COMMUNITY PATTERNS AND PREY CONSUMPTION OF ARTHROPOD PREDATORS AT THE AQUATIC-TERRESTRIAL INTERFACE

by

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All you need is faith, trust and a little pixie dust

Peter Pan (1953)

Declaration

I hereby declare that I independently conducted the work presented in this thesis entitled “Community patterns and prey consumption of arthropod predators at the aquatic-terrestrial interface”. All used assistances are mentioned, and involved contributors are either co-authors of or are acknowledged in the respective publication.

I declare that while I used large language models (LLM: e.g., ChatGPT, DeepL) to assist with phrasing and improving the clarity of the text, all ideas, arguments, interpretations, and conclusions presented in this thesis are entirely my own. The LLM was used solely for language support and not for generating original content or shaping the intellectual substance of this work.

This thesis has never been submitted elsewhere for an examination, as a thesis or for evaluation in a similar context to any department of this university or any scientific institution. I am aware that a violation of the aforementioned conditions can have legal consequences.

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Abstract

Riparian areas serve as integrative landscapes that connect riverine and terrestrial ecosystems, shaping hydrological, geomorphological, and biological interactions. Further, longitudinal changes in hydrology and aquatic communities characterise rivers and streams. Consequently, aquatic insects can function as important subsidies that alter the diet composition of riparian consumers. In addition, riparian communities might also show characteristic changes along the longitudinal gradient based on their strong connection towards the waterbody. However, little is known about the effect of riparian area characteristics on the arthropod communities and their diet composition along the river course. In this dissertation, I first systematically reviewed the importance of aquatic subsidies for riparian arthropod predators. Furthermore, I examine potential variations in community characteristics and the dietary contributions of carabid beetles in three river sections: upstream, midstream and downstream.

The literature review (Chapter 2) revealed that aquatic subsidies contribute substantially to the diet of riparian arthropod communities. Factors such as seasonality, riverine characteristics, riparian habitat structure, distance from the water, predator body size and life stage influence the extent to which aquatic subsidies are incorporated into their diets. However, most of the studies conducted have focused on spiders and mainly the horizontal web-building spider *Tetragnatha.* This reveals a research gap for other riparian arthropods than spiders.

Based on this finding, a field study was conducted. Chapter 3 evaluates the effect of the river section to the carabid community. This showed significant differences in the carabid beetle community between upstream, midstream and downstream sections with the highest richness, individual number and functional trait diversity in the downstream section. Chapter 4 assesses the dietary spectrum using DNA metabarcoding for a subset of carabid beetles collected in the field. A dominance of terrestrial prey items was found which differs between carabid beetle tribes and riparian habitat specialists and generalists. Despite the minimal use of aquatic subsidies, the proportion of this food source was highest in the downstream section.

The results indicate that carabid beetles serve as reliable bioindicators of riparian habitat characteristics along streams, despite exhibiting a largely generalist diet. Additionally, the use of DNA metabarcoding revealed unexpected predator-prey species interactions. This knowledge should be considered for further conservation and restoration projects.

Chapter 1:

Introduction

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Chapter 1.1: Interactions between aquatic and terrestrial ecosystems

Riparian food webs

Ecosystems do not function in isolation; they are linked through the exchange of energy and nutrients via subsidy fluxes (Tansley, 1935). The flux of organic carbon from terrestrial to aquatic ecosystems serves as a prominent example of cross-ecosystem nutrient transfer, while aquatic subsidies are of high nutritional quality (Marcarelli et al., 2011; Twining et al., 2016) because of polyunsaturated fatty acids (PUFAs) – especially arachidonic acid (ARA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3) – which are exclusively produced by aquatic primary producers and usually not found in exclusively terrestrial feeding animals (Twining et al., 2019; Twining et al., 2021). Therefore, nutrients from aquatic resources are important for health and physiological functioning of terrestrial consumers (Martin-Creuzburg et al., 2017; Twining et al., 2019).

Important components of this high-quality source are aquatic emerging insects; insects with an aquatic larval stage and a terrestrial adult phase (Baxter et al., 2005; Schulz et al., 2015; Kraus et al., 2020; Schulz et al., 2024). Based on their high abundance and mass emergent events (Salvarina et al., 2017) aquatic insects serve as a prominent food source for a variety of terrestrial predators such as birds (e.g., Swallows; Jackson and Fischer, 1986; McCathy, 1997), bats (e.g, Daubenton's bat: Vaughan, 1997), lizards (e.g., *Sceloporus* sp., Sabo and Power, 2002) and riparian arthropods like spiders (e.g., Bollinger et al., 2023) or carabid beetles (e.g.,Paetzold and Tockner, 2005; Nash et al., 2023).

Riparian arthropods occur in high abundance in these transitional habitats (Stanley et al., 1991) and therefore are important predators. Consequently, the intake of aquatic subsidies by riparian arthropods has emerged as a focal point of considerable scientific interest (Lafage et al., 2019) which also shows that aquatic subsidies can affect predator communities, influencing e.g. the abundance and survival rate (Gallé and Schwéger, 2014). For example, spider populations respond to fluctuations in aquatic insect emergence in their web density and abundance (Burdon and Harding, 2008). However, the impact and utilisation of aquatic subsidies by riparian arthropods can be influenced by extrinsic factors (e.g. distance from the waters' edge, seasonality, and interspecific dietary competition) and intrinsic factors (e.g. hunting strategy, web orientation, body size), leading to a great variation. Variation in stream size, also within one stream from upstream towards downstream, was associated with differences in the intake of aquatic subsidies, as demonstrated in spiders (Chari et al., 2020).

Natural river characteristics as drivers of riparian patterns

Although cross-boundary resource flows are important, riparian zones hold additional ecological value beyond their role as recipients of aquatic subsidies. These areas function as transitional habitats between the adjacent aquatic and terrestrial systems. Despite their limited spatial extent (Downing et al., 2012), rivers and their adjacent riparian zones host striking high biodiversity, featuring both habitat specialists and threatened species (Naiman et al., 1993; Andersen and Hanssen, 2005; Hauer et al., 2016; Denux et al., 2017; González et al., 2017; Bauspiess et al., 2024). This is influenced, not least, by the structural heterogeneity and unstable characteristics of the riparian habitat (Merritt, 2022), which are shaped by temporal variations in water availability. However, riparian areas not only vary temporarily but also along the river, caused by biological, physical, and chemical processes such as the deposition of sediments and the transfer of chemicals and nutrients (Capon and Pettit, 2018).

In addition, floodplain types vary along the river course (Globevnik et al., 2021). At higher elevations, floodplains are narrow and steep, shaped by active geomorphic processes and coarse soils, whereas in lowland areas they become wide and flat with finer sediments and reduced relief dynamics (Koenzen, 2005; Globevnik et al., 2021). In the flow direction, there is an increase in inundation duration and availability of freshwater-driven resources (Ramey and Richardson, 2017) while decreases in grain size of the sediment and the slope of the river are common (Petts et al., 2000; Sadler et al., 2004; Baiocchi et al., 2012). Local factors such as sediment and nutrient content, water discharge and disturbance regime may have a greater impact than longitudinal changes (Ward et al., 2002). The midstream zone often exhibits the highest biodiversity in terms of flora (Tabacchi et al. 1996), due to its intermediate flooding frequency, which can be equated with intermediate disturbance (Connell, 1978; Malanson, 1984).

Moreover, aquatic communities characteristically change from upstream to downstream (Živić et al., 2001; Santos et al., 2010; Figueroa and Rios-Escalante, 2021). For macroinvertebrates, the feeding types of shredders and collectors dominate the upstream region, while collectors and grazers are the most prevalent feeding types in midstream, and collectors alone dominate the downstream section (Vannote et al., 1980). Fish communities also change along the flow direction. In Europe, rivers can be divided into four zones based on the fish communities, starting in the upstream region with the trout zone, transitioning into the grayling, barbel and bream zones depending on the size of the downstream region (Aarts and Nienhuis, 2003).

However, there is limited research on how riparian arthropod communities might change along the river. Furthermore, it is expected that terrestrial communities will change due to the strong cross-boundary linkages between rivers and their adjacent habitats. In Australia, for example, the composition and densities of ground-dwelling riparian arthropods - including carabid beetles and wolf

spiders - differ notably between the upstream and downstream sections. However, the geographical region and the taxa lead to variations in the response intensity (Framenau et al., 2002). Among the two most abundant riparian arthropod predators, spiders and carabid beetles (Kühnelt, 1943; Plachter, 1986), spiders exhibit dietary shifts along the river course as they are predominantly generalist feeders and can adjust their diet according to the prey available (Kato et al., 2004; Radermacher et al., 2020; Twining et al., 2021), whereas for carabid beetles, knowledge of their use and reliance on aquatic subsidies across this gradient remains limited.

Carabid beetles

Contrary to their limited dietary knowledge, carabid beetles in riparian areas are established as reliable indicators of bank characteristics (Januschke et al., 2011; Sprössig et al., 2022). They are particularly responsive to (micro-)climate variations, which are influenced by factors such as vegetation density, illumination level, soil substrate composition, thermal profile and moisture content (Lambeets et al., 2008; Lambeets et al., 2009; Schirmel et al., 2015; Schirmel and Gerlach, 2022). The high habitat diversity – they also occur e.g. in grassland (Blake et al., 1994), forests (Halme and Niemelä, 1993) and agricultural landscapes (Kromp, 1999) - is accompanied by a broad spectrum of functional traits in carabid beetles, such as body size, feeding strategy, flight ability and habitat specialisation (Kotze et al., 2011). The combination of habitat sensitivity and various trait characteristics, including dispersal abilities, make carabid beetles effective indicator species, particularly in riparian ecosystems (Januschke et al., 2014; Sprössig et al., 2022). Beyond this, small and long-winged carabid beetle species can rapidly (re-)colonise river banks (Sadler et al., 2004; Lambeets et al., 2009). Therefore, carabid beetles are used to indicate the status and condition of river banks (Jachertz et al., 2019; Sprössig et al., 2022; Bauspiess et al., 2024) and to evaluate river restoration initiatives (Januschke et al., 2011). However, even though the habitat preferences for riparian carabid beetles are well studied, the possibility of community changes along rivers and the diet of carabid beetles in this environment has not been studied extensively (Hering and Plachter, 1997; Hering, 1998; Paetzold and Tockner, 2005; O'Callaghan et al., 2013).

Methods to analyse gut content

Methods to analyse the consumed items of carabid beetles adjusted to water are mainly Stable Isotope Analyses (SIA) (Paetzold and Tockner, 2005; O'Callaghan et al., 2013) and the visual determination of food fragments in the digestive tract (Hering and Plachter, 1997; Hering, 1998). Even though SIA is a valuable method for identifying the original orientation – aquatic or terrestrial – incorporating DNA metabarcoding for analysing the consumed prey will bring broader insights. DNA gut content analyses have the potential to outperform other methods by simultaneously identifying multiple prey items with enhanced taxonomic detail (Dunshea, 2009; Pompanon et al., 2012). Although visual gut content

analysis offers certain advantages, such as distinguishing between larval and adult prey, it is limited to relatively well-preserved prey items, thereby constraining the detection of highly digested material. DNA metabarcoding is particularly effective for this type of analysis (Eitzinger et al., 2019). Besides, the use of DNA metabarcoding will help for a deeper understanding of trophic linkages (Symondson, 2002; Sheppard and Harwood, 2005; King et al., 2008; Pompanon et al., 2012), which might bring important implications for biodiversity conservation (Clare, 2014). Consequently, investigating the diet and community changes of carabid beetles living in riparian areas represents a research gap in understanding the structure and dependence of the aquatic-terrestrial linkages. This dissertation will contribute to the very limited research using DNA metabarcoding to investigate the diet spectrum of various riparian carabid beetles (Kamenova et al., 2018).

Chapter 1.2: Research questions

Despite numerous studies addressing the research question of how important aquatic subsidies are for riparian arthropod predators, a systematic review incorporating methods beyond stable isotope analyses was lacking. To address this and identify other research gaps, I first conduct a systematic literature review. Building on this and the well-studied within-stream community changes along the river course and drawing on the gap identified in the literature regarding changes within the adjacent riparian community and their trophic linkages across this longitudinal gradient, I aim to contribute filling these gaps. I conducted a field study focusing on carbide beetles, their community and diet. I decided to focus on the following overall research question in my dissertation (Figure 1):

In what ways do arthropod predator communities and their prey consumption vary at the aquatic–terrestrial interface?

The constant energy and nutrient exchange between adjusted ecosystems and the high number of insect emergences suggest an intense use of this prey source for arthropod predators in riparian areas. However, the different strata these predators appear in (e.g. ground, vegetation) and the various hunting strategies (e.g. fee-hunting, horizontal webs, vertical web) might affect the amount of consumed aquatic insects. In addition to the predator characteristics, the availability of aquatic insects might also affect the intake. The season, the distance to the water and the position along the river are examples of factors affecting the intake of aquatic subsidies, other than the predator characteristics.

To address the overall research question, this dissertation draws on three complementary approaches: literature review, field study and DNA metabarcoding analyses. Together with my co-authors, I systematically reviewed the available literature to investigate the state of research. Based on the apparent research gaps, the co-authors and I conducted a field study along forest streams using the representative arthropod predator carabid beetles in riparian areas. With this study, we mainly aimed to investigate expected changes in the community and their functional traits in different stream sections. We also analysed the diet of these carabid beetles, using DNA metabarcoding to investigate the overall composition and its connection towards water (the supplementary material for Chapters 2 to 4 is available in digital format). Thus, the overall research question is divided into three subsections (Figure 1).

1.1 *To what extent do riparian arthropod predators rely on aquatic subsidies and what factors shape this reliance?* (Chapter 2)

Aquatic insects are widely recognised as a common vector of energy and nutrients from aquatic to terrestrial habitats. We systematically reviewed the literature to assess the importance of aquatic insects for riparian arthropod predators and the factors affecting their intake. We also evaluated the methods used to quantify this consumption and the effect of variation in the availability on the abundance of riparian arthropod predators. This highlights a clear bias towards spiders. Aside from that, the horizontal web-building *Tetragnatha* was the most present taxon in this kind of literature. In addition, stable isotope analyses were the most used methods for investigating the proportion of aquatic subsidies in the predators' diet. It has become apparent that there is a notable lack of studies in the southern hemisphere. Because of the limited research in carabid beetles regarding this topic, we focus on this group for the following research questions.

1.2 *Is stream zonation mirrored in the community composition of carabid beetles?* (Chapter 3)

Due to the high sensitivity and common use as bioindicators in evaluating river restoration projects of carabid beetles, as well as the lack of information about their community composition along rivers, we assessed the relationship between river sections and carabid beetle communities at five streams sampled at three near-natural river sections each (upstream, midstream, downstream). We evaluated differences in species composition and number, individual number, trait composition and indicator species regarding the different stream sections and their environmental characteristics.

1.3 What is the prey spectrum of various riparian carabid beetles and to what extent do aquatic subsidies contribute to their diet? (Chapter 4)

The results of the previous chapters indicate that the river course affects both aquatic and riparian communities with potential consequences for their diet. We applied DNA metabarcoding to analyse gut contents of multiple carabid beetle species, making this among the earliest studies to employ this approach in 28 different carabid beetle species. This method enables a more detailed taxonomic understanding by allowing simultaneous detection of multiple prey species, potentially revealing unexpected species interactions. We assumed that the diet would differ between their tribe, habitat specialisation and river section positions. We further hypothesised that the use of aquatic subsidies would be the highest for riparian specialists and in the downstream area of the river.

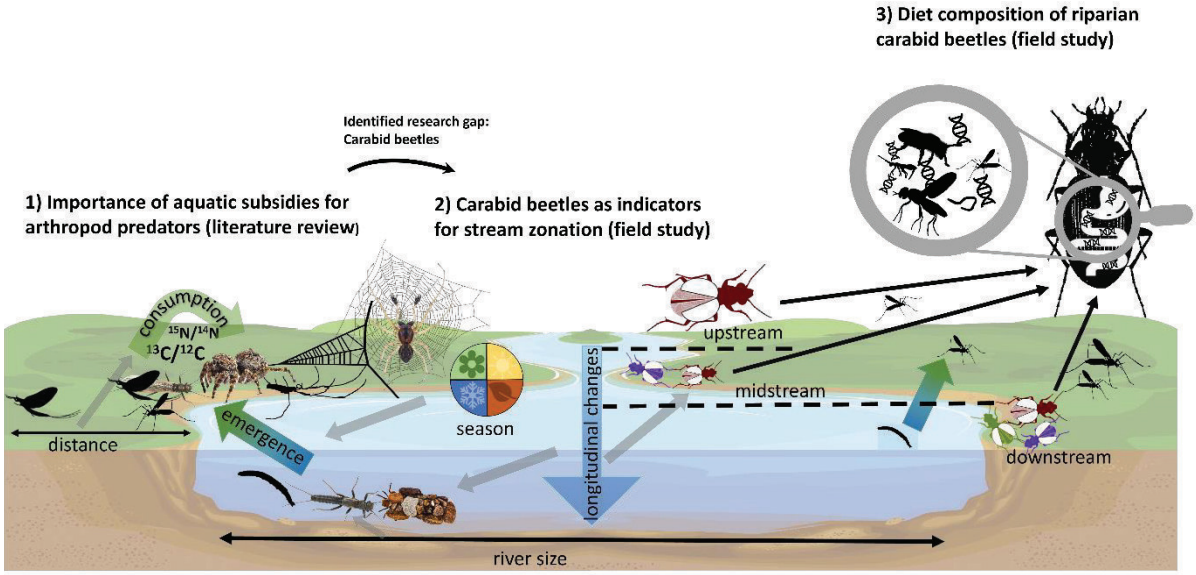


Figure 1: Graphical summary of topics covered in the dissertation, represented by the studied elements of the riparian habitat. Grey arrows on the left side of the graph represent direct or indirect effects on the emergence/ productivity of the river and therefore have an effect on the intake of aquatic subsidies by riparian arthropod predators. The grey arrow on the right side of the graph indicates direct or indirect effects of the river on the riparian zone, which in turn influence the carabid beetle community. Numbered text on top of each part of the graph refers to the various research included in this work.

Chapter 1.3: Methodological approach

We addressed the research questions through a threefold strategy: reviewing existing literature (Chapter 2), conducting field studies (Chapters 3) and performing DNA metabarcoding analyses (Chapter 4). For the literature review, we searched for studies that exclusively integrate arthropod predators consuming aquatic metazoans, in ISI Web of Science (https://www.webofscience.com/) and Google Scholar (https://scholar.google.de/) without time restrictions. Therefore, we used different keyword combinations with a last search on 8th January 2025. Based on the results of the literature review, we decided to focus on carbide beetles in the following field study as they appeared to be insufficiently explored.

The field study took place at five different streams in Rhineland-Palatinate, Germany. Each stream was sampled at three sections: upstream, midstream and downstream, resulting in a total of 15 sample points. The streams flow from the Palatinate Forest in the west into the River Rhine in the east. Every sampling point was surrounded by forest and had near-natural features, namely a gentle bank slope, absence of bank reinforcement and moderate meander. Sampling only took place at slip-off slobs or straight sections and ran from 8th to 19th May and 11th to 21st September 2023. In both periods, comprehensive catching methods were conducted. Pitfall traps were installed for one week and additional hand catches were performed on the same day as the traps were set. To analyse the carabid beetle community the individuals were morphologically identified while the diet analysis was conducted by DNA metabarcoding of the digestive tract.

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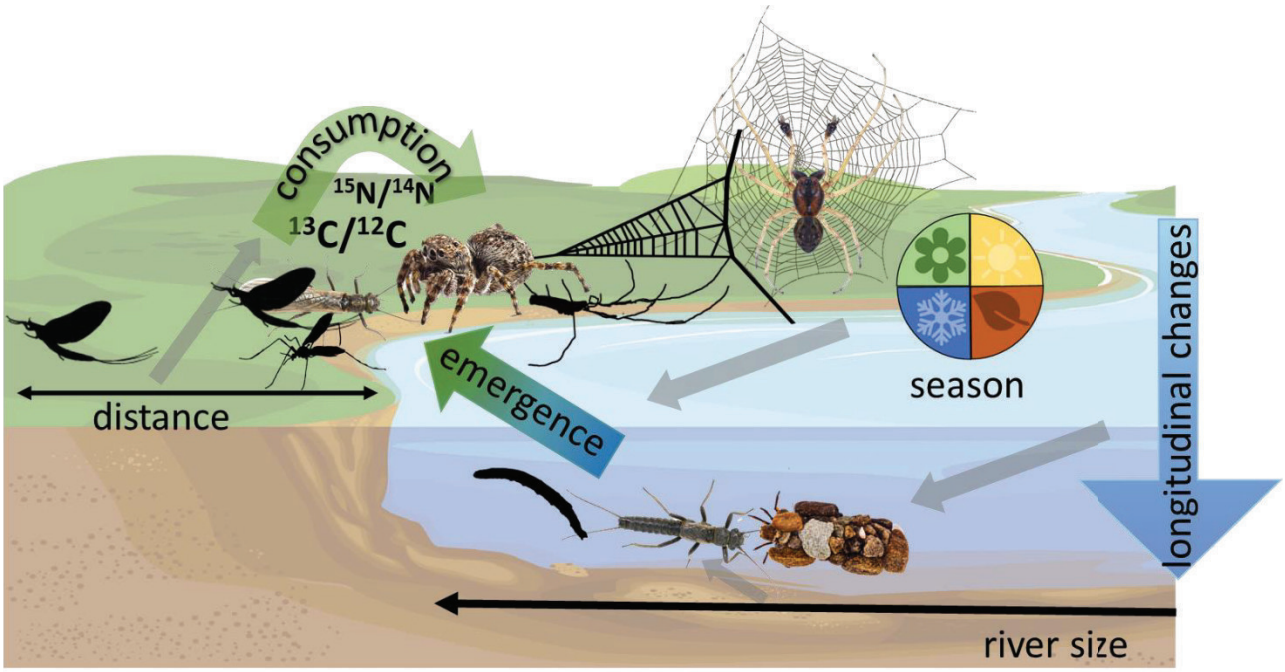
Chapter 2:

Review of the importance of aquatic prey for riparian arthropod predators

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REVIEW ARTICLE

Review of the importance of aquatic prey for riparian arthropod predators

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ABSTRACT

Aquatic-derived resources can be important subsidies for riparian consumers. We systematically reviewed variations in the use of aquatic prey by riparian predators and investigated whether changes and differences in the availability of emerging insects influence riparian arthropod predator communities. We found that aquatic subsidies present a major food source for several riparian arthropod predators. However, the role of aquatic prey in the diet varied between predator groups and hunting strategies. Riparian web-building spiders, especially horizontal web-builders such as *Tetragnatha*, showed the highest proportion of aquatic insects in their diet (70 %). However, free-hunting spiders and riparian beetles also benefited substantially from these subsidies. The use of aquatic prey decreased with increasing distance from the water, varied throughout the year in line with the emergence peaks, and was affected by habitat characteristics. Our review also shows that the abundance and biomass of riparian arthropod predators can be influenced by variations in the availability of aquatic subsidies. This was particularly evident for riparian web-building spiders, especially horizontal web-building spiders such as *Tetragnatha*. Despite the considerable research activity over the past two decades, we identified several research gaps and present opportunities for future studies. First, there is a clear geographical bias, with a marked lack of studies in the Southern Hemisphere and Asia, with the exception of Japan. Second, most studies have focused on a few families of mostly web-building spiders while only a few have considered spiders with different hunting modes (e.g., ambush hunters or free hunters on vegetation), carabids and other beetles. Third, most studies used stable isotope analysis (SIA) for prey analysis. Additional methods, such as polyunsaturated fatty acid (PUFA) analysis, molecular gut content analysis, or combinations of these methods with SIA, should give a clearer picture of the reliance of riparian arthropods on aquatic prey.

