Impact across ecosystem boundaries – Does Bti application change quality and composition of the diet of riparian spiders?

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HIGHLIGHTS

- Mesocosm study linking anthropogenic impact on aquatic subsidy to riparian predator.
- Emergence dynamics altered by Bti could transfer to subsidized terrestrial spiders.
- Bti treatment did not affect the nutrient content of aquatic subsidy.
- Stable isotope analyses implied a shift in spider diet to replace Bti-sensitive prey.
- Altered diet suggests indirect effects of Bti on spider fitness and populations.

GRAPHICAL ABSTRACT
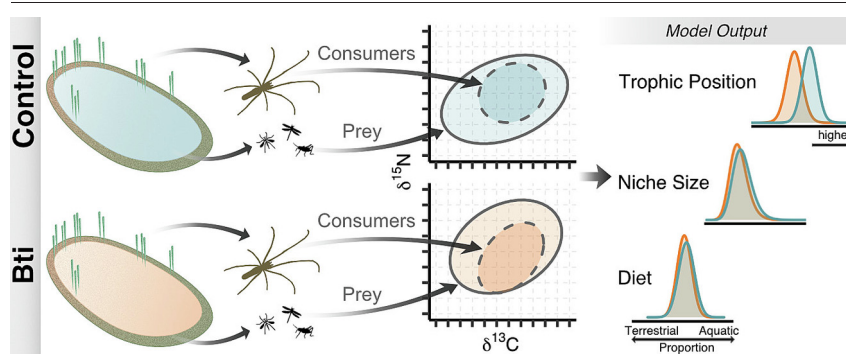
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ABSTRACT

Emerging aquatic insects link aquatic and adjacent terrestrial food webs by subsidizing terrestrial predators with high-quality prey. One of the main constituents of aquatic subsidy, the non-biting midges (Chironomidae), showed altered emergence dynamics in response to the mosquito control agent *Bacillus thuringiensis* var. *israelensis* (Bti). As riparian spiders depend on aquatic subsidy, they may be affected by such changes in prey availability. Thus, we conducted a field study in twelve floodplain pond mesocosms (FPMs), six were treated with Bti (2.88×10^9 ITU/ha, VectoBac WDG) three times, to investigate if the Bti-induced shift in chironomid emergence dynamics is reflected in their nutritional value and in the diet of riparian spiders. We measured the content of proteins, lipids, glycogen, and carbohydrates in emerged Chironomidae, and determined the stable isotope ratios of female *Tetragnatha extensa*, a web-building spider living in the riparian vegetation of the FPMs. We analysed the proportion of aquatic prey in spiders' diet, niche size, and trophic position. While the content of nutrients and thus the prey quality was not significantly altered by Bti, effects on the spiders' diet were observed. The trophic position of *T. extensa* from Bti-treated FPMs was lower compared to the control while the aquatic proportion was only minimally reduced. We assume that spiders fed more on terrestrial prey but also on other aquatic organisms such as Baetidae, whose emergence was unaffected by Bti. In contrast to the partly predaceous Chironomidae, consumption of aquatic and terrestrial primary consumers potentially explains the observed lower trophic position of spiders from Bti-treated FPMs. As prey organisms vary in their quality the suggested dietary shift could transfer previously observed effects of Bti to riparian spiders conceivably affecting their populations. Our results further support that anthropogenic stressors in aquatic ecosystems may translate to terrestrial predators through aquatic subsidy.

1. Introduction

The reciprocal flow of resources between aquatic and terrestrial habitats, as it naturally occurs in riparian zones, links food webs and increases their respective productivity (Polis et al., 1997). While the input of terrestrial organic matter is well known to support aquatic food webs, flying insects of aquatic origin are especially relevant for riparian predators, such as spiders (Baxter et al., 2005; Marczak and Richardson, 2007; Richardson et al., 2010). Emergence of adult aquatic insects (e.g., midges or mayflies) increases the prey availability in terrestrial habitats and supports riparian predators. At the same time, these insects of aquatic origin serve as high-quality prey due to their significant nutritional value for these spiders (Akamatsu et al., 2004; Kato et al., 2003).

