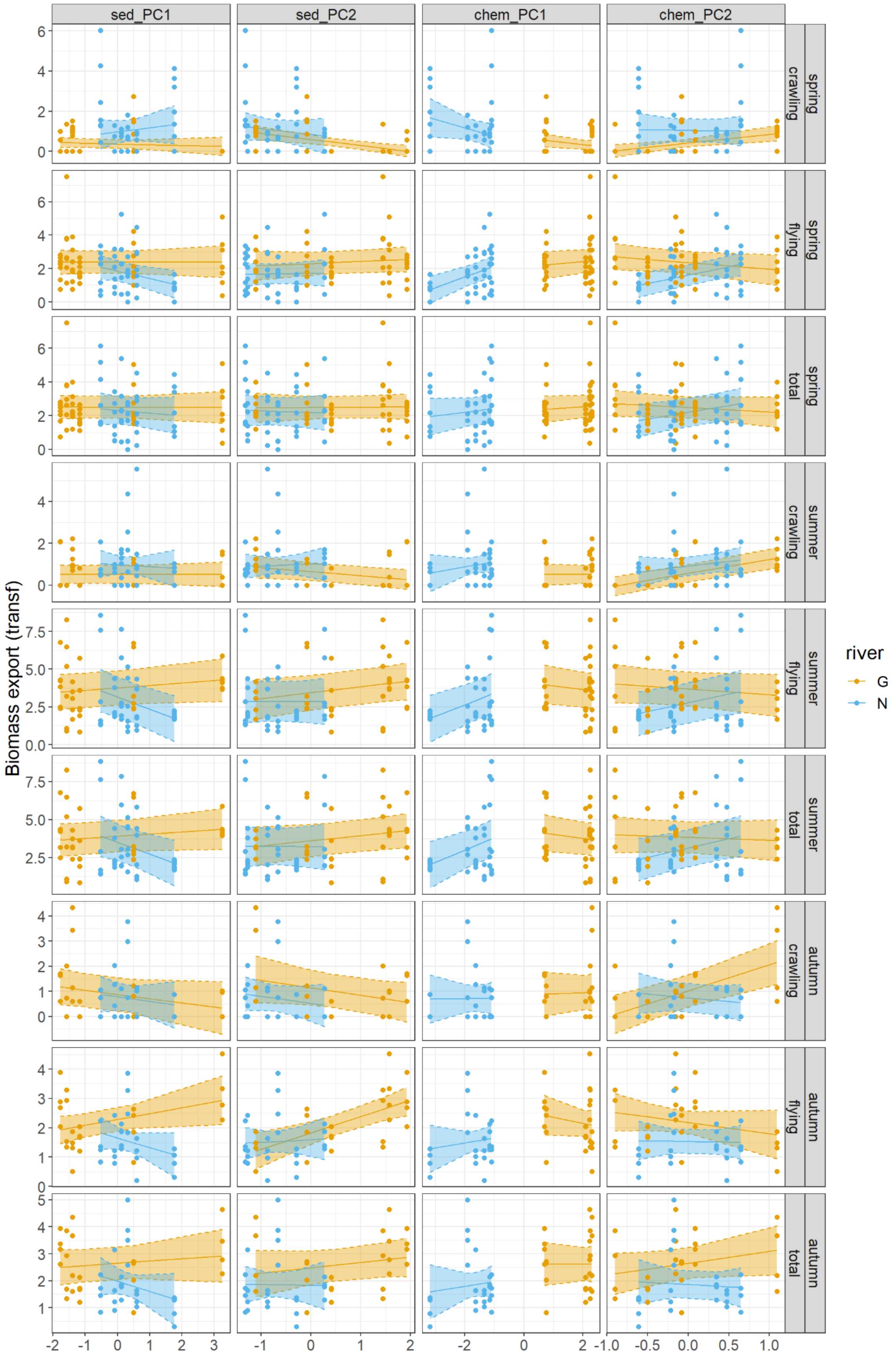


Figure 1: Results from mixed model testing the association of environmental principal component axes scores by season for crawling, flying and total biomass export, with sampling date fitted as a random effect.