Introduction

Ecosystems are interconnected by flows of energy and nutrients in the form of subsidy fluxes. Interest in the importance of aquatic-derived resources for terrestrial systems has grown strongly over time (Baxter et al., 2005; Kraus et al., 2020; Schulz et al., 2015, 2024). Although the mass transfer of organic carbon from terrestrial to aquatic ecosystems is typically higher, the nutritional quality of the flux from water to land is superior (Marcarelli et al., 2011; Twining et al., 2016). In particular, the export of essential polyunsaturated fatty acids (PUFAs) – such as arachidonic acid (ARA 20:4n-6) or eicosatetraenoic acid (EPA, 20:5n-3) – from aquatic ecosystems is vital for physiological processes in terrestrial consumers (Martin-Creuzburg et al., 2017; Twining et al., 2019).

Terrestrial animals that directly benefit from aquatic subsidies include fish-eating mammals and birds such as bears (e.g., *Ursus arctos*; Levi et al., 2020), raccoon dogs (e.g., *Nyctereutes procyonoides*; Kauhala et al., 1998), kingfisher (e.g., *Alcedo atthis*; Nessi et al., 2021), and cormorants (e.g., *Phalacrocorax carbon*; Klimaszyk & Rzymski, 2016). In addition, aquatic insects – i.e. species with aquatic larvae and terrestrial adults such as biting and non-biting midges, stoneflies and caddisflies – are an important food source for bats (e.g. Daubenton's bat; Vaughan, 1997), birds (e.g., Swallows; Jackson & Fisher, 1986; McCarty, 1997) and lizards (e.g. *Sceloporus* sp.; Sabo & Power, 2002). Importantly, the quantity and quality of the resource flows between aquatic and terrestrial systems are highly dependent on river structure (Füreder et al., 2005; Vannote et al., 1980) and environmental conditions, such as nutrient enrichment (Davis et al., 2011; Galloway & Winder, 2015; Kelly et al., 2019). These cross-ecosystem fluxes are also determined by season

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(Ahlgren et al., 1997) as well as top-down (Collins et al., 2020) or bottom-up processes (Allen et al., 2012).

Arthropods play a key role among riparian consumers due to their high diversity and abundance. Several riparian arthropod predators, such as spiders (Arachnida), ground beetles (Coleoptera: Carabidae) and rove beetles (Coleoptera: Staphylinidae), feed on aquatic subsidies, particularly aquatic insects (Nash et al., 2023; Paetzold & Tockner, 2005). For example, the diet of riparian web-building spiders – such as species of the genus *Tetragnatha*, the most diverse genus of long-jawed orb weavers (Tetragnathidae) – may be dominated by prey of aquatic origin Bollinger et al. (2023); Gergs et al. (2014); Krell et al. (2015). Aquatic subsidies can affect the abundance, biomass, density and fitness of riparian arthropod predators: Burdon and Harding (2007) showed that the abundance of web-building spiders was positively associated with biomass of emerging stream insects, suggesting that low predator abundance are due to low emergence rates. Similarly, Uno (2016) found that body sizes and juvenile survival rates of spiders were negatively affected by reduced abundances of aquatic insects. Earlier studies found that species with different web orientations (horizontal, vertical, sheet) responded differently to aquatic subsidy availability (Kato et al., 2003). Given the numerous studies published since, it is worth investigating whether these findings still hold.

Driven by the growing interest in this area, Lafage et al. (2019) conducted a meta-analysis of aquatic subsidy consumption by riparian arthropod predators based on 21 stable isotope analysis (SIA) studies and found that the aquatic subsidy usage was higher than expected. Our review expands on these findings by incorporating additional studies across diverse ecosystems and water body types, integrating non-SIA methods, and considering various spider hunting strategies. Our understanding of the variation in the usage of aquatic prey between different riparian arthropods and the consequences of changes and differences in the quality and quantity of emerging insects for riparian predators is still incomplete. This systematic review aims to fill these gaps by including studies that extend beyond SIA and by considering data on diet composition as illustrated by molecular gut content analysis and PUFA, as well as community data of riparian predator species. We addressed the following research questions: (i) Which riparian arthropod predator taxa and habitats were predominantly studied and in which geographical regions? (ii) Which approaches (e.g., SIA, molecular gut content, biomass, abundance) were used to study carnivore consumer-resources relationships for aquatic-derived resources? (iii) To what extent do aquatic subsidies contribute to the diet of riparian arthropod predators, and what internal (e.g., life stage, body size) and external (e.g., season, distance from the water) factors influence this? (iv) How can the availability and abundance of aquatic subsidies affect the abundance of riparian arthropod predators?

Literature search and data usage

We systematically searched the literature on the linkage between aquatic subsidies and riparian arthropod predators. We performed multiple searches without time restrictions in ISI Web of Science (https://www.webofscience.com/) and Google Scholar (https://scholar.google.de/), with the last search on 08 January 2025, using combinations of the following keywords: “terrestrial* arthropod*" OR "terrestrial* insect*" OR carabid* OR beetle* OR coleoptera* OR spider OR arachnid* OR "ground* dwelling" OR ”Opiliones” OR “harvestmen” OR “ant*” OR “Formicidae” AND "riparian* area*" OR stream* OR river* OR shore* OR "water* edge*" OR drift* OR flood* OR "wash* margin” AND "aquatic insect*" OR emergence* OR "aquatic* subsid*" OR drift* AND feeding* OR "food web*" OR diet* OR prey* OR predator*. The searches yielded 353 peer-reviewed studies, and the references in these publications were screened for additional potentially relevant literature (White, 2009). For our review, we focused on freshwater habitats that reported the use of aquatic subsidies by riparian arthropod predators. We included brackish waters because, similar to freshwater systems, they host aquatic insects, mainly chironomids, as potential prey for terrestrial arthropods (Fuentes et al., 2005; Parma & Krebs, 1977). Therefore, by screening titles and abstracts, we excluded studies that (I) studied only riparian herbivores or vertebrates, (II) investigated marine environments (except brackish), (III) studied only the pollution transfer from aquatic to terrestrial areas via aquatic subsidies, and (IV) analyzed the effects of terrestrial subsidies for aquatic consumers. A total of 109 studies were retained for this systematic literature review (see Appendix A Table 1). From these studies, we gathered information on the taxa of riparian arthropod predators and their prey studied, the methods used to identify the relation between riparian arthropod predators and aquatic subsidies (e.g., community analyses, SIA, gut content analyses), the study area (country), the waterbody studied (lentic, lotic or brackish) and the adjacent terrestrial habitats (e.g., forest, urban area, pasture). In some studies, more than one riparian arthropod predator taxon was observed, so the number of observations may exceed the number of studies.

Our qualitative review is based on all articles found ($N_{studies} = 109$). An exception is the presentation of the results related to the proportion of consumed aquatic subsidies by riparian arthropods (research question iii). To estimate the average proportions of consumed aquatic subsidies in the diet of riparian arthropod predators, only studies using SIA were used ($N_{studies} = 57$), because only these studies included proportions of consumed prey. Where sampling sites in these studies were arranged along lateral gradients from the shore to the adjacent terrestrial habitat, we only used data from the sampling site closest to the aquatic ecosystem. This limitation was made to allow for better comparability and because only a few observations were available at greater distances from waterbodies, but we have included distance information in the discussion. By measuring the ratios of stable isotopes such as nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$), SIA provides information on the trophic level of the consumer and the proportion of aquatic subsidy in the diet (Inger & Bearhop, 2008; Peterson & Fry, 1987; Post, 2002). However, the use of this method may over- or underestimate the aquatic prey based on the consumed carbon source of the prey or the challenge of determining the appropriate trophic discrimination factor (Mellbrand & Hambäck, 2010; Stephens et al., 2023). DNA-based gut content analysis is a powerful tool for understanding short-term dietary patterns by identifying recently consumed organisms down to species level. In particular, metabarcoding of gut content allows to characterize the collective prey community of single predator specimen. Yet, a correct quantification of ingested prey is hampered due to several methodological factors (e.g., primer bias Lamb et al., 2019) which is why most studies use frequency of prey occurrence across many predator individuals to measure prey proportions (e.g., Huszarik et al., 2024). While methods to quantify prey sources with DNA metabarcoding are being developed (Luo et al., 2023; Uiterwaal & DeLong, 2024), such information is not yet available for the diet of riparian predators. PUFA analysis provides short-term, high-resolution dietary information and tracks essential nutrients. As the PUFA content of aquatic organisms is higher than that of terrestrial organisms (which cannot synthesise them themselves), the occurrence of certain PUFAs in terrestrial consumers is a good proxy for aquatic sources (Hixson et al., 2015; Twining et al., 2016). Moreover, two studies have analyzed the gut content of riparian beetles by visual examination (Hering, 1998; Hering & Plachter, 1997). This method provides a direct indication of the midgut content, but it is very time-consuming and requires a high level of expertise in identifying insect fragments. However, calculating the actual amount of aquatic subsidies consumed remains a challenge (Heissenberger et al., 2010). It is not the aim of this literature review to compare the different methods; for further discussion, see Kuhrt et al. (2024) and Nielsen et al. (2018). Instead, we draw on findings from various studies to analyze prey use, acknowledging that different methods may yield varying results. Notably, we did not integrate results from different methods in our calculations, as numerical data from SIA were more prevalent than those from other approaches.

Overview of studies

The use of aquatic subsidies by riparian arthropod predators was studied predominantly in the Northern Hemisphere, particularly in Europe (37.7 %) and North America (35.8 %), followed by Asia (16.0 %; Fig. 1A). Only a few studies were conducted in the Southern Hemisphere, including Australia, Brazil, New Zealand, and South Africa (Fig. 1A). Since 2000, the number of studies per year has steadily increased, while earlier publications appeared less frequent (Fig. 1B).

Representation of predator taxa, habitats and aquatic subsidies

Among the studies included in this systematic literature review, 78 specifically targeted spiders (Araneae) (Fig. 1A and 2A). Of these, 33 focused on web-building spiders, 15 on free-hunting spiders, and 30 either included both or did not provide identification beyond spiders. At the family level, Tetragnathidae ($N_{Studies}$ = 46, all belonging to the genus *Tetragnatha*), Araneidae ($N_{Studies}$ = 28), and Linyphiidae ($N_{Studies}$ = 22) were the most studied web-building spiders, whereas Lycosidae ($N_{Studies}$ = 42) and Pisauridae ($N_{Studies}$ = 11) were the most studied free-hunting spiders (see Appendix A Table 2). Remaining studies often combined spiders with other taxa, such as carabid beetles (Coleoptera: Carabidae) or rove beetles (Coleoptera: Staphylinidae), or combined different beetle species (Fig. 2A). Only five studies exclusively investigated carabid beetles (Fig. 2A).

Most of the studies ($N_{Studies}$ = 66) investigated riparian arthropod predators adjacent to lotic waters, ranging from large rivers, such as the Scioto River (Ohio, USA: 372 km length) (Alberts & Sullivan, 2016), to small streams such as the Buizin (France: 12 km length) (Barthélémy et al., 2022). Among these studies, 29 did not consider the river section as an influencing factor, and detailed information on the location of the study sites along the watercourses was often not provided. However, 23 studies focused on a specific river section (headwater: $N_{Studies}$ = 11 middle reach $N_{Studies}$ = 12), and 14 studies took place along the river course. The linkages between aquatic subsidies and riparian arthropod predators were studied much less frequently in lentic ($N_{Studies}$ = 13) and brackish waters ($N_{Studies}$ = 3). Three out of the four studies on brackish waters were conducted on the southern Swedish coast, adjacent to the Baltic Sea, and one in Florida (USA) at a brackish creek (Moon & Silva, 2013). Across all water body types, the most common adjacent terrestrial habitat studied was forest ($N_{Studies}$ = 45), followed by grassland ($N_{Studies}$= 10). In addition, only seven of all the studies provided specific details on the distance from the water's edge at which they sampled, while most others simply described the sampling sites as "close to the water's edge".

As our systematic review focused on arthropod predators, only animals were considered as aquatic subsidies. In most studies (83 %), the aquatic insects considered as food sources were not further analyzed, while Chironomidae were identified as aquatic prey in 10 % of the studies, followed by Ephemeroptera, Plecoptera and Diptera (7 %). In most cases, only live insects were interpreted as prey, while only four studies argued that dead washed-ashore insects are a potential food source.

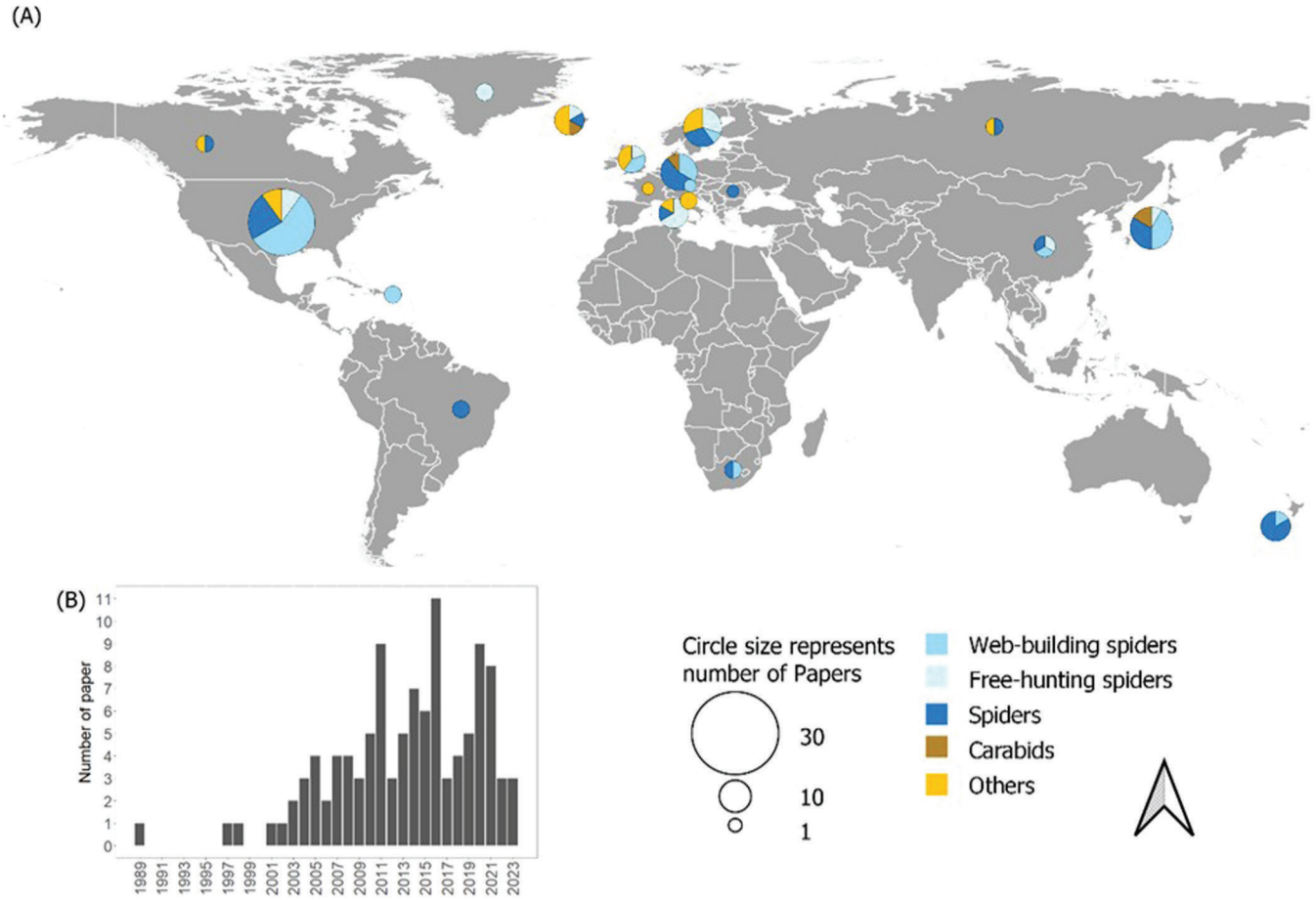


Fig. 1. (A) Spatial pattern of publications (circle size indicates number of studies) and studied organism groups (web-building spiders = exclusively investigating web-building spiders; free-hunting spiders = exclusively investigating free-hunting spiders; spiders = investigating spiders in general; carabids = exclusively investigating carabids; others = studies which did not fit in one of the other categories including roove beetles, harvestman, ants, common woodlouse and click beetles). (B) Number of publications per year between 1989 and 2023.

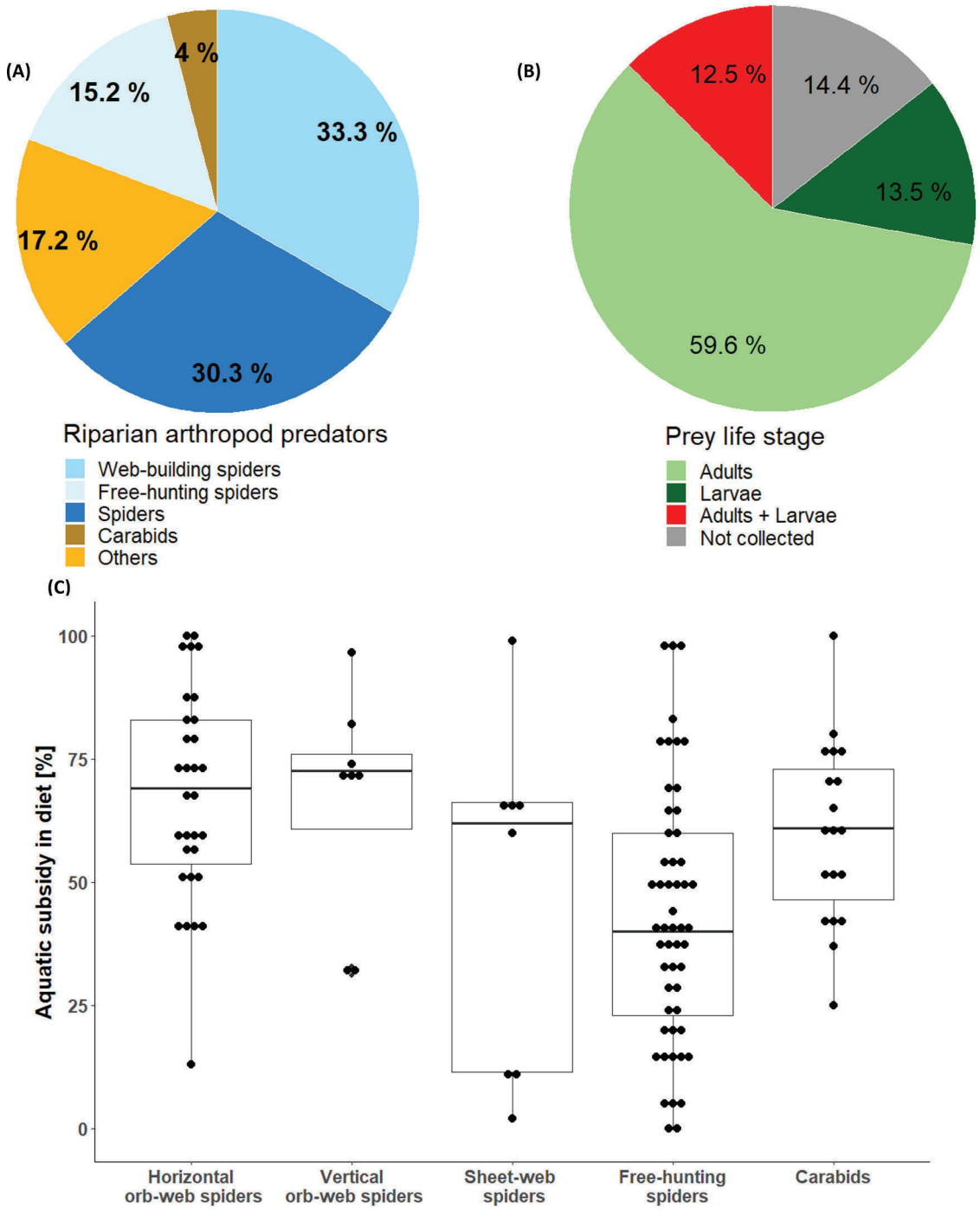


Fig. 2. Proportion of studies for (A) investigated riparian arthropod predator groups and (B) life stages of the sampled aquatic prey sources. (C) Proportion of consumed aquatic subsidies by horizontal orb-web spiders ($N_{studies} = 24$, $N_{observations} = 31$), vertical orb-web spiders ($N_{studies} = 7$, $N_{observations} = 8$), sheet-web spiders ($N_{studies} = 7$, $N_{observations} = 8$), free-hunting spider ($N_{studies} = 44$, $N_{observations} = 54$), carabids ($N_{studies} = 5$, $N_{observations} = 19$).

Approaches to study aquatic subsidies to riparian arthropod predators

The approaches to examine the use of aquatic subsidies by riparian arthropod predators can be divided into two main groups: (1) approaches to quantify the amount of aquatic prey in the diet of the target organism ($N_{studies} = 68$), and (2) approaches to analyze the abundance (including biomass, density or related measures) of the riparian arthropod predators as a response to the (altered) availability of the aquatic subsidies ($N_{studies} = 29$). Some studies examined other response variables such as feeding behavior or body condition. These studies will not be further discussed.

Various analytical methods have been used to study the diet of riparian arthropod predators (group 1). Stable isotope analysis is the most common method to quantify the proportion of aquatic and terrestrial food sources by analyzing the amount of nitrogen (^{15}N) and carbon (^{13}C) in riparian arthropod predators, sometimes in combination with PUFA ($N_{Studies} = 3$) or gut metabarcoding ($N_{Studies} = 1$). In addition, 10 studies used PUFA and two studies used DNA metabarcoding exclusively (Northam et al., 2012; Verschut et al., 2019). The relatively large number of SIA studies found in our review is due to the fact that this method was used much earlier than PUFA or molecular gut content analysis. Finally, two studies used microscopic gut content analysis of

carabids (Hering, 1998; Hering & Plachter, 1997). Sampling of the potential aquatic prey is required for SIA and PUFA analyses to establish a baseline. A variety of methods were used, with sampling of emerging insects ($N_{Studies}$ = 75) being the most common approach, followed by sampling of aquatic insect larvae ($N_{Studies}$ = 27; Fig. 2B). Emerging insects were mainly collected using pyramidic floating emergence traps, sticky traps, or sweep nets. Aquatic larvae were mainly sampled with kick net techniques or Surber sampling (Surber, 1937). However, even when larvae were collected for SIA, the majority of studies considered only the flying adult stage as a potential food source for riparian arthropod predators (Paetzold et al., 2005; Raikow et al., 2011).