However, the linkage through aquatic subsidy may transfer disturbances from aquatic to terrestrial systems through changes in timing, nutritional value, or abundance of adult life stages of aquatic insects (Schulz et al., 2015; Stepanian et al., 2020). Indeed, various forms of human activity, spanning from hydromorphological modifications and invasive species to pollution, can modify the subsidy of terrestrial food webs by aquatic resources (Gergs et al., 2014; Greig et al., 2012; Kraus et al., 2020). Among those disturbances, the mosquito control agent *Bacillus thuringiensis* var. *israelensis* (Bti) directly targets parts of the aquatic subsidy, mainly mosquitoes and blackflies, when deliberately applied to freshwater bodies (Boisvert and Boisvert, 2000). As biological alternative to chemical insecticides with an assumed taxa-specific mode of action against the larvae of several nematocerous dipterans, Bti is considered relatively environmentally friendly and safe for humans explaining its worldwide application (Després et al., 2011). The larvicidal activity is based on δ-endotoxins which are solubilized and activated in the larval midgut after ingestion. Binding to specific receptors impairs the midgut epithelium resulting in damage of the gut walls and ultimately mortality within hours (Dylo et al., 2014; Vachon et al., 2012).

Despite the presumed high specificity of Bti against target dipterans, several studies demonstrated negative responses of phylogenetically closely related non-target organisms (Boisvert and Boisvert, 2000; Brühl et al., 2020; Jakob and Poulin, 2016). Especially non-biting midges (Diptera: Chironomidae) were identified as a sensitive member of aquatic macroinvertebrate communities (Allgeier et al., 2019; Bordalo et al., 2021, 2020; Gerstle et al., 2023; Kästel et al., 2017). Unintended negative impacts on Chironomidae, which often dominate aquatic subsidy with shares of up to 90 % to total emergence, hold the potential for aquatic-to-terrestrial propagation as suggested by Kolbenschlag et al. (2023). In this predecessor publication, the authors report a significant alteration of the emergence dynamics over 3.5 months as a consequence of Bti applications (Kolbenschlag et al., 2023). In contrast to chironomids, the second most abundant taxon (Ephemeroptera: Baetidae) did not show any response to Bti. In fact, the emergence of Chironomidae from treated floodplain pond mesocosms (FPMs) peaked 10 days earlier at an approximately 26 % reduced abundance. At the same time, the overall number of emerged chironomids (i.e., over the entire study duration) was slightly reduced by 14 % (Kolbenschlag et al., 2023) reflecting the lower larval densities in Bti-treated FPMs observed in parallel (Gerstle et al., 2023). Although the study by Kolbenschlag et al. (2023) points towards Bti-induced shifts in the insect emergence and thus temporal changes in the quantity of aquatic subsidy, it lacks the consideration of the quality, i.e., the nutritional value of adult chironomids for terrestrial predators. Reduced larval densities could relax resource competition enabling surviving individuals to build up larger reserves of energy and nutrients (Arrese and Soulages, 2010). Furthermore, potential alterations on the linked terrestrial food web are not yet assessed but are of substantial ecological relevance.

To close this gap of knowledge, we expanded on published studies by analysing energy reserves (i.e., macronutrients and glycogen) of emerged chironomids from control and Bti-treated FPMs. In addition to prey quality, we investigated the consequences of Bti treatments on subsidized riparian spiders. We focused on the web-building *Tetragnatha extensa*, as it is known to strongly rely on aquatic prey (Kato et al., 2003; Wieczorek

et al., 2015) and thus responds to changes in its availability (Graf et al., 2020). Adults are usually abundant between May and August, and reproduce in late summer (Bellmann, 2016). Their diet as opportunistic feeders includes small flying insects caught in their web such as diptera, aphids and mayflies but also e.g., damselflies (Nyffeler and Benz, 1981; Wieczorek et al., 2015). By measuring stable isotope ratios, we compared the spiders' diet, trophic level, and niche size between control and Bti-treated FPMs. We hypothesised that the Bti-induced changes in the emergence dynamics are reflected (1) in higher levels of nutrients in adult chironomids from Bti-treated FPMs due to lower resource competition in the aquatic habitat. Furthermore, the changed emergence dynamics caused an intermittently lower availability of aquatic subsidy. Given that spiders' diet often follows the relative availability of food sources (Ryabov et al., 2015; Wilder, 2011) and the mentioned dependence of *T. extensa* on flying aquatic insects, we hypothesised (2) a shift to higher proportions of terrestrial prey for *T. extensa* in Bti-treated FPMs relative to controls detectable via their stable isotope ratios.

