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Research

Dietary availability determines metabolic conversion of long-chain polyunsaturated fatty acids in spiders: a dual compound-specific stable isotope approach

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Consumers feeding at the aquatic–terrestrial ecosystem interface may obtain a mixture of aquatic and terrestrial diet resources that vary in nutritional composition. However, in lake riparian spiders, the relative significance of aquatic versus terrestrial diet sources remains to be explored. We investigated the trophic transfer of lipids and polyunsaturated fatty acids (PUFA) from emergent aquatic and terrestrial insects to spiders at varying distances from the shoreline of a subalpine lake in Austria, using differences in fatty acid profiles and compound-specific stable carbon ($\delta^{13}C$) and hydrogen ($\delta^{2}H$) isotopes. The omega-3 PUFA content of emergent aquatic insects was higher than that of terrestrial insects. Emergent aquatic insects contained on average 6.6 times more eicosapentaenoic acid (EPA) and 1.2 times more α-linolenic acid (ALA) than terrestrial insects, whereas terrestrial insects contained on average 2.6 times more linoleic acid (LIN) than emergent aquatic insects. Spiders sampled directly on the lake and in upland habitats had similar EPA contents, but this EPA was derived from different diet sources, depending on the habitat. The $\delta^{13}C_{EPA}$ and $\delta^{2}H_{EPA}$ values of 'lake spiders' revealed an aquatic diet pathway (i.e. EPA of aquatic origin). In contrast, EPA of spiders collected in terrestrial habitats was depleted in both ^{13}C and ^{2}H compared to any potential food sources, and their ALA isotopic values, suggesting that EPA was partly bioconverted from its dietary precursor ALA (i.e. internal pathway). The $\delta^{2}H$ values of fatty acids clearly indicated that diet sources differed depending on the spider's habitat, which was less evident from the $\delta^{13}C$ values of the fatty acids. Our data highlight that spiders can use two distinct pathways (trophic versus metabolic) to satisfy their physiological EPA demand, depending on habitat use and dietary availability.

Keywords: bioconversion, carbon isotopes of fatty acids, eicosapentaenoic acid, emerging aquatic insects, hydrogen isotopes of fatty acids, riparian consumers

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Introduction

Nutrient and energy transfer across ecosystem boundaries is a key process ensuring ecosystem functioning and food web stability (Barnes et al. 2018). Insects that spend their juvenile life stages in aquatic ecosystems before emerging (i.e. emergent aquatic insects) into terrestrial ecosystems as adults are important vectors of dietary nutrients, including omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA; ≥ 20C). In aquatic ecosystems, n-3 LC-PUFA are mostly synthesized by aquatic primary producers and subsequently transferred across trophic levels within the aquatic food web, while these dietary nutrients are generally lacking in terrestrial food webs (Twining et al. 2016a, 2019). During their larval stages, emergent aquatic insects acquire n-3 LC-PUFA, such as eicosapentaenoic acid (EPA, 20:5n-3), from aquatic resources (Torres-Ruiz et al. 2007, Martin-Creuzburg et al. 2017, Scharnweber et al. 2019, Twining et al. 2019). The n-3 LC-PUFA are considered to be essential for animals because they are required to maintain vital physiological processes but cannot be synthesized de novo by many taxa (Cook and McMaster 2002, Twining et al. 2016a, Fritz et al. 2017). However, many consumers are able to synthesize n-3 LC-PUFA from dietary C18 PUFA precursors, albeit in quantities that are often insufficient to cover their needs (Twining et al. 2018). Even when bioconversion from dietary precursors is possible, it is likely more energetically demanding than dietary acquisition of specific n-3 LC-PUFA (Parrish 2009). Therefore, many consumers can benefit from obtaining n-3 LC-PUFA through their diet to meet their physiological demands. The differences in fatty acid profiles between aquatic and terrestrial insects can help reveal aquatic–terrestrial linkages, e.g. by comparing fatty acid profiles of consumers, like spiders, with their potential prey (Fritz et al. 2017, Chari et al. 2020).

Spiders and other insectivorous riparian consumers prey upon a wide range of insects and thus obtain a mixture of resources differing in nutritional quality (Paetzold et al. 2005, Fritz et al. 2017, Twining et al. 2019, Chari et al. 2020). Web-constructing spiders are relatively stationary predators and mostly insectivorous (Nyffeler 1999); they occur along broad ecological gradients and can disperse by means of ballooning (Bonte 2013). In spiders, random colonisation by ballooning can be elicited by kin competition (Berger-Tal et al. 2016) or poor resource availability (Mestre and Bonte 2012). This strategy is risky and energetically costly (Bonte 2013), but can provide access to high quality resources (e.g. emergent aquatic insects rich in n-3 LC-PUFA). In contrast to spiders that drift within terrestrial habitats, spiders that incidentally drift to floating structures (e.g. docks, boats, emergence traps) on aquatic habitats likely encounter food sources that are richer in n-3 LC-PUFA, i.e. aquatic insects. The availability of n-3 LC-PUFA from aquatic resources can affect the performance of riparian insectivores (Twining et al. 2016b, 2018, 2019), including spiders (Fritz et al. 2017). However, how spiders process diets from aquatic or terrestrial origin has not been studied yet.

Bulk carbon and nitrogen stable isotopes as well as fatty acid profiles have been successfully used to reveal food sources and the degree to which aquatic or terrestrial resources are used by different consumers (Vander Zanden et al. 1999, Iverson et al. 2004, Perga et al. 2006, Iverson 2009, Galloway et al. 2015), even though their application is constrained by the fact that several resources may share similar stable isotope/fatty acid compositions (Cloern et al. 2002, Guo et al. 2018, Twining et al. 2020, Ebm et al. 2021). Compound-specific stable isotope analysis (CSIA) offers an innovative and promising approach to these limitations. The stable isotope values of fatty acids can indicate their dietary or metabolic origin (Bec et al. 2011, Burian et al. 2020, Kühmayer et al. 2020, Pilecky et al. 2021). Twining et al. (2020) recently proposed that stable hydrogen isotopes of fatty acids can be used at much finer-scale resolutions to assess the origin of dietary resources and trophic interactions than it would be possible from stable carbon isotopes, especially in systems in which the carbon stable isotope values of potential resource fatty acids overlap (Pilecky et al. 2021). Here, we applied these methods to determine whether spiders obtain physiologically important n-3 LC-PUFA, such as EPA, from aquatic or terrestrial sources.

Few analyses of spiders' responses to variations in diet composition and quality at small spatial scales have been conducted so far, and such studies have largely focused on river ecosystems (Paetzold et al. 2005, Chari et al. 2020, Siebers et al. 2021). In addition, in spite of studies suggesting that aquatic-derived n-3 LC-PUFA can be beneficial for riparian spiders (Fritz et al. 2017), previous studies have not resolved whether lake riparian spiders acquire n-3 LC-PUFA from aquatic or terrestrial sources. In this context, the aim of our study was to characterize the food resources and origin of n-3 LC-PUFA in lake riparian consumers as well as their metabolic fate. We used carbon and hydrogen stable isotopes of fatty acids to investigate the origin of fatty acids and demonstrate their potential as dietary tracers in field studies. To understand how habitat changes affect resource use by spiders, we assessed PUFA sources of (long-jawed) orb-weaving and sheet-weaving spiders (Tetragnathidae, Araneidae and Linyphiidae) within the riparian zone of a subalpine lake. We collected potential insect prey emerging from the lake and the surrounding terrestrial habitat as well as Tetragnathid, Araneid and Linyphiid spiders. Based on total lipid content, fatty acid and stable isotope profiles, as well as CSIA of fatty acids, we tested the following hypotheses: 1) total lipid and n-3 LC-PUFA contents of emerging aquatic insects are higher than those of terrestrial insects; 2) spider fatty acid and bulk stable isotope profiles reflect those of their insect prey; 3) the origin of spider fatty acids differs between spiders collected directly on the lake and those collected around the lake.