In studies of subsidy effects on predator abundance (group 2), predators were mostly collected by hand-picking ($N_{Studies}$ = 21). Each of the remaining methods e.g., sweep netting, counting webs, and suction sampling using an adapted leaf vacuum, was used in less than four studies.

Aquatic insects in the diet of riparian arthropod predators

Variation of aquatic subsidies in the diet between riparian arthropod predator groups

Aquatic insects are high-quality food for riparian predators due to their PUFA composition (Pietz et al., 2023). In our literature review, we found that aquatic insects are often an important component of the diet of many riparian arthropod predators (Fig. 2C). While the SIA data collected from the literature showed a high variation in the intake of aquatic food sources both within and between predator groups, the mean intake of aquatic resources was higher in horizontal web-building spiders (70.0 % ± 19.1; $N_{studies}$ = 24) compared to vertical web-building spiders (e.g., Araneidae; 66.5 % ± 21.4; $N_{studies}$ = 7), sheet-web weavers (Linyphiidae; 47.1 % ± 21.4; N = 7), free-hunting spiders (e.g., Lycosidae 46.0 % ± 23) and carabids (60.0 % ± 17.9; $N_{studies}$ = 5) (Fig. 2C). The high ingestion of aquatic prey by horizontal web-building spiders, as observed in our review, aligns with the findings of Gillespie (1987) and Tagwireyi and Sullivan (2014). These studies show that such spiders typically build their webs in overhanging vegetation near water, increasing their chances of capturing emerging aquatic insects.

The remarkably large variation in aquatic prey intake suggests interspecific differences in riparian arthropod predators and reflects the heterogeneity in sampling design, season, waterbodies, habitat types and geographical regions examined between studies. Importantly, prey choice is influenced not only by the availability of aquatic prey in the environment but also by the physical conditions of the predator species (e.g., life stage), as well as local and landscape-scale habitat and environmental conditions (e.g., waterbody size, riparian habitat type, surrounding land use and associated water pollution). Our review identified several factors that influence the proportion of aquatic prey in the diet of riparian arthropod predators, which are discussed in the following paragraphs.

Sex, life stage and body size

Our review demonstrates that prey selection in riparian spiders and beetles can be influenced by sex, life stage and body size. For example, males of the spider *Trichonephila clavata* (Nephilidae), which are substantially smaller than females (Chuang et al., 2023), feed relatively more on aquatic insects than females, even when the number of aquatic and terrestrial prey in the webs was constant (Akamatsu et al., 2007). Furthermore, juvenile wolf spiders have been shown to feed more on aquatic prey, while adults shift to terrestrial prey, even when aquatic prey such as chironomids are abundant (Verschut et al. 2019). This pattern may be explained by a more favorable predator-prey body size ratio, with aquatic prey often being smaller than terrestrial prey (Akamatsu & Toda, 2011). Similarly, smaller spiders are less efficient at capturing larger terrestrial prey due to their morphological characteristics and web structure (Collier et al., 2002). Consistent with this, smaller carabid beetles (e.g., *Bembidion* spp.) have been shown to feed more on aquatic subsidies (90 %) than larger carabids (e.g., *Nebria picicornis*; 51 %), despite sharing the same riparian habitat (Hering & Plachter, 1997). However, it should be noted that factors other than body size, such as agility, may contribute to the reduced aquatic uptake in larger beetles.

Distance from the water

Across different groups of riparian arthropod predators, the proportion of aquatic subsidies in the diet of predators decreases with increasing distance from the water (e.g., Hering & Plachter, 1997; Lafage et al., 2019; Surber, 1937; Verschut et al., 2019). Three out of four studies on web-building spiders showed a clear decrease of aquatic subsidies in the diet with increasing distance, including lentic and lotic waters as well as brackish waters (Henschel et al., 2001; Mellbrand et al., 2011; Raikow et al., 2011). Similarly, the proportion of aquatic prey in the diet of free-hunting spiders decreased with increasing distance from the water (Briers et al., 2005; Fritz et al., 2017; Paetzold & Tockner, 2005) and carabid beetles sampled more than 50 m from the water's edge fed exclusively on terrestrial food sources (Paetzold & Tockner, 2005). It appears logical that this pattern occurs because the availability of aquatic insects decreases with increasing distance from the water's edge. In a meta-analysis, Muehlbauer et al. (2014) showed that aquatic prey activity was highly concentrated within 1.5 m of the waterbody, while only 10 % of the insect activity was found at a 500 m distance.

The reduced proportion of aquatic subsidies in the diet of predators with increasing distance from the water may also be due to lateral shifts in the composition of the predator community, with the abundance of riparian specialists decreasing with distance from the shoreline (Gallé & Schwéger, 2014; Scheidler, 1990). This is discussed in the chapter "Aquatic subsidies and the abundance riparian arthropod predators". However, a recent study found no difference in aquatic prey consumption between syntopic pairs of riparian specialist and generalist spiders (Bollinger et al. 2023).

Seasonal effects

The uptake of aquatic subsidies can vary with season (Chan et al., 2007; Gratton et al., 2008). However, it should be considered that this may interact with the ontogenetic stage of the predator and associated changes in dietary requirements (see above; Akamatsu et al., 2007). Nevertheless, a clear relationship between the seasonal peak of emergence and the number of aquatic insects in the diet was reported for web-building spiders (e.g., Chan et al., 2009; Chari et al., 2020; Kelly et al., 2015; Kowarik et al., 2021). For example, along tropical forest streams in China, the emergence of aquatic insects was lower in the dry season, resulting in 50 % fewer aquatic insects in the diet of web-building spiders (Chan et al., 2007, 2009). Furthermore, *Tetragnatha* spiders in Switzerland had the highest concentration of Eicosapentaenoic acid (EPA) – a long-chain fatty acid associated with aquatic sources (Hixson et al., 2015; Kowarik et al., 2021) – in spring, the season with the highest emergence of aquatic insects (Salvarina et al., 2017). Chironomids are often the most abundant emerging insects in aquatic habitats (Kautza & Sullivan, 2016). Indeed, feeding of web-building spiders on chironomid midges increases with prey density (Diesburg et al., 2021; Gergs et al., 2014). Similar to web-building spiders, the feeding behavior of free-hunting spiders has been shown to be altered by seasonal variation in the abundance of aquatic insects (Siebers et al., 2021; Verschut et al., 2019), with aquatic insects accounting for a higher proportion in the diet as the abundance of emerging aquatic insects increases. The diet of riparian carabids may be affected by seasonality in different ways, with some species showing temporal variation in the intake of aquatic subsidies, as described for spiders, while other carabids

are highly dependent on aquatic insects throughout the season (Gratton et al., 2008; Terui et al., 2017). This may indicate that some carabid beetles actively hunt for aquatic insects, whereas other riparian carabids and spiders often are more generalist feeders whose diet is more dependent on what prey is available.

Size and trophic productivity of waterbodies

The primary productivity in river networks generally increases from headwaters (energized by the input of carbon particulate organic matter from adjacent terrestrial areas) towards the middle and lower reaches (Vannote et al., 1980). As a result, the biomass and transfer of aquatic insects to adjacent terrestrial habitats typically increases towards the highly productive middle and lower reaches (Vannote et al., 1980). The literature confirms that changes in the size and trophic productivity of waterbodies lead to changes in the availability of aquatic subsidies and consequently affect the proportion of aquatic insects in the diet of riparian arthropod predators ($N_{studies} = 7$). For example, high transfers of aquatic subsidies in middle and lower reaches increased the proportion of aquatic prey in the diet of web-building spiders (Chari et al., 2020). Consistent with this, Marker et al. (2023) reported a higher proportion of aquatic prey in riparian spiders from larger, more productive rivers compared to smaller, less productive streams. However, this pattern may also be influenced by the shoreline-to-water surface ratio, as lower ratios tend to facilitate higher aquatic subsidies (Gratton & Zanden 2009). Additionally, it must be noted that the isotope signal of aquatic insects in headwater regions is often more terrestrially driven (Kraus & Vonesh, 2012; Scharnweber et al., 2014), potentially weakening the aquatic signal in these areas. This complexity makes it more challenging to interpret the proportion of aquatic prey in riparian arthropod predators' diets in relation to waterbody size and productivity.

Riparian habitat, surrounding land-use and associated water pollution

Riparian habitat type and surrounding land use may influence the use of aquatic prey, although no consistent pattern has yet emerged within and across riparian arthropod predator groups. For web-building spiders, there are some indications that riparian forests may promote a more aquatic-based diet compared to open riparian habitats (Alberts & Sullivan, 2016; Ramberg et al., 2020). Spider communities in riparian forests are often diverse and include many web-building species that utilize aquatic prey (Jackson et al. 2016). Furthermore, the availability of alternative terrestrial prey may be higher in productive open grasslands (high primary productivity due to light and nutrient availability), making spiders in riparian forests more dependent on the presence of aquatic subsidies (Alberts & Sullivan, 2016). In contrast, Krell et al. (2015) observed higher proportions of aquatic prey in web-building spiders (Tetragnatidae) in grasslands (80–100 %) than in forests (40–60 %). Presumably, higher tree canopy cover in this study reduced the occurrence of emerging aquatic insects, as shown by Kautza and Sullivan (2015). However, Krell et al. (2015) also found only a small difference in the carbon stable isotope signature between aquatic and terrestrial insects in the forest areas, because aquatic insects may feed more on allochthonous food sources (leaf litter), which are more abundant in forest streams than in streams surrounded by open grassland. This may have underestimated the proportion of aquatic insects in the diet. An overestimation of the aquatic signal in riparian carabids due to the consumption of springtails feeding on aquatic algae was shown by Mellbrand and Hambäck (2010). This shows the limitations of SIA studies and highlights the importance of integrating additional methods (e.g., DNA-metabarcoding of gut content) for a better understanding of the origin of food sources. Furthermore, the use of PUFA could provide valuable insights into the quality of aquatic subsidies. For example, the FA quality of riparian *Tetragnatha* was shown to be higher in forested streams, even though the biomass of emerging insects was higher in agricultural surroundings. Consequently, shading and the stream pool quality appear to be more important determinants of FA quality than agricultural stressors (Ohler, 2024). In contrast to web-building spiders, free-hunting spiders and carabid beetles have been shown to rely more heavily on aquatic subsidies in open than in woody riparian habitats (Briers et al., 2005; Greenwood, 2014; Krell et al., 2015; Lafage et al. 2019; Siebers et al., 2021). Free-hunting spiders and carabid beetles are predominantly active on the ground, and higher abundances of soil-dwelling springtails, which are a high-quality food source, may explain the more terrestrial diet in forests (Krell et al. 2015). In addition, woody vegetation such as shrubs might act as a barrier for flying aquatic insects, restricting their availability in more distant areas around forest streams (Greenwood, 2014).

The surrounding land use, which strongly influences water pollution through the discharge of nutrients, pesticides and metals (Paetzold et al., 2011; Paul & Meyer, 2001; Tong & Chen, 2002), can also affect the diet of riparian arthropods. Water pollution can shift aquatic communities towards smaller insects (often chironomid midges) at the expense of larger species such as caddisflies, mayflies and stoneflies (Jones & Clark, 1987). Therefore, water pollution may explain the low use of aquatic subsidies by riparian web-building spiders in landscapes surrounded by agricultural or urban land use (Graf et al., 2020; Hunt et al., 2020). These aquatic communities may be less favorable for spiders, resulting in a more terrestrial diet of web-building spiders (Graf et al., 2020; Hunt et al., 2020). Thus, web-building spiders tend to have a higher proportion of aquatic insects in their diet in more natural riparian habitats and under more pristine conditions (Graf et al., 2020; Hunt et al., 2020; Kautza & Sullivan, 2016).

Invasive species

Invasive species can profoundly alter food web structure (David et al., 2017). Invasive aquatic predators can reduce the abundance of emerging aquatic insects, potentially affecting the diet of riparian predators. Jackson et al. (2016) found a 51 % trophic niche overlap between riparian web-building spiders and invasive trout, likely due to trout feeding on chironomid larvae (Kautza & Sullivan, 2016). In contrast, the overlap between spiders and native trout was only 5 %, suggesting greater competition for the same food between invasive fish and web-building spiders. Similar results were shown for the invasive amphipod *Dikerogammarus villosus* (Amphipoda: Gammaridae), which reduced the proportion of aquatic insects in the diet of *Tetragnatha* spiders from 60 % to 10 % (Gergs et al., 2014). However, niche overlap between invasive and native species need not necessarily result in competition, especially if prey is not a limiting factor, such as mass emergence events of midges (Corbet, 1964). In addition, invasive aquatic species could potentially provide an additional prey source for riparian arthropod predators – as has been shown, for example, for ibis feeding on invasive apple snails (Bertolero & Navarro, 2018) – but we did not find any such study with our search. However, due to the limited number of studies, it is too early to draw general conclusions about the influence of invasive aquatic species on the diet of riparian arthropod predators.

Aquatic subsidies and the abundance of riparian arthropod predators

Investigating the relationship between aquatic subsidies and riparian predator communities is challenging due to several confounding or interacting variables. For example, habitat structure and land use may influence the emergence rate of aquatic insects, which in turn may affect the population size of riparian predators (Krell et al., 2015). At the same time, habitat structure and land use can also directly influence riparian spiders (Alberts & Sullivan, 2016; Graf et al., 2019; Hunt et al., 2020). Nevertheless, our literature review suggests that variation in the availability of aquatic subsidies may indeed influence the abundance of riparian arthropod predators.

We have found evidence that the abundance of riparian web-building spiders is positively related to the availability of aquatic subsidies in running waters (e.g., Kato et al., 2003; Wesner, 2012). As a result, natural or anthropogenically driven reductions (e.g., increased metal content (Kraus et al., 2014)) in rivers, in aquatic insects are often reported to have a negative impact on the abundance of web-building spiders. Such a pattern has been found in studies analyzing spider abundance at different distances from the water along lateral transects. For example, Burdon and Harding (2007) reported that the web abundance (as a proxy for spider abundance) in a forest decreased with increasing distance from the water edge, while the forest structure did not change along this lateral gradient. Consequently, this could be related to the reduced availability of aquatic insects with increasing distance from the shoreline (Muehlbauer et al., 2014). However, the reduced abundance of web-building spiders could also reflect the shift from riparian forest to inland forest, together with a change in the associated spider species composition. Henschel et al. (2001) contradict this by attributing the loss of spider biomass with increasing distance from the water to a reduced density of aquatic insects. Densities were 4.5–7.5 times lower 30–60 m from the shore compared to areas directly adjacent to the shore. However, physiological constraints, such as desiccation tolerance - which varies among spider species (Nentwig et al., 2025) - may also influence abundance patterns along the gradient from the moist riparian zone to the drier terrestrial area. Additionally, some studies show a positive relationship between seasonal peaks in the emergence rate and the abundance of web-building spiders (Kato et al., 2003; Wojan et al., 2014). However, seasonal variation of spider abundance is also driven by their life cycle, making it difficult to draw conclusions about the causal relationship with emergence. In general, riparian consumers in temperate regions show a greater dependence on periodic emergence fluxes than in the tropics, because of the greater climate-driven seasonal variation in emergence in temperate regions compared to the tropics (Nash et al., 2023). The abundance of web-building spiders (Tetragnathidae and Linyphiidae) has also been shown to reflect the variation in emergence rates between river sections of varying geomorphology and productivity (Iwata, 2007) and in response to invasive aquatic species. For example, the presence of the invasive rainbow trout in a Japanese river was associated with a 35 % reduction in emerging aquatic insects, which in turn reduced the abundance of *Tetragnatha* spiders by 65 % (Baxter et al., 2004).

Unlike web-building spiders, the abundance of free-hunting spiders often appears to be unaffected by changes in aquatic emergence rates, or patterns are not uniform (Recalde et al., 2016). In line with that, no changes in the abundance of *Pardosa glacialis* and other free-hunting spiders were observed with distance to ponds in Greenland (Culler et al., 2021) or the Baltic Sea (Mellbrand et al., 2010). In contrast, Yuen and Dudgeon (2015) found that the abundance and biomass of free-hunting spiders are higher directly adjacent to running waters than at further distances. Obviously, distance effects on riparian free-hunting spiders related to the availability of aquatic subsidies seem to be context-dependent and may vary with factors such as climate, type of water bodies, and habitat structure.

Effects of aquatic subsidies on the abundance of riparian beetles have rarely been studied, and the results are mixed. Hering and Plachter (1997) found no relationship between food availability and *Bembidion* density along a river. In a field experiment on gravel bars along a river, beetle abundance and richness were also not related to aquatic subsidies, but rather differed between wet and dry plots (Henshall et al., 2011), suggesting a stronger habitat effect on carabids than the availability of aquatic resources. In contrast, a similar field experiment found that beetle abundance was higher in plots with additional aquatic subsidies (Paetzold et al., 2006). Both studies were conducted on gravel banks along rivers and manipulated the amount of aquatic subsidies for riparian consumers. The contrasting results could be explained by differences in patch size, patch distance from the water and, most importantly, in the amount of aquatic subsidies added. Henshall et al. (2011) added 5 mg of dry weight of chironomids in a 4 m^2 plot, while Paetzold et al. (2006) added 50–100 mg of aquatic subsidies to 1 m^2, representing a multiple times higher subsidy concentration. In a study where salmon carcasses were added to riparian patches, the abundance of staphylinids in particular responded positively to the added aquatic subsidy. However, this effect was dependent on local habitat conditions and stronger in a vegetated (four times higher abundance than in the control) than in an unvegetated patch (Collins & Baxter, 2014).

Conclusion

Our literature review confirms that aquatic subsidies, mainly aquatic insects, are an important food source for riparian arthropod predators. At the same time, the role of aquatic prey is highly variable between and within arthropod predator groups, and is strongly dependent on the physical conditions of predators, river structure, habitat and environmental conditions. Riparian web-building spiders, especially horizontal web-builders such as *Tetragnatha*, showed the highest reliance on aquatic prey, but also free-hunting spiders and riparian beetles can make strong use of these inflows. The literature also shows that the abundance of riparian arthropod predators can be influenced by the availability of aquatic inflows. In particular, the abundance of web-building spiders was positively related to the abundance of emerging insects. Despite considerable research activity over the last two decades, there remain several research gaps and opportunities for future studies:

- There is a clear geographical bias, with a lack of studies in the southern hemisphere and Asia, except Japan. Future studies should attempt to fill these gaps. This is particularly interesting as the aquatic prey source may vary between ecozones, as has recently been shown for tropical and temperate forests (Nash et al., 2023).
- Most studies have focused on web-building spiders (mainly *Tetragnatha* spp.), while only a few studies have considered spiders with different hunting modes (e.g., ambush hunters or free hunters on vegetation), carabids and other beetles. Other riparian arthropod predators such as rove beetles, harvestmen (Opiliones) or ants (Formicidae) are severely underrepresented. Future studies should therefore investigate the potential importance of aquatic prey subsidies for riparian arthropod predators other than spiders and carabid beetles. Similarly, potential aquatic subsidies other than emerging aquatic insects should be considered, as ground-dwelling riparian predators also use dead organisms washed ashore (Hering & Plachter, 1997). We are not aware of any study analyzing aquatic prey subsidies in the diet of canopy-dwelling predators, reflecting the general lack of studies in this vegetation layer (Albacete et al., 2020).
- To analyze the proportion of aquatic insects in the diet of predators, most studies used SIA. However, differences in stable isotope signatures between freshwater and adjacent terrestrial ecosystems are often small, limiting the reliability of mixing models (Krell et al., 2015; Phillips et al., 2014). Thus, additional DNA-based prey analysis could give a clearer impression of the variety of food consumed (Verschut et al., 2019), while PUFA would add valuable information on the food quality (Ohler, 2024).

By identifying these research gaps, we hope to stimulate future research efforts that will ultimately strengthen our understanding of aquatic-terrestrial meta-ecosystems.

Data availability statement

Data is presented in supplementary material.

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CRediT authorship contribution statement

Franziska Middendorf: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mirco Bundschuh:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Bernhard Eitzinger:** Writing – review & editing, Supervision, Conceptualization. **Martin H Entling:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Jens Schirmel:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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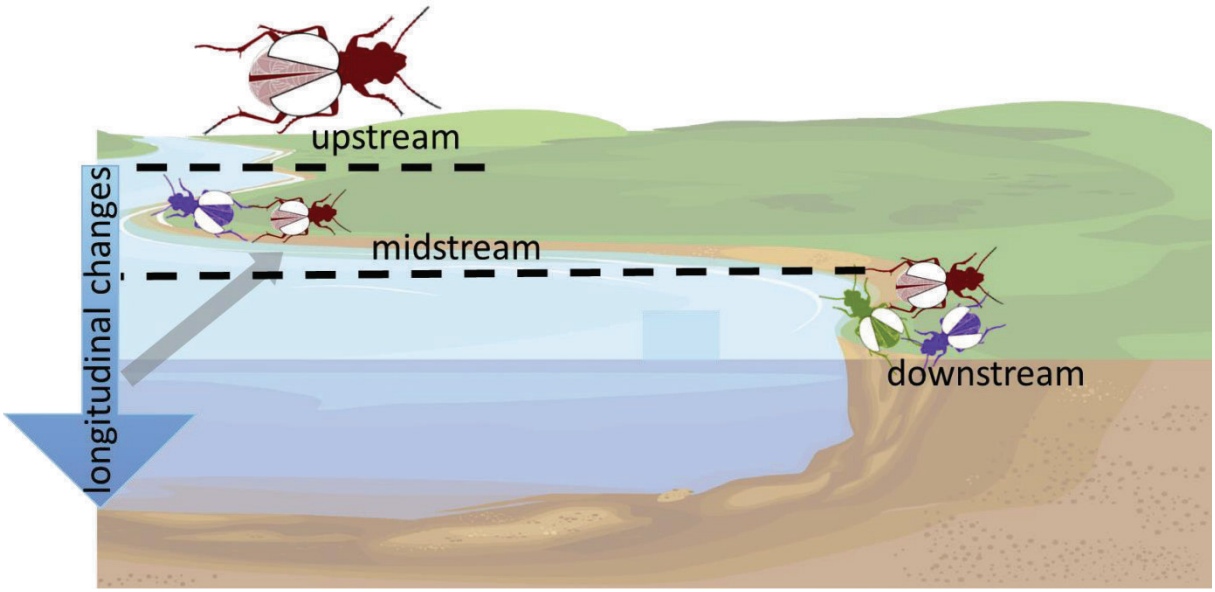
Chapter 3:

Carabid beetles as indicators of stream zonation

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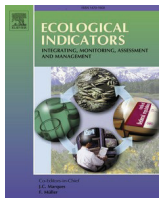
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Carabid beetles as indicators of stream zonation

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ABSTRACT

Rivers and streams are characterised by longitudinal changes in hydrology and aquatic communities. Due to the strong connectivity between watercourses and adjacent terrestrial ecosystems, riparian communities might also show characteristic changes along stream courses. Although carabid beetles are often used as ecological indicators in riparian habitats (e.g., to monitor restoration success), little is known about how they indicate stream zonation. We conducted a study of riparian habitats at five streams, each sampled in three near-natural sections upstream, midstream and downstream, to analyse changes in carabid beetle abundance, species richness, indicator species and functional trait composition along stream courses. Carabid beetles were sampled using pitfall traps and hand collections in May and September 2023. Carabid abundance, species richness and functional diversity increased significantly from upstream to downstream. Carabid communities shifted from a dominance of large-bodied and short-winged species in the upstream zone towards smaller and long-winged species downstream. In addition, 11 indicator species were identified in the downstream zone, compared to only two in the upstream and one in the midstream zone. In contrast, the percentage of riparian habitat specialists peaked midstream. Changes in carabid communities were likely driven by increasing size of the riparian area and soil pH along the course of the streams, likely reflecting the increasing hydrological connectivity and sediment deposition. Our findings clearly showed longitudinal changes in the community structure and trait composition of carabid beetles along streams. We conclude that carabids are therefore excellent ecological indicators of the zonation of the riparian zone of small streams.

