## Environmental Toxicology

# Subsidy Quality Affects Common Riparian Web-Building Spiders: Consequences of Aquatic Contamination and Food Resource

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**Abstract:** Anthropogenic stressors can affect the emergence of aquatic insects. These insects link aquatic and adjacent terrestrial food webs, serving as high-quality subsidy to terrestrial consumers, such as spiders. While previous studies have demonstrated that changes in the emergence biomass and timing may propagate across ecosystem boundaries, the physiological consequences of altered subsidy quality for spiders are largely unknown. We used a model food chain to study the potential effects of subsidy quality: *Tetragnatha* spp. were exclusively fed with emergent *Chironomus riparius* cultured in the absence or presence of either copper (Cu), *Bacillus thuringiensis* var. *israelensis* (Bti), or a mixture of synthetic pesticides paired with two basal resources (Spirulina vs. TetraMin®) of differing quality in terms of fatty acid (FA) composition. Basal resources shaped the FA profile of chironomids, whereas their effect on the FA profile of spiders decreased, presumably due to the capacity of both chironomids and spiders to modify (dietary) FA. In contrast, aquatic contaminants had negligible effects on prey FA profiles but reduced the content of physiologically important polyunsaturated FAs, such as 20:4n-6 (arachidonic acid) and 20:5n-3 (eicosapentaenoic acid), in spiders by approximately 30% in Cu and Bti treatments. This may have contributed to the statistically significant decline (40%–50%) in spider growth. The observed effects in spiders are likely related to prey nutritional quality because biomass consumption by spiders was, because of our experimental design, constant. Analyses of additional parameters that describe the nutritional quality for consumers such as proteins, carbohydrates, and the retention of contaminants may shed further light on the underlying mechanisms. Our results highlight that aquatic contaminants can affect the physiology of riparian spiders, likely by altering subsidy quality, with potential implications for terrestrial food webs. *Environ Toxicol Chem* 2023;42:1346–1358. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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## INTRODUCTION

Freshwater ecosystems worldwide are impacted by anthropogenic activities in their catchment (Stoate et al., 2001; Tilman et al., 2001; Walsh et al., 2001). Many of these

activities lead to constant or repeated exposure of aquatic organisms to a wide range of contaminants (Kumar et al., 2019; Morrissey et al., 2015; Wolfram et al., 2018; Zubrod et al., 2019), with potential negative effects on their fitness and subsequent community turnover (Liber et al., 1996; Liess et al., 2021; Liess & Schulz, 1996; Wei et al., 2021). Against this background, research addressing the response of aquatic insect larvae to contaminant exposure has recently gained interest, motivated by the quantitative and qualitative importance of aquatic insects subsidizing terrestrial food webs in adjacent (riparian) ecosystems (Nakano & Murakami, 2001; Schindler & Smits, 2017; Twining et al., 2019). In situations in which anthropogenic activity affects aquatic

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emergence, top-down or bottom-up effects in terrestrial ecosystems can occur (Schulz et al., 2015).

Indeed, contaminants in aquatic ecosystems (e.g., metals and synthetic pesticides) have been linked to the decline of aquatic insect emergence (Kraus et al., 2014; Stepanian et al., 2020) and declines in terrestrial consumers (Graf et al., 2019; Kraus et al., 2014). The latter may at least partly be attributed to reduced fluxes, and hence availability, of physiologically important nutrients (Pietz et al., 2023; Twining et al., 2019). Metals, for example, can alter the fatty acid (FA) composition and content of aquatic midges and reduce their emergent biomass (Pietz et al., 2023). Some of these FAs, such as the polyunsaturated FAs (PUFAs) eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (ARA; 20:4n-6), promote immune function, growth, and breeding success in terrestrial consumers (Fritz et al., 2017; Twining, Shipley, & Winkler, 2018; Twining et al., 2019). Because animal's capacity to biosynthesize such long-chain PUFAs from precursors (e.g., 18:2n-6 and 18:3n-3) is often insufficient to meet their physiological demands, they must obtain these (essential) nutrients through their diet (Twining et al., 2016; Twining, Lawrence, et al., 2018). At the aquatic–terrestrial interface, FA profiles of aquatic primary producers strongly differ from those of their terrestrial counterparts (Hixson et al., 2015; Twining et al., 2016). However, even within the group of aquatic primary producers FA profiles vary substantially. Cryptophytes and diatoms, for example, are rich in long-chain PUFAs, whereas green algae and cyanobacteria generally contain low levels of these nutrients (Taipale et al., 2016). Consequently, aquatic insects contain—depending on their diet—up to 10 times higher levels of long-chain PUFAs compared to terrestrial insects (Hixson et al., 2015), making them a valuable resource for consumers in both aquatic and subsidized terrestrial food webs.