Supplement C – PUFA content per dry mass

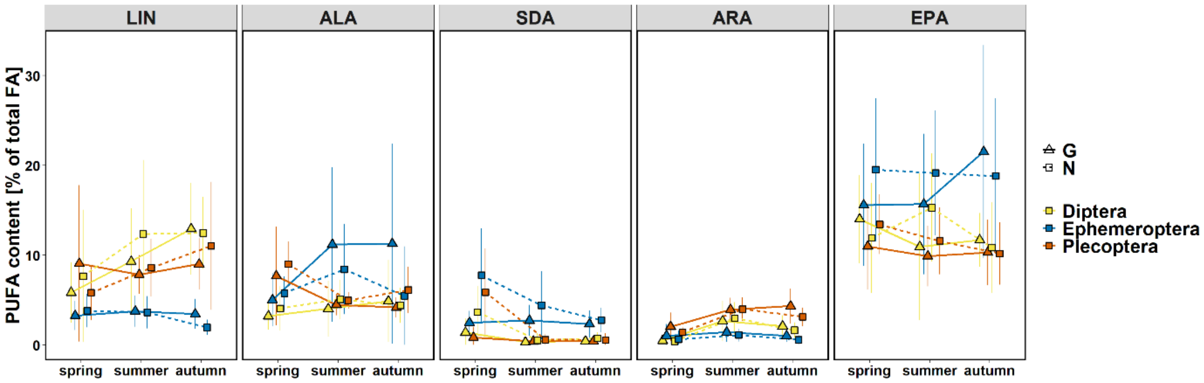


Figure 1: Individual PUFA content (mg/g dry mass) in Diptera, Ephemeroptera and Plecoptera by stream and season. Points represent mean content and error bars represent standard deviation.

Supplement D - Seasonal differences in PUFA content

EPA content did not significantly differ among seasons ($p > 0.05$). SDA content in Diptera was highest at both streams in spring (Necker: $p < 0.001$; Glatt: $p < 0.05$). SDA content in Plecoptera at the Necker was also significantly higher in spring than summer and autumn ($p < 0.001$). We found no significant differences in SDA content in Ephemeroptera among seasons ($p > 0.05$). In contrast, ARA content was lower in Diptera and Plecoptera in spring than summer and autumn ($p < 0.001$). ARA content in Ephemeroptera was at low levels during all seasons.

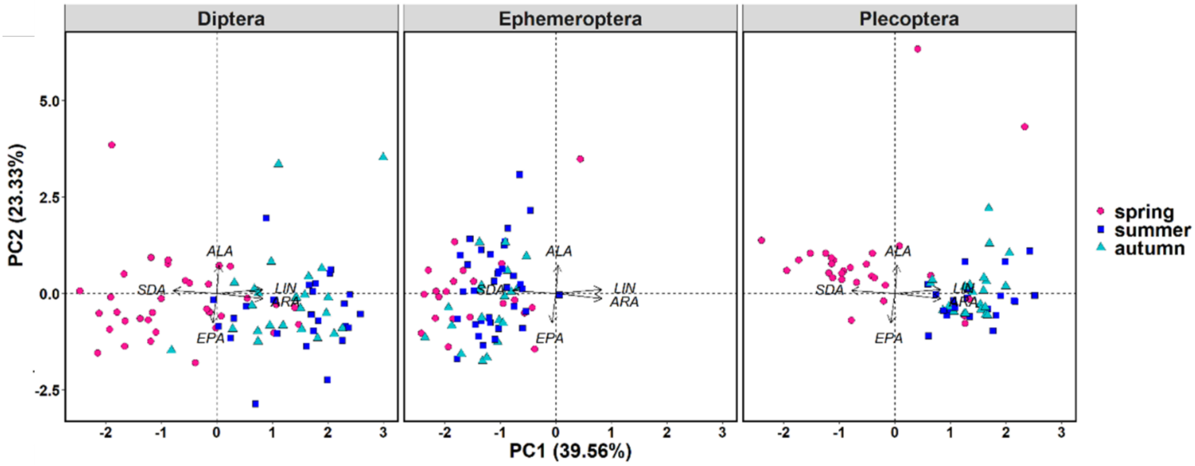


Figure 1: Principal component analysis of PUFA profiles of emergent aquatic insects, highlighting differences among seasons. *LIN* - linolenic acid, *ALA* - alpha-linolenic acid, *SDA* - stearidonic acid, *ARA* - arachidonic acid, *EPA* - eicosapentaenoic acid.