Material and methods

Study sites and sampling

Insects and spiders were collected twice a week, from mid-June to the end of September 2019, at the subalpine Lake

Lunz, Austria and in its adjacent terrestrial habitat (47°85′N, 15°05′E). Six floating emergence traps were deployed on Lake Lunz (Fig. 1A–B), to collect emerging aquatic insects. Araneidae, Tetragnathidae and Linyphiidae spiders colonizing emergence traps ('lake spiders' hereafter) were collected by hand. Nine window/malaise hybrid traps were placed along three different terrestrial transects, each along a distance gradient from the lake shore (1, 70 and 150 m; Fig. 1A, C). Two transects of three window traps each were deployed in an orchard (roughly corresponding to CLC 2.4.2 Complex Cultivation Patterns), while the third transect was installed in a coniferous forest (CLC 3.1.2), all along the southern margin of Lake Lunz; again, Araneidae, Tetragnathidae and Linyphiidae spiders colonizing window traps were collected by hand.

Traps collected a broad range of taxa that were grouped as 'aquatic insects' and 'terrestrial insects'. 'Aquatic insects' comprised Chironomidae and Trichoptera (Phryganeidae and Limnephilidae) from emergence traps on Lake Lunz, while Cicadidae and a set of terrestrial Diptera (mostly Muscidae, Phoridae, Psychodidae, Sciaridae and Dolichopodidae) from window traps were considered 'terrestrial insects'. Each month, Araneidae, Tetragnathidae and Linyphiidae spiders (total n = 5 per habitat) from the outside of traps were collected in each habitat. Correspondingly, spiders collected on the lake traps are considered 'lake spiders', while spiders collected on terrestrial traps are considered 'terrestrial spiders'.

Each emergence trap placed on Lake Lunz consisted of four floatable tubes covering a surface area of 0.36 m^2 forming a pyramid-shaped construction covered with extra fine mosquito net (mesh size ~500 µm) (Martin-Creuzburg et al. 2017, Fig. 1B). Window traps (0.36 m^2 area) were covered with same nets as the lake traps. Terrestrial traps were suspended from trees and equipped with collecting vials (Fig. 1C).

All collected insects and spiders were transported to the laboratory within 1 hour, frozen at −80°C, then freeze-dried for 24 h and identified to order or family level. Both spiders and insects were counted and put in pre-weighed tin cups, weighed and stored at −20°C until further analyses.

Fatty acid analysis

After freeze-drying, a minimum of 2 mg of insect (n = 10 per order and per habitat) and spider (n = 5 per habitat) samples were homogenized and lipids were extracted according to the method described by Guo et al. (2016). Extracted lipids were transmethylated to obtain fatty acid methyl esters (FAME) that were subsequently analyzed on a gas chromatograph (Trace GC; Thermo Scientific; FID 250°C, carrier gas: He: 1 ml min^{-1}, detector gases: H_2: 35 ml min^{-1}, make-up gas flow 30 ml min^{-1}, air: 350 ml min^{-1}, temperature ramp of the oven: 140°C at 20°C min^{-1} for 5 min, to 170°C at 4°C min^{-1} and to 240°C at 2°C min^{-1} for 8 min), equipped with a flame-ionization detector (FID, set at 250°C). FAME were separated by a Supelco SP-2560 column (100 m, 0.25 mm i.d., 0.2 mm film thickness), identified by comparison of their retention times with standards (37-component FAME Mix, Supelco 47885-U; Bacterial Acid Methyl Ester Mix, Supelco 47080-U) and quantified with reference to seven-point calibration curves based on known standard dilution raw concentrations. All fatty acids were measured and reported as FAME, and their contents are expressed in mass fractions (i.e. mg FAME g dw^{-1}), and in percentages (%) of total fatty acids.

Bulk and compound-specific stable isotope analyses

Freeze-dried and homogenized samples (ca 0.3 mg) of insects (n = 10 per order and per habitat) and spiders (n = 5 per habitat) were put into tin capsules. Their bulk stable isotope

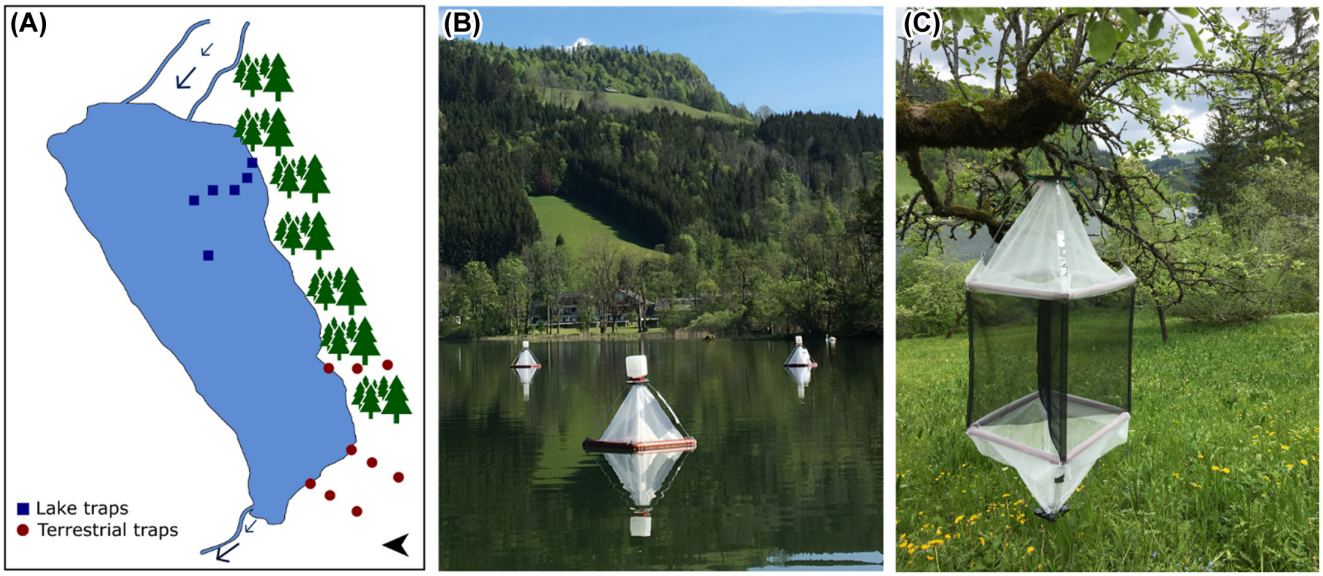

Figure 1. Location of the sampling habitats around Lake Lunz (A; 47°85′N, 15°05′E; Austria). Lake traps are represented by dark blue squares, and terrestrial traps by red squares. Floating traps on the lake (B) and window trap on terrestrial habitat (C).