While field studies have demonstrated the transfer of contaminants as well as nutrients from aquatic to terrestrial food webs through emerging insects (Kraus et al., 2021; Martin-Creuzburg et al., 2017; Previšić et al., 2021; Twining, Razavi, et al., 2021), the physiological consequences for terrestrial consumers (e.g., riparian spiders) remain unclear. The goal of the present study was to explicitly investigate this linkage through a two-step process. First, we conducted a fully factorial laboratory study testing the importance of food quality (two levels) and aquatic contamination (three stressors plus a control) on a model aquatic insect, *Chironomus riparius* (Diptera: Chironomidae). Differences in food quality were simulated using Spirulina and TetraMin® as basal resources, both of which differ in their FA profile but support the development of chironomids (Goedkoop et al., 2007; Strandberg et al., 2020). Copper (Cu), the biocontrol agent *Bacillus thuringiensis* var. *israelensis* (Bti), and a mixture of synthetic pesticides were used as model stressors because of their wide application to, or frequent detection in, freshwaters (Becker et al., 2018; Brühl et al., 2020; Feiler et al., 2013; Halbach et al., 2021). In addition, they affect the lipid profile of invertebrates (Pietz et al., 2023; Zubrod et al., 2011), or their mode of action compromises lipid membranes in the gut of nematocerous dipterans (Vachon et al., 2012). The selection of chironomids is motivated by their quantitative importance for aquatic emergence and as prey for many terrestrial consumers (Leeper & Taylor, 1998; Martin-Creuzburg et al., 2017; Ohler, Schreiner, Link, et al., 2023; Regester et al., 2008). In a second step, the insects that emerged from each of these eight treatments were fed to a model web-building spider, *Tetragnatha* spp., a common taxon in riparian areas that is strongly dependent on insects of aquatic origin (Kato et al., 2004; Krell et al., 2015; Walters et al., 2018). We characterized the nutritional quality of basal resources as well as the physiology of adult life stages of chironomids and spiders by assessing FA profiles. Moreover, the number and body mass of emerged chironomids as well as spider growth were examined.

We hypothesized that contaminant exposure and food quality shape the FA profile of chironomids and that these differences would be reflected in the FA profile of spiders. Based on findings from previous studies (Pietz et al., 2023; Scharnweber et al., 2020), we expected sex to be another discriminating factor for the FA profile of chironomids and potentially spiders. Overall, we expected our model stressors to reduce the emergent biomass of chironomids and, in turn, FA flux. The biomass fed to spiders was kept constant across treatments to ensure that potential effects on spider growth were related to qualitative, and not quantitative, shifts in aquatic subsidy.

## MATERIALS AND METHODS

### Prey quantity and quality: Culturing chironomids

Chironomid larvae were reared in sediment–water microcosms (for details, see Roodt et al., 2022). Briefly, each microcosm consisted of a glass vessel ($\sim32 \times 22 \times 7$ cm) containing a 2-cm layer of artificial sediment (see Organisation for Economic Co-operation and Development [OECD], 2004) topped by a 4-cm column of test medium (SAM-5S; Borgmann, 1996). Each microcosm was placed in a mesh cage ($38 \times 23 \times 24$ cm, 0.6 mm mesh size) to keep emerged adults separated by treatment and replicate (four replicates per treatment).

Chironomid larvae were cultured under one of eight treatments, characterized by the absence or presence of either Cu, Bti, or a mixture of synthetic pesticides (i.e., seven fungicides and two herbicides; Supporting Information, Table S1) paired with two basal resources, that is, Spirulina (*Arthrospira platensis*; Bionutra; BTG Berlin Trade) and TetraMin (Tetra). Copper was applied to the sediment, whereas Bti and the synthetic pesticide mixture were spiked into the test medium. The sediment application of Cu is described in Pietz et al. (2023). Briefly, a Cu solution was prepared in distilled water and added to the dry artificial sediment to achieve an environmentally relevant nominal Cu content of 200 mg kg$^{-1}$ dry weight (1.0–3100 mg kg$^{-1}$ dry wt; Arambourou et al., 2020; Feiler et al., 2013; Steinmetz et al., 2017). Actual Cu sediment contents measured at the end of the bioassay were $151 \pm 8$ mg kg$^{-1}$ dry weight and are presented in Supporting Information, Table S2. The spiked sediment was evenly distributed to the respective microcosms, and the test medium was slowly added to minimize sediment disturbance.