Supplement E – EPA export

Table 1: Estimated EPA export (µg m^{-2} day^{-1}) at the different sites in spring, summer and autumn

Season	river	site	crawling	flying	total
spring	Glatt	G_A	12.72 ± 8.35	63.83 ± 52.57	76.55 ± 48.05
		G_B	0.75 ± 1.5	60.67 ± 34.2	61.42 ± 33.65
		G_D	2.81 ± 7.44	263.38 ± 320.59	266.19 ± 319.38
		G_F	0 ± 0	116.43 ± 132.33	116.43 ± 132.33
	Necker	N_B	128.85 ± 180.73	83.9 ± 86.37	212.76 ± 189.86
		N_D	4.33 ± 5.99	48.43 ± 52.3	52.75 ± 54.39
		N_E	4.67 ± 9.22	56.01 ± 60.63	60.68 ± 67.97
		N_F	9.64 ± 14.4	37.66 ± 39.29	47.31 ± 46.99
summer	Glatt	G_A	21.23 ± 18.66	142.59 ± 79.02	163.82 ± 68.35
		G_B	15.68 ± 24.29	238.45 ± 216.73	254.13 ± 207.83
		G_D	0 ± 0	441.17 ± 547.72	441.17 ± 547.72
		G_F	6.28 ± 9.33	199.32 ± 78.73	205.61 ± 73.74
	Necker	N_B	11.25 ± 18.75	390.56 ± 513.62	401.81 ± 528.25
		N_D	75.28 ± 159.75	97.79 ± 106.68	173.07 ± 172.4
		N_E	2.98 ± 3.71	129.87 ± 90.02	132.85 ± 89.29
		N_F	62.57 ± 96.74	188.95 ± 210.38	251.52 ± 177.63
autumn	Glatt	G_A	87.89 ± 96.29	25.73 ± 19.53	113.63 ± 78.61
		G_B	17.69 ± 9.84	169.28 ± 115.9	186.97 ± 106.5
		G_D	13.79 ± 23.47	85.55 ± 73.86	99.34 ± 81.13
		G_F	4.76 ± 5.51	187.31 ± 97.73	192.07 ± 102.17
	Necker	N_B	3.94 ± 5.03	25.47 ± 16.33	29.41 ± 16.11
		N_D	4.67 ± 5.47	8.86 ± 7.32	13.53 ± 3.41
		N_E	20.3 ± 25.98	24.97 ± 26.97	45.27 ± 51.71
		N_F	51.2 ± 59.44	137.25 ± 84.86	188.44 ± 91.71

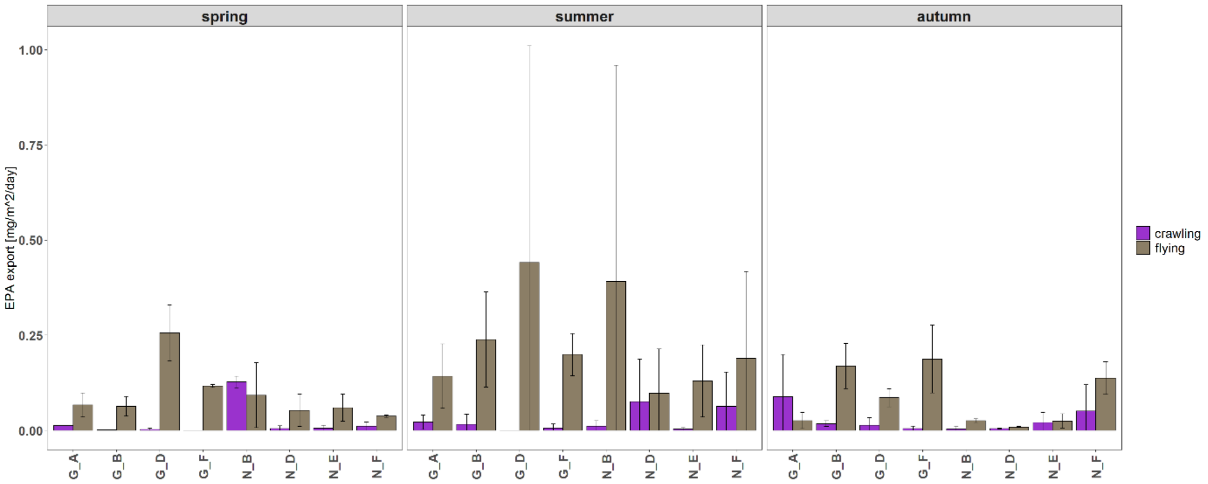


Figure 1: Estimation of PUFA export via flying (grey) and crawling (purple) emergence from different sites at the Glatt and Necker in spring summer and autumn. Bars represent estimated mean export, error bars represent standard deviation.