1. Introduction

Riparian zones are transitional habitats between adjacent aquatic and terrestrial ecosystems and are considered as biodiversity hotspots worldwide (Naiman et al. 1993; Hauer et al., 2016). Shaped by frequent flooding events and temporal variations in water availability, riparian zones are characterised by their heterogeneity and instability, harbouring distinct biological communities adapted to disturbances (Merritt, 2022). Hence, riparian zones play a disproportionate role for supporting biodiversity at the landscape scale and supporting highly specialised, rare and endangered species (Andersen and Hanssen, 2005; Denux et al., 2017; González et al., 2017; Bauspiess et al., 2024).

Riparian habitats vary along river course from its head water to its mouth shaped by the adjacent running water system and associated physical, chemical and biological processes such as sediment deposition, chemical transfer and nutrient loads (Capon and Pettit, 2018). Longitudinal changes from upstream to downstream river courses include an increase of inundation duration and freshwater-derived resource availability (Ramey and Richardson, 2017) while the grain-size of sediments (Sadler et al., 2004; Baiocchi et al., 2012; Petts et al., 2000) and river slope is decreasing (Tomanova et al., 2007). This is accompanied by a shift in adjacent riparian habitats and floodplain types (see Globevnik et al., 2020). In general, floodplain types change along river courses according to abiotic factors with a transition from narrow and steep floodplains characterised by dynamic relief-forming processes and substrates with larger soil particle sizes at higher elevations, to wide and very flat floodplains with reduced dynamic of relief building processes and smaller soil particle sizes in the lowlands ((Koenzen, 2005; Globevnik et al., 2021). Changes in habitat will eventually translate to changes in biodiversity and species assemblages. For example, riparian floral diversity is often highest in mid reaches (Tabacchi et al., 1996), mainly explained by the intermediate disturbance hypotheses (Connell, 1978; Malanson, 1984). However, regional (e.g., sediment and nutrient content) and temporal (e.g., water discharge and disturbance regime) effects can outweigh these longitudinal effects (Ward et al., 2002).

Many studies have shown that aquatic communities undergo

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characteristic changes along the river course (Figueroa and Rios-Escalante, 2021; Živić et al., 2001; Santos et al., 2010). For example, aquatic macroinvertebrate communities are dominated by the feeding types of shredders and collectors in upper reaches while collectors and grazers dominate in middle reaches and collectors in downstream reaches (Vannote et al., 1980). Longitudinal respective altitudinal zones are an important component also for general classifications of river types (Jupke et al., 2022). Similarly, fish communities show distinct changes along river courses. For example, European rivers can be divided into four zones, starting with the trout zone in the upper reach, followed by the grayling, barbel and bream zone if the river reaches a sufficient size within downstream lowland areas (Aarts and Nienhuis, 2003). In contrast to aquatic organisms, riparian organisms received less attention. Characteristic changes of terrestrial communities in riparian areas can be expected due to the strong cross-boundary linkages between rivers and adjacent riparian habitats (e.g. Schulz et al., 2015). For example, the species and trait composition of carabids and spiders varied along the river course of the Common Meuse (border river between the Netherlands and Belgium), which was mainly attributed to differences in flooding duration (Lambeets et al., 2008b; Lambeets et al., 2009). Framenau et al. (2002) found substantial differences in riparian arthropod composition and densities (including wolf spiders and carabid beetles) between upland and lowland rivers in Australia. However, the intensity of changes in the terrestrial arthropod communities was dependent on the geographical region and taxa.

Carabid beetles (Coleoptera: Carabidae) are widespread and abundant in terrestrial habitats and contain species strongly varying in size, feeding strategy, flight ability and habitat specialisation (Kotze et al., 2011). Based on their high sensitivity to environmental conditions, carabid beetles are very useful ecological indicators. They respond strongly to changes in (micro)habitat conditions such as vegetation density, light intensity, substrate composition, temperature regime and moisture content (Lambeets et al., 2008a; Lambeets et al., 2009; Schirmel and Gerlach, 2022; Schirmel et al., 2015). Some carabids are known for their rapid (re-)colonization of river banks, mostly by specialised, small and full-winged species (Lambeets et al., 2009; Sadler et al., 2004) This makes carabid beetles particularly good indicators of riparian habitats, where they have been used to indicate the status and condition of river banks (Jachertz et al., 2019; Sprössig et al., 2022; Bauspiess et al., 2024) and to monitor the success of river restoration measures (Januschke et al., 2011).

To test community changes of carabid beetles in riparian areas from up- to downstream, investigations along multiple streams in the same geographical regions are necessary. Therefore, our study focused on how carabid species and trait composition varied along stream courses. We conducted a field study at five similar tributary streams of the river Rhine, southwest Germany, and sampled carabid beetles in near-natural riparian habitats in three zones: upstream, midstream and downstream. We hypothesised that (i) the species richness, the number of individuals and the functional diversity of carabid beetles increase from upstream to downstream sections and that (ii) the different stream zones are characterised by distinct species compositions with different indicator species. Thereby, we assumed that riparian habitat specialists increasingly dominate towards downstream zones. We further hypothesised that (iii) the different stream zones are also characterised by specific trait compositions of carabid beetles.

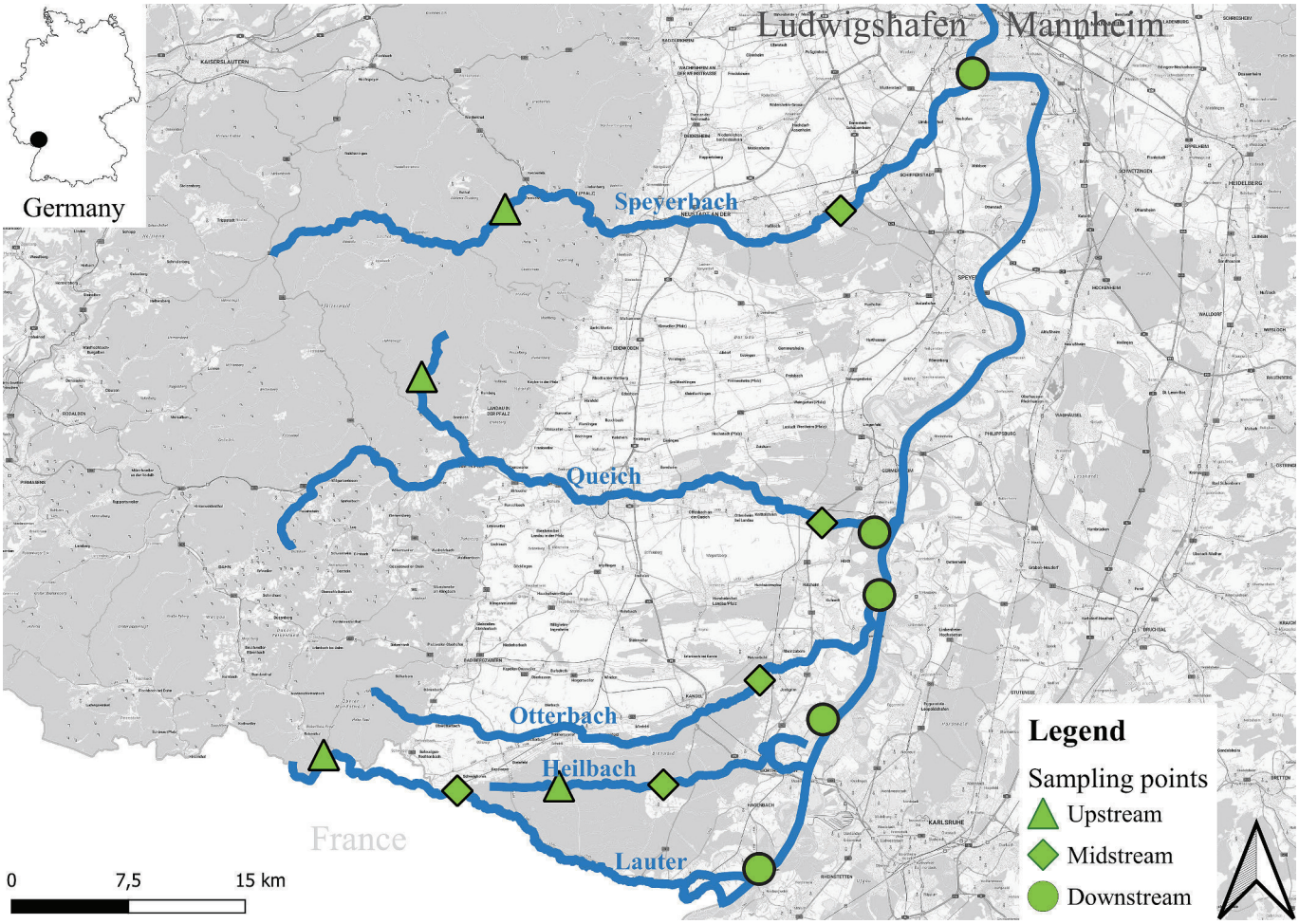


Fig. 1. Location of the study area in Germany (inlet) and the 14 sampling sites (upstream, midstream, downstream) at the 5 streams.

2. Material and methods

2.1. Study area, stream and site selection

The study was conducted in summer 2023 in south-west Germany, in the federal state of Rhineland-Palatinate. The study area includes the low mountain ranges of the Palatinate Forest in the west and the upper Rhine Valley in the east (Fig. 1). The climate in the region is characterised by an average annual air temperature of 9.3 °C (long-term medium 1971–2000, Klimawandelinformationssystem Rheinland-Pfalz (2024)). Precipitation intensity decreases from west to east, with higher annual mean precipitation in the Palatinate Forest (850 mm) than in the upper Rhine Valley (505 mm) (reference period 1991–2020, Landesamt für Umwelt Rheinland-Pfalz (2023)).

We selected five streams with near-natural sections, all originating in or close to the Palatinate Forest and entering the river Rhine (catchment size: 213,300 km^2; Fig. 1). The catchment areas of the sampled streams range from 108 km^2 (Otterbach) to 251 km^2 (Speyerbach) and the length of the streams varies from 24 km (Heilbach) to 114 km (Wieslauter/Lauter) (Table 1). In this study, a stream was defined as a continuous watercourse including its lateral flows. For example, we included a near-natural section of the Katzenbach as an upstream site for the Queich river, which had no suitable sections in its own headwaters that met our selection criteria.

At each stream we selected three riparian sampling sites, each one in upstream, midstream and downstream, except for the Otterbach, where only midstream and downstream were sampled (N_{total} = 14 sampling points). Riparian sampling sites were selected for their near-natural shore characteristics, including a gentle slope and lack of bank reinforcement. In addition, cut off slope orientated banks were excluded. In addition, we only considered riparian sites with a minimum bank length of 2.5 m which are located within forest areas to minimise impact of agriculture, roads and human settlements. Finally, the downstream zones have been chosen so that they are close to point of entry into the Rhine. As the upstream section of the Otterbach did not meet the requirements of the targeted stream bank characteristics and the surrounding forest, no sampling was carried out here. In Germany, streams are classified into 25 types based on size and morphological, physico-chemical, hydrological and biocenological characteristics (LAWA types; Pottgiesser 2018). The 14 sampled stream sections belong to five out of the eight LAWA types specifically identified in the lower mountain ranges (Table 1). The riparian areas can be classified into three main floodplain types (Koenzen 2005; Table 1). The upstream sections are located in low-gradient floodplains of the overburden, while the midstream sections are found in low-gradient floodplains of lowland and hilly regions. All downstream sections are located in low-gradient floodplains that experience summer floods. With the exception of the upstream and midstream sections of the Heilbach, which are characterised by organic matter, all other stream sites are characterised by sandy substrates (Koenzen, 2005).

During the study period, the average water level of the streams varied between 20.3–87.4 cm and 3.2–78.5 cm in May and September, respectively (data from nearest water level stations provided by the Ministry of the Environment of the Federal State of Rhineland-Palatinate, Landesamt für Umwelt Rheinland-Pfalz (2023) (Table 1). In general, the most drastic water level fluctuations occurred at the downstream sampling sites close to the Rhine (Fig. A1).

2.2. Environmental parameters

In total, 14 environmental parameters were measured. The length and width of the riparian area was measured three times both in May and September using a measuring tape. In this study the term "riparian area" refers to the clearly defined natural shallow bank structure. An inclinometer (Creative development for all, 2022; Mobile App) was used to identify the bank slope. Measurements took place at 10 random spots per sampling point at the same dates as the size of the riparian area was measured.

On the same dates, the cover (%) of the total vegetation, bare ground, mosses, herbs and shrubs within a representative plot of 1×2 m were estimated. Tree canopy cover (%) was estimated from the centre of the plots by eyesight. A drop disc (cardboard disc with glued fibre disc on a measuring stick) was used to measure the vegetation height (cm) by dropping the disc from 1 m above ground along a scaled stick (Douglas and Crawford, 1994) at 10 random locations within the riparian area.

Soil moisture was measured once in May at 10 random points per

Table 1
Characteristics of the investigated streams and stream zones. Stream type (LAWA, German Working Group on water issues of the Federal States and the Federal Government 2021): 5.1: small fine substrate dominated siliceous highland rivers, 6: small fine substrate dominated calcareous highland rivers, 9.1: mid-sized fine to coarse substrate dominated calcareous highland rivers, 11: small, organic substrate-dominated rivers, 19: small steams in riverine floodplains. [1] Data from Landesamt für Umwelt Rheinland-Pfalz (2023), [2] Data based on the profile of surface waters provided by Landesamft für Umwelt Rheinland-Pfalz (2024), [3] upstream and midstream dried out in September. [3] Floodplain type according to Koenzen (2005): A = low-gradient floodplains of the overburden, B = low-gradient floodplains of lowland and hilly regions, C = low-gradient floodplains with summer floods.

Stream	Catchment size (km^2)	Length (km)	Water level[1] (cm)	Stream zone	Stream type[2]	Floodplain type[3]	Coordinates
Wieslauter	247.13	114.1	Bobenthal:May: 87.4September: 78.5	Upstream	5.1	A	49.0483, 7.8787
			Salmbacher Passage	Midstream	9.1	B	49.0286, 7.9966
			May: 20.3September: 13.2	Downstream	9.1	C	48.9856, 8.2623
Heilbach	110.27	24	Wörth 2	Upstream	11	A	49.0321, 8.0858
			May: 30.0	Midstream	11	B	49.0335, 8.1775
			September 3.2[3]	Downstream	11	C	49.0719, 8.3174
Otterbach	107.88	40.9	Rheinzabern SüdMay: 43.2September: 23.5	–			
				Midstream	9.1	B	49.0943, 8.2613
				Downstream	9.1	C	49.1440, 8.3659
Queich	157.73	72.3	SiebeldingenMay: 43.3September: 32.6	Upstream	5.1	A	49.2671, 7.9604
				Midstream	19	B	49.1854, 8.3147
				Downstream	19	C	49.1802, 8.3612
Speyerbach	251.25	112.3	DudenhofenMay: 37.0September: 21.7	Upstream	5.1	A	49.3632, 8.0320
			NeustadtMay 32.3September: 37.5	Midstream	9.1	B	49.3654, 8.3288
				Downstream	9.1	C	49.4453, 8.4446

sampling section using a soil moisture meter (ProCheck Decagon Devices with the EC-5, Water content sensor; measurements in % volume water content (VWC)). Soil pH and grain size was determined in the lab with soil samples taken in September. Five soil cores (diameter 53 mm, hight 51 mm) were randomly taken at each sampling point and were frozen in plastic bags at −20 °C until further processing. Before measurements, the soil samples were dried at 30 °C for two weeks. Before soil pH measurements (protocol [Der Titel "DIN ISO 10390 2022 08 – Boden (Boden (2022)), behandelter Bioabfall und Schlamm" kann nicht dargestellt werden. Die Vorlage "Kurznachweis im Text – Norm – (Standardvorlage)" beinhaltet nur Felder, welche bei diesem Titel leer sind.]), we sieved the soil with a 2 mm sieve. Five grams of sieved soil were mixed with 25 ml $CaCl_2$ and gently shaken for 1 h. Afterwards the samples rested for 1 h before measuring the pH (WTW pH 3110). The soil fractions were identified by using the dried soil in a sieve vibration machine (Retsch AS 200 control; amplitude: 1.5; times 10 min; sieves: 4 mm, 2 mm, 630 µm, 200µ, 63 µm). Finally, the soil fraction was divided in three different classes: coarse (>4 – 2 mm), middle (<2 – 0.2 mm) and fine (<0.2 mm). For statistical analysis values for each environmental parameter were averaged to obtain one data point per sampling site.

2.3. Carabid beetles

Carabid beetles were sampled from 8 to 19 May and from 11 to 21 September 2023. Ground-active carabids were collected using pitfall traps for seven days at both time points. At each sampling site, ten pitfall traps (0.2 ml plastic cubs filled to 1/3 with 50:50 water: propylene glycol and a drop of odour-free sop) were installed within the riparian area with a maximum distance of 1.5 m to the water. In September, the traps at the downstream sections were placed in exactly the same sampling locations as in May, although the water level was lower in September and therefore the distance from the water was up to 10 m at the downstream sites. This decision was made because great water level fluctuations were also expected in September. Upon collection the trap contents were sieved and transferred to 80 % denatured ethanol.

In addition, standardised hand catches within the riparian area were made for 2 h in May and 1.5 h in September to find species that are difficult to catch with pitfall traps, in order to cover as much of the local carabid beetle fauna as possible. The decision to reduce the capture time by half an hour was made due to the increased experience and efficiency of the sampler. Nitril gloves and an exhaustor were used to catch individuals. In the search for carabid beetles, stones and wood were turned over and the surface of the ground was scratched loose. Individuals were separately stored in reaction tubes and kept in cooling boxes. Carabid beetles collected by hand were placed in a freezer at −20 °C on the same day after field work.

Carabid beetles were determined to species level using the identification key of Müller-Motzfeld (2004) and the digital identification key of Lompe (2002). Carabids of the genus *Bembidion* were mainly identified with the identification key of Götz (2023). To ensure that the frozen beetles do not defrost during the identification process the identification took place on ice. Trait information for the carabid beetles were gathered from Klaiber et al. (2017). The following traits were considered: wing morphology (short-winged, wing-dimorphic, long-winged), habitat specialism (generalist, specialist), moisture preference (ranging from 1 to 5; 1 = no moisture preference, 5 = strongest moisture preference) and body size (continuous in cm) (Table A1). For further statistical calculations the community weighted mean (CWM) was calculated for continuous data by multiplying each trait value by its community abundance and then divided by the abundance. Further, percentages were calculated for categorical data.

2.4. Data analysis

Statistical analyses were performed using the R software (R version 4.3.2, R Core Team, 2023) in the integrated development environment RStudio (2024.04.1+748). Functional diversity measures of carabid beetles included functional richness, functional evenness, functional divergence and functional dispersion which were calculated using the FD package (Laliberté et al., 2023). Visualisation of the plots was conducted using the ggplot2 package (Wickham et al., 2024).

To calculate the relative abundance of each species present in the different stream zones (upstream, midstream, downstream), a rank-abundance analysis was performed with the BiodiversityR package (Kindt, 2024). Indicator species for the three stream zones were calculated using the indicator value analysis implemented in the IndVal package (Roberts, 2023). A species was considered as an indicator species if the condition $p < 0.05$ was met.

In a first step, we analysed the effect of stream zone (upstream, midstream, downstream) on dependent variables (species richness, number of individuals, functional richness, functional evenness, and proportions or CWM for various traits) with linear mixed effect models (LMMs) with stream ID as a random effect. The significance of stream zone was calculated using type-II analysis-of-variance-tables based on Wald Chi^2 tests (R package car, (Fox et al., 2023).

In a second step, we related the dependent variables to environmental variables. We have chosen this two-step approach because many environmental variables were highly correlated with the stream zones (Table 2). Of the 14 measured environmental variables, only those with correlation coefficients less than ±0.7 were included in the full models. Correlations were analysed with the "corr" function included in the basic R functions. When deciding which of the highly correlated variables to retain, the one with the assumed highest ecological relevance for carabid beetles was chosen (see Table 2). For automated model selection, we used an information-theoretic approach to multimodel inference (Burnham & Anderson, 2002) using the "dredge" function in the MuMIn package (Bartoń, 2010); the delta for AICc was set to <4 in order to select the top-ranked models. In the full models we included stream ID as a random factor. Averaged parameter estimates from the

Table 2
Comparison of environmental variables (mean and standard deviation) between upstream, midstream and downstream zones. Differences were tested with linear mixed model with river ID as a random effect. Significant effects ($p < 0.05$) are shown in bold.

Environmental variable	Upper	Middle	Lower	Chi^2	p
Riparian area length (m)*	9.2 ±3.3	8.2±4.3	16.5 ±2.1	**17.56**	**<0.001**
Riparian area width (m)	4.0 ±1.8	3.0±1.5	7.6 ±1.5	**21.96**	**<0.001**
Bank slope (°)	21.1 ±12.8	20.0 ±6.7	14.5 ±5.6	2.34	0.310
Vegetation height (cm)	7.7 ±9.0	8.7±5.2	9.5 ±5.5	0.14	0.933
Canopy cover (%)*	57.3 ±35.6	68.7 ±9.5	70.8 ±17.1	0.94	0.630
Shrub layer (%)	10.5 ±6.4	9.8±6.5	11.5 ±6.8	0.16	0.920
Herb layer (%)	25.4 ±16.8	27.0 ±2.9	29.2 ±4.4	0.6716	0.7148
Moss layer (%)	19.7 ±4.4	2.6±2.9	4.0 ±4.4	**8.89**	**0.010**
Bare ground (%)*	43.9 ±31.3	50.6 ±36.9	54.3 ±11.8	0.30	0.860
Soil pH*	5.1 ±0.9	5.7±1.1	7.1 ±0.2	**15.74**	**<0.001**
Soil moisture (% VWC)	39.2 ±4.7	32.7 ±1.7	36.0 ±4.7	1.11	0.574
Coarse soil fraction (>4 – 2 mm)*	5.6 ±6.7	2.4±2.8	14.1 ±8.4	**9.45**	**0.009**
Middle soil fraction (<2 – 0.2 mm)	39.5 ±14.9	46.6 ±5.5	37.6 ±14.0	1.90	0.390
Fine soil fraction (<0.2 mm)	55.0 ±20.0	51.0 ±7.1	48.3 ±18.2	0.53	0.770

* Used as explanatory variables in the multiple models.

top-ranked models were then produced using the 'model.avg' function. We used the full model average, where parameter estimates are averaged over all top-ranked models, which reduces model selection bias and is appropriate to determine which factors have the strongest effect on the response variable (Burnham & Anderson, 2002; Nakagawa & Freckleton, 2011).