Microcosms containing Cu were prepared 14 days prior to the introduction of chironomid larvae; thus, equilibrium conditions of Cu between different environmental media (OECD, 2004; Simpson et al., 2004) were assumed when chironomids were introduced. To allow for a direct comparison between all treatments, each non-Cu microcosm was prepared following the same procedure but without Cu addition. All microcosms were aerated throughout the equilibration period.

After the equilibration period of 14 days, Bti was applied to the test medium shortly after the introduction of larvae. For this, 50 ml of a stock suspension (5760 international toxic units [ITU] $L^{-1}$) was prepared from the product VectoBac WDG (Valent BioSciences) and evenly applied over the surface of the test medium. This procedure ensured an exposure of the chironomid larvae equivalent to 10% of the recommended field rate (i.e., 144 ITU $L^{-1}$). As highlighted in Gerstle et al. (2023), reliable analytical methods confirming sterilized Bti toxin concentrations were not available at the time of the present study. Consequently, nominal concentrations were used assuming accurate application.

The synthetic pesticide mixture was applied to the respective replicates once, namely 24 h prior to the introduction of chironomid larvae (for details, see Roodt et al., 2022). A pesticide stock solution (700 µg $L^{-1}$) was prepared using the marketed products (i.e., formulations) and napropamide analytical standard. The stock solution was evenly applied to the surface of the test medium to achieve a nominal concentration of 35 µg $L^{-1}$ for each of the nine pesticides. The measured concentration of each active ingredient was 24.5 ± 6.4 µg $L^{-1}$ and is presented in Supporting Information, Table S1 (for details, see Roodt et al., 2022).

These stressors in aquatic systems were paired with two basal resources (i.e., Spirulina and TetraMin) reflecting a different nutritional quality as confirmed by their FA composition (Strandberg et al., 2020). Independent of the basal resource, larvae were fed every second day at a rate of 0.5 mg per larva and day. The volume of food added to each microcosm was consequently reduced over time, informed by the cumulative number of emerged adults. Because larval mortality could not be assessed without destructive sampling, this parameter was disregarded in our study.

Each treatment consisted of four replicates (32 microcosms in total). All microcosms were kept in a climate-controlled chamber at 20 ± 1 °C with 70% relative humidity, under a 16:8-h light:dark cycle and continuous aeration. Microcosms were randomly distributed within the climate chamber. Larvae originated from an in-house culture maintained according to OECD guidelines (OECD, 2004). Egg masses were collected from this culture and evenly distributed to crystallizing dishes filled with 500 ml SAM-5S medium. Crystallizing dishes were gently (i.e., ≤1 bubble $s^{-1}$) aerated, and 400 freshly hatched larvae (≤2 days old) were carefully and randomly allocated to each microcosm, resulting in approximately 2 cm² surface area per larva. Aeration was suspended for 24 h following the introduction of larvae, to facilitate their settlement. Emerged adults from each replicate were collected daily, separated by sex, counted, and stored at −80 °C. Adult chironomids used for the FA analysis were weighed to the nearest 0.01 mg to determine their wet weight, freeze-dried, and weighed again to determine their dry weight. Thereby a conversion factor was obtained to estimate the dry weight of chironomids fed to spiders, for which only the wet weight could be determined.

## Spider bioassay

Adult spiders of *Tetragnatha* spp. were collected at the Eußerthal Ecosystem Research Station (49°15′14″N, 7°57′42″E), Germany, between May 14 and 16, 2020. Spiders were sampled by hand in close proximity (≤1 m) to a restored section of the stream Sulzbach, upstream of any settlement to reduce anthropogenic interferences. Individuals were identified to species level (*Tetragnatha extensa* Linn. and *Tetragnatha montana* Simon), sorted by sex, and transported to the laboratory, where they were kept in individual plastic terrariums (22.5 × 16.5 × 7 cm). Spiders were maintained in a climatic chamber with a 16:8-h light:dark cycle, at 21 ± 1 °C, and with 70% relative humidity throughout the acclimation phase (1 week) and the subsequent bioassay period of 3 weeks. In addition, water was sprayed every second day into each terrarium using a pump spray bottle to maintain a high humidity and prevent dehydration of spiders. Replicate terrariums were randomized to avoid any confounding effects. During the acclimation period, spiders were fed a total of three to four adult chironomids (mix of females and males) sampled from our in-house culture. Adult chironomids were handled with a tweezer and held within reach in front of the spider, which usually caught the animal(s) within a few seconds.