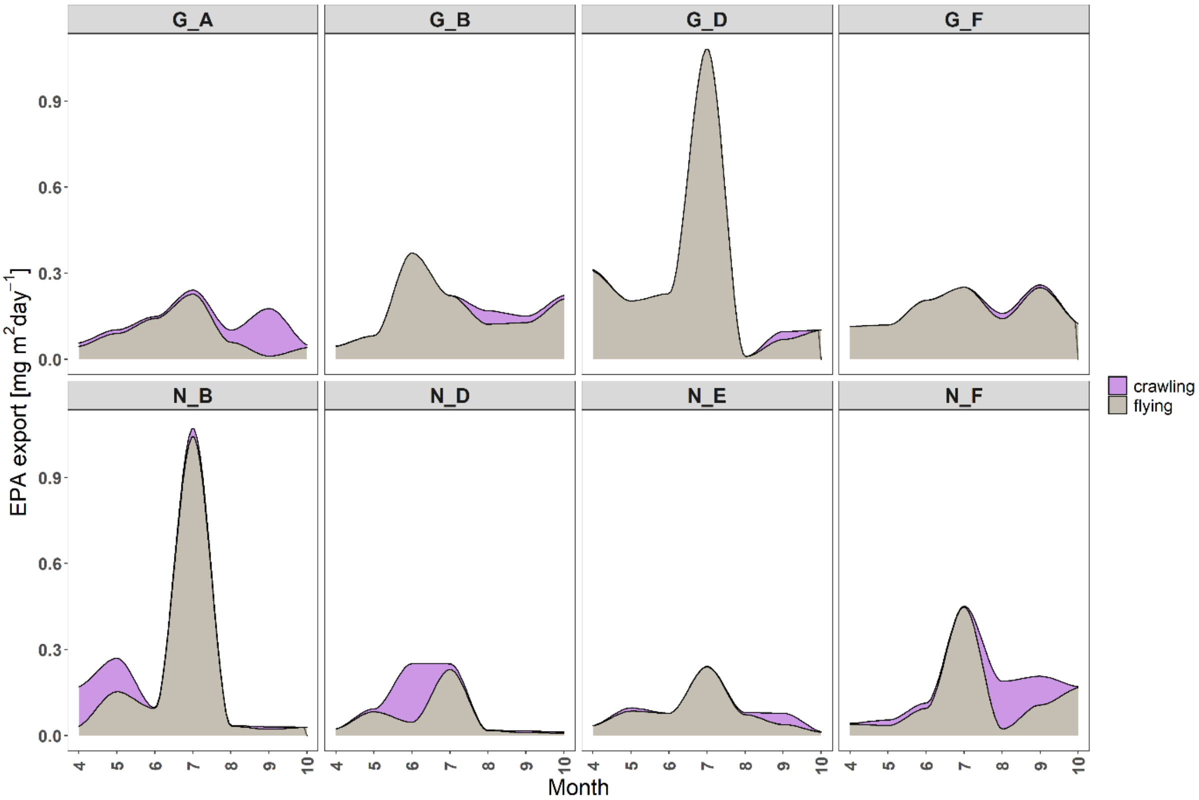


Figure 2: EPA mass export via emerging aquatic insects, via two different emergence modes “flying” (grey) and “crawling” (purple) for the Glatt (upper row) and the Necker (lower row). Curves represent estimation of EPA export based on a local smoother function.