2. Materials and methods

2.1. Study setup

This study was conducted between April and July 2020 in twelve artificial floodplain pond mesocosms (FPMs) located at the Eußerthal Research Station (EERES), Germany, in parallel to other studies (Gerstle et al., 2023; Kolbenschlag et al., 2023). These FPMs were designed to assess implications of stressors on aquatic subsidy in an environmentally realistic and at the same time replicated manner. Therefore, natural colonization and succession were allowed to occur in these mesocosms since their construction in 2017 (Stehle et al., 2022). The water level of each FPM can be regulated via adjustable in- and outflows directly connected to an adjacent stream of high ecological quality. One shore in each FPM represents a floodplain area with a flat slope (3°), leading to differences in the surface area with changes in water level. When the water level reached 50 cm, which is the maximum used in the present study, the water surface of each FPM measured about 104 m^2 (~20 m × 5.2 m). All FPMs were vegetated mostly by waterweeds, coontails, green algae, bulrushes, and rushes. During the study, the pH was regularly measured while dissolved oxygen and temperature were continuously logged. Data suggest similar conditions between the treatments as detailed in the Supplementary Material (Fig. S1).

2.2. Bti application

The hatching of the target mosquito larvae in the Upper Rhine Valley, mainly *Aedes vexans*, is initiated by flooding and hence the application of Bti is temporally linked to such events (Becker et al., 2018). To simulate flooding, the water height of every FPM was increased by 20 cm (from 30 to 50 cm) three times in an interval of three weeks which corresponds to a realistic frequency of Bti applications in the Upper Rhine Valley (Becker et al., 2018). Three days after the onset of each flood (i.e., 14th April, 4th May, 25th May), the maximum field rate (i.e., 2.88 × 10^9 ITU/ha) of the mosquito control agent *Bacillus thuringiensis* var. *israelensis* (Bti) was applied to six out of twelve FPMs (Fig. S2). For this, the formulation VectoBac WDG (Valent BioSciences, Illinois, USA) was suspended in pond water and homogeneously applied to the water surface using a conventional knapsack sprayer (prima 5, GLORIA, Germany). All Bti products applied in Germany need to be sterilized by gamma radiation to annihilate viable spores or cells which lowers long-term persistence of Bti (Becker et al., 2018; Poulin et al., 2022). Applications of Bti were exclusively done on windless days in order to prevent any unintended contamination of control (unexposed) FPMs. The water levels were reset to 30 cm one week after each application (see for details Kolbenschlag et al., 2023).

Since the successful application of Bti could not be verified analytically, a bioassay, which uses the toxicity of Bti towards mosquito larvae (i.e., *Culex* sp.) as response variable, was employed as an alternative method. In parallel, the rate of larval survival in control FPMs was used

to rule out any cross-contamination. Following this procedure, we indirectly verified a successful Bti application by observing at least 90 % mortality of *Culex* sp. compared to the controls.

2.3. Arthropod sampling and identification

Over the entire study period, emerging aquatic insects were caught using three floating emergence traps per FPM, each covering an area of 0.33 m^2 (Cadmus et al., 2016). These traps were emptied once or twice a week by vacuuming all living insects from the inside with a self-constructed hand-operated suction sampler (Fig. S3). For stable isotope analyses, terrestrial arthropods were collected in the first two weeks of June twice a week from the floodplain vegetation of control and treated FPMs using a suction sampler (modified Stihl SH 86; Stihl, Waiblingen, Germany) for about 2 min per FPM. Additionally, five *T. extensa* females were gathered from the riparian vegetation of each FPM on June 15, 2020. All arthropods were conserved in liquid nitrogen and stored at −80 °C until further analyses. Aquatic insects and terrestrial arthropods were identified to family or order level (Klausnitzer, 2011; Köhler et al., 2015), respectively, using a stereomicroscope (SZX 9, Olympus) with a transmitted brightfield illumination base (SZX-ILLB200, Olympus).

2.4. Nutrient composition

Chironomids caught in the emergence traps were pooled in eight periods of approximately two weeks, representing the emergence of the respective sampling dates (Table S5). This pooling was done for each replicate (i.e., FPM) separately. Chironomid samples were freeze-dried for at least 48 h and ground in a mixer mill by adding a stainless-steel ball. From the pulverised biomass, 1.9 mg (± 0.1 mg) were weight into 2-mL Eppendorf tubes to analyse proteins, lipids, glycogen, and carbohydrates largely following Foray et al. (2012) but adapted to Chironomidae (see Supplementary Material for further details). Briefly, proteins were solubilized in a phosphate buffer and their content was measured using the Bradford assay. In a second step, chloroform-methanol and sodium sulphate were added to the remaining sample allowing the quantification of glycogen and carbohydrates using an anthrone-based assay while the lipid content was determined utilizing phospho-vanillin reactions. To account for recovery rates of each endpoint, larvae of darkling beetles, i.e., *Zophobas morio* and *Tenebrio molitor*, were measured and compared to their known nutrient contents.