At the start of the bioassay period, spiders were divided into eight groups, each group being fed exclusively with adult *C. riparius* that had been cultured under one of the eight treatments (i.e., combination of contaminant exposure and basal resource) described above. All spiders were weighed alive to the nearest 0.1 mg. Based on the spiders' species, sex, and weight, 10 spiders were allocated to each treatment (n = 80): six *T. montana* (five females, one male) and four *T. extensa* (two females, two males)—a selection driven by their respective availability. In addition, 10 spiders (same species and sex distribution) were euthanized in liquid nitrogen and stored at −80 °C until FA analysis.

Each spider was fed with five adult chironomids per week (either two females and three males or vice versa), for a total of 3 weeks. To allow for a direct comparison among all treatments, each spider received the same number of females and males during each of the three weekly feedings. For this, chironomids were taken from the −80 °C freezer and weighed as described above. Following thawing, chironomids were handled with a tweezer and held within reach in front of the spiders. If spiders did not catch and consume the individual(s) offered within 5 min, they were placed in the spiders' web for later consumption. Leftovers from the previous feeding were removed from the terrarium and weighed to estimate the net consumption of each spider (disregarding loss of weight due to desiccation). In addition, terrariums were cleaned of feces to

prevent microbial growth and kept moist to prevent dehydration of spiders. All replicates were checked daily for dead individuals, which were removed and stored at −80 °C. At the end of the bioassay, live spiders were again weighed to the nearest 0.1 mg. Afterward, spiders were euthanized in liquid nitrogen and stored at −80 °C until FA analysis.

## FA analysis

We analyzed the FA profile of both basal resources (Spirulina and TetraMin) as well as adult chironomids (females and males) and adult spiders (different species, females and males) from each of the eight treatments. To characterize the resource quality for spiders, total FAs were quantified in basal resources ($n = 5$–6 samples, respectively; $n = 11$) and adult chironomids ($n = 4$ samples per treatment and sex; $n = 64$; Fink, 2013). Briefly, lipids from freeze-dried and weighed samples (7–14 mg dry wt and 3–6 mg dry wt for basal resources and chironomids, respectively) were extracted in 5 ml of a chloroform/methanol mixture (2:1; v:v). A triacylglycerol with three deuterated 18:0 FAs (Tristearin-D105; Larodan) serving as internal standard (Konschak et al., 2020) was added, and the samples were stored at −20 °C. On the following day, samples were dried under a stream of nitrogen at 40 °C. Hydrolysis of lipids and subsequent methylation of FAs to fatty acid methyl esters (FAMEs) was achieved with 3 N methanolic HCl (Sigma-Aldrich) at 70 °C for 20 min; FAMEs were extracted using isohexane, dried under a stream of nitrogen at 40 °C, and subsequently dissolved in 100 μl of dichloromethane.

For spiders, we determined neutral lipid FAs (NLFA; $n = 6$–10 samples per treatment; $n = 70$) because they constitute an important energy storage in invertebrates (Azeez et al., 2014) and because their composition and content is altered relatively fast, compared to phospholipid FAs, in response to shifts in food quality (Iverson, 2009; Koussoroplis et al., 2014). As described in Konschak et al. (2020), freeze-dried and weighed (3–12 mg dry wt) spiders were manually crushed with glass Pasteur pipettes in a chloroform/methanol/Milli-Q water (1:2:0.8; v:v:v) mixture. By addition of chloroform and Milli-Q water, a final volume of 8.7 ml with a mixture ratio of 2:2:1.8 (v:v:v) was achieved. Tristearin-D105 was added as internal standard, and lipids were extracted overnight at 4 °C. Neutral lipids were separated from glycolipids and phospholipids via solid-phase extraction by elution with 4 ml chloroform through preconditioned (4 ml chloroform) polar modified polystyrene/divinylbenzene copolymer cartridges (Chromabond® easy polypropylene columns; Macherey-Nagel). Samples were subsequently dried under a stream of nitrogen at 40 °C and redissolved in 100 μl of dichloromethane. Hydrolysis of lipids and subsequent methylation of FAs to FAMEs were achieved with trimethylsulfonium hydroxide (Sigma-Aldrich).