Supplement F - Water temperature

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Supplement G – Biomass export via different groups

Table 1: Biomass export via different groups during spring, summer and autumn

season	river	site	Baetidae	Heptageniidae	Leuctridae	Nemouridae	Taeniopterygidae	Chironomidae	Tipulidae	Simuliidae	Psychodidae
spring	Glatt	G_A	1.8	0	0.26	0.31	0.86	2.07	0	0.06	0.14
		G_B	0.5	1.91	0.14	0.05	0	2.67	0.2	0	0.18
		G_C	2.01	2.62	0.07	0	1.82	3.19	1.24	0.08	0.04
		G_D	0.63	2.59	0	0	0.26	12.06	0.25	0.14	0.09
		G_E	1.15	0.64	0	0	0	1.9	0.16	0	0.01
		G_F	0.32	0.36	0	0	0	5.97	0.97	0	0.03
	Necker	N_A	0.12	0	0.21	0.26	7.28	0.44	0.07	0	0
		N_B	2.96	0	0.26	0.34	9.34	2.46	0	0	0
		N_C	2.25	0	0.08	0.19	0	4.88	0	0.14	0
		N_D	2.06	0	0	0.5	0	4.27	0	0.06	0.06
		N_E	1.23	0	0	0.32	0	1.5	0	0.36	0
		N_F	1.42	0	0.14	0.38	0.31	1.26	0	0.08	0.02
summer	Glatt	G_A	1.87	1.38	1.96	0	0	3.73	0.23	1.53	0.02
		G_B	2.88	8	1.45	0	0	3.11	2.6	0.44	0.02
		G_C	2.51	1.53	0.45	0	0	12.66	1.55	0.05	0.03
		G_D	2.25	10.88	0	0	0	11.06	0	0.15	0.01
		G_E	2.56	3.02	0.11	0	0	2.72	0.05	0.43	0.03
		G_F	3.31	5.08	0.81	0	0	8.5	0.07	1.09	0
	Necker	N_A	0.9	0.42	0.37	0	0	1.35	0.32	0.07	0
		N_B	4.3	5.71	0.59	0	0	8.99	0	0.58	0
		N_C	4.08	8.14	1.36	0	0	5.07	0.15	1.06	0
		N_D	2.09	0.52	0.65	0	0	3.68	0.08	0.14	0
		N_E	0.9	0.94	0.33	0	0	4.36	0.04	0.64	0.08
		N_F	1.22	5.59	4.82	0	0	2.51	0.05	0.12	0.02
autumn	Glatt	G_A	1.01	0	8.06	0	0	0.55	0	0	0
		G_B	4.14	1.09	2.22	0	0	0.93	0.25	0.25	0.04
		G_C	1.02	1.53	0.53	0	0	0.69	0.11	0	0
		G_D	2.27	0	1.14	0	0	2.5	0.03	0	0
		G_E	1.89	0	0.09	0	0	1.77	0	0	0.08
		G_F	2.21	1.84	0.49	0	0	4.99	0.17	0	0.03
	Necker	N_A	0.58	0	0.19	0	0	0.23	0	0.06	0
		N_B	1.91	0	0.42	0	0	0.91	0	0	0
		N_C	1.88	0	0.31	0	0	0.62	0.11	0.12	0
		N_D	0.15	0	1.1	0	0	0.39	0.18	0	0
		N_E	0	0	1.48	0	0	0.97	0	0	0
		N_F	0.37	10.53	6.21	0	0	2.5	0.12	0	0