The carabid beetle species composition was related to the stream zone and environmental variables using redundancy analysis (RDA) (R library 'vegan'; (Oksanen et al., 2024). Therefore, Hellinger transformed community data was used. The selection of the environmental variables for the RDA followed similar criteria as for the LMM. Consequently, the same environmental factors as those used in the model selection process were chosen but including stream section.

3. Results

3.1. Differences in environmental variables between stream sections

Five out of the 14 measured environmental variables differed significantly between the different stream zones (Table 2). The size of the riparian area (length and width), the proportion of coarse soil fraction and the soil pH were all highest in the downstream sections. In contrast, the moss cover was highest in the upstream zone compared to midstream and downstream zones.

3.2. General results of carabid beetles

In total, we collected 86 species with 2,495 individuals (Table A1). *Bembidion elongatum* (n = 314), *Nebria brevicollis* (n = 310), *B. tetracolum* (n = 203) and *Limodromus assimilis* (n = 203) were the most abundant species across all sites and stream zones. The upstream carabid beetle

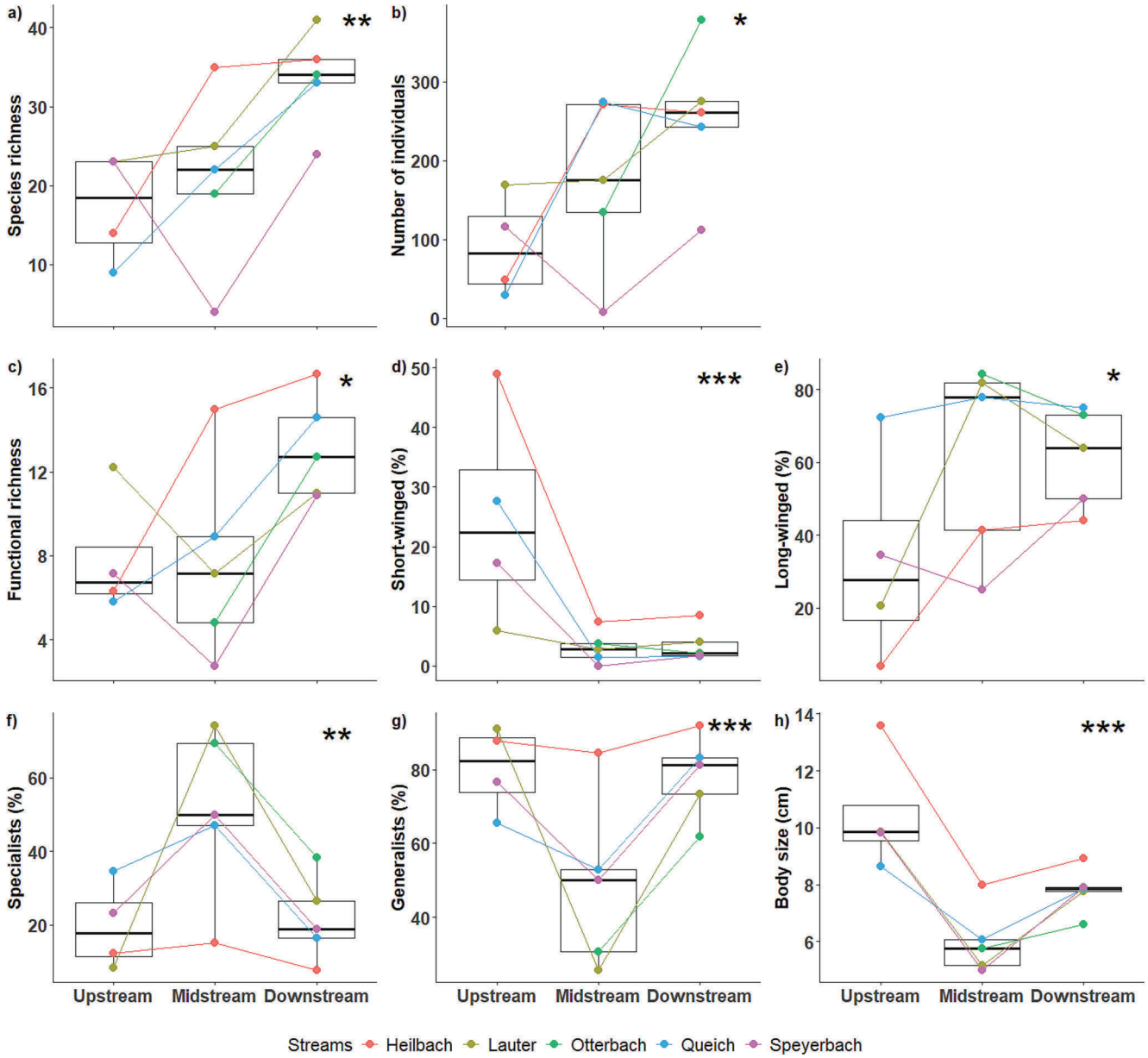


Fig. 2. Comparison of a) species number, b) number of individuals, c) functional richness, d) proportion of short-winged individuals, e) proportion of long-winged individuals, f) proportion of specialist individuals, g) proportion of generalist individuals and h) community weighted mean (CWM) of body size between upstream, midstream and downstream zones. Differences were tested using linear mixed effect models with river ID as random effect.

community was dominated by *N. brevicollis* (33.9 %) while *B. elongatum* was the most dominant species midstream (33.8 %). The downstream section was not dominated by a single species, with *B. tetracolum* (11.0 %), *L. assimilis* (10.4 %), *Agonum emarginatum* (10.2 %) and *B. dentellum* (10.1 %) being the most frequent species making up about 40 % of the total individuals in this zone. The highest number of species was found downstream (n = 65) followed by midstream (n = 51) and upstream (n = 37, Fig. A2).

3.3. Carabid beetle diversity and traits between stream zones

Significant differences between the stream zones were found for carabid beetle species richness (Chi^2 = 10.33, p = 0.006) and the numbers of individuals (Chi^2 = 6.83, p = 0.030). Both were highest downstream, with more than twice as many species number and about three times as many individuals downstream than upstream (Fig. 2a and b). The stream zone also significantly affected the functional richness of carabid beetles (Chi^2 = 8.14, p = 0.020), again increasing towards the downstream zones (Fig. 2c). In contrast, functional evenness (Chi^2 = 3.34, p = 0.19) showed no significant relationships with the stream zone.

The proportion of short-winged individuals was highest in the upstream zone (Chi^2 = 17.45, p < 0.001, Fig. 2d), where consequently the proportion of long-winged individuals was lowest (Chi^2 = 7.27, p = 0.030; Fig. 2e). The proportion of wing-dimorphic individuals remained unaffected by the stream zone (Chi^2 = 2.14, p = 0.340). Habitat specialists, i.e. species preferring riparian habitats, dominated in the midstream zone (Chi^2 = 15.03, p < 0.001; Fig. 2f). The community weighted mean of body size was also significantly affected by stream zone and highest for upstream followed by downstream and midstream (Chi^2 = 43.79, p < 0.001; Fig. 2g). The community weighted mean of moisture preference remained unaffected (Chi^2 = 0.87, p = 0.650) with hygrophilic individuals dominating at all stream sections (upstream: mean: 89.1± standard deviation: 5.4 %, midstream: 91.2±11.9 %, downstream: 87.5±14.5 %).

3.4. Relation of carabid beetle diversity and traits to environmental variables

Species richness was significantly positively affected by the length of

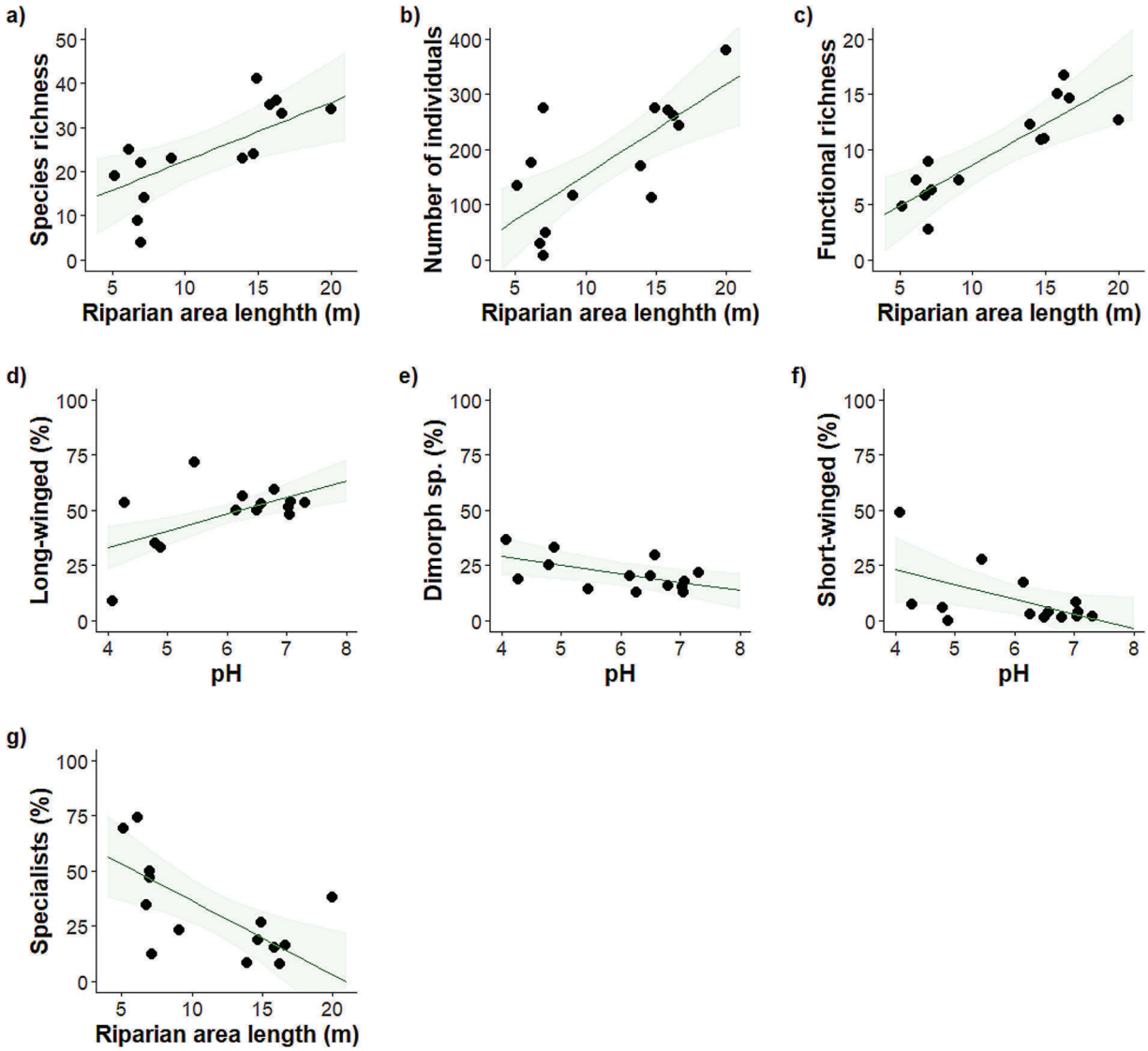


Fig. 3. Significant relationships between carabid beetles and environmental variables. a) species richness and riparian area length, b) number of individuals and riparian area length, c) functional richness and riparian area length (m), d) proportion of long-winged individuals and soil pH, e) proportion of wing-dimorph individuals and soil pH, f) proportion of short-winged individuals and soil pH, g) proportion of species of habitat specialists and riparian area length. For statistics see text and Table A2.

the riparian area (z = 2.357, p = 0.018; Fig. 3a). Similarly, the number of individuals significantly increased with the riparian area length (z = 3.144, p = 0.002; Fig. 3b). Functional richness significantly increased with the riparian area length (z = 6.305, p < 0.001; Fig. 3c), whereas functional evenness showed no significant relations to any environmental variable (Table A2).

The proportion of long-winged individuals was significantly positively related to the soil pH (z = 3.013, p = 0.003; Fig. 3d). In contrast, the proportion of dimorphic individuals was negatively affected by the soil pH (z = 2.512, p = 0.012; Fig. 3e). This pattern was also shown for the proportion of short-winged individuals (z = 2.011, p = 0.044; Fig. 3f). In contrast, the proportion of habitat specialists was negatively affected by the length of the riparian area (z = 2.919, p = 0.004; Fig. 3g). The CWM of body size and the moisture preference were unaffected by any measured environmental variable (Table A2).

3.5. Community composition of carabid beetles and indicator species

The carabid beetle species composition was significantly affected by stream zone (p = 0.026) and soil pH (p = 0.033), although the RDA model itself was not significant (F = 0.108). The environmental variables explained 66 % of the variation in the carabid community composition, with axis one and two explaining 29.6 % and 22.1 % of the variation, respectively (Fig. 4).

Each stream zone was characterised by one or more indicator species. While the upstream zone was characterised by the common forest species *Cychrus attenuatus* (p = 0.001) and *Abax ovalis* (p = 0.040), the only indicator species for midstream was *Bembidion elongatum* (p = 0.036), a typical riparian species preferring vegetated riverbanks. The eleven indicator species for the downstream zone include a mixture of typical riparian forest species (*Patrobus atrorufus*, *Pterostichus strenuus*, *Agonum emarginatum*) and generalist species of grasslands and agriculture (*Clivina fossor*, *Pterostichus melanarius*; Table 3) and about 55 % of the indicator species belong to riparian specialists (e.g., *B. semipunctatum*, *P. artrorufus*, *Agonum micans, A. emarginatum*). Additionally, the downstream zone comprised the only steno-hygrophilic species (*B. dentellum* and *Oxypselaphrus obscurus*) as indicator species. All other indicator species were hygrophilic, except *Clivina fossor* which is mesophilic.

Table 3
Indicator species of carabid beetles (ordered by indicator value) for the stream zones upstream, midstream and downstream calculated with the IndVal package in R.

Indicator species	Indicator value	p
Upstream		
Cychrus attenuatus	1.00	0.001
Abax ovalis	0.66	0.044
Midstream		
Bembidion elongatum	0.72	0.036
Downstream		
Bembidion dentellum	0.85	0.010
Oxypselaphus obscurus	0.83	0.002
Pterostichus melanarius	0.80	0.009
Bembidion semipunctatum	0.78	0.015
Agonum micans	0.77	0.018
Agonum emarginatum	0.71	0.044
Pterostichus strenuus	0.69	0.015
Patrobus atrorufus	0.66	0.043
Leistus fulvibarbis	0.65	0.047
Clivina fossor	0.65	0.032
Limodromus assimilis	0.63	0.022

4. Discussion

4.1. Stream zone and environmental factors

As expected and in line with the floodplain typology (Koenzen, 2005, Globevnik et al., 2021), the size of the near-natural and shallow bank (here referred to as "riparian area") was clearly highest in the downstream zone near the Rhine which is likely related to the shallower topography of the former River Rhine floodplain compared to the hillier upstream and midstream areas. The streams studied originated in or near a lower mountain range of sandstone and therefore their riverbeds are dominated by fine sediment. The grain size consequently did not decrease from upstream to downstream, as it is often found in mountain or alpine streams (e.g., Sadler et al., 2004; Baiocchi et al., 2012; Petts et al., 2000). The strong increase in the soil pH from upstream and

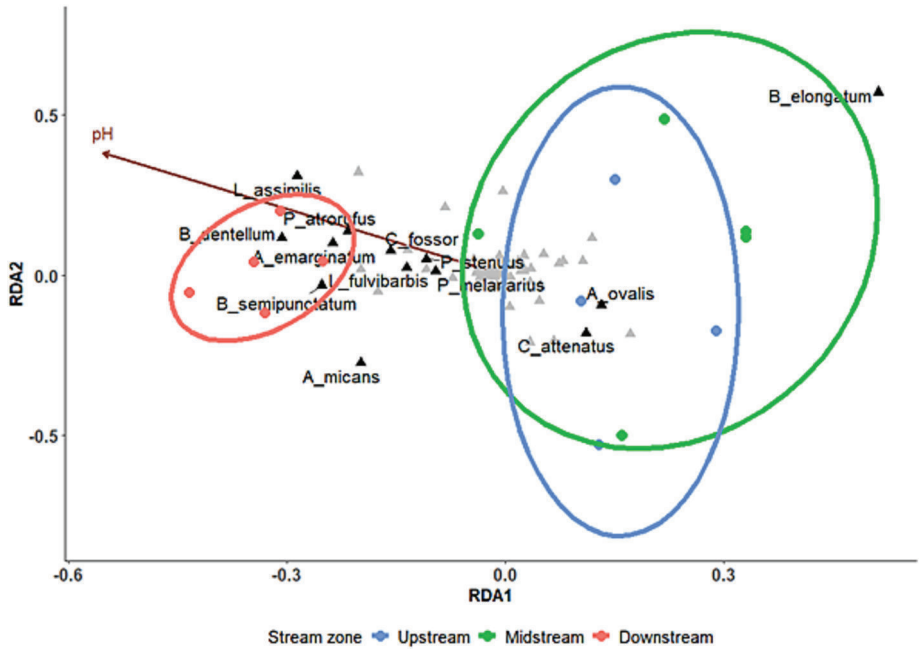


Fig. 4. RDA of Hellinger transformed community data of carabid beetles at five streams with visualisation of the significant effect of soil pH and stream zone. Grey triangles represent species with labelled black ones representing the indicator species.

midstream to downstream can be explained by the transport of calcareous sediments by the River Rhine (originating from calcareous areas in the Swiss Alps), which dominate the soils of its floodplain (Hauenstein and Goldschmitt, 2022). In contrast, the sandy soil in midstream and upstream is dominated by humus-rich forest soil and therefore characterised by a lower pH (Amelung, 2018; Hauenstein and Goldschmitt, 2022). The observed higher moss layer in the upstream zone can be explained by the more humid condition in the Palatinate Forest and the lower disturbance by flooding, which both favour the occurrence of mosses (Ortlepp and Mürle, 2003; Stefańska-Krzaczek et al., 2022).

4.2. Species richness and number of individuals of carabid beetles

In line with our hypothesis, we found that the species richness and number of individuals of carabid beetles increased along the stream course from upstream to downstream. This finding is consistent with the results in the study by Framenau et al. (2002) who showed that the density of carabid beetles was higher in downstream than in upstream riparian habitats in the northern hemisphere. In contrast, they also showed that the carabid abundance in the southern hemisphere did not differ between river zones. It might be that the increase in species richness and individual numbers towards the Rhine floodplain is enhanced by the floodplain characteristics with its shallower topography and would be weaker if the streams would enter in smaller rivers outside of a tectonic basin. The observed increase in diversity and abundance of carabid beetles is associated with changes in environmental conditions from upstream to downstream (Table 2) and is reflected in the species-environment relationships.

Most importantly, we found that the species richness and the number of individuals increased with the size of the shallow and near-natural bank area ("riparian area"). This indicates that carabid community changes along streams with fine sediments found in our study, align with findings for gravel banks along larger river courses. In this respect, Smit et al. (1997), for example, showed that carabid abundance is positively correlated with gravel bank size along the flow direction. In conjunction with the larger riparian area in the present study, a higher water level fluctuation was observed in the downstream zones. The associated flooding may result in increased disturbance, which could lead to increased vegetation diversity, (micro)habitat and microclimate heterogeneity, and food sources (Ramey and Richardson, 2017) that support carabid beetle diversity in the downstream zone.

Finally, the higher diversity and abundance of carabids in downstream zones might be explained by the potentially higher food availability. Larger rivers and downstream zones usually contain a higher biomass of emerging and washed ashore drifting aquatic insects compared to smaller streams. Similarly terrestrial detritivores such as collembolans and snails, which benefit from washed ashore algae and leaf litter, follow this pattern. All of these resources can serve as an important food source for some riparian carabids (Hering, 1998; Hering and Plachter, 1997; Ramey and Richardson, 2017).

4.3. Community and trait composition and indicator species

In addition to species richness and number of individuals, the species composition in the downstream zone differed obviously from the mid- and upstream zone, supporting our second hypothesis. This may simply reflect differences in the regional species pool of landscapes and floodplain types (i.e. Rhine Valley vs. Palatinate Forest). Moreover, this can partly be explained by differences in environmental conditions (e.g., riparian area size, soil, pH) between the stream zones. Based on the RDA, the soil pH and stream zone itself turned out to be the main driver for the variation in the community composition. Similarly, other studies have shown that local habitat conditions in riparian habitats can strongly influence the structure of carabid beetle communities. For example, Bauspiess et al. (2024), who studied carabid beetles in floodplains in the same geographic region, found that the carabid beetle communities differed between floodplain and deeply incised banks of small streams, with pH also having an influence. It is known that carabids show species-specific preferences for certain pH ranges, often with a tendency to prefer values around 7 (Paje and Mossakowski, 1984), as it was the case in our sampled downstream zones. However, it is more likely that the soil pH itself might not be the main factor explaining carabid beetle occurrence (Paje and Mossakowski, 1984; Antvogel and Bonn, 2001). Rather, soil pH appears as an indirect factor in our study, as it reflecting changes in landscapes and floodplain types with which it shows a strong association (Table 2). Assmann and Terlutter (1999) reported that the carabid beetle community in floodplains along the river Ems (Germany) varied with changes in the local soil conditions, with strong differences between sandy and brackish conditions.