Gas chromatography (GC) with flame-ionization detection (Trace GC Ultra; Thermo Fisher Scientific), using a Restek FAMEWAX capillary GC column (30 m × 0.25 mm, 0.25 μm film thickness) and helium (1.4 ml min$^{-1}$) as carrier gas, was used to analyze FAMES. For each sample, FAMEs were identified

based on different retention times using FAME standards (37-component FAME Mix; Supelco CRM47885) and quantified (micrograms of FA per milliliter) using external standard calibration. In addition, the FAME of the internal standard (Methyl D-35 Octadecanoate; Larodan) was added directly to the standards of the calibration series in the same concentration as that of the lipid internal standard (Tristearin-D105) in the samples. Concentrations of FAs were adjusted for inaccuracies of the instrument (e.g., variations in the detector performance), by the recovery rate of the lipid internal standard and by blank correction. The corrected FA concentrations were normalized to the total volume and the dry weight of the sample (microgram FA per milligram dry wt).

## Data analysis

A generalized linear model (GLM) with a negative binomial distribution and log link function was used to analyze the effects of our explanatory variables (i.e., *exposure* and *food*) and their interaction on the number of emergent chironomids. Effects on the dry weight of adult chironomids were evaluated through linear models (LMs) using *exposure*, *food*, their interaction, and *sex* as explanatory variables. The specific growth rate (SGR) for each spider was calculated as

$$\text{SGR} = \frac{[\ln(ww_t) - \ln(ww_0)] \times 100}{t} \qquad (1)$$

where $ww_0$ is the wet weight before and $ww_t$ is the wet weight after the 21-day ($t$) bioassay. Spiders' SGR were analyzed using a linear model with *exposure*, *food*, and their interaction as explanatory variables. The consumed biomass by spiders is autocorrelated to spider growth and was therefore also included in the model.

We used redundancy analysis (RDA) to assess and display the effect of *exposure*, *food*, and *sex* on the FA composition in chironomids and spiders. Detrended correspondence analysis of FA data was used to check the gradient lengths of axes. Gradient lengths were always below one standard deviation, suggesting a monotonic response and that the use of an LM was appropriate (ter Braak & Prentice, 1988). The RDA was performed on scaled (i.e., standardized to unit variance) data. A permutation test was used to evaluate the significance of the RDA model and canonical axes. Scores were calculated in the same ordination space but are separated between female and male chironomids and spiders, respectively, in the ordination to improve clarity. Similarly, only FAs that jointly account for ≥70% of the total content of the respective FA group (e.g., saturated FA [SAFA], monounsaturated FA [MUFA], PUFA) or are physiologically important are shown. Differences in the FA content (micrograms per milligram dry wt) among different treatments were assessed using null hypothesis significance testing. Effects of our explanatory variables (i.e., *exposure* and *food*) and their interaction were evaluated using two-way analysis of variance (ANOVA). Data normality and homoscedasticity were checked by visual inspection of model residuals. Two-way ANOVA was performed on ranks in case data did not satisfy

assumptions of parametric testing, and Brunner-Dette-Munk tests (Brunner et al., 1997) were applied for data with heterogeneous variances. Significant effects of *exposure* or interactions were further evaluated by one-way ANOVA followed by Student's *t* test, if assumptions were met, and Wilcoxon rank sum test or Welsh's *t* test, if assumptions were not met.

Statistical analyses were performed with R (Version 4.1.0 for Windows; R Core Team, 2021). Detailed information on statistical tests for investigated endpoints is provided in Supporting Information, Tables S3 and S4. The significance level α was set at 0.05, but the interpretation of the data was also based on effect sizes (i.e., percentage of difference between the mean of each treatment and the control's mean) as a measure of the relative importance of an effect.

## RESULTS

### Basal resources

Spirulina and TetraMin differed significantly in their FA profile (Supporting Information, Table S5). The total FA content in Spirulina was 40%, but nonsignificantly, higher than in TetraMin. These higher FA levels were related to elevated levels of SAFAs (~110% higher, $p = 0.008$, Brunner-Dette-Munk) and PUFAs (~70% higher, not significant). In Spirulina, the group of PUFAs consisted almost exclusively of omega-6 PUFAs, particularly gamma-linolenic acid (GLA; 18:3n-6), which was approximately 160 times higher than in TetraMin. However, TetraMin contained higher levels of the long-chain omega-6 PUFA ARA and all omega-3 PUFAs, for example, alpha-linolenic acid (ALA; 18:3n-3), EPA, and docosahexaenoic acid (DHA; 22:6n-3; Supporting Information, Table S5). TetraMin was also relatively rich in MUFAs (~30% of total FAs), while they contributed <10% to the total FA content in Spirulina (Supporting Information, Table S5).