($\delta^{13}C$ and $\delta^{15}N$) values were quantified using an A flash HT Plus CNSOH elemental analyzer interfaced with a Conflo IV device to a continuous flow stable isotope ratio mass spectrometer (Delta V Advantage IRMS). Values were normalized against reference gas injections of N_2 and CO_2 and standardized using international standards using international standards IAEA-N-1, and IAEA-N-2 for nitrogen, and USGS24, and IAEA-CH-7 for carbon.

Compound-specific stable isotope analyses (CSIA) were performed to assess the isotopic composition of selected FA. We selected insect samples (n = 5 per order, i.e. n = 10 per habitat) collected in both habitats in August, and spiders (n = 5 per habitat) of each habitat from June to September. FAME were separated using a gas chromatograph linked to the Delta V Advantage IRMS via Isolink 2 and Conflo IV. A Split/Splitless Liner with Single Taper (4 × 6.3 × 78.5 mm, vat. no. 453A1355) was used, the injector temperature was kept at 250°C and all samples were injected in splitless mode. For $\delta^{13}C$, FAME were separated on a VF-WAXms 60 m/0.25 mm i.d./0.25 µm film thickness column (Agilent Technologies) at a flow rate of 1.2 ml min^{-1}, followed by oxidation to CO_2 in a combustion reactor, filled with Ni, Pt and Cu wires, at a temperature of 1000°C. For δ^2H, FAME were separated on a VF-WAXms 30 m/0.32 mm i.d./1 µm film thickness column) at a flow rate of 1.0 ml min^{-1}, followed by reduction to H_2 by passing through a high thermal conversion reactor (empty ceramic tube) kept at 1420°C. The temperature gradient for $\delta^{13}C$ analysis started at 80°C, which was kept for 2 min, after which the temperature was raised by 30°C min^{-1} to 175°C, by 5°C min^{-1} to 200°C and finally by 2.4°C min^{-1} to 250°C, which was maintained for 30 min. The temperature gradient for δ^2H analysis started at 80°C, which was kept for 2 min, after which the temperature was raised by 30°C min^{-1} to 175°C, and then by 5°C min^{-1} to 240°C, and held for 35 min. FAME were identified as for GC-FID using 37-component FAME Mix. Results are expressed in delta (δ) units with respect to international standards (Vienna Standard Mean Ocean Water for δ^2H, Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$), following the equation: δ^2H, $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$ (expressed in ‰), where R is $^2H/^1H$, $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The 16:1n-7 and 16:1n-9, as 18:1n-7 and 18:1n-9 coelute on the column, therefore they are simultaneously analyzed and reported as Σ16:1 and Σ18:1.

Statistical analysis

Normality distribution and homoscedasticity of data were analyzed using Shapiro–Wilk's and Bartlett's tests; both prerequisites were not met and thus data were analyzed using non-parametric tests. First, we tested for differences in spiders collected along a distance gradient (i.e. aquatic, riparian and more distant terrestrial). As there were no significant differences in $\delta^{13}C$ and δ^2H values of fatty acids among these three sites (due to low number of replicates in different sites), we combined riparian and distant terrestrial spiders and compared 'lake' versus terrestrial spiders.

To assess the difference of nutritional values of insects depending on their habitats of origin, Kruskal–Wallis (KW) tests followed by Conover–Iman multiple comparisons with Bonferroni adjustment method (post hoc tests) allowed us to compare the mean seasonal lipid content; as well as LIN, ALA, EPA, total PUFA contents and for each other single fatty acid found in insects of each habitat (in %, Table 1).

The diet of the spiders was characterized according to their habitats. For that, we first compared the diets' lipid and PUFA contents using KW tests. To represent lipid contents, we combined the 'violin' and 'box' plots to show the density distribution of our data together with the median, and quartiles of it, where dots represent outliers. Violin plots show the probability density of the total lipid contents (Hintze and Nelson 1998; Fig. 2). Particular fatty acid contents (mg FAME g^{-1}) of interest are depicted in histograms (Fig. 3).

The KW tests also allowed to test for differences in the bulk stable isotope values (carbon and nitrogen) between habitats of spiders and between their potential preys, for each month independently. Bulk stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of insects and spiders (i.e. including only Tetragnathidae) in August were then used to assess differences in dietary sources and consumers between both habitats (95% confident ellipses for spiders were estimated using the *stat_ellipse* function of ggplot2. Fig. 4).

Finally, the mean $\delta^{13}C$ and δ^2H values of 'terrestrial' and 'aquatic' spider fatty acids were compared using the same method of KW tests (Supporting information), yielding differences of PUFA origins between habitats. Then, to trace the origin of EPA in spiders, $\delta^{13}C$ and δ^2H values of EPA and ALA were compared between spiders (i.e. consumers including only Tetragnathidae) and insects (i.e. prey) from August in each habitat, and represented by boxplots (fig. 5).

All statistical analyses were performed and visualized with R ver. 3.6.1 (<www.r-project.org>), using vegan, stat and PMCMR, lm4 packages.

Results

Differences in the nutritional value of emergent aquatic versus terrestrial insects

The total lipid content of emergent aquatic insects was on average 1.2 times higher than in terrestrial insects (KW test, H25 = 6.00, $p < 0.05$, Fig. 2). The total PUFA content of insects did not differ significantly between aquatic and terrestrial habitats (KW test, H25 = 3.40, p = 0.06; Fig. 3D). On average, terrestrial insects contained 2.6 times more LIN than emergent aquatic insects (KW test, H25 = 21.06, $p < 0.001$; Fig. 3A), whereas emergent aquatic insects contained 1.2 times more ALA than terrestrial insects (KW test, H25 = 9.10, $p < 0.01$; Fig. 3B). In particular, the EPA content of emergent aquatic insect was on average 6.6 times higher than that of terrestrial insects (KW test, H25 = 63.40, $p < 0.001$; Fig. 3C). However, emergent aquatic insects had higher n-3 PUFA, but lower n-6 PUFA than terrestrial

Table 1. Total lipids (TL mean ± SE; mg g^{-1}) and fatty acids contents (mean ± SE; mass percentage of total FA, %) of insects and spiders from both aquatic and terrestrial habitats. Only the fatty acids accounting for > 1% in at least one sample are shown. Different letters indicate significant difference between insect and spider (KW tests and Conover–Iman multiple comparisons, significant level $p < 0.05$), for each environment.