Supplement H – PUFA content in stoneflies

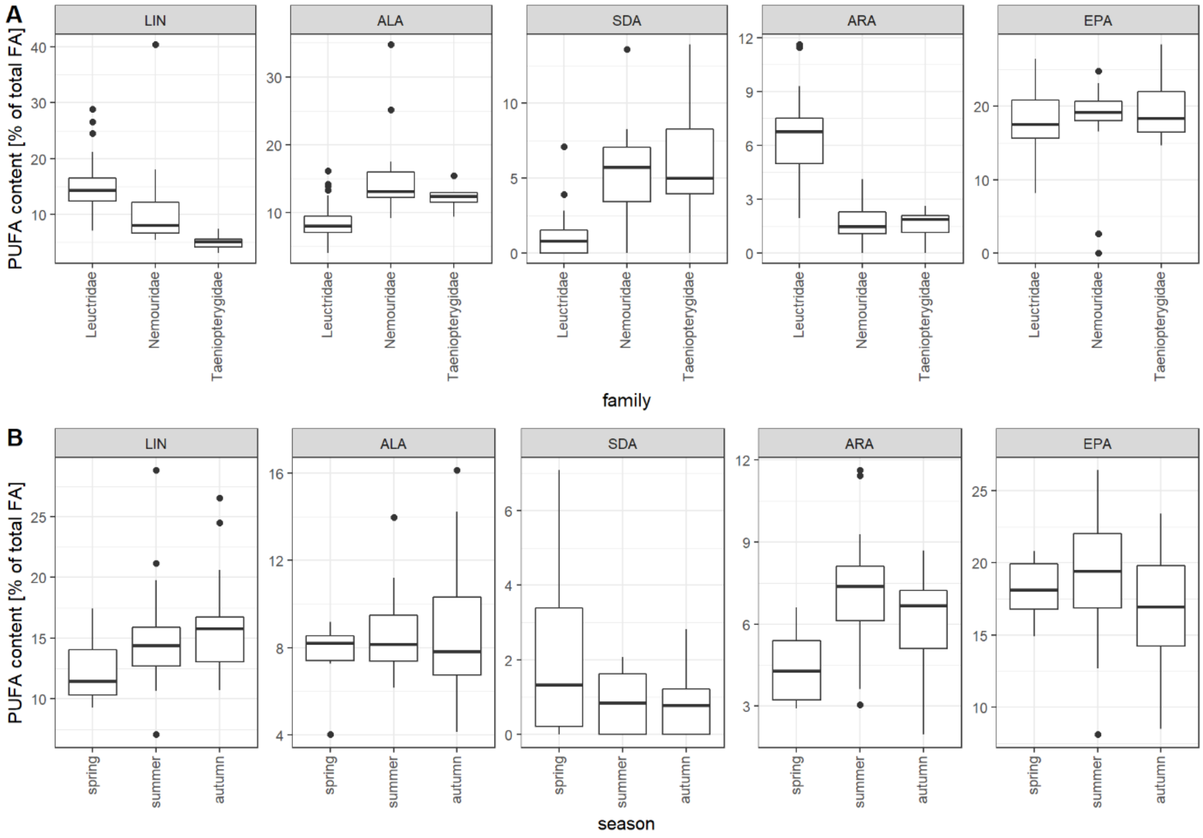

Figure 1: A) Differences in PUFA content between stonefly families B) seasonal differences in Leuctridae

Supplement I – Differences in EPA content in periphyton

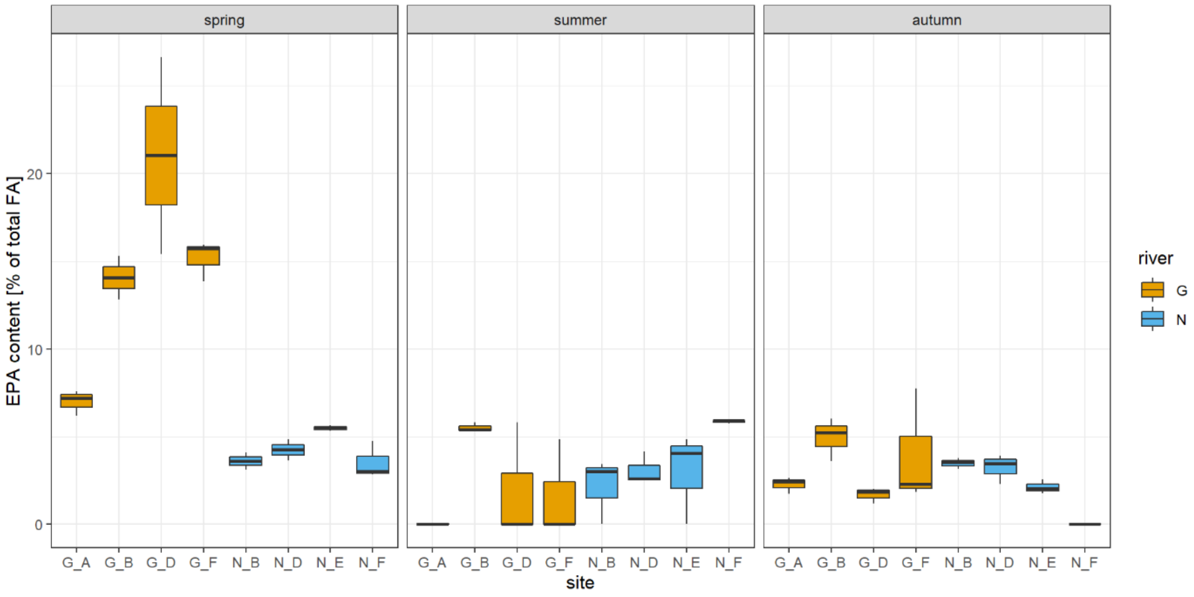

Figure 1: Differences in EPA content in periphyton (% of total fatty acids) among sites in spring, summer and autumn