### Chironomid emergence and nutritional quality

Contaminant exposure (i.e., Cu, Bti, and the pesticide mixture) during aquatic life stages nearly significantly reduced adult emergence compared to the control, a pattern driven by the approximately 40% decrease in the presence of Cu ($p = 0.054$, GLM; Figure 1; Supporting Information, Table S3). Basal resource did not significantly affect adult emergence, though the Spirulina treatment exhibited an approximately 15% lower emergence relative to TetraMin (Supporting Information, Figure S1 and Table S3). The dry weight of emerged chironomids differed significantly between sexes, with males being approximately 50% lighter than females ($p < 0.001$, LM; Supporting Information, Figure S2 and Table S3). We observed a tendency toward lower dry weight in females (reduction by up to 12%) when larvae were fed with Spirulina compared to TetraMin, whereas this remained similar in males (Supporting Information, Figure S2 and Table S3).

*Food* and *sex* were the most important predictors of the chironomid FA profile according to RDA (Figure 2). The first two RDA axes were significant ($p = 0.001$, detrended
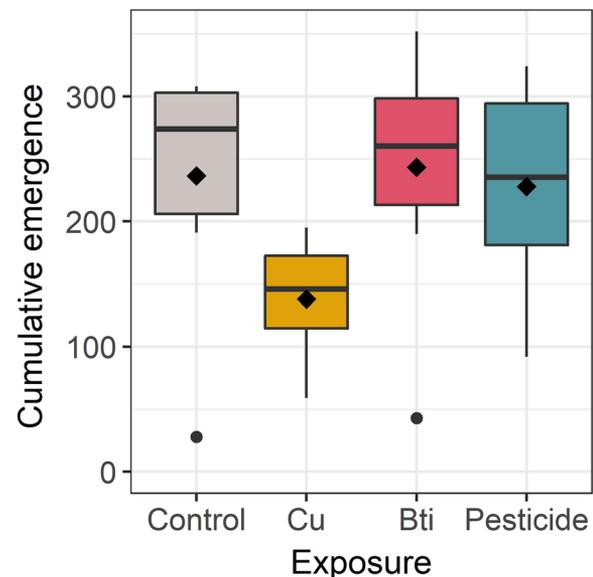


**FIGURE 1:** Cumulative emergence of chironomid adults under different exposure conditions aggregated across basal resources. Lines in boxes are medians; box ends are 25th and 75th percentiles. Whiskers and black dots show data points outside the lower and upper quartiles. Black diamonds show the mean cumulative emergence. Bti = *Bacillus thuringiensis* var. *israelensis*.

correspondence analysis; Supporting Information, Table S3), explaining 34% and 13% of the total FA variation (69% and 27% of the constrained variation), respectively. Axis RDA1 reflected differences in the FAs of adult chironomids when larvae were fed with Spirulina or TetraMin (i.e., *food*). Emergent adults fed with Spirulina during their aquatic life stage generally contained higher levels of 18:3n-6 (GLA), while they had a lower content of 18:0, 18:1n-9, 18:3n-3 (ALA) and 20:5n-3 (EPA) compared to individuals fed with TetraMin (Figure 2). The observed increase in 18:3n-6 (GLA) content by 940% drove the significant increase in omega-6 PUFA and highly unsaturated FA (HUFA, i.e., three or more double bonds) levels by 25% and 21%, respectively ($p < 0.001$, two-way ANOVA; Table 1; Supporting Information, Table S3). In contrast, total FA, MUFA, and omega-3 PUFA contents in adults were significantly lower (10%–80%) when larvae were fed with Spirulina compared to TetraMin ($p < 0.05$, two-way ANOVA and Brunner-Dette-Munk test; Table 1; Supporting Information, Table S3). Axis RDA2 separated the FA profiles between female and male chironomids (i.e., *sex*; Figure 2). Females were rich in 16:0 and 16:1n-7 and thus had significantly higher total FA, SAFA, and MUFA contents than males ($p < 0.05$, *t* tests; Tables 1; Supporting Information, Table S3). Male adults contained significantly higher levels of omega-3 PUFA (26%) and HUFA (29%), the latter being driven by a 60% increase in 20:4n-6 (ARA; $p < 0.034$, Wilcoxon rank sum test, $p < 0.001$, *t* test; Table 1; Supporting Information, Table S3).