2.5. Stable isotope analysis

The opisthosomas of *T. extensa* were separated from the body and analysed for their stable isotope signature. Spiders' opisthosoma and prosoma show significant differences in their isotopic signatures (Wieczorek et al., 2015) due to differences in their elemental turnover rate. The focus on opisthosomas is justified by the relatively fast turnover rate (i.e., 8 days) compared to other body parts which reflects the resources sampled 1–2 weeks before the spiders (Belivanov and Hambäck, 2015). The resources were selected based on abundance and relevance for *T. extensa* as a web-building spider. Thus, Chironomidae, Baetidae, and Coenagrionidae were selected as aquatic prey. As terrestrial prey, Auchenorrhyncha, Sternorrhyncha (Aphidina, Psyllina), and Lepidoptera as herbivores, Nabidae, and Linyphiidae as carnivores, and Collembola as detritivores were chosen due to their size and their ability of jumping, flying or ballooning – all traits that allow the species to be caught by *T. extensa*. All samples, except *T. extensa* and Coenagrionidae, consisted of several individuals due to the low body weight of individuals.

All samples were dried for at least 48 h at 60 °C and ground to fine powder as detailed above for the energy reserves. For each sample and replicate, a mass of 0.6 mg (± 0.1 mg) was packed in tin capsules (5 mm × 9 mm, IVA, Meerbusch, Germany) using an ultrafine balance (Sartorius, Goettingen, Germany) with an accuracy of 0.1 µg. Elemental contents and isotope ratios of C and N were measured by an elemental analyzer (EA, Flash 2000 HT, Thermo Scientific, Bremen, Germany) coupled to an isotope ratio mass spectrometer (IRMS, Delta V Advantage, Thermo Scientific, Bremen, Germany). Values are reported in the delta notation:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 1000‰ \qquad (1)$$

where δX is either $\delta^{13}C$ or $\delta^{15}N$, and R_{sample} and $R_{standard}$ are the abundance ratios of the heavy to the light isotope of the sample and the international standard, respectively (Vienna Pee Dee Belemnite for C, atmospheric air for N). A working standard (i.e., casein) was measured in duplicate every ten samples with a precision of <0.06 ‰.

2.6. Calculations and statistics

The contents of each of the four nutrients were calculated from the respective calibration curves. To investigate the influence of Bti on the different nutrients and their dynamics, autoregressive mixed effect models (LME) were generated with FPM as random effects to account for repeated measurements and autocorrelation. Treatment, time period and their interaction were included as explanatory variables. In order to identify statistically significant differences among the factor levels, analyses of variance (ANOVAs) were used with an alpha level set at 0.05. All variables were checked for normality and homoscedasticity.

Prior to further use in stable isotope mixing models, terrestrial sources were grouped by k-means clustering with the optimal number of clusters being determined based on within sum of squares. This step was done to reduce the number of sources included in the mixing models and thus lower their underdetermination (Phillips et al., 2014). The proportions of aquatic and terrestrial resources in the diet of *T. extensa* were estimated with Bayesian mixing models for each FPM applying a generalist prior (i.e., α = 1) using the R package "MixSIAR" (chainLength = 300,000, burn = 200,000, thin = 100, chains = 3, resid_err = TRUE, process_err = TRUE, version 3.1.12, Stock et al., 2018). Since stable isotope signatures are systematically increasing through the food web (i.e., trophic enrichment), resources were adjusted by 0.5 ± 0.19 ‰ for $\delta^{13}C$ and 2.3 ± 0.24 ‰ for $\delta^{15}N$ (McCutchan et al., 2003). Niche sizes were assessed as Bayesian standard ellipse area (SEA) using the R package "SIBER" (Version 2.1.6, Jackson et al., 2011) and normalized to the SEA of resources for comparability between treatments. The trophic position of the spiders was estimated with a two-baseline model using the R package "tRophicPosition" (Version 0.8.0, Quezada-Romegialli et al., 2022) with Baetidae and Aphidina as baseline organisms for the aquatic and terrestrial habitat, respectively, and the same trophic enrichment factors as specified above. All calculations, statistics, and data visualizations were conducted with R (4.2.1, R Core Team, 2022) using the packages "ggplot2" (Wickham, 2016), "ggpubr" (Kassambara, 2020), and "nlme" (Pinheiro et al., 2021).