	Lake Lunz		Terrestrial	
	Insects	Spiders	Insects	Spiders
ΣBFA	6.6 ± 0.6	8.1 ± 0.8	2.1 ± 0.3[a]	4.3 ± 0.7[b]
14:0	2.5 ± 0.3[a]	1.8 ± 0.4[b]	1.9 ± 0.4[a]	2.6 ± 0.4[b]
16:0	17.2 ± 0.5[a]	13.4 ± 0.8[b]	15.8 ± 0.7	15.1 ± 0.6
18:0	6.5 ± 0.2[a]	12.3 ± 0.6[b]	6.6 ± 0.6[a]	8.1 ± 0.2[b]
ΣSFA	28.2 ± 0.7	30.0 ± 1.0	25.8 ± 1.1	27.2 ± 0.9
16:1n-7	9.8 ± 0.8[a]	6.8 ± 0.8[b]	9.3 ± 1.4	7.8 ± 0.7
18:1n-9	13.3 ± 1.1[a]	16.2 ± 1.5[b]	23.4 ± 1.3	24.3 ± 1.3
18:1n-7	3.8 ± 0.4[a]	6.1 ± 0.8[b]	0.8 ± 0.2[a]	3.5 ± 0.6[b]
ΣMUFA	28.1 ± 1.1	30.0 ± 2.0	34.7 ± 1.9	36.7 ± 1.5
18:2n-6 (LIN)	10.2 ± 0.7[a]	18.0 ± 1.1[b]	25.8 ± 2.1	21.2 ± 1.3
18:3n-3 (ALA)	9.7 ± 1.1[a]	3.9 ± 0.5[b]	7.3 ± 1.5[a]	5.1 ± 0.6[b]
18:4n-3	1.2 ± 0.2[a]	0.2 ± 0.0[b]	0.0 ± 0.0[a]	0.2 ± 0.1[b]
20:4n-6	2.5 ± 0.2	3.8 ± 0.6	2.1 ± 0.4	2.3 ± 0.2
20:5n-3 (EPA)	14.9 ± 1.4	12.2 ± 1.3	2.2 ± 0.5[a]	6.5 ± 0.8[b]
ΣPUFA	41.9 ± 1.2	39.6 ± 2.6	38.6 ± 1.8	35.9 ± 1.5
Σn-3	26.1 ± 1.7[a]	17.1 ± 1.6[b]	9.7 ± 1.4[a]	11.9 ± 0.9[b]
n-3 HUFA	15.3 ± 1.4	13.0 ± 1.5	2.3 ± 0.5[a]	6.6 ± 0.9 [b]
Σn-6	13.6 ± 0.8[a]	23.0 ± 1.6[b]	28.6 ± 1.9	24 ± 1.2
n-3/n-6	2.7[a]	0.8[b]	0.5	0.5
TL	182.7 ± 7.8	165.8 ± 13.1	155.49 ± 8.7[a]	246.6 ± 18.2[b]

SFA: saturated fatty acids; MUFA: monosaturated fatty acids; PUFA: polyunsaturated fatty acids; BFA: Bacterial fatty acids (sum of 15:0, iso15:0, anteiso15:0, iso16:0, 17:0, iso17:0 and anteiso17:0, 18:1n-7, 18:1n-6); Σn-3: sum of n-3 fatty acids; Σn-6: sum of n-6 fatty acids; n-3 HUFA: n-3 highly unsaturated fatty acids (sum of 20:3n-3, 20:4n-3, 20:5n-3, 22:3n-3, 22:5n-3, 22:6n-3); n-3/n-6: sum of n-3 fatty acids/sum of n-6 fatty acids.

insects, indeed the n-3/n-6 ratios of emergent aquatic insects were on average 5.4 times higher than those of terrestrial insects (Table 1).

Diet of spiders after dispersal in different habitats

Unlike insects, spiders collected on the lake ('lake spiders') had on average 1.5 times lower lipid contents than spiders from the terrestrial habitat (KW test, $H25 = 10.40$, $p < 0.01$, Fig. 2). Total PUFA (KW test, $H25 = 13.10$, $p < 0.001$), LIN (KW test, $H25 = 14.30$, $p < 0.001$) and ALA (KW test, $H25 = 11.40$, $p < 0.001$) contents were on average 1.7, 2.2 and 2.3 times higher in terrestrial than in 'lake spiders', respectively (Fig. 3). The EPA contents of spiders did not differ significantly between the two habitats (KW test, $H25 = 0.03$, $p = 0.87$; Fig. 3C). The n-3/n-6 ratios of spiders from both habitats were < 1, with 'lake spiders' showing a significantly higher ratio (0.8) compared to terrestrial spiders (0.5) (KW test, $H25 = 7.90$, $p < 0.001$). The EPA content of emergent insects did not differ compared to 'lake spiders' (KW test, $H25 = 1.86$, $p = 0.17$; Table 1), while the terrestrial spiders have higher EPA contents than terrestrial insects (KW test, $H25 = 21.86$, $p < 0.001$; Table 1). Terrestrial spiders accumulated higher contents of n-3 fatty acids (included n-3 HUFA) compared to terrestrial insects (KW test, $H25 = 5.77$, $p < 0.05$; Table 1). In comparison, emergent insects had higher n-3 HUFA than 'lake spiders' (KW test, $H25 = 10.22$, $p < 0.01$; Table 1).

Emergent aquatic insects from Lake Lunz were ^{13}C depleted compared to insects from terrestrial habitats throughout the study period (KW tests, $p < 0.05$; Table 2), and compared to spiders from both habitats (KW tests, $p < 0.05$). Despite the differences between insects, bulk carbon stable isotope values did not differ significantly between 'lake spiders' and spiders collected from terrestrial traps (Table 2) with the exception of July, when 'lake spiders' were more ^{13}C depleted than riparian spiders (KW tests, $H25 = 5.80$, $p < 0.05$; Table 2). The bulk nitrogen stable isotope values of spiders from both habitats were also similar over the season, as well as when comparing insects with spiders (Table 2, Fig. 4). For example, in August, spiders from both habitats did not differ in their bulk stable isotope values (Fig. 4), but inter-individual variation within 'lake spiders' was larger compared to inter-individual variation within terrestrial spiders.

Stable carbon and hydrogen values of PUFA in spiders

The $\delta^{13}C_{ALA}$ values in emergent aquatic insects were lower compared to those in terrestrial insects (Supporting information), but the $\delta^{13}C_{EPA}$ values did not significantly differ between habitats (Supporting information). The $\delta^{2}H_{EPA}$ values were lower in emergent aquatic insects than in terrestrial insects ($\Delta\delta^{2}H = 121.9‰$; Supporting information). The $\delta^{13}C_{EPA}$ values in 'lake spiders' were higher compared to those of terrestrial spiders ($\Delta\delta^{13}C = 3.8‰$; KW test, $H25 = 4.56$, $p < 0.05$; Supporting information), while the $\delta^{13}C$ values of other PUFA were not significantly different between habitats (Supporting information). The $\delta^{2}H_{ALA}$ values were higher in 'lake spiders' than in terrestrial spiders ($\Delta\delta^{2}H = 60.6‰$; Supporting information) and the $\delta^{2}H_{EPA}$ values not significantly different between habitats, but 'lake spiders' were isotopically slightly depleted compared to terrestrial spiders (Supporting information).