The presence of contaminants during chironomid development had marginal effects on the FA profile of adults. The contents of total FAs, SAFAs, MUFAs, and PUFAs and of individual FAs were comparable to the control. Only the content
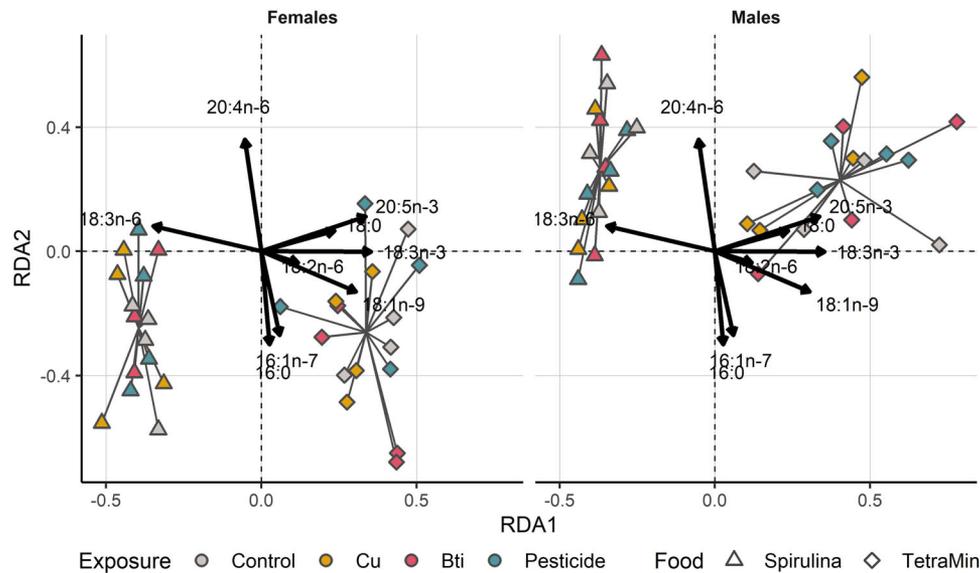
**FIGURE 2:** Redundancy analysis using fatty acid profiles (micrograms per milligram dry wt) of emergent chironomid females and males cultured under one of eight treatments (i.e., combination of contaminant exposure and basal resource). Basal resources are indicated by triangles (Spirulina) and diamonds (TetraMin). Color represents larval exposure to control (gray), Cu (yellow), Bti (red), and synthetic (blue) pesticides. Scores were calculated in the same ordination space but are separated between females and males in the ordination to avoid cluttering of the figure. RDA = redundancy analysis; Bti = *Bacillus thuringiensis* var. *israelensis*.

of 20:4n-6 (ARA) tended to decrease relative to the control, driven by a reduction (25%) following Cu exposure (Table 1).

## Spider physiology

Spiders were offered the same number of female and male chironomids at each feeding. Mean total chironomid biomass consumed by spiders was approximately 15% lower in male than female spiders but similar among treatments (Supporting Information, Figure S3 and Table S4). The wet weight of spiders was similar across treatments at the start and end of the bioassay but tended to increase during the bioassay period, irrespective of treatment (Figure 3). Spider SGR was significantly reduced by 40%–50% relative to the control when individuals were fed chironomids cultured in the presence of one of the contaminants ($p = 0.024$, LM; Figure 3). These reductions were generally stronger when chironomid larvae were fed Spirulina ($p = 0.063$, LM). Furthermore, the total chironomid biomass consumed by spiders significantly affected their SGR ($p = 0.031$, LM), which was approximately 45% lower in males than in females.