Supplement J – PUFA profiles of periphyton among sites

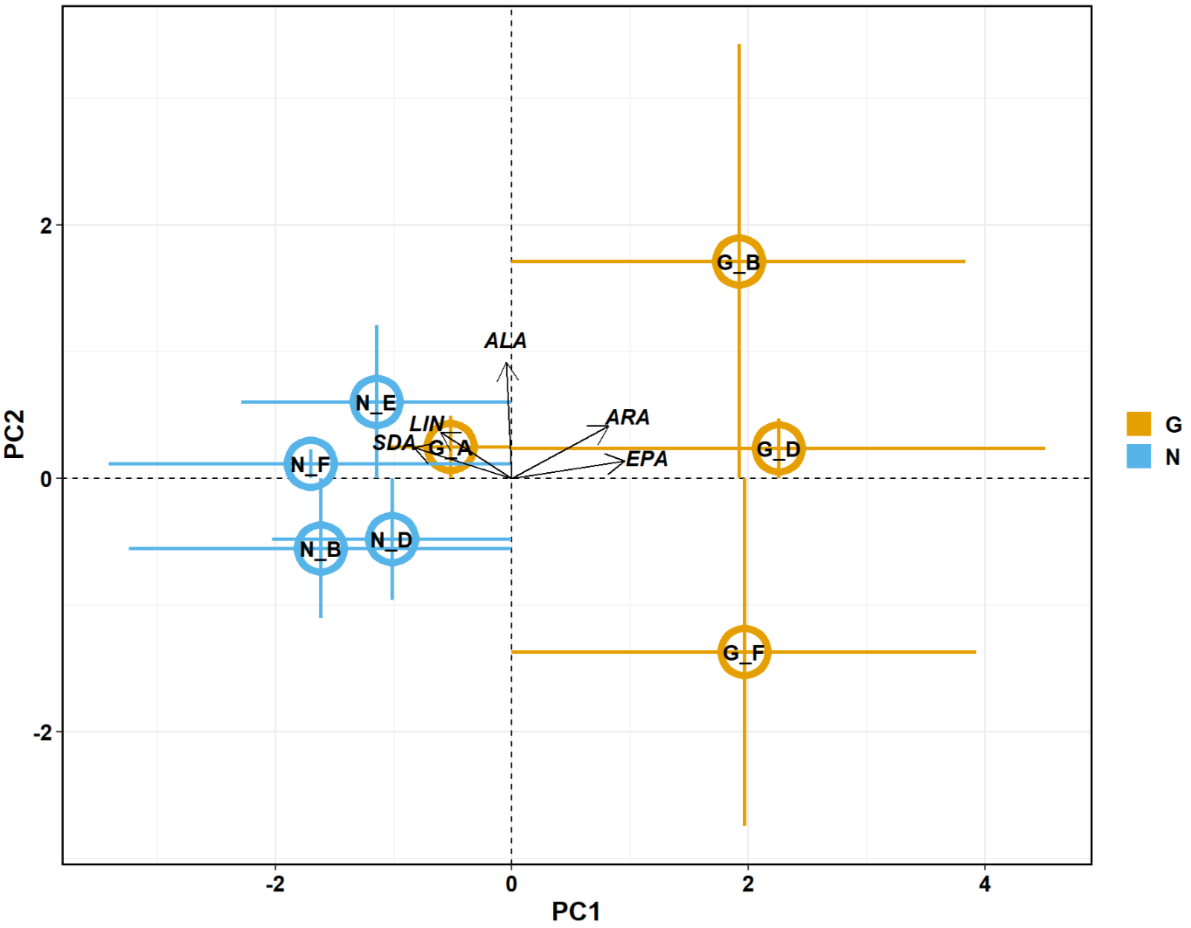

Figure 1: Principal component analysis of PUFA profiles of periphyton in spring (per site). *LIN* - linolenic acid, *ALA* - alpha-linolenic acid, *SDA* - stearidonic acid, *ARA* - arachidonic acid, *EPA* - eicosapentaenoic acid.