The stream zones also differed in the functional diversity and trait composition of carabids, supporting our third hypothesis. The upstream zone was characterised by large-bodied and short-winged beetles. In carabid beetles, there is a general connection between increasing body size and decreasing flight ability (Homburg et al., 2013), with large-bodied flightless species (such as *Carabus* spp.) dominating in more stable, late-successional environments (Desender, 1989). This corresponds both with the larger area of continuous, late-successional, forest and the reduced lateral extent of flooding in upstream areas. In contrast, the carabid beetle communities midstream and downstream were characterised by small and long-winged species. Both zones were dominated by species of the genus *Bembidion*, which are typically small-sized and flyable carabids. According to Lambeets et al. (2008a), many *Bembidion* species increase in body size with increasing flood intensity (Lambeets et al., 2008b). This, together with the occurrence of larger species typical for agricultural habitats (see below), might explain why we found a slightly larger community weighted mean (CWM) of body size in the downstream zone, which gets flooded more frequently than midstream (own observations). Higher flood intensity is also associated with more dynamic environmental conditions, which in turn would induce a higher emigration pressure. Therefore, carabid beetles with a good flight ability might be more successful in downstream areas, as we could show in our study. This is line with Rainio and Niemelä (2003), who showed, that lager species with a weak dispersal ability decreased with increasing disturbance frequency, while smaller species with a strong dispersal ability increased. We found the highest proportion of (riparian) habitat specialists in the midstream zone. According to Lambeets et al. (2008b), specialised riparian carabids are highest at an intermediate flooding frequency. This finding, combined with the high number of specialised species in midstream in our study, also indicate that the mid- and downstream zones are flooded, but the downstream zones are flooded more frequently. In general, the occurrence of specialised species – especially in the midstream zone – and the dominance of hygrophilic species indicate natural or near-natural river bank conditions at our sampling sites (Sprößig et al., 2020; Jachertz et al., 2019; Lambeets et al., 2008b). Therefore, it is plausible that the variation in the functional traits is driven by variations in flooding events and associated increased habitat heterogeneity (Sadler et al., 2004; van Looy et al., 2005), which is also reflected in the higher functional richness in the downstream zone.

Our results of the indicator species analyses matched very well with the findings of the trait composition: The upstream zone was characterised by relatively large and short-winged forest species (*Cychrus attenuates* and *Abax ovalis*) indicating a stable environment with a strong impact of the surrounding forest. The midstream and downstream zones contained habitat specialists for river banks, which were smaller and predominantly had long wings. Here, several species from the genus *Bembidion* were identified as indicator species, which are often well adapted for floodplain sediments and characteristic for river banks (Bonn et al., 2002), (Januschke et al., 2011), (Desender, 1989). Thus, *Bembidion* species are regarded as particularly useful indicators for habitat condition in riparian habitats such as river slope, soil substrate or vegetation density (Jachertz et al., 2019; Kirichenko-Babko et al.,

2020; Manderbach and Hering, 2001). The only indicator species detected for midstream was *Bembidion elongatum*, a species known for its preference for sandy and vegetated banks (Klaiber et al., 2017), which, however, is not very widespread throughout Germany. The high numbers of this habitat specialist underline the importance of near-natural midstream zones for species conservation. Further, *Bembidion dentellum* was identified as an indicator species for the downstream zone, which support the findings of (van Looy et al., 2005; van Looy et al., 2007), who found a positive relationship between the abundance of *B. dentellum* and the size of river banks. The downstream indicator species were not only typical riparian carabids but also species which prefer moist grassland and crop fields. This mixture of habitat preferences might be explained by the heterogeneous habitat conditions in the lager riparian areas (Ward et al., 1999). Moreover, arthropods well adapted to agriculture, such as the wolf spider *Pardosa agrestis*, are often originally found in highly disturbed, water-influenced open habitats such as coastal salt marshes (Arachnologische, 2024). Therefore, carabids typically found in agricultural habitat such as *P. melanarius* might have a similar history of pre-adaption.

5. Conclusion

Our results underline that carabid beetle communities change characteristically along stream courses. Species richness, number of individuals and functional richness each increased towards the downstream zone. Furthermore, the stream zones exhibited differences in species composition, with each zone displaying distinct indicator species. This led to significant variations in trait composition. While large and short-winged species were typical upstream, small and long-winged habitat specialist dominated in midstream and downstream, indicating more dynamic (flood intensity) conditions and increasing hydrological connectivity and sediment deposition in these zones. The increasing size of the near-natural and flat banks as well as the increasing soil pH were the main environmental factors explaining the changes in the carabid community. Overall, our study highlights that carabid beetles are excellent ecological indicators for riparian habitats across different stream zones and should be integrated in future indicator-based river and stream monitoring and restoration programs.

CRediT authorship contribution statement

Franziska Middendorf: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bernhard Eitzinger:** Writing – review & editing, Validation, Supervision. **Martin H. Entling:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition. **Jens Schirmel:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.113036.

Data availability

Data will be made available on request.

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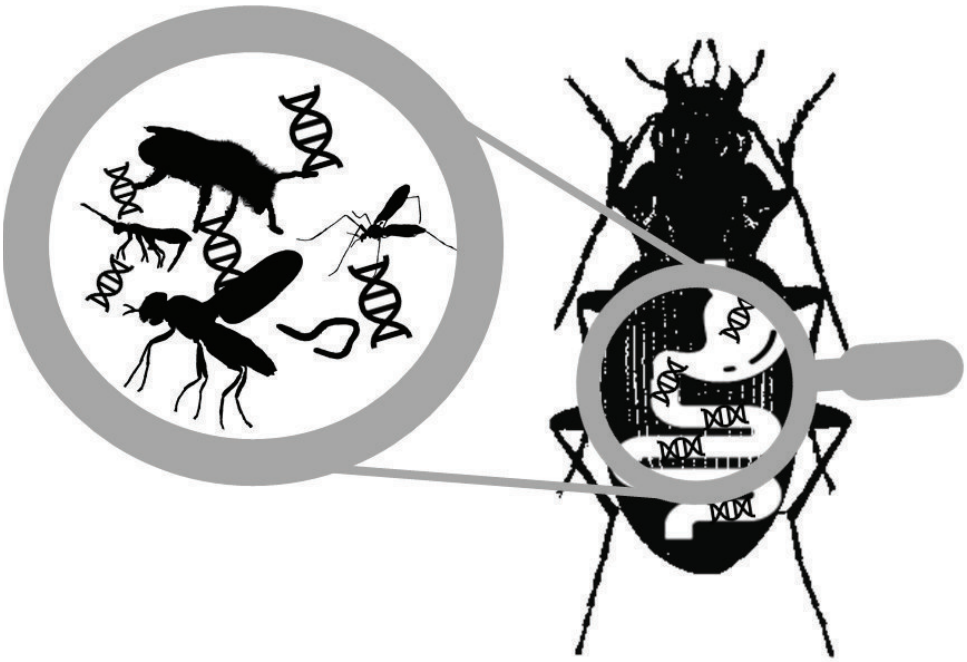
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Chapter 4:

Diverse terrestrial but limited aquatic prey in riparian carabid beetles

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Diverse terrestrial but limited aquatic prey in riparian carabid beetles

Abstract

Riparian areas are transitional zones between aquatic and terrestrial ecosystems, linking their food webs. Although carabid beetles are frequently used as ecological indicators, little is known about their prey consumption. In this study, we investigated the prey spectrum of riparian carabid beetles using DNA metabarcoding of gut contents. Carabid beetles were sampled at five different streams across upstream, midstream, and downstream sections. The diet of carabid beetles was dominated by terrestrial prey (primarily insects), while aquatic prey accounted for only about 12.1 % on average. Prey composition varied among carabid beetle tribes and between riparian habitat specialists and generalists, driven by a higher detection of earthworms in habitat generalists. We also observed differences among stream sections: Aquatic subsidies contributed twice as much to beetle diets in downstream compared to upstream reaches. Importantly, however, the role of carabid beetle in linking aquatic and terrestrial food webs appears to be minor at small forestry lowland streams compared to previous results from larger rivers on mountain streams. Future studies across broader geographic regions, stream sizes, and seasonal contexts are needed to capture the full variability of riparian carabid diets and to clarify their role in linking aquatic and terrestrial food webs.

Keywords: aquatic subsidies, diet, DNA metabarcoding, food web

1 Introduction

Ecosystems operate as open systems, constantly exchanging energy and biotic components with neighbouring ecosystems (Tansley, A., G. 1935). Riparian areas along rivers exemplify this role, functioning as transition zones between aquatic and terrestrial ecosystems (Naiman et al. 1993). These ecosystems are linked by bilateral nutrient flows that sustain diverse biogeochemical and ecological interactions (Collins et al. 2020; Gary A. Polis et al. 1997; Richardson and Sato 2015). For example, insects emerging from aquatic habitats provide vital nutritional subsidies to riparian zones, linking the food webs of both ecosystems (Twining et al. 2016; Twining et al. 2019).

Riparian habitats support exceptionally high biodiversity, including many specialised, rare, and endangered species (Naiman et al. 1993; Hauer et al. 2016; Andersen and Hanssen 2005; Denux et al. 2017; González et al. 2017; Bauspiess et al. 2024). Variation in riverine processes – such as sediment deposition, chemical transfer and nutrient loading – shapes riparian zones (Capon and Pettit 2018). (Koenzen 2005; Globevnik et al. 2021), which in turn influence riparian biodiversity and species composition (e.g., carabid beetles: (Middendorf et al. 2025a).

For riparian predators, however, changes in community structure are not only driven by habitat variation but may also be linked to prey availability (Ramey and Richardson 2017). A recent review by Middendorf et al. (2025b) highlighted that many riparian arthropod predators – particularly spiders, but also carabid beetles – rely on aquatic food resources. Because the abundance and composition of aquatic organisms vary along river courses (Vannote et al. 1980b), the prey and community composition of riparian arthropod predators can be directly affected (Middendorf et al. 2025b). For example, variation in river size has been shown to affect spider diets: larger rivers with higher aquatic emerging insect rates provide a greater proportion of aquatic subsidies, reflecting the natural increase in river size from upstream to downstream (Chari et al. 2020). The response of riparian arthropod predators to aquatic subsidies is thereby highly dependent on taxonomic and functional group. Horizontal web-building riparian spiders, for instance, often rely more heavily on aquatic prey than ground-hunting spiders (Middendorf et al. 2025b). This may explain why horizontal web-building spiders – primarily *Tetragnatha* spp. – have been the most extensively studied group regarding dependence on aquatic subsidies (Iwata 2007; Alberts and Sullivan 2016; Wieczorek et al. 2015; Middendorf et al. 2025b). Habitat specialisation may further shape the use of aquatic subsidies; riparian specialists are expected to rely more heavily on aquatic prey than generalists (Ramey and Richardson 2017), although this pattern has not been consistently demonstrated in spiders (Bollinger et al. 2023). In contrast to spiders, relatively little research has examined the prey composition of other riparian arthropods, such as carabid beetles, and even less is known about how their diet changes along the river courses.

Carabid beetles are among the most dominant arthropod predators in riparian ecosystems (Kühnelt 1943; Plachter 1986) and are widely recognised as ecological indicators in these habitats (Januschke et al. 2011; Sprößig et al. 2020; Lambeets et al. 2008; Lambeets et al. 2009),(Gerisch et al. 2006; van Looy et al. 2005). Although their habitat requirements are well known, comparatively little attention has been given to their diet, particularly the contribution of aquatic subsidies. The limited studies available on riparian carabid beetle diets rely largely on visual identification of prey fragments in the digestive tract (Hering and Plachter 1997) or stable isotope analysis (O'Callaghan et al. 2013; Paetzold et al. 2005). DNA-based gut content analyses are a powerful tool to study predator-prey interactions (Symondson 2002; King et al. 2008; Pompanon et al. 2012; Sheppard and Harwood 2005) even under difficult circumstances, e.g., when semi-digested prey remains do not allow for a visual identification (Eitzinger et al. 2019). In particular, DNA metabarcoding - that is, the simultaneous amplification and identification of consumed prey

DNA through high-throughput sequencing (Pompanon et al. 2012; Dunshea 2009; Meyer et al. 2007) – offers unprecedented insights in the feeding ecology of predators and has become a crucial tool for understanding the structure and dynamics of food webs that interconnect species communities (Clare 2014). While there is a growing number of studies using metabarcoding to study trophic interactions of riparian spiders (Middendorf et al. 2025b) there are no such studies investigating prey spectra of carabid beetles from stream shores.

In this study, we aimed to investigate the prey composition of riparian carabid beetles in different sections (upstream, midstream, downstream) along near-natural medium-sized streams by using metabarcoding of gut contents. We hypothesised that (i) the prey composition of carabid beetles will differ between taxonomic tribes and (ii) between riparian habitat specialists and generalists. In addition, we hypothesize that (iii) carabid beetle communities from different stream sections (upstream, midstream, downstream) feed on various prey communities. Finally, we (iv) assumed that the aquatic prey component differs between tribes, is higher for riparian specialists than for generalists and is highest in the downstream section.

2 Methods

2.1 Sampling sites and collection of carabid beetles

Carabid beetles were sampled at five medium-sized (Strahler-Oder ranging from 1 to 6) streams, with three sections per stream: upstream, midstream, and downstream (N_{total} = 15 sampling sites). All streams are tributaries of the Rhine River, located in southwest Germany within the federal state of Rhineland-Palatinate. The catchment areas of the streams range from 108 km^2 (Otterbach) to 251 km^2 (Speyerbach), and stream lengths vary from 24 km (Heilbach) to 114 km (Wieslauter/Lauter). To minimise artefacts by different human impact levels, we selected sampling sites in forests, which also features gently sloping shores. Downstream sites were situated near the confluence with the Rhine. For additional details on the streams and sampling sites, see Middendorf et al. (2025a).

We collected carabid beetles at two time points (8th–19th May, 11th–21st September 2023) using two complementary methods: pitfall trapping and hand collection (Middendorf et al. (2025a). At each sampling site, ten pitfall traps (0.2 ml transparent plastic cups filled to one third with a solution of 50:50 water: propylene glycol and a drop of odourless soap) were installed within the first 2.5 m of the water´s edge. An exception was made in the downstream section only in September, when traps were placed up to 10 m away from the shoreline due to anticipated higher fluctuations in water level. Traps were left open for seven days per point in time, after which their contents were sieved and preserved in 80 % denatured ethanol on the same day. In addition, we collected carabid beetles by hand for 1.5–2 hours on the same days these traps were set. Nitrile gloves were worn to avoid contamination, and an exhaustor was used to capture small beetles efficiently. Hand-collected specimens were kept in individual vials and temporarily stored in cooling boxes during fieldwork and subsequently frozen at -20°C in the laboratory on the day of collection.

Carabid beetles were morphologically identified using a stereo microscope and the identification keys Müller-Motzfeld (2004), Lompe (2002), and Götz (2023). Specimens collected by hand were identified using sterile instruments while maintained on ice to minimise DNA degradation due to thawing.

2.2 DNA metabarcoding of carabid gut contents

For gut content analysis, we selected carabid beetle species represented by at least seven individuals in a stream section. To minimize potential bias from individual sampling sites, we included specimens from as many streams

and stream sections as possible. In total, 579 individuals from 28 species were analysed, representing 6 genera and 9 tribes.

To increase the yield of prey DNA during the DNA extraction process, large individuals (>8 mm) were thawed, and their digestive systems were removed by carefully twisting and detaching the head, allowing the digestive tract to be extracted intact. To prevent cross-contamination, new gloves and heat-sterilised tweezers were used for each specimen. Extracted digestive systems were placed individually in 2.5 ml Eppendorf tubes. Small beetles (<8 mm) were processed as a whole following a bleaching procedure to remove external contaminations (Huszarik et al. 2023). This procedure, adapted from Jüds et al. (2023), involved two pre-washing steps: first with water and gently inversion for 2 minutes, followed by washing with ethanol for an additional 2 minutes. Specimen were then exposed to 1000 µl of >5% household bleach (DanKlorix) and gently inverted for 5 minutes. To remove any residual bleach, beetles were washed twice with distilled water, gently shaking the tubes for 2 minutes each time. Ultimately individuals were placed on clean absorbent paper to air-dry for 5 minutes before being transferred to fresh tubes.

All samples were subjected to the same DNA extraction protocol, based on a modified high-salt extraction method (Aljanabi and Martinez 1997). Carabid beetle tissues were homogenised in ATL extraction buffer using high-grade steel beads (Ø 3 mm, Ecooe) with a TissueLyser II (Qiagen, Hilden, Germany) for three cycles of 1 minute at a frequency of 30.0 Hz. Proteinase K (Zymo Research, Irvine CA, USA) was then added, and samples were incubated overnight at 56°C on a heated rocking platform (Eppendorf Thermomixer Comfort). The following day, 5M NaCl was added, and the mixture was centrifuged. The supernatant was mixed with an equal volume of ice-cold isopropanol and stored at –20°C for at least 2 hours. After a second centrifugation, the DNA pellet was washed with ethanol, air-dried, and resuspended in 100 µl of MQ water. Extracted DNA was stored at –20°C until further analysis.

To reduce the content of high-molecular-weight predator DNA, we followed the protocol by Krehenwinkel et al. (2017), which recommends a DNA concentration of 20–40 µg μL^{-1} for optimal removal. When necessary, samples were diluted with double-distilled water to achieve this concentration. Magnetic beads (AMPure XP, Beckman Coulter, Brea CA, USA) were then added at 0.75 x the sample volume (40 µl). High-molecular-weight DNA binds to the beads, while the targeted lower-molecular-weight prey DNA remains in the supernatant. Supernatants were transferred to new tubes, mixed with isopropanol and 0.5 µl of glycogen solution (Qiagen), and centrifuged. The resulting DNA pellet was washed with ethanol, centrifuged again, and air-dried for 5–10 minutes before being resuspended in 20 µl of double-distilled water.

A two-step metabarcoding approach was employed following the methodology of Zizka et al. (2019). For the subsequent PCR, a 10 µl reaction volume was prepared, consisting of 5 µl of MyTaq HS Red Mix, 2X (Meridian Bioscience, Cincinnati OH, USA), 0.5 µl of bovine serum albumin (BSA; 10 mg/ml; Honywell Fluka, Buchs, Switzerland), 0.5 µl each of forward and reverse primers (fwhF2 and FwhR2n; Vamos et al., 2017), 2.5 µl of double-distilled water, and 2 µl of extracted DNA. While these primers were originally designed for aquatic macroinvertebrates (Vamos et al. 2017), they have also been tested successfully for terrestrial invertebrates (Elbrecht et al. 2019). For each 96-well plate of samples, primers were tagged with a unique combination of forward and reverse tags and contained a universal binding site for index primers used in the second PCR to anneal. The PCR started with an initial denaturation at 95°C for 5 minutes, followed by 34 cycles of 95°C for 30 seconds, 48°C for 30 seconds, and 72°C for 2 minutes, with a final elongation at 72°C for 5 minutes.

In the second PCR, individual indices were attached following the protocol of the first-step PCR but which consisted of only 12 cycles with an annealing temperature of 61°C. The reaction volume was the same as in the first-step PCR, except that it included 1 µl of a 10 µM mixture of forward and reverse index primers and a 1:10 dilution of the first-step PCR product. PCR amplification success was assessed using gel-electrophoresis on a 1.5 % agarose gel.

For library preparation we prepared two pools of 300 samples each, including negative controls. To balance DNA concentration between samples, we used 4 µl of samples with strong PCR bands and 6 µl of samples with weak bands. An aliquot of each library was purified using SPRIselect magnetic beads (Beckman Coulter, Brea CA, USA) at 1:1 ratio, following the manufacturer's protocol. Sequencing was performed at CeGaT GmbH (Tübingen, Germany) on an Illumina MiSeq platform using the V3 kit with 600 cycles, generating 300-bp paired-end reads.

2.3 Bioinformatic

Initial bioinformatics processing was performed separately for the two libraries and subsequently merged in R (R version 4.3.2; R Core Team 2023) using the RStudio development environment (2024.04.1+748; Posit team 2023), following taxonomic assignments.

CeGat provided the raw sequences demultiplexed by index reads. Further demultiplexing based on primer tags was performed using the Python package 'demultiplexer' (v 1.2.0 https://github.com/DominikBuchner/demultiplexer). Primers were removed using 'cudadapt' (v 3.7 Martin 2011), and reads were processed with the DADA2 pipeline (v 1.30.0 Callahan et al. 2016) in R. Optimal truncation lengths were determined and set to 140 bp for forward reads and 80 bp for reverse reads. Chimeras were identified and removed with 'vsearch' (version v2.29.1; Rognes et al. 2016), resulting in 2.7 % and 3.1 % chimeras in runs A and B, respectively.

Erroneous amplicon sequence variants (ASVs) were filtered using LULU (Frøslev et al. 2017) in R, which merges highly similar sequences based on co-occurrence patterns. Parameters were set to a minimum match of 96 % and a minimum relative co-occurrence of 0.95.

For taxonomic assignment, the resulting fasta file was processed using BOLDigger3 (Buchner and Leese 2020), which identifies DNA sequences in BOLD System v5 (Ratnasingham and Hebert 2007). Both the public and private animal databases were used in the Genus and Species Search mode. Default threshold settings were applied throughout the analysis: 97% for species, 95% for genus, 90% for family, and 85% for order. Sequences flagged by BOLDigger3 – indicating uncertainty in the selected hit, such as multiple taxonomic assignments among the top 100 hits within the threshold or matches to private data – were subsequently subjected to BLAST searches in GenBank for further validation using BlastN (NCBI N V 2.16.0+; Camacho et al. 2009). In these cases, the top 10 hits were retrieved, applying a minimum similarity threshold of 80 %. If BLAST results provided a better match than BOLD, based on identity score, alignment length, and E-value, the BLAST assignment was adopted.

At this stage, the two data sets were merged and processed as a single dataset. Only sequences with 205 ± 10 bp were kept for further analyses. Taxonomic matches were cross-checked against geographic distributions using occurrence records from the Global Biodiversity Information Facility (GBIF.org, accessed April 2025), including all species documented in Germany and neighbouring countries.

In this study various approaches have been applied to address errors arising from PCR amplification and sequencing (summarized in Drake et al. 2022). Specifically, we applied a filtering strategy by removing all reads representing less than 3% of the total reads per ASV. Remaining reads in the PCR negative controls were subtracted per ASV

per 96 well-plate. Notably, reads remaining in the negative controls were exclusively associated with carabid beetle sequences. ASVs assigned to the same species were then merged to reflect the presence of each prey taxon within individual predator samples. Sequences representing carabid beetles, non-metazoans and parasites were then removed, leaving only prey taxa. Following bioinformatic processing, 209 of the 579 carabid beetle samples (36.1 %) were retained in the final dataset. Finally, read counts were converted to presence-absence data.

2.4 Data analysis

Carabid beetles were assigned to riparian habitat specialists and generalists according to Klaiber et al. (2017). Prey species were categorised into aquatic (i.e. organisms with a fully aquatic lifestyle or an aquatic developmental stage) and terrestrial based on Schmidt-Kloiber and Hering (2015).

All statistical analyses were conducted in the R software (R version 4.3.2 R Core Team 2023) using the development environment RStudio (2024.04.1+748). For a comprehensive overview of the prey consumed by carabid beetles on all sites and to account for the uneven sample size between the two sampling periods (May: 157 samples, September: 52 samples), samples of carabid gut contents from both sampling periods was analysed as a single dataset.