To assess the origin of EPA in spiders, the $\delta^{13}C$ and $\delta^{2}H$ values of ALA and EPA were compared between insects and spiders in each habitat (Fig. 5). The $\delta^{13}C_{ALA}$ values of 'lake spiders' and emergent aquatic insects did not differ significantly (KW test, $H25 = 5.40$, $p = 0.07$; Fig. 5A). The $\delta^{2}H_{ALA}$ values of 'lake spiders' were significantly higher than those of Trichoptera and similar to those of Chironomidae (KW test, $H25 = 7.30$, $p < 0.05$; Fig. 5B). The $\delta^{13}C_{EPA}$ values of 'lake spiders' were similar to those of emergent Chironomidae, and significantly higher than those of Trichoptera (KW test, $H25 = 9.12$, $p < 0.05$; Fig. 5C), while the $\delta^{2}H_{EPA}$ values of 'lake spiders' were significantly higher than those of Trichoptera and similar to those of Chironomidae (KW test, $H25 = 5.90$, $p < 0.05$; Fig. 5D). Terrestrial spiders had

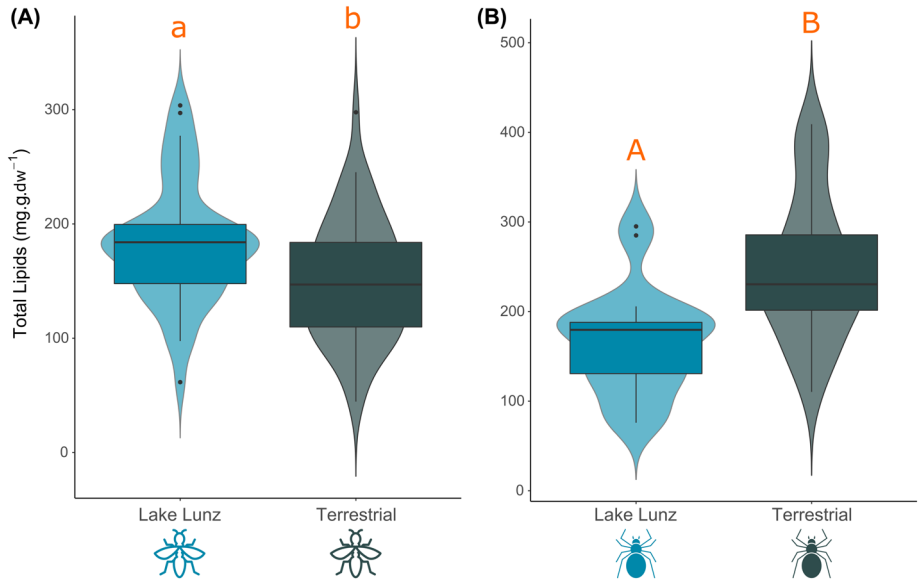

Figure 2. Total lipid contents (mg g dw^{-1}) of (A) insects and (B) spiders from aquatic and terrestrial habitat. Significant differences (KW tests and Conover–Iman multiple comparisons) between origin of insects (i.e. aquatic versus terrestrial) are indicated with lowercase letters, while differences between origin of spiders (i.e. aquatic versus terrestrial) are indicated with capital letters. The violin plots present the probability density distribution of the total lipids (therefore the 'distribution' of the data). In the boxplots, the median is represented by the thick horizontal line; the box limits are the 25% (lower part) and the 75% (upper part) quartiles of the dataset; the vertical bars represent 1.5 times the interquartile range (IQR (i.e. the difference between the first and third quartile) above the upper quartile and below the lower quartile; and dots represent outliers which are therefore the observations that are above q0.75 + 1.5 × IQR or below q0.25 − 1.5 × IQR.

significantly lower $\delta^{13}C_{ALA\ \&\ EPA}$, $\delta^{2}H_{ALA\ \&\ EPA}$ values than terrestrial Diptera (KW tests, H25 = 7.30, 6.20 and 4.2, $p < 0.05$, respectively; Fig. 5).

Discussion

Emerging aquatic insects were richer in n-3 LC-PUFA, especially EPA, than terrestrial insects that contained more of the n-6 PUFA LIN, indicating the higher nutritional value (i.e. n-3 LC-PUFA) of emergent aquatic insects. Our data revealed that riparian spiders used dietary sources from both ecosystems and obtained their n-3 LC-PUFA via two distinct pathways. 'Lake spiders' collected from Lake Lunz acquired their EPA directly via the consumption of emergent aquatic insects, most likely from Chironomidae, while terrestrial spiders biosynthesized their EPA from dietary precursors, i.e. ALA and/or stearidonic acid (18:4n-3). The combined use of stable hydrogen and carbon isotopes of fatty acids emphasized the significance of these two distinct pathways for spiders in the acquisition of EPA, which was not possible to reveal from fatty acid profiles or bulk stable isotopes.

PUFA of terrestrial and emergent aquatic insects as food sources

The higher n-3 LC-PUFA contents, in particular EPA, in emergent aquatic insects compared to terrestrial insects, are in line with previous findings (Twining et al. 2019). These differences in the nutritional quality of insects appear to be a result of ecosystem-based differences in the feeding history of insect larvae (Guo et al. 2018, Scharnweber et al. 2019). Emergent aquatic insects likely obtain their ALA and EPA from dietary algae rich in PUFA, especially EPA (Hixson et al. 2015, Twining et al. 2016a). In contrast, terrestrial insects mainly derive their LIN and ALA from the base of the terrestrial food web (Gladyshev et al. 2013, Twining et al. 2019), which provides mainly ALA, but only traces of EPA (Gladyshev et al. 2009, Hixson et al. 2015, Taipale et al. 2015). Because n-3 LC-PUFA are important for development, somatic growth and reproduction of animals (Twining et al. 2016b, 2019), their higher contents in emergent aquatic insects compared to terrestrial insects results in differences in dietary quality supplied to riparian insectivores. Therefore, emergent aquatic insects may generally be of higher nutritional value, in terms of n-3 PUFA content, than terrestrial insects for insectivorous riparian consumers, such as spiders.

Interestingly, the high ALA contents in both emerging aquatic and terrestrial insects indicate ALA to be a poor diet source biomarker in this study. This fatty acid can be synthetized by both aquatic and terrestrial primary producers (Twining et al. 2016a). ALA is a physiologically important fatty acid (e.g. as cell membrane component), and serves as a potential precursor for n-3 LC-PUFA (Brenna et al. 2009, Hixson et al. 2015). The capacity to convert C18 to C20 PUFA (e.g. ALA to EPA) differs among consumers (Bell and Tocher 2009). In addition, this conversion is metabolically cost-intensive (Parrish 2009). Hence, riparian spiders that have direct access to dietary EPA may have a metabolic