3. Results & discussion

3.1. Quality of aquatic subsidy: Nutrient composition

The composition of macronutrients (i.e., protein, lipids, carbohydrates) and the proportion of glycogen were not affected by the Bti treatment relative to the control (Table 1, Fig. S6). These parameters changed over the course of the study, however with no statistically significant interaction with Bti (Table 1, Fig. 1). While the variations in the macronutrients did not follow a consistent pattern over time (Fig. 1a), average glycogen content tended to decrease when progressing from spring to summer (Fig. 1b). This decrease in the glycogen content might be linked to increasing temperatures as this energy reserve is known to be mobilized under temperature stress to produce cell-protecting disaccharides (Watanabe et al., 2002). Nevertheless, the temporal patterns showed no obvious link to the shift in the emergence dynamics of Chironomidae from Bti-treated FPMs, that is an earlier and reduced emergence (Kolbenschlag et al.,

Table 1

Treatment, time, and interaction effects on contents of glycogen, lipid, protein, and carbohydrates analysing the variance (ANOVA) of the respective mixed effect models. Significant *p*-values are printed in bold.

| Component	Factor	numDF	denDF	F-value	p-Value
Glycogen	Treatment	1	10	0.1764	0.6834
	Time	7	69	12.2092	**<0.0001**
	Treatment:time	7	69	1.0871	0.3811
Lipid	Treatment	1	10	0.0459	0.8347
	Time	7	70	6.9741	**<0.0001**
	Treatment:time	7	70	0.7090	0.6645
Protein	Treatment	1	10	2.4393	0.1494
	Time	7	70	40.5383	**<0.0001**
	Treatment:time	7	70	0.9324	0.4873
Carbohydrates	Treatment	1	10	3.2538	0.1014
	Time	7	70	6.3603	**<0.0001**
	Treatment:time	7	70	1.3311	0.2488
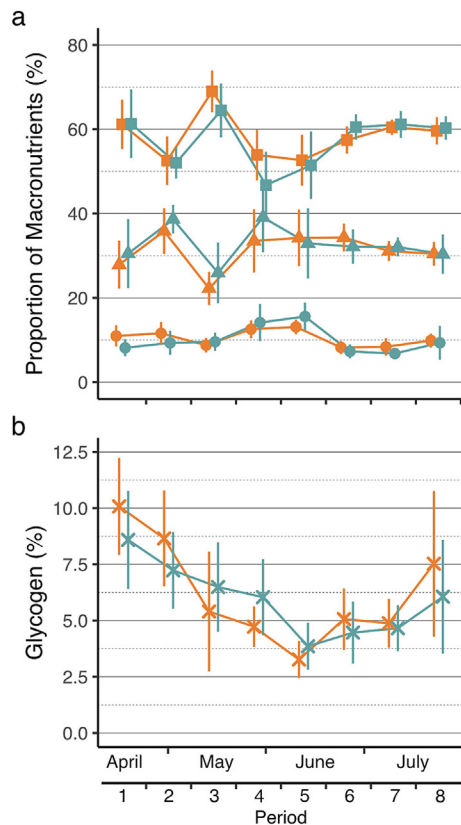
Fig. 1. a) Mean (± 95 % CI) composition of macronutrients (● carbohydrates, ▲ lipids, ■ proteins) and b) mean (± 95 % CI) proportion of glycogen in Chironomidae from control (cyan; n = 6) and Bti-treated (orange; n = 6) FPMs between April and July, measured in eight time periods. Bti was applied three times during April and May.

2023). This is in contrast to our hypothesis, which was built on the assumption that Bti reduced the number of sensitive chironomid larvae leading to increased resource availability for surviving chironomids. Ultimately, accelerated larval development could explain the documented earlier emergence (Kolbenschlag et al., 2023) which should also be reflected in the nutrient composition of adults. Recolonization of the FPMs via ovipositing multivoltine species during the study could have partly mitigated any Bti-induced effect. To further understand the discrepancy between the observation and our hypothesis, we need to recognize that insect larvae have to meet size checkpoints to enter metamorphosis

(Nijhout, 1975). More specifically, reaching the *minimal viable weight* assures sufficient nutrient storage in larvae to survive metamorphosis while the *critical weight* influences the adult size and can be affected by environmental conditions (Davidowitz et al., 2003; Mirth and Riddiford, 2007). Against this background, we conclude that the presumed increased resource availability in Bti-treated FPMs indeed promoted an earlier accomplishment of metamorphosis checkpoints supported by the earlier emergence (Kolbenschlag et al., 2023). However, this might not necessarily change the critical weight and thus does not mean that substantially more macronutrients and glycogen are accumulated during the larval development as reflected in our data. Alternatively, metamorphosis is an energetically costly process (Arrese and Soulages, 2010), which might have masked any energetic advantage chironomids might have acquired during their aquatic life stage in the Bti-treated systems. Considering that the few studies on the nutritional value of adult dipterans suggest lower contents with increasing larval density (Morimoto et al., 2019; Nguyen et al., 2019; Takken et al., 2013), we propose targeted studies on the nutrient content of larval and adult Chironomidae reared at different larval densities to elucidate the mechanism behind our observations. Additionally, analysing the nutrient content of the collected chironomids grouped for their feeding habits (i.e., filter-feeding or predatory) could help to develop a more sophisticated understanding of the Bti-induced changes in this ecologically highly relevant family.