In order to relate the prey composition to carabid beetle tribes (Bembidiini, Carabini, Nebriini, Notiophilini, Omophoroninii, Patrobini, Platynini, Pterostichini, Scaritini), habitat specialisation (riparian specialists vs. generalists) and stream section (upstream, midstream, downstream), we performed a redundancy analysis (RDA). We used the Jaccard distance on presence-absence data of prey families and combined individuals of the same carabid beetle species per site. For further food web analysis, the niche overlap and connectance was calculated using the bipartite package (Dormann et al. 2007) and its default settings. Niche overlap indicates the average shared prey community (range 0–1), while connectance measures how many of the possible interactions in a bipartite network are actually realised (range 0–1). The relationships between carabid beetles at the tribe level and prey (class level, and order level for insects) were visualised using the circlize package (Gu et al. 2014).

Generalised linear models with a binomial error distribution was fitted to test for differences in the proportion of aquatic prey detections (lme4 package (Bates et al. 2003). Therefore, individuals of the same species per site were pooled. The response variable was specified as a two-column matrix of aquatic prey detections and terrestrial prey detections, with carabid beetle tribes, specialisation, and stream section as explanatory variables. Model significance was assessed using a Type II Wald chi-square test of the car package (Fox et al. 2001). Estimated marginal means were calculated using the emmeans package (Lenth 2017) and presented on the response scale as probabilities for interpretability. For visualisation of aquatic prey usage, we created a stacked bar plot with ggplot2 (Wickham et al. 2007).

3 Results

3.1 General prey consumption

The final dataset of 209 carabid beetle individuals included 28 species with numbers of individuals ranging between one (*Bembidion lunulatum*) and 22 (*Nebria brevicollis;* APPENDIX). In total, 112 prey items were identified in these samples, including 78 species, 71 genera, 61 families, 22 orders and 10 classes. The number of prey items per carabid beetle sample ranged from one to four, with an average of 1.5 prey items. Across all carabid beetle species, prey was dominated by Insecta (60.2 %), followed by Clitellata (13.3 %) and Collembola (9.7 %). Other groups

contributed less than 5% each, including Gastropoda (5.3 %), Malacostraca (4.4 %), and Diplopoda (2.7 %) (Fig. 1a).

Among Insecta, Diptera accounted for the majority of prey taxa detected (48.5 %) followed by Hymenoptera (16.2 %) and Coleoptera (13.2 %). Hemiptera (7.4 %), Lepidoptera (4.4 %), and Psocodea (4.4 %) were detected at lower frequencies (Fig. 1b).

Of all prey species, 84.8 % were terrestrial and 13.4 % aquatic (1.8 % could not be classified into one of these categories). Limoniidae (Diptera) dominated the aquatic prey (34.3 %), followed by Lumbricidae (28.6 %) represented by a single aquatic-oriented species, *Eiseniella tetraedra*.

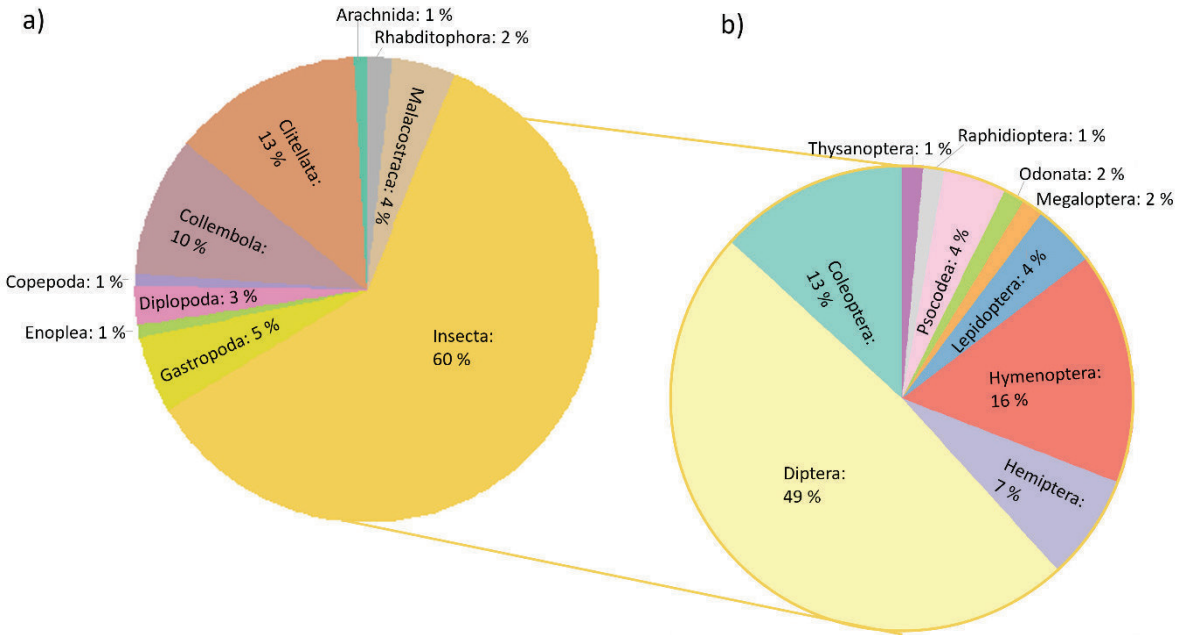


Fig. 1: Proportion of detected prey in riparian carabid beetles (N=209 individuals) shown at A) the class level and B) the order level for insect prey.

3.2 Differences between carabid beetle tribes, specialisation and stream section

The prey composition at family level differed significantly between the carabid beetle tribes (Table 1, Fig. 2). In Bembidiini, Diptera constituted the largest share of prey items (32.6%), followed by Rhabditophora (13.7%), the latter being detected in only one other carabid beetle tribe, Platynini (1.5%). As in Bembidiini, Diptera were also the most frequently detected prey in Carabinii (33.3%), Nebriini (31.6%), Patrobini (40%), Platynini (28.1 %), and Pterostichini (32.6 %). In contrast, Notiophiliini showed the highest prey detection for Hymenoptera (28.6 %), which also represented the most frequently detected prey in Scaritini (57.9%) (Fig. 2a). The niche overlap was 0.49, indicating a moderate dietary similarity among species. The connectance was 0.27, reflecting a moderately dense web of realised trophic links and suggesting that carabid beetles exploit a fairly broad range of prey rather than being highly specialised.

Differences in prey composition among tribes were also evident at the insect family level (Fig. 2b). In Bembidiini, the most frequently detected dipteran family was Stratiomyidae (12.5%), a pattern shared by most other carabid beetle tribes. In the carabid tribe Nebriini, Stratiomyidae were also detected as prey, while the most frequently detected dipteran family was Limoniidae in Pterostichini (10.9%) and Agromyzidae (5.7%). Scaritini was the only

tribe in which Stratiomyidae were not detected. Andrenidae was the most frequently detected hymenopteran family in Notiophilini (20.0%) and all other carabid tribes, except Nebriini where Andrenidae were absent.

Table 1: Relation of prey composition to carabid beetle tribes, specialisation of carabid beetles, and stream section using redundancy analysis (RDA). We used the Jaccard distance on presence-absence data of prey families and combined individuals of the same carabid beetle species per site.

	F	P	R^2
Carabid beetle tribe	1.91	0.001	0.07
Specialisation (riparian specialist vs. generalist)	2.96	0.001	**0.01**
Stream section (upstream, midstream, downstream)	0.97	0.520	**0.01**

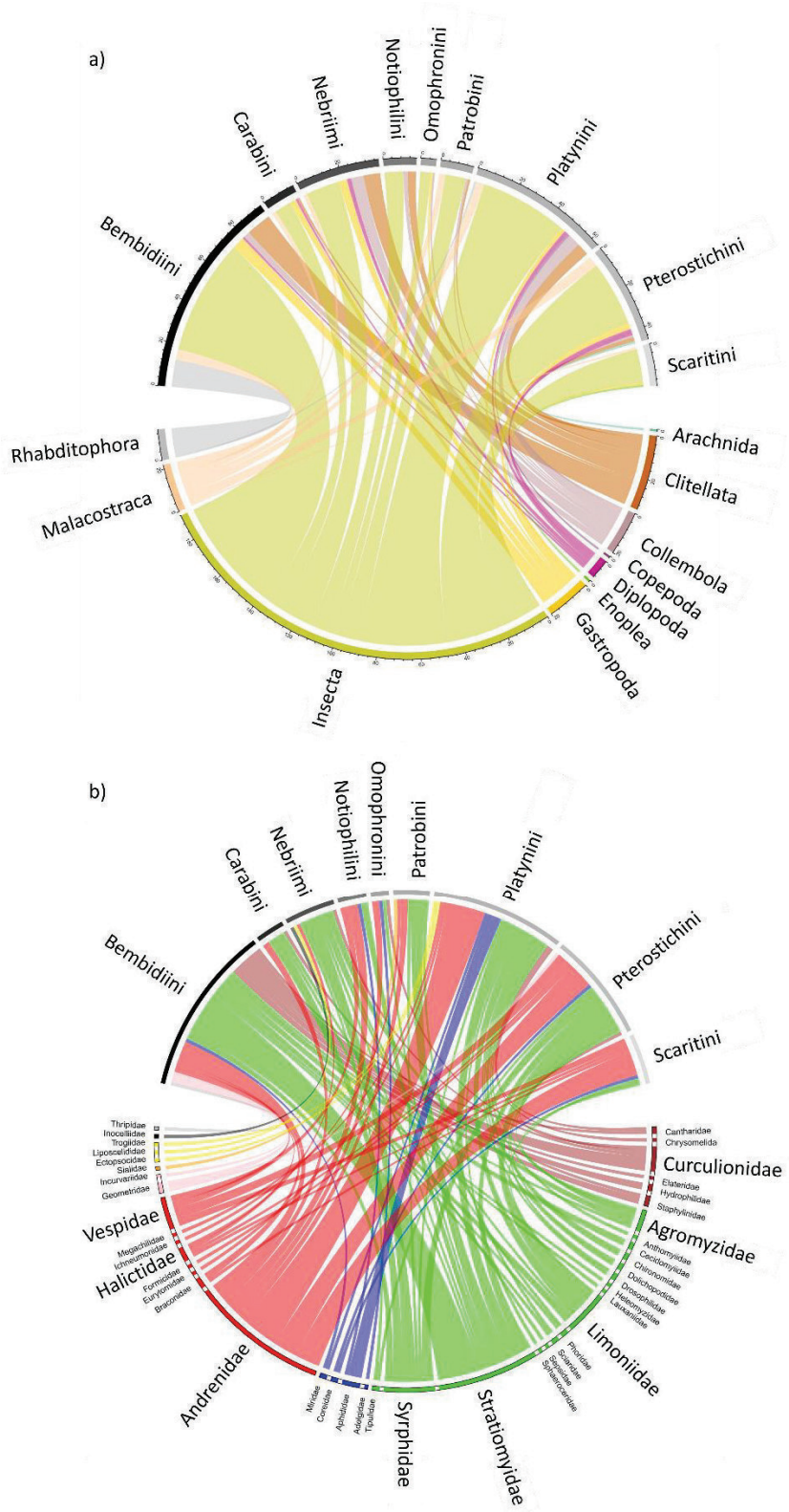


Fig. 2: Circular representation of trophic interactions between carabid beetles (tribe level) and their invertebrate prey (a: class level, b: insect family level). The width of curved bars denotes the proportional representation of predators (top) and prey (bottom). Trophic interactions are represented by links between predator and prey. From left to right: Thysanoptera (grey), Raphidioptera (black), Psocodea (yellow), Megaloptera (orange), Lepidoptera (pink), Hymenoptera (red), Hemiptera (blue), Diptera (green), Coleoptera (brown).

Although the overall prey composition of riparian specialists and generalists was relatively similar in terms of niche overlap (0.72) and connectance (0.72), the RDA revealed a significant difference in the detected prey community (Table 1). This difference was primarily driven by Lumbricidae, which dominated the prey of generalists (15.3 %) but accounted for only 1.6 % of prey detected in riparian specialists. On the other hand, the hymenopteran suborder Apocrita was detected in 9.8 % of the riparian specialists, while it was less frequently detected in generalists (5.1 %) (Fig. 3).

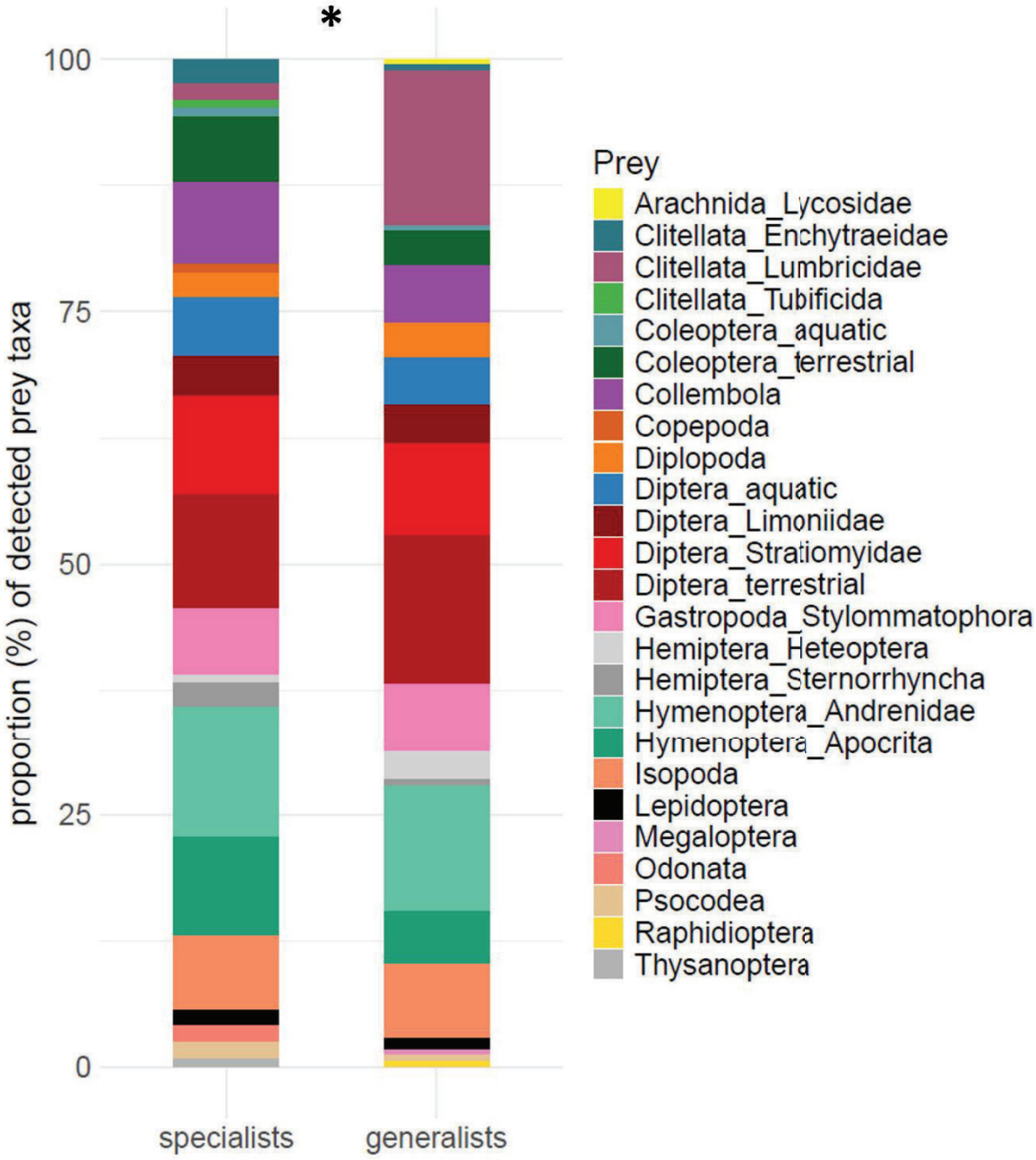


Fig. 3: Stacked bar plots showing the prey composition of riparian specialists and generalists. Differences in prey composition was tested at the family level (Table 1), but prey taxa were grouped in the graph at higher taxonomic levels (primarily classes for insects and orders for non-insects) to enhance clarity and reduce visual complexity. However, particularly prominent or ecologically relevant taxa (e.g., certain families) were retained at finer taxonomic resolution where appropriate.

In contrast, the prey composition was not significantly different between carabid communities at the different stream sections (Table 1). The high similarity between the detected prey items along the stream sections was also indicated by the large niche overlap of 0.87 and a connectance of 0.58. Indicating a rather similar diet between the stream sections, high dietary redundancy and increased robustness of the food web to the loss of individual prey species.

In all stream sections, Andrenidae (upstream: 12.6 %, midstream: 13.0 %, downstream: 13.7 %) and Lumbricidae (upstream: 11.5 %, midstream: 8.0 %, downstream: 9.8 %) belonged to the most dominant prey items.

3.3 Aquatic prey proportions

Aquatic prey was detected in 33 out of the 209 predator individuals. Eighteen carabids contained only aquatic prey. The average aquatic prey proportion between the carabid beetle tribes ranged between 0 % (Notiophilini and Omphronini) and 20.0 % (Patrobini) with a mean value of 12.1 % across the tribes (Figure 4a). However, this difference was statistically not significant ($Chi^2 = 7.37$, $P = 0.50$). Similarly, the proportion of aquatic prey was not significantly different between riparian specialists (8.08 %) and generalists (12.36 %) ($Chi^2 = 0.01$, $P = 0.92$)(Fig. 4b). In contrast, the proportion of aquatic prey was significantly different between the three stream sections ($Chi^2 = 11.04$, $p = 0.004$). Estimated probabilities for aquatic prey detection were 18.7 % (95 % CI: 12.4–27.2) in downstream, 7.0 % (95 % CI: 3.4–14.0) in midstream and 9.0 % (95 % CI: 4.6–17.0) upstream. A higher proportion of aquatic prey was associated with a wider range of consumed aquatic organisms: eight aquatic-oriented prey families were identified in the downstream section, whereas five and three were detected in the midstream and upstream sections, respectively. Also, the relative contribution of aquatic prey families differed across the upstream, midstream, and downstream sections e.g., Limoniidae dominated (50 %) the aquatic prey items in the upstream section, while the most dominant aquatic prey item in the midstream section was Lumbricidae (*Eiseniella tetraedra*; 43 %). Limonoiidae (35 %) and Lumbricidae (25 %) dominated the downstream aquatic prey (Fig. 4c).

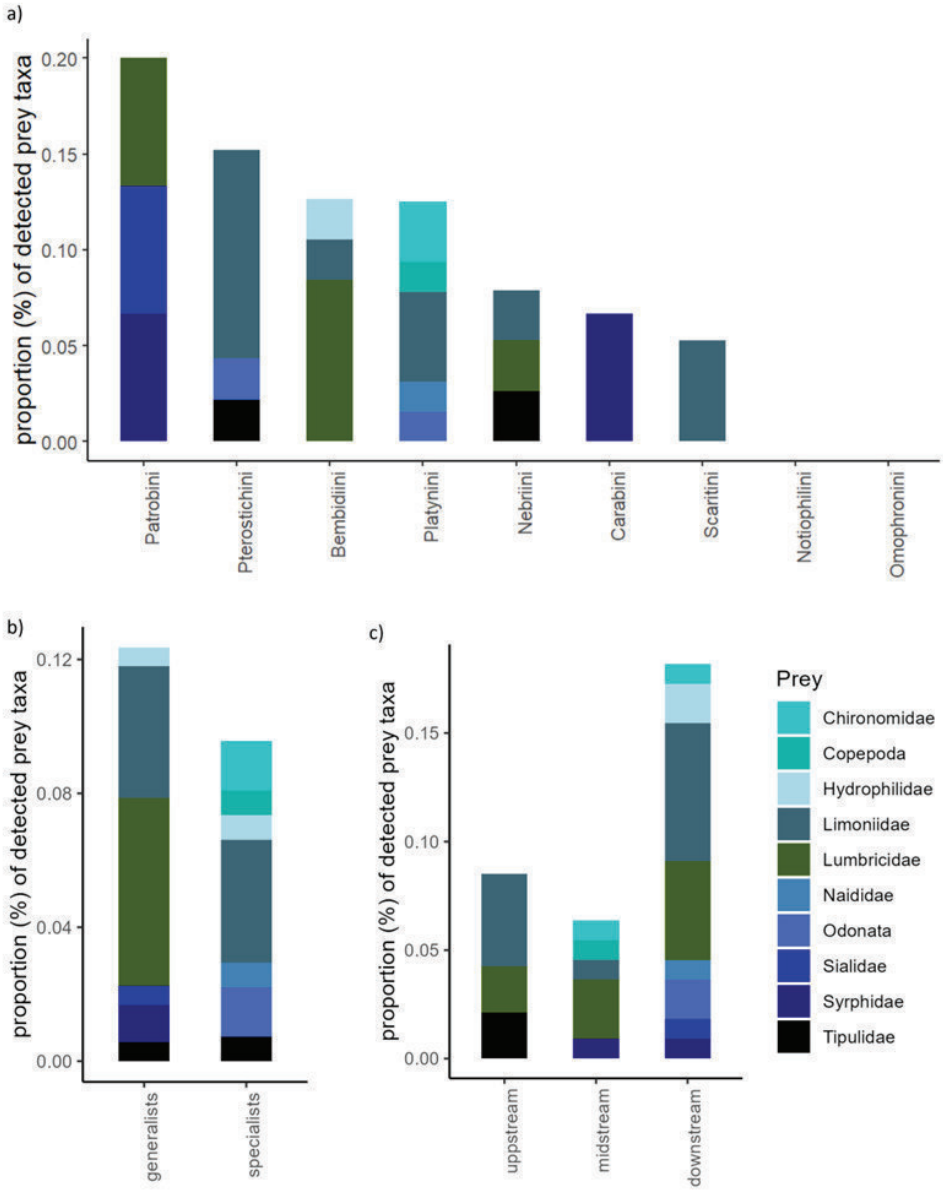


Fig. 4: Stacked bar plots showing the composition of aquatic prey (family level) detected in carabid beetles. The bar height indicates the proportion of aquatic prey of total detected prey grouped by a) carabid beetle tribe, b) specialisation, and c) stream section.

4 Discussion

4.1 General prey consumption of riparian carabid beetles

To our knowledge, this is the first application of DNA metabarcoding to investigate the prey composition in carabid beetles from riparian habitats. Only one previous study has addressed carabid diets using this approach, examining 14 species in agroecosystems (Kamenova et al. 2018). Our results demonstrate that riparian carabids exploit a broad prey spectrum, dominated by insects but also including Clitellata, Collembola, Malacostraca, and Gastropoda. This pattern aligns with earlier non-molecular studies indicating that carabid beetles are generalist feeders with relatively unspecific food requirements (Hering and Plachter 1997; Kulkarni et al. 2015; Penney 1996). Notably, the prey of

riparian carabid beetles was highly dominated by terrestrial organisms, while the use of aquatic prey was rather limited (only 12.1 % on average).