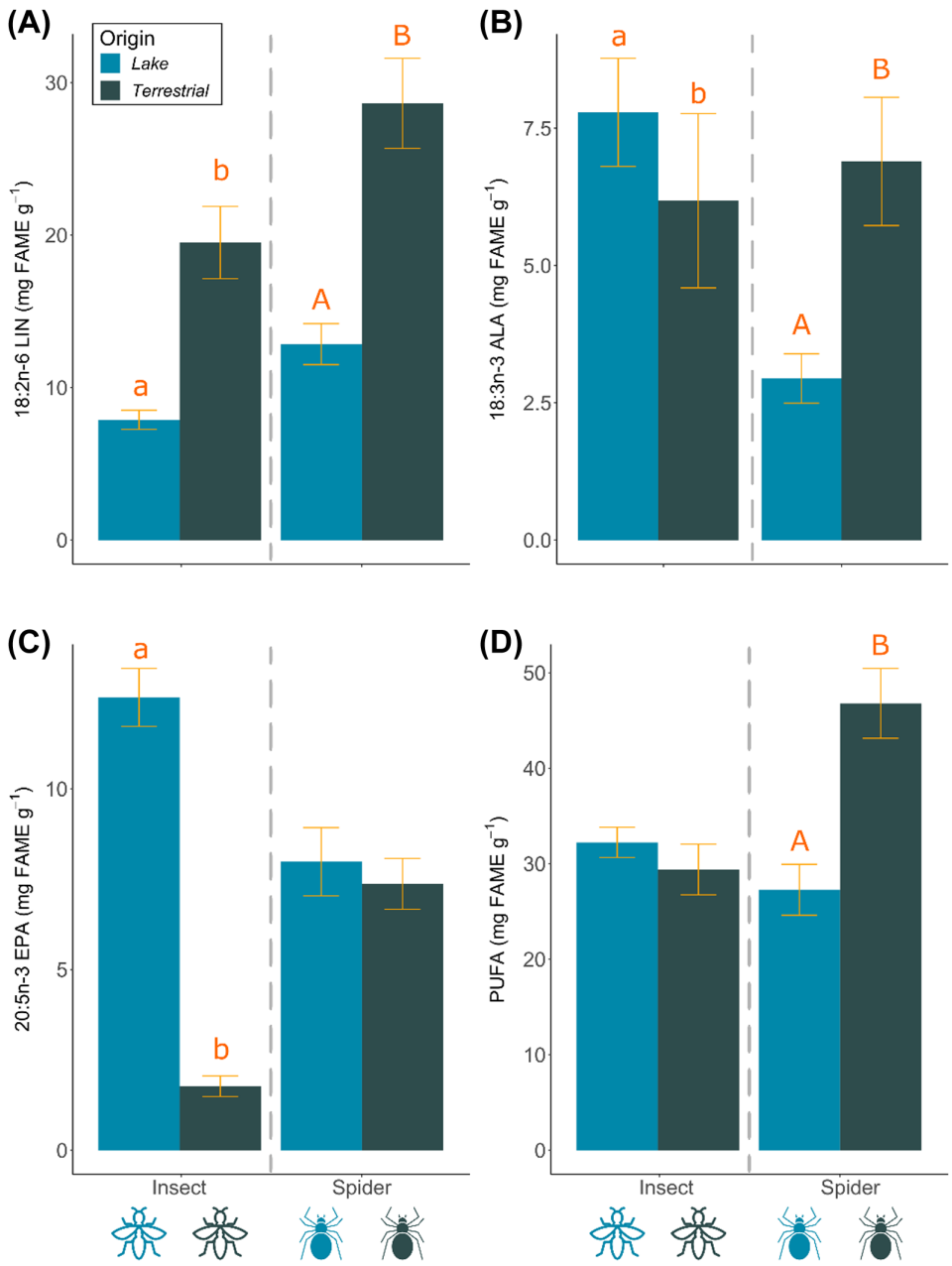

Figure 3. Contents in (A) LIN, (B) ALA, (C) EPA and (D) total PUFA of insects and spiders from aquatic and terrestrial habitats (mean ± SE; mg FAME g^{-1}). Significant differences (KW tests and Conover–Iman multiple comparisons) between origin (i.e. aquatic versus terrestrial) of insects are indicated with lowercase letters, while differences between origin (i.e. aquatic versus terrestrial) of spiders are indicated with capital letters.

advantage over spiders that need to biosynthesize EPA from dietary ALA.

Diet of spiders after random colonization of different habitats

Dietary sources of spiders were difficult to discern based on bulk carbon and nitrogen stable isotope values. Bulk $\delta^{13}C$ and $\delta^{15}N$ values of 'lake spiders' and terrestrial spiders did not differ and thus no dietary distinction was possible between spiders from aquatic and terrestrial habitats based on bulk stable isotope data. The turnover of the stable isotopes in spiders ranged between one and three weeks (Belivanov and Hambäck 2015). Therefore, it is most likely that 'lake spiders' have recently dispersed to the aquatic environment, as reflected by the stable isotope values closer from the terrestrial habitat, where they have been feeding before dispersal. In a recent study, bulk stable carbon isotope values of stream riparian spiders did not match the ones of aquatic source, suggesting that they did not obtain carbon from dietary

Table 2. Bulk carbon and nitrogen isotope composition (mean ± SD; $\delta^{13}C$ and $\delta^{15}N$, ‰) of insects and spiders from aquatic (lake) and terrestrial habitats. Different letters indicate significant differences (KW tests and Conover–Iman multiple comparisons) between habitats; $\Delta\delta^{13}C$ = difference in $\delta^{13}C$ values (‰) between aquatic and terrestrial habitats.

	Insects					Spiders				
	$\delta^{13}C$ (‰)		$\Delta\delta^{13}C$	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)		$\Delta\delta^{13}C$	$\delta^{15}N$ (‰)	
	Lake Lunz	Terrestrial	(‰)	Lake Lunz	Terrestrial	Lake Lunz	Terrestrial	(‰)	Lake Lunz	Terrestrial
June	-34.2 ± 1.6^{b}	-27.7 ± 2.0^{a}	6.5	3.0 ± 1.4	2.4 ± 2.3	-28.7 ± 1.9	-28.5 ± 1.3	0.2	2.0 ± 1.4	1.7 ± 0.8
July	-31.0 ± 3.1^{b}	-27.9 ± 1.6^{a}	3.1	1.8 ± 1.8	2.0 ± 2.5	-29.3 ± 1.1^{b}	-27.3 ± 0.7^{a}	2.0	2.4 ± 1.0	1.5 ± 1.6
August	-30.5 ± 1.8^{b}	-26.8 ± 1.9^{a}	3.7	1.3 ± 2.2	1.8 ± 3.0	-27.9 ± 2	-29.0 ± 0.8	1.1	1.7 ± 1.6	1.3 ± 1.4
September	-30.2 ± 2.0^{b}	-27.5 ± 2.2^{a}	2.7	-0.6 ± 3.6^{b}	2.1 ± 2.2^{a}	-27.7 ± 1.4	-28.5 ± 0.6	0.8	1.4 ± 2.5	3.2 ± 0.9

aquatic resources (Siebers et al. 2021). Chitin and proteins represent up to 90% of the material found in spider cuticles (Sewell 1955, Nentwig 2012), therefore their nitrogen isotopic composition should reflect the one of the habitat in which they were hunting before moulting. However, these limitations can be overcome by combining elemental analysis (e.g. bulk $\delta^{13}C$) with molecular (e.g. fatty acids) biomarkers (Perga et al. 2006, Jardine et al. 2015), and/or compound-specific stable isotope analyses to trace dietary sources and specific compounds within food chains (Kohl et al. 2015, Taipale et al. 2015, Twining et al. 2020).

Spiders collected on the lake traps had lower total lipid, PUFA, LIN and ALA contents than terrestrial spiders, but the equal EPA contents suggest preferential retention of this fatty acid. The particular retention of EPA (%) and n-3 HUFA by terrestrial spiders compared to terrestrial insects highlights the importance of long-chain PUFA in riparian consumers. However, the PUFA composition of terrestrial spiders was characterized by higher C18 PUFA contents, i.e. LIN and ALA, compared to 'lake spiders'. These differences in PUFA contents suggest that terrestrial spiders are part of a distinct terrestrial food chain rich in LIN and ALA, but poor in EPA (Budge and Parrish 1998, Twining et al. 2016a). The lower lipid content observed in 'lake spiders' may have resulted from a lower food availability on the floating lake traps, and/or before their dispersal. In contrast, terrestrial spiders may have been able to maintain higher lipid contents because of a higher prey density in the riparian zone and the absence of dispersal costs. It is also possible that 'lake spiders' may have used C18 PUFA preferentially for gaining energy, but retained EPA in their cell membranes, suggesting a hierarchy in the use of biochemical components when facing changing resources. Moreover, the high EPA content in emergent aquatic insects supports the hypothesis that spiders that drifted to aquatic habitats via ballooning likely have higher dietary access to this n-3 LC-PUFA. Our results corroborate that EPA is an excellent biomarker of aquatic-derived subsidies in riparian consumers (Chari et al. 2020), and highlight the importance of n-3 LC-PUFA for both riparian spiders . In particular, these data reveal two different trophic trajectories through which spiders, or riparian insectivores in general, obtain PUFA.