Though not obviously linked to the Bti treatment, fluctuations in macronutrient and glycogen levels over time could be relevant for arachnid consumers (Wilder, 2011; Wilder et al., 2010). While herbivores and omnivores possess a selective nutrient intake to maintain nutritional homoeostasis (Persson et al., 2010), carnivores (e.g., spiders) seem to be more heterogeneous in their ability to respond to variations in the nutrient composition (Mayntz et al., 2005). Wandering spiders such as wolf spiders or jumping spiders could behaviourally and physiologically adapt to varying nutrient content in prey (Jensen et al., 2011; Koemel et al., 2019; Toft et al., 2010). In contrast, web-building spiders, to which *T. extensa* belongs, showed limited regulation of their nutrient intake based on their prey (Hawley et al., 2014). This may be related to regular limitations in prey amount and quality over the year. Due to their web-based foraging, *T. extensa* fully depend on insects caught in their webs presumably leading to an opportunistic and less selective feeding behaviour compared to free-hunting spiders (Hawley et al., 2014; Jensen et al., 2011). Consequently, if the consumed prey does not match spiders' nutritional demands, their fitness and performance may be negatively affected (Mayntz and Toft, 2001; Wilder, 2011). Vice versa, high-nutrient prey is expected to increase survival, reproductive success as well as the tolerance towards toxic substances and to accelerate juvenile growth (Mayntz and Toft, 2001). Thus, the observed temporal fluctuations in macronutrient and glycogen levels of aquatic insects and their relevance for terrestrial predators should be addressed further – particularly in situations in which disturbances in aquatic systems are affecting besides the quantity also the quality of this subsidy (Pietz et al., 2023).

3.2. Spider diet

The structures of the analysed food webs were comparable between Bti-treated and control FPMs in terms of raw stable isotope signatures of C and N, and the size of the resource polygons (Fig. 2a; Bayesian SEA_{Bti} = 9.07 (7.58–10.94) ‰^{2}, Bayesian SEA_{Ctrl} = 9.04 (7.52–10.98) ‰^{2}, median with 95 % equal-tail interval). Aquatic and terrestrial prey were well separated with higher $\delta^{13}C$ values in the latter allowing for a reasonable distinction between resources of different origins (Fig. 2a). In accordance with literature (Akamatsu et al., 2004; Krell et al., 2015), aquatic insects were an important resource for *T. extensa*, contributing approximately 50 % to their diet (Fig. 2b). Based on several studies reporting a negative impact of Bti on Chironomidae (reviewed in Brühl et al., 2020), which is with around 90 % the largest contributor to aquatic subsidy in the FPMs, a lower abundance of Chironomidae was expected, resulting in increased consumption of terrestrial prey by spiders. Contrary to this hypothesis, Bti
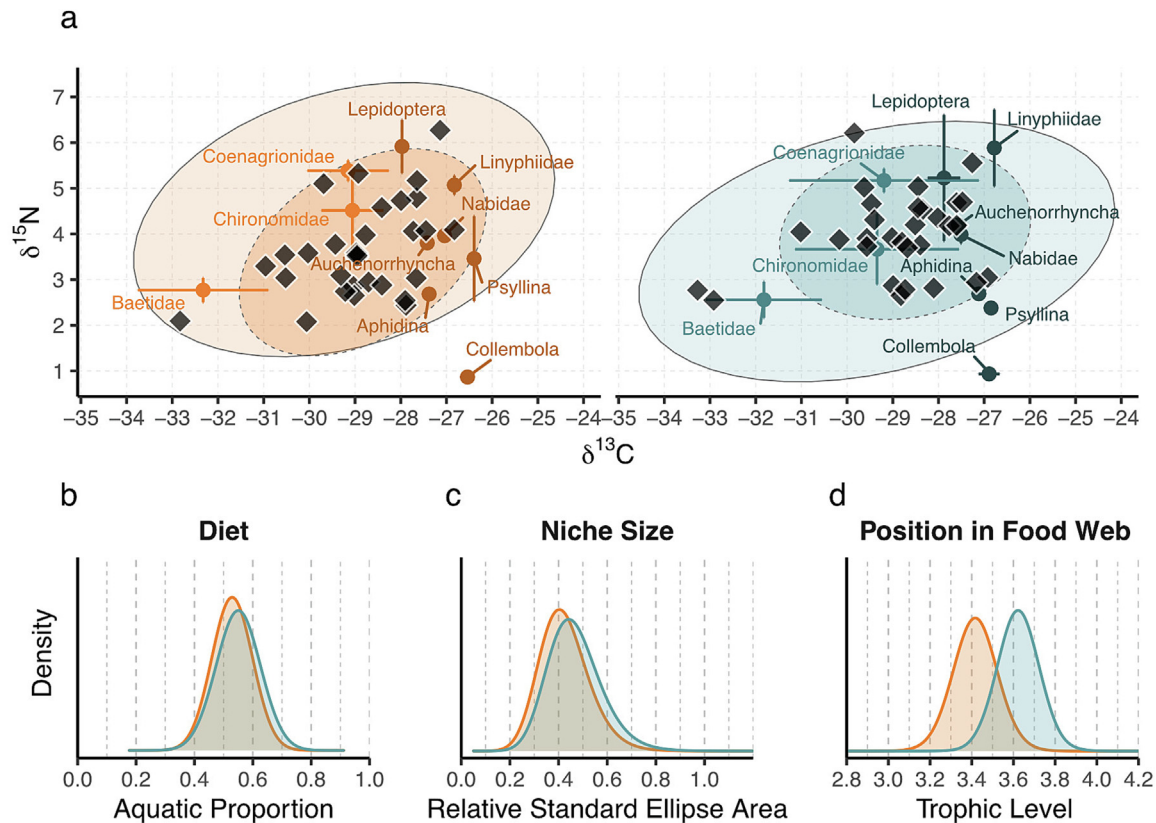
Fig. 2. a) 95 %-confidence ellipse of *T. extensa* (dark shade) determined from the stable isotope ratios of C and N (black diamonds) and 95 %-confidence ellipse (bright shade) of resources adjusted for trophic enrichment, for Bti-treated (orange) and control (cyan) FPMs ($n = 6$). Means ± SD of aquatic and terrestrial prey organisms ($n = 5$) are plotted as bright and dark dots, respectively. b) aquatic proportion in diet of *T. extensa*, c) niche size of *T. extensa* determined from relative SEA, and d) the trophic position of *T. extensa*. b), c), and d) show probability densities which are unitless.