Forest carabid beetles are known to feed on soil-dwelling Collembola, as well as taxa such as Lepidoptera, Lumbricidae, and Isopoda (Šerić Jelaska et al. 2014). Consistent with these observations, our study also detected these prey groups in the diets of carabid beetles from riparian forests. However, Collembola were detected at unexpectedly low levels in our study, particularly for the specialised Collembola feeder *Notiophilus biguttatus* (Baulechner et al. 2022). One possible explanation is that Collembola may be less dominant in riparian habitats than forests (Lek-Ang et al. 1999). Further, the primer set used may be suboptimal for amplifying degraded Collembola DNA. Alternatively, Collembola may be more difficult to capture than other prey, making them a less profitable resource if the energetic cost outweigh the benefits (Baulechner et al. 2022). Supporting this interpretation, Penney (1996) showed that Collembola can be underrepresented in the diet relative to their availability, as observed for *Nebria brevicollis* using visual gut content analysis. Future evaluations of the fwhF2 and FwhR2n primer set (Vamos et al. 2017) with respect to Collembola DNA could help clarify potential methodological limitations.

The high frequency of Diptera DNA detected in our study is consistent with previous findings (Penney 1996). The dominant Diptera family identified was Stratiomyidae, whose larvae develop in decaying plant and animal material as well as in wet moss (Kovac and Rozkošný), all of which are accessible to ground-dwelling carabids. This suggests that carabid beetles may primarily feed on the fly larvae rather than on adults. Supporting this interpretation, visual gut content analyses have revealed both adult and larval fragments in carabid diets (Hering and Plachter 1997a).

In addition, DNA of the mining bee *Andrena* was detected across major carabid beetle tribes. *Andrena* species inhabit diverse environments, including forest edges, floodplains, and sparsely vegetated areas (Szczepko-Morawiec et al. 2024), which overlap with our sampling sites. Given this habitat overlap, their occurrence in carabid diets is not unexpected. As with Diptera, consumption likely includes immature stages (eggs, larvae, pupae).

Aphids and snails, both previously documented as prey of carabids with important implications for pest control (Kromp 1999), were also detected in our metabarcoding analysis. Snails are more frequently consumed as carrion, since their mucus serves as a defence mechanism that forces predators to clean their mandibles after each attack (Mair and Port 2001). Accordingly, the snail DNA detected in our study likely reflects scavenging rather than predation.

Our results raises the question of the extent to which scavenging contributes to carabid feeding. The method applied here does not allow to distinguish between scavenging and predation. Previous research, however, has shown reduced RNA detection in beetle regurgitates from decayed food compared to fresh food (Neidel et al. 2022). As this finding is based on a single-food feeding experiment, its applicability to field samples remains limited. Nevertheless, expanding this approach or developing complementary methods could help estimate the contribution of decomposed food in natural diets. Traditional visual gut content analysis may also serve as a useful complement. For instance, Hering and Plachter (1997) suggested that *Bembidion* species are predominantly scavengers, as the fragments identified in their guts closely resembled the remains of organisms washed ashore along rivers.

4.2 Differences in prey consumption between tribes, riparian specialists and generalists, and stream sections

Prey composition differed markedly among carabid beetle tribes. The sand bee *Andrena*, one of the most frequently detected prey taxa, was absent from Nebriini, likely because adult bees exceed their typical prey size range of 0.4–4 mm (Penney, 1996). Other tribes may capture bees entering nests, scavenge dead individuals, or feed on early life stages (eggs, larvae, pupae). Bembiini were unique as they exhibited a large portion of 10% of Platyhelminthes (Rhabditophora) comprise over 10% of its diet. This likely reflects greater habitat overlap, as carabids are sensitive to habitat structure and have specific habitat requirements (Kotze et al., 2011). Rhabditophora inhabit moist forest soils and are poor dispersers, unable to survive dry conditions (Álvarez-Presas et al., 2012), making them more accessible to Bembiini. Although not statistically significant, the proportion of aquatic prey items varied among tribes, consistent with the differences in the use of aquatic subsidies observed across spider groups (Middendorf et al. 2025b)

Riparian habitat specialists and generalists also differed in their prey consumption. Contrary to our expectation and to Ramey and Richardson (2017), who reported greater reliance of riparian specialists on aquatic subsidies, these differences were not primarily driven by aquatic prey. Although chironomids – an abundant and important aquatic subsidy (Kautza and Sullivan, 2016) – were detected exclusively in riparian specialists, the main difference was the higher consumption of Lumbricidae by generalists, which were absent from the diets of specialists. These patterns likely reflect differences in hunting strategies between specialists and generalists and are consistent with species-level variation previously reported for Collembola consumption (Baulechner et al., 2022).

Although overall prey family composition of riparian carabid beetle communities did not differ between stream sections, the contribution of aquatic prey increased downstream. Our results therefore support the assumption that carabid beetles along smaller streams rely primarily on terrestrial prey, while aquatic prey becomes increasingly important with larger stream size, consistent with observations by Hering and Plachter (1997). Beyond stream size, additional factors such as riparian habitat productivity may also shape the extent of aquatic prey consumption. For instance, carabids inhabiting low-productivity habitats, such as alpine streams with extensive gravel beds, may depend more strongly on allochthonous aquatic subsidies than those in more productive environments (Hering and Plachter 1997; Middendorf et al. 2025b). Our results therefore indicate that changes of riparian carabid beetle communities along longitudinal stream gradients (Middendorf et al. 2025a) may indeed be partly driven by food supply and consumption.

5 Conclusion and outlook

Our molecular gut content analysis revealed a diverse terrestrial prey spectrum in riparian carabid beetles, with only low proportions of aquatic prey. As one of the first studies to apply prey DNA metabarcoding to multiple riparian carabid species, our work underscores both the potential and the challenges of this approach. In particular, distinguishing between scavenging and active predation remains unresolved, yet our findings suggest that both strategies may play important roles in riparian food webs. In addition, future progress could be made by developing primers that reduce predator DNA amplification while enhancing prey detection, as demonstrated for spiders (Lafage et al. 2019). Moreover, extending such studies across broader geographic regions, stream sizes, and seasonal contexts will be essential to capture the full variability of riparian carabid diets. Despite these limitations, our study provide novel insights into the prey consumption of riparian carabid beetles. Their role in linking aquatic and terrestrial food webs at small forested lowland streams appears limited compared to findings from larger mountain rivers,

underscoring the importance of river characteristics and environmental context in shaping aquatic subsidy use by carabid beetles.

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Chapter 5:

Synthesis and outlook

Franziska Middendorf



Overarching discussion

This thesis shows that variations in river and bank characteristics affect riparian arthropod communities and their use of aquatic subsidies, underscoring the role of habitat heterogeneity in riparian systems.

In the first step, our results show that riparian spiders in the norther hemisphere, especially the horizontal web-building *Tetragnatha*, are the most well-studied taxa regarding to the use of aquatic subsidies. The proportion of aquatic subsidies, mostly analysed using SIA, varies strongly. For example, smaller spiders tend to use more aquatic subsidies than larger ones (Akamatsu et al., 2007). There is a general agreement that increasing the distance from the water body has a negative effect on the intake of aquatic subsidies (Henschel et al., 2001; Mellbrand et al., 2011; Raikow et al., 2011). Similar to that, the seasonal impact was identified in several studies (Chan et al., 2009; Krell et al., 2015; Chari et al., 2020; Kowarik et al., 2021), with higher use of aquatic subsidies during higher emergence rates related to the season. Though, this might co-occur with changes in ontogenetic stages of the predator. The influence of the surrounding habitat on aquatic subsidy use was less consistent: some studies report higher use in riparian forests compared to grasslands (Alberts and Sullivan, 2016), while others report the opposite pattern (Krell et al., 2015). The use of aquatic subsidies increases in larger rivers, both along the downstream gradient (Chari et al., 2020) and across rivers of different sizes (Marker et al., 2023). Based on the results of the literature review and the resulting research gap for arthropods other than spiders, we focused on carabid beetles in a further step.

We detected that carabid beetle communities differ between various river sections (upstream, midstream, downstream; Chapter 3). The river sections themselves vary significantly in riparian area size – riparian area is here defined as a part of the bank with a gentle slope well connected with the adjusted waterbody – as well as in the proportion of moss, soil pH and the amount of coarse soil. In particular, the size of the riparian area influenced the carabid community positively, which contained significantly more species and individuals in the downstream section, as well as the highest functional diversity. However, the community-weighted mean was highest in the upstream section, where short-winged traits were also most prevalent. This pattern suggests a more stable environment, as such traits are generally less suited for rapid dispersal. In contrast, the smaller body sizes and long-winged characteristics observed in the midstream and downstream sections point to a more mobile carabid beetle community, likely linked to frequent flooding events along these banks, which in turn impose stronger migration pressures on the midstream and downstream communities.

The greater species and functional richness observed on downstream banks is likely driven by more frequent flooding events, which generate disturbance and, in turn, promote (micro)habitat and microclimate heterogeneity, enhance vegetation diversity and increase food availability (Ramey and

Richardson, 2017). Furthermore, there is also evidence that the availability of aquatic subsidies has a positive effect on the abundance of riparian arthropod predators (Kato et al., 2003; Wesner, 2012). This availability and therefore the abundance of the predator can change e.g. with the river section (Iwata, 2007), which usually has a higher emergence rate towards the downstream section (Vannote et al., 1980).

Despite that, two studies on riparian carabid beetles, both manipulating the availability of dead chironomid larvae on bank patches, yielded contradictory results. These inconsistent findings might reflect the considerable variation in the quantity of chironomid larvae offered per square meter (1.25 mg vs. 50 -100 mg), with the lower number showing no effects on the abundance of riparian carabid beetles, while the higher number had a positive effect (Paetzold et al., 2006; Henshall et al., 2011). In addition, our DNA metabarcoding results hint that those areas with higher availability of aquatic subsidies may support larger carabid populations, as reflected by the downstream sections showing both peak carabid beetle abundance (Chapter 3) and elevated detection of aquatic prey in their diet (Chapter 4). Based on these results, it is not possible to confirm this idea, as, for example, the actual availability of prey was not sampled. Moreover, separating the effects of habitat from those of food availability remains highly challenging. As environmental conditions - e.g. distance from the water, size and trophic productivity of the water body, food competition with invasive species and the surrounding habitat, as well as physiological conditions e.g. sex, size and life stage (Middendorf et al., 2025) - affect the intake of aquatic subsidies as well.

Further, it was shown that different carabid beetle genus use aquatic subsidies in various amounts, as e.g., *Bembidion* sp. feeds more on aquatic subsidies than the larger *Nebria* sp., despite sharing the same habitat (Hering and Plachter, 1997). Yet, this could be due to the more favourable size ratio between predator and prey, as aquatic prey is often smaller than terrestrial prey (Akamatsu and Toda, 2011). On the other hand, it is possible that, despite the similar habitat, *Bembidion* occurs closer to the water's edge because they are shore specialists, and *Nebria* are not (Klaiber et al., 2017). In line with the variation between the taxa, the DNA metabarcoding study included in this dissertation (Chapter 4) reveals a significant difference in the diet composition between different carabid beetle tribes. Variation in body size is affecting the intake of aquatic subsidies for spiders, with smaller spiders consuming a higher amount than larger spiders (Verschut et al., 2019; Chuang et al., 2023). In our field study (Chapter 3), the community weighted mean (CWM) body size of the carabid beetle community was highest in the upstream section, while the CWM body size was relatively similar between the midstream and downstream sections. The proportion of detected aquatic prey was highest in the downstream section but similar between midstream and upstream sections, which might indicate that body size is not an important factor regarding the consumption of aquatic subsidies for carabid beetles.

Nevertheless, there was a generally low proportion of aquatic subsidies in the diet of the riparian carabid beetles (Chapter 4). This also could indicate that environmental conditions, e.g. riparian area size and flooding frequency, have a higher effect on the apparent predator characteristics than the available prey. In addition, the relatively low proportion of aquatic subsidies in the diet of riparian carabid beetles at forest streams, as surrounded by forests, is in line with earlier research on ground-welling spiders and carabid beetles, which feed more on terrestrial prey than in grassland habitats (Krell et al., 2015). In forest habitats, Krell et al. (2015) argue that the availability of Collembola as high-quality food is comparatively high, which might be a reason why aquatic subsidies are less consumed. But Collembola was detected at low frequency in our DNA metabarcoding study, which might indicate that the importance of the consumption of this organism might be overestimated, at least for carabid beetles. This prey may be less available in riparian forest areas (Lek-Ang et al., 1999). Also, Capturing Collembola is hard due to their fast movements (Baulechner et al., 2022), and therefore, the energy costs to catch this potential prey might be too high. Indeed, in our study the Hymenoptera Aranea and the Diptera Stratyominidae were detected at a high frequency in the diet of the carabid beetles. These two groups are also frequently present on ground level, as they also nest and develop in this stratum (Kovac and Rozkošný, 2004; Szczepko-Morawiec et al., 2024). They might be easier to prey on than Collembola and, therefore, are more frequent in the diet of carabid beetles.

Conclusively, our field study indicates that river characteristics may shape the consumption of aquatic subsidies for carabid beetles, with higher proportions detected in the larger downstream sections compared to the smaller midstream and upstream sections. In contrast, we detected that ground-dwelling carabid beetles appear to rely more heavily on terrestrial prey along forest streams. This is contrary to the diet of riparian web-building spiders, as they can be strongly dominated by aquatic subsidies, as demonstrated in Chapter 2, although several factors may influence this proportion e.g. river size and river section, distance to the water and surrounding habitat.

Open questions

Our study advances the understanding of the diet of arthropods beyond spiders. Nevertheless, this area remains underexplored, with the diets of canopy-dwelling riparian arthropods, for instance, are still are underrepresented (Albacete et al., 2020).

Although the use of aquatic insects was minor in our study for riparian carbide beetles, the results from the systematic literature review suggest that many factors, such as season, river size, distance from the water and surrounding habitats, can affect the amount of consumed aquatic subsidies. Therefore, it would be revealing to incorporate these factors in future studies to get a more comprehensive insight into the importance of aquatic subsidies for various riparian carabid beetles. While DNA metabarcoding provides detailed identification of diverse prey items at a fine taxonomic

resolution, prey abundance can only be inferred indirectly, as the method primarily yields occurrence data (i.e., the frequency of prey items across individuals) rather than quantitative measures of biomass or consumption. Accordingly, incorporating additional stable isotope analyses (SIA) might bring further insights into the ratio of aquatic to terrestrial food sources. Based on the ratio between different carbon isotopes it is possible to calculate the proportion of aquatic subsidies in the diet of the predator. The $\delta^{13}C$ is higher for terrestrial plants than for aquatic ones; this baseline is then detectable through the food web and higher trophic niches (Krell et al., 2015; McClain-Counts et al., 2017). This method, while being useful, is also prone to misinterpretation, as aquatic insects may primarily feed on terrestrial leaf litter, or terrestrial herbivores may consume washed-ashore algae, which could lead to an incorrect assessment of the aquatic versus terrestrial origin of resources at higher trophic levels, since the baseline would be misleading (Mellbrand and Hambäck, 2010; Krell et al., 2015). This demonstrates the added value of employing these two complementary methods in tandem. Though, obtaining reliable results from SIA requires the concurrent sampling of potential food sources. As our study represents the first large-scale metabarcoding analysis of the diet of various riparian carabid beetles, their exact feeding habits were previously unclear and the likelihood of not sampling the appropriate food sources was high. Under such circumstances, the additional use of SIA would have provided only minimal benefits. For future studies it would be advisable to use both methods. In addition, sampling potential prey would provide valuable insights into the availability of specific food sources. This, in turn, would enable the assessment of dietary preferences by comparing the prey availability with the actual occurrence of consumed prey in the diet of the predator community. To further improve the DNA analysis of gut contents, it would be beneficial to develop primers that do not efficiently amplify the predator's own DNA. This would increase the read depth of prey sequences, as in our study, the majority of reads was assigned to the predator. Such an approach has already been applied in dietary analyses of spiders (Lafage et al., 2019). PUFA analyses would also add valuable information about the food quality (Ohler et al., 2024). As demonstrated in the literature review, this approach is still underrepresented in research on cross-boundary food-web interactions involving riparian arthropod predators.

Thus far, no method has successfully addressed the approach distinguishing between active predation and scavenging or for differentiating the consumption of different developmental stages. Though, the results of this study, consistent with prior studies (Hering and Plachter, 1997), suggest that all these food sources were consumed. It would be valuable to explore whether one of the established dietary analysis methods can be further developed to differentiate among prey stages, such as freshly captured individuals, carcasses, larvae, and adults. Furthermore, there are already studies which demonstrate that the RNA concentration of one specific carcass in regurgitates of one Carabid species is lower than that of freshly consumed prey (Neidel et al., 2022). However, based on the limited prey

variation tested, it is probably not yet sufficiently developed to be applied to field studies. Early studies did visual gut content analyses, in which, based on the found prey fragments, it was possible to distinguish between adult and larval life stages. Despite this, only relatively well-preserved fragments in the digestive tract can be identified visually, which inevitably limits the representation of the full prey spectrum. Nevertheless, distinguishing between the different prey stages (adult, larvae, fresh/alive, carcass) would bring valuable information about the food web and interaction between species. For example, the consumption of early life stages (larvae, pupae, eggs) is particularly critical for populations, as individuals at these stages are not yet capable of reproduction. In case that carabid beetles preferentially feed on early life stages, this could explain the minimal use of aquatic subsidies and, consequently, the weaker linkage between terrestrial and aquatic food webs along smaller forest streams. Washed ashore prey might be a pathway, which has been rather neglected in existing riparian arthropod dietary literature. Hering and Plachter (1997) argue that carabid beetles exploit this food source, which may explain the higher use of aquatic subsidies observed at larger rivers, where increased insect emergence and therefore density (Vannote et al., 1980; Marker et al., 2023) enhances the likelihood of larvae being washed ashore. Further research is needed to clarify the importance of washed-ashore organisms in the aquatic–terrestrial food web.

In addition, if carabid beetles predominantly consume dead rather than living slugs and snails, the pest-control potential of carabid beetles could have been overestimated. This dietary study focused on the carnivorous aspect of carabid beetles; therefore, it cannot be excluded that a considerable proportion of individuals that did not contain any DNA other than carabid DNA may have fed exclusively on plant material. It may also be worthwhile to conduct additional DNA analyses with plant-specific primers to assess the dietary contribution of plant material in riparian carabid beetles.

The field studies in this dissertation focused on smaller rivers in southwest Germany surrounded by forest during the summer months. It would be interesting to conduct similar field studies in different geographical regions, since aquatic-terrestrial food-web research is already biased toward the Northern Hemisphere (Chapter 2, Middendorf et al., 2025). Additionally, the increasing riparian area in the downstream region is characteristic for the flat river Rhine Valley. The effect of the river section might be less pronounced if the investigated stream was to enter a smaller adjacent river.

Assessing the diet of riparian carabid beetles throughout the year, and potentially over multiple years, would provide a better understanding not only of variation in carabid beetle communities but also of their food intake, thereby offering valuable insights into their habitat and dietary requirements and informing conservation efforts. In addition, studies at river sites surrounded by grassland as well as agriculture could bring further insights into the diet of these arthropod predators. For instance, previous research has shown no consistent pattern in how spiders use aquatic subsidies across habitats

(Krell et al., 2015; Alberts and Sullivan, 2016; Ramberg et al., 2020). This result cannot simply be transferred to carabid beetles, as they often react more sensitively to habitat changes (Lambeets et al., 2008; Lambeets et al., 2009; Schirmel et al., 2015; Schirmel and Gerlach, 2022), and perhaps this can also be seen in their diet. Although this was not the case in our study as the diet composition did not differ between the stream sections, even though the carabid community clearly changed from upstream towards downstream. Dietary shifts may be driven more by habitat type (forest, grassland, agriculture) than by the longitudinal position of a river, even though the proportion of aquatic subsidies was indeed higher in the downstream section. In addition, it would be interesting to investigate whether changes in carabid beetle communities and trait composition are consistent across different habitat types, for example by comparing grassland communities at various positions along rivers as well as across rivers of different sizes. Future studies across diverse habitats and regions are needed to test the generality of these findings and to inform effective riparian conservation.

Implications for conservation biology

Based on the significant variation in carabid communities among the stream sections, we identified distinct indicator species for each river zone, reflecting different habitat demands. In the upstream region forest specialists, *Cychrus attenuatus* and *Abax ovalis,* were characteristically. The midstream section was represented by *Bembidion elongatum*, a species not widely distributed in Germany but typical for vegetated riverbanks. In the downstream zone, eleven indicator species were detected, comprising both typical riparian forest carabid beetles (*Patrobus atrorufus*, *Pterostichus strenuus*, *Agonum emarginatum*) and generalist species associated with grasslands and agriculture (*Clivina fossor*, *Pterostichus melanarius*). Together with observed differences in richness and trait composition, these findings suggest that carabid beetle communities are characteristically of each river section, which should be considered for future monitoring and restoration programs, as they are also effective indicators. While our results show that feeding choices do not reflect their sensitivity, carabid beetles appear to be food generalists. Nevertheless, DNA metabarcoding of their gut contents proves to be highly valuable for uncovering species interactions e.g. the surprisingly high frequency of Andrena detections as well as the low detection frequency of Collembola.

Conclusively, characterising trophic interactions, food webs and habitat requirements represents a key step in explaining ecosystem functioning. In addition, climate change brings further challenges, such as more frequent extreme events, e.g. flooding or drought. Therefore, assessing these effects is crucial for a better understanding of the interactions between the environment and its fauna, as well as species interactions, to develop effective management and conservation plans (Bradley et al., 2007; Roslin and Majaneva, 2016).

Conclusions

The aquatic–terrestrial linkages examined in this dissertation show that the river characteristics affect the riparian habitat, riparian arthropod community and associated food-web structures. Further, the contribution of aquatic subsidies to the diet of carabid beetles varies longitudinally, although the overall diet of riparian carabid beetles remains similar across stream sections while their community structure is subjected to changes in the bank characteristics. This underscores their sensitivity to habitat changes, despite their generalist feeding behaviour. This connection between the two habitats should be strongly considered in future re-naturalisation and conservation projects. In addition, further investigations are necessary as many factors can impact the diet and aquatic subsidy proportion in it. The studies incorporated in this dissertation could only cover a small part of it, but they nevertheless show the high value of carabid beetles as indicator species and reveal their interactions with various invertebrates.

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Appendix

Publication list

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Only first author contributions

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Poster Presentation

Middendorf, F., Eitzinger, B., Schirmel, J. (2024). Carabid beetle communities exhibit distinct composition along streams.

GfÖ. 53 Annual Conference. 09.September

Middendorf,F., Eitzinger, B., Schirmel, J. (2024). Diet or riparian carabid beetles along stream courses.

BES. 12.December

Author contribution

The authors' contribution to the peer-reviewed articles contained in the cumulative dissertation was as follows:

Chapter 2:

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Franziska Middendorf: Writing – review & editing, Writing – original draft, Visualisation, Methodology, Investigation, Formal analysis, Data curation, Conceptualisation

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Chapter 3:

Middendorf, F., Eitzinger, B., Entling, M., H., Schirmel, J. (2025). Carabid beetles as indicator stream zonation. *Ecological indicators*. 170. 113036. *https://doi.org/10.1016/j.ecolind.2024.113036*

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