Origin of PUFA in spiders

The CSIA data suggest that spiders acquired their EPA from different sources. 'Lake spiders' likely obtained their EPA directly

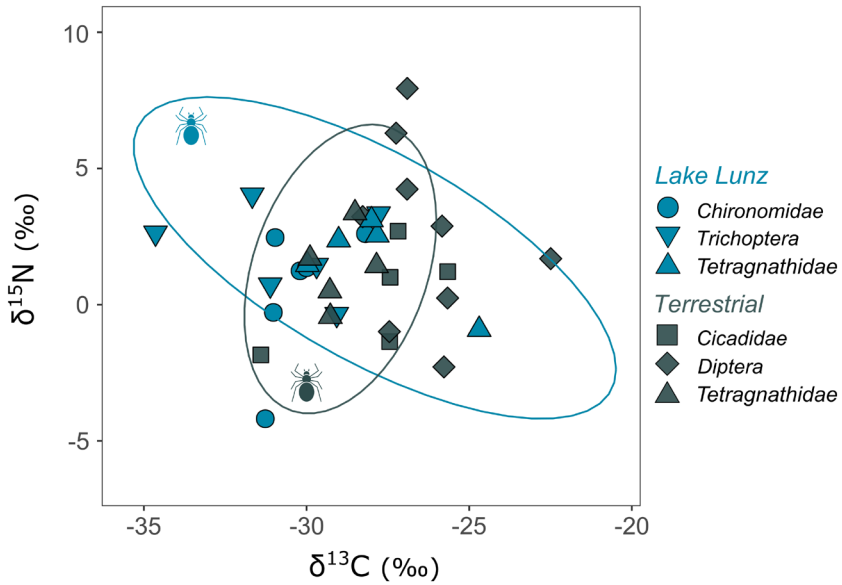

Figure 4. Bulk stable isotopes carbon and nitrogen ratios ($\delta^{13}C$ and $\delta^{15}N$, ‰) of insects and spiders from aquatic and terrestrial habitats in August. The 95% normal ellipse from aquatic spiders is represented in blue, and from terrestrial spiders in dark green.

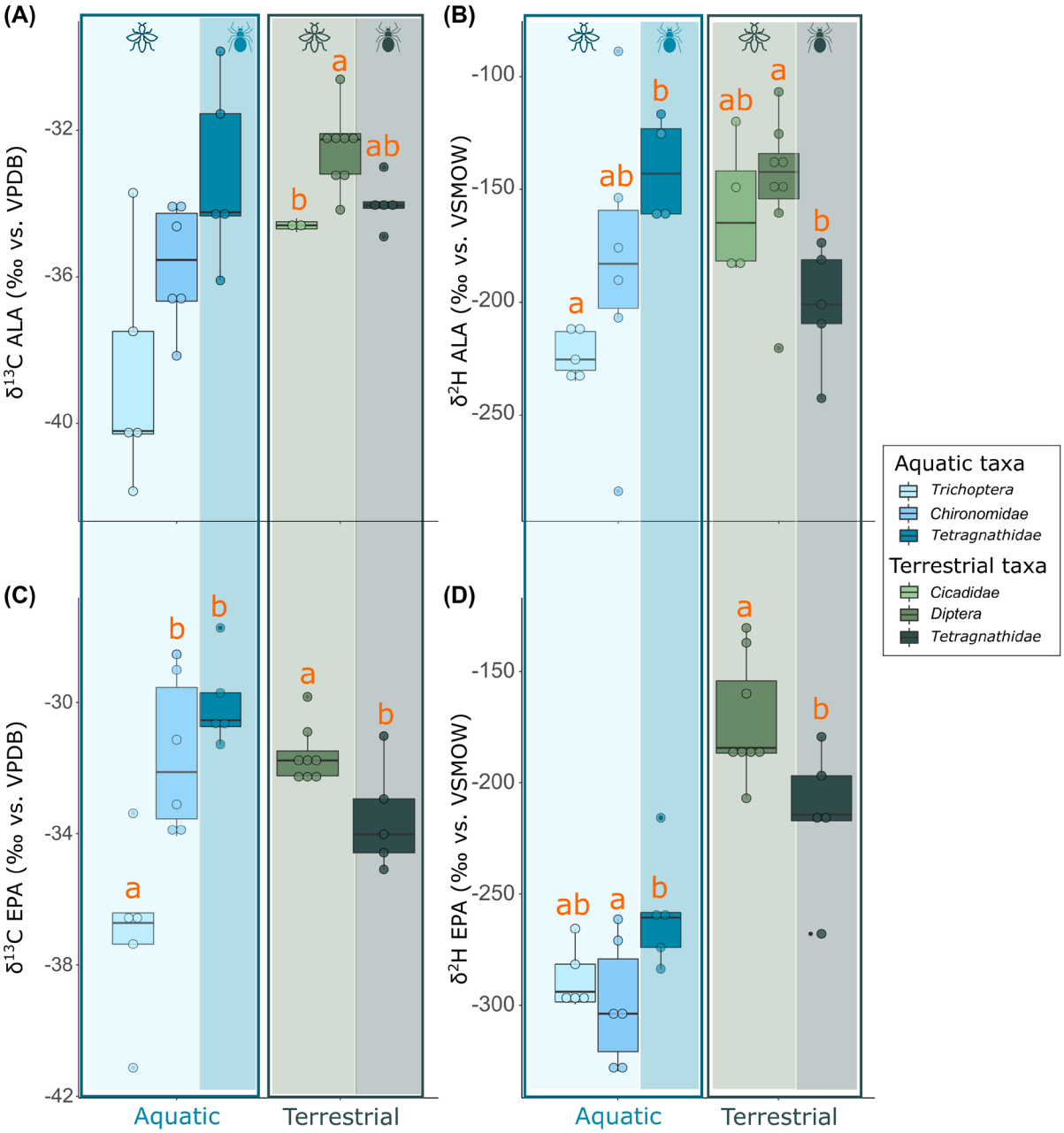

Figure 5. Stable carbon and hydrogen isotope values ($\delta^{13}C$ and $\delta^{2}H$, ‰) of the n-3 polyunsaturated fatty acids ALA (A and B, respectively) and EPA (C and D, respectively) of insects and spiders collected from aquatic (Lake Lunz) and terrestrial habitats in August. The letters indicate significant differences (KW tests and Conover–Iman multiple comparisons) between stable isotope values of n-3 PUFA between taxa from aquatic and terrestrial habitats.

from their aquatic diet, while terrestrial spiders likely obtained EPA from both diet and bioconversion (i.e. they converted dietary precursors to EPA intrinsically). Based on $\delta^{13}C_{EPA}$ values, 'lake spiders' obtained their EPA from Chironomidae, one of the most abundant emergent aquatic insect taxa found in lakes (Armitage 1995, Martin-Creuzburg et al. 2017, Selene et al. 2020, Mathieu-Resuge et al. 2021b), making them a likely food source for 'lake spiders'. Because the $\delta^{13}C_{EPA}$ values of emergent aquatic insects overlapped with those from terrestrial insects, it was not possible to trace the origin of spider EPA using $\delta^{13}C_{EPA}$ values alone. However, the $\delta^{2}H_{EPA}$ values of 'lake spiders' were close to those of emergent aquatic insects, which were clearly distinct from those of terrestrial insects ($\Delta\delta^{2}H = 121.9‰$). Thus, the $\delta^{2}H_{EPA}$ values provided isotopic evidence for the aquatic origin of EPA in 'lake spiders', highlighting the potential of using dual compound-specific stable isotope approaches in tracing the origin of fatty acids in natural ecosystems.