reduced the contribution of aquatic prey to *T. extensa*'s diet by only 2 % (52.9 % (43.6–62.4)) compared to control FPMs (55 % (43.9–66.3)) suggesting only marginal changes in the spiders' diet (Fig. 2b). This relatively low shift may be driven by the varying effect of Bti on the emergence dynamics of aquatic insects (Kolbenschlag et al., 2023). In fact, during the period which is reflected in the spiders' stable isotope signature, chironomid emergence was reduced by only roughly 10 % in the Bti-treated FPMs. Consequently, sampling spiders at a later stage of the experiment, during which the number of emerging chironomids was decreased by up to 50 %, might have yielded a more prominent change in the diet.

Despite the only marginal reduction of aquatic prey in spiders' diet (Fig. 2b), the dietary niche of *T. extensa* (i.e., relative niche size) differed slightly between treatments (Bti = 0.42 (0.28–0.63) ‰^{2}, Ctrl = 0.47 (0.31–0.70) ‰^{2}; Fig. 2c) indicating an altered food intake. This was mainly attributed to an expansion of the niche of spiders from Bti-treated FPMs towards lower $\delta^{15}N$ values compared to controls ($\Delta^{15}N_{Bti-Ctrl} = -0.34$ ‰), which strongly suggests a higher consumption of prey lower in $\delta^{15}N$ than Chironomidae. Such values were observed in most terrestrial prey but also in Baetidae, the second most abundant taxon in aquatic emergence. As ^{15}N accumulates with higher trophic level the stable isotope ratio of N can be used as an indication of food chain length (McCutchan et al., 2003). Chironomidae vary strongly in their feeding habits including also predaceous species (Allan et al., 2021; Armitage et al., 1995) leading to high $\delta^{15}N$ (Fig. 2a). In contrast, larvae of Baetidae consume algae and organic detritus and are thus lower in $\delta^{15}N$. The same applies to terrestrial herbivores and detritivores as primary consumers. Consequently, the lower trophic level estimate of *T. extensa* (Fig. 2d) in Bti-treated FPMs (3.42 (3.23–3.61)) compared to controls (3.62 (3.45–3.80)), supports the suggested dietary shift towards an increased feeding on both Baetidae and terrestrial prey (herbivorous or detritivorous). In addition, we

measured 0.9 ‰ higher $\delta^{15}N$ values in Chironomidae from Bti-treated FPMs (Fig. 2a) indicating alterations in the species composition of this family towards more predaceous species. Thus, even in a situation of equal chironomid consumption by spiders of both treatments, the higher $\delta^{15}N$ in chironomids from Bti-treated FPMs would increase the spiders' trophic level estimate. As we observed the opposite (Fig. 2d), this is additional evidence for the assumption of a dietary replacement of chironomids by Baetidae and terrestrial prey.