The FA content of spiders fed during the bioassay was significantly higher (50%–130%) than that of individuals preserved at test initiation (Supporting Information, Tables S4 and S6). The two spider species did not differ in their FA profile (Supporting Information, Tables S4 and S7). *Sex* and *food* were the most important predictors of the spider FA profile according to RDA (Figure 4). The first two RDA axes were significant ($p = 0.001$, detrended correspondence analysis; Supporting Information, Table S4), explaining 16% and 11% of the total FA variation (52% and 36% of the constrained variation), respectively. The FA

profiles of female and male spiders were separated along the first RDA axis. Males contained significantly higher levels of most individual FAs (e.g., 25% more 20:5n-3, EPA) and all FA groups (up to ~35% for SAFA, $p < 0.001$, Wilcoxson rank sum test, and omega-6 PUFA, $p = 0.003$, *t* test; Figure 4, Tables 1; Supporting Information, Table S4). The second RDA axis reflected the differences in the spider FA profile based on the basal resource offered to the consumed chironomids, that is, Spirulina or TetraMin (Figure 4). Spiders had significantly higher levels of omega-6 PUFA (~25%, 0.023, two-way ANOVA; Supporting Information, Table S4), such as 18:3n-6 (GLA, ~720%) and 20:4n-3 (ARA, ~35%), when chironomids were fed with Spirulina relative to being fed with TetraMin, while they had significantly lower levels of omega-3 PUFA (~25%, $p = 0.008$, Brunner-Dette-Munk test; Supporting Information, Table S4), such as ALA (~50%) and EPA (20%; Table 1).

The FA content in spiders was altered by the presence of contaminants during chironomid development (Supporting Information, Table S4). Total FA and PUFA contents decreased nearly significantly ($p = 0.078$, Brunner-Dette-Munk test, $p = 0.057$, two-way ANOVA) by approximately 20% and 30%, respectively, and the HUFA content decreased significantly ($p = 0.034$, Brunner-Dette-Munk test) by approximately 30%, when larvae were cultured in the presence of Bti or Cu. Similarly, ARA and EPA contents were approximately 30% lower in Bti and Cu treatments than in the control, while levels of DHA were comparable to (Cu) or higher than (Bti) the control. Effects on the FA content were less pronounced when larvae were cultured in the presence of the synthetic pesticide mixture (reduction by ~10% compared to the control). Reductions in the FA content were generally stronger in female than in male spiders. Total FA,

**TABLE 1:** Mean (±95% confidence interval) fatty acid content (in micrograms per milligram dry wt) of adult chironomids and spiders by sex, food, and contaminant exposure

| FA (group) | Sex | | Food | | Exposure | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Females | Males | Spirulina | TetraMin | Control | Cu | Bti | Pesticide |
| *Chironomus riparius* | | | | | | | | |
| 16:0 | **15 ± 2** | **10 ± 1** | 12 ± 2 | 13 ± 2 | 12 ± 3 | 12 ± 2 | 14 ± 2 | 13 ± 2 |
| 18:0 | 4.4 ± 0.4 | 4.7 ± 0.5 | **3.8 ± 0.2** | **5.3 ± 0.4** | 4.8 ± 0.8 | 4.3 ± 0.5 | 4.7 ± 0.7 | 4.4 ± 0.6 |
| ΣSAFA | **22 ± 2** | **18 ± 1** | 19 ± 2 | 21 ± 2 | 19 ± 3 | 19 ± 2 | 21 ± 3 | 20 ± 2 |
| 16:1n-7 | **8.6 ± 1.1** | **6.1 ± 0.8** | 6.8 ± 1.1 | 7.9 ± 1.0 | 7.2 ± 1.9 | 7.0 ± 1.6 | 8.1 ± 1.6 | 7.1 ± 1.2 |
| 18:1n-9 | **3.2 ± 0.60** | **2.7 ± 0.4** | **1.7 ± 0.1** | **4.1 ± 0.4** | 3.0 ± 0.9 | 3.0 ± 0.7 | 3.1 ± 0.8 | 2.7 ± 0.7 |
| ΣMUFA | **13 ± 1** | **9.6 ± 1.1** | **9.5 ± 1.2** | **13 ± 1** | 11 ± 2 | 11 ± 2 | 12 ± 2 | 11 ± 2 |
| 18:2n-6 | 9.9 ± 1.1 | 9.6 ± 0.8 | **8.9 ± 0.7** | **11 ± 1** | 9.2 ± 1.6 | 9.5 ± 1.5 | 10 ± 1 | 9.9 ± 1.2 |
| 18:3n-6 (GLA) | 2.6 ± 0.9 | 3.0 ± 0.9 | **5.2 ± 0.4** | **0.50 ± 0.09** | 2.8 ± 1.3 | 2.7 ± 1.2 | 2.8 ± 1.5 | 2.9 ± 1.4 |
| 18:3n-3 (ALA) | 0.90 ± 0.