Spiders from terrestrial habitats appear more likely to have obtained their EPA partially from diet sources and partially

through bioconversion from dietary ALA. As it was the case for 'lake spiders', $\delta^{13}C_{EPA\ \&\ ALA}$ values of aquatic and terrestrial insects overlapped, precluding us from drawing strong inferences about fatty acid origin for terrestrial habitat spiders based upon $\delta^{13}C_{EPA\ \&\ ALA}$. Terrestrial spiders had $\delta^{2}H_{EPA\ \&\ ALA}$ values between aquatic and terrestrial insects, suggesting that EPA can come from both aquatic (i.e. direct assimilation and accumulation) and terrestrial (i.e. bioconversion from precursors) sources. However, in contrast to the $\delta^{2}H_{EPA}$ values of 'lake spiders', the $\delta^{2}H_{EPA}$ values of terrestrial spiders were substantially higher than those of emergent aquatic insects ($\Delta\delta^{2}H = 79‰$), making it highly unlikely that they only obtained their EPA directly from feeding on emergent aquatic insects. As terrestrial insects contained very little EPA, compared to terrestrial spiders and because the $\delta^{2}H_{EPA}$ values of terrestrial spiders were lower than those of terrestrial insects ($\Delta\delta^{2}H = -43‰$) it is also unlikely that spiders obtained their EPA directly through diet from terrestrial insects. Instead, terrestrial spiders had isotopically lighter values of $\delta^{13}C_{EPA\ \&\ ALA}$ and $\delta^{2}H_{EPA\ \&\ ALA}$ compared to terrestrial insects, pointing towards innate PUFA conversion, as suggested for other taxa (Bec et al. 2011, Burian et al. 2020, Twining et al. 2020). Moreover, the $\delta^{2}H_{EPA}$ values were lower than the $\delta^{2}H_{ALA}$ ($\Delta\delta^{2}H = 13‰$), supporting the idea of internal bioconversion from ALA to EPA. Indeed ALA (%) was high in insects from both environments, but was also specifically accumulated by terrestrial spiders. Because isotopically lighter ALA should be faster converted to EPA (Bec et al. 2011, Twining et al. 2020), the remaining pool of ALA remains isotopically more enriched compare to the EPA bioconverted. Lower dietary access to emerging aquatic resources may select for increased bioconversion in spider populations in terrestrial habitats, or increased bioconversion may be the result of plasticity in response to the relative availability of EPA and its precursors. Future laboratory feeding studies will be necessary to distinguish between these possibilities, by characterising fatty acid fractionations depending on dietary and/or metabolic acquisition. Therefore, these results imply that terrestrial spiders do not necessarily rely on dietary EPA from aquatic sources, but given that bioconversion is costly this suggests that riparian spiders preferentially use dietary EPA whenever it is available.

Compound-specific stable isotope analysis on fatty acids is a powerful tool to better understand trophic transfer of dietary energy in and across ecosystems (Pilecky et al. 2021). The simultaneous use of stable hydrogen and carbon isotopes of fatty acids allows to better discriminate the origin of fatty acids. Even if both hydrogen and carbon stable isotopes allow discrimination among food sources at the compound-specific level (Burian et al. 2020, Twining et al. 2020), the isotopic discrimination between source and consumer is greater for hydrogen than carbon stable isotopes (Pilecky et al. 2021). In our study, the distinction between terrestrial and 'lake spiders' and their food sources were much more evident for $\delta^{2}H_{EPA}$ ($\Delta\delta^{2}H = 42.9‰$ and 121.9‰, respectively) than for $\delta^{13}C_{EPA}$ values ($\Delta\delta^{13}C = 3.80‰$ and 2.50‰, respectively). However, this study lacks information on how H stable isotopes are processed during lipid metabolism (i.e. elongation, desaturation and retro-conversion) and, more precisely, what are the specific values of hydrogen isotopic fractionations during incorporation and internal biosynthesis of fatty acids. One way to solve these uncertainties and fully characterise the fractionation processes is by conducting laboratory feeding experiments (e.g. knowing the food source fatty acids' stable isotope composition). Such controlled experiments can effectively characterise precisely the changes in isotopic fractionations of fatty acids between dietary and metabolic pathways. Our study therefore illustrates the added value that can be gained from this new method in trophic ecology, allowing us to explore how the fatty acid requirements of consumers change with habitat and how bioconversion is triggered by dietary availability.

Conclusion

This field study underlines the importance of aquatic resources in terrestrial consumer diets (e.g. n-3 PUFA) and thus the interconnectivity between aquatic and terrestrial habitats. Our results highlight the intrinsic hierarchy of biochemical compounds in spiders, with a preferential retention of EPA, compared to LIN and ALA. The dual use of stable hydrogen and carbon isotopes of fatty acids is a novel and promising approach for trophic ecology to characterize various diet sources and pathways to consumers. Results of this field study suggest that spiders acquire EPA from both terrestrial and aquatic resources using two pathways: 1) direct dietary acquisition (trophic pathway), and/or, 2) bioconversion (intrinsic pathway). Specifically, spiders collected from Lake Lunz acquired their EPA directly from emergent aquatic insects – most likely from Chironomidae – while terrestrial spiders seemed to have obtained their EPA partially from diet sources (i.e. from aquatic insects) and partially through bioconversion from dietary precursors (e.g. ALA and/or stearidonic acid). Thus, spiders appear to be capable of biosynthesizing their n-3 LC-PUFA from dietary precursors depending on dietary PUFA availability. Finally, the combined use of stable hydrogen and carbon isotopes of fatty acids provides better understanding of dietary energy transfer in aquatic and terrestrial food webs than it would be possible from using only fatty acid profiles or bulk stable isotope values, but it will be necessary to better calibrate these methods with laboratory feeding experiments to better understand fractionation along dietary and metabolic pathways.

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Author contributions

Margaux Mathieu-Resuge: Conceptualization (equal); Formal analysis (equal); Investigation (lead); Methodology (equal); Validation (equal); Visualization (lead); Writing – original draft (lead). **Matthias Pilecky**: Formal analysis (equal); Methodology (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Cornelia W. Twining**: Investigation (equal); Methodology (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Dominik Martin-Creuzburg**: Conceptualization (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (lead); Supervision (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Tarn-Preet Parmar**: Methodology (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Simon Vitecek**: Methodology (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Martin J. Kainz**: Conceptualization (equal); Funding acquisition (lead); Investigation (lead); Methodology (equal); Project administration (lead); Resources (lead); Supervision (lead); Validation (equal); Visualization (equal); Writing – original draft (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.crjdfn34q> (Mathieu-Resuge et al. 2021a).

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