Beyond this, we acknowledge that an increased contribution of Baetidae to the diet of spiders from Bti-treated FPMs could cause an underestimation of the proportion of terrestrial prey and thus explain the only marginal difference in aquatic proportion between treatments (Fig. 2b). More specifically, a higher dietary intake of Baetidae, which were low in $\delta^{13}C$ (-32.6 ‰, $\Delta^{13}C_{Baetidae-spiders} = -3.4$ ‰), would shift spiders residing at Bti-treated FPMs towards lower $\delta^{13}C$ values (i.e., to the "aquatic side"; Fig. 2a) compared to control spiders. Conversely, an equally increased consumption of terrestrial prey would rise spiders' $\delta^{13}C$ values (i.e., to the "terrestrial side") to a much lesser extend owing to only slightly higher $\delta^{13}C$ than *T. extensa* ($\Delta^{13}C_{Terr. prey-spiders} = 1.7$ ‰). Since $\delta^{13}C$ values of spiders did not differ between treatments, a higher consumption of terrestrial prey by spiders from Bti-treated FPMs might be masked by the increased contribution of Baetidae leading to artificially obscured treatment effects on the dietary proportions of prey origins. This could also be partly attributed to a slightly increased relative density of Baetidae in aquatic subsidy (Factor of 1.13) since they did, in contrast to Chironomidae, not experience Bti-induced reductions. As mentioned above, prey availability mainly determines the diet of web-building spiders as opportunistic feeders (Kato et al., 2003; Nakano and Murakami, 2001).

Since we could only partially detect the mentioned potential changes in the diet using mixing models, the inclusion of additional tracers (e.g., fatty

acids; Twining et al., 2020) or DNA-based analyses of the spiders' diet (Piñol et al., 2014) could shed light on the underlying processes. By providing more details or a better separation of the resources in complex food webs the above-discussed potential underestimation of dietary shifts could be resolved and our understanding of the ecological interactions might be increased (Pacioglu et al., 2019; Stock et al., 2018).

Despite that dietary shifts may have been masked in mixing models, even small reductions in the aquatic proportion of spiders' diet induced by anthropogenic disturbances hold the potential for relevant ecological consequences. A shift in consumed prey can also involve a change in the available nutrients for the predator (Toft, 2013). In fact, the nutrient content varies depending on the insect order, the mating system and the dispersal strategy (Gerber et al., 2022), which may affect spider performance including the web quality (Blamires et al., 2009) or the reproductive success (Salomon et al., 2008), parameters especially related to the availability of proteins and lipids, respectively. Such an impact on the individual level may, in the long run, have consequences for the development of spider populations: densities of web-building riparian spiders usually decrease with altered or reduced aquatic subsidy (Kato et al., 2003; Marczak and Richardson, 2007; Paetzold et al., 2011) which could lead to propagating effects on consumers feeding on spiders (Recalde et al., 2020).

4. Conclusion

The present study points towards limited changes in the nutrient transfer from aquatic to terrestrial ecosystems via adult chironomids as a consequence of Bti application. However, extending the analyses by, partly essential, amino and fatty acids could be a valuable amendment to increase the understanding of changes in the aquatic-terrestrial meta-ecosystem. Despite no detectable effects of Bti on the nutrient content of aquatic subsidy, consequences of Bti for riparian spiders are suggested in terms of an altered composition of their diet. While the observed change in the proportion of aquatic prey was rather low due to a potential underestimation, the analyses of the trophic position indicated shifts to alternative prey which suggest ecologically relevant consequences. Altered performance and density of spider populations could propagate to higher as well as lower trophic levels due to their linking role in terrestrial food webs functioning as both consumers and prey.

CRediT authorship contribution statement

Sara Kolbenschlag: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Eric Bollinger:** Investigation, Formal analysis, Visualization, Writing - review & editing. **Verena Gerstle:** Methodology, Writing - review & editing. **Carsten A. Brühl:** Methodology, Writing - review & editing. **Martin H. Entling:** Methodology, Writing - review & editing. **Ralf Schulz:** Conceptualization, Resources, Writing - review & editing. **Mirco Bundschuh:** Conceptualization, Resources, Supervision, Writing - review & editing.

Data availability

Data will be made available on request.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162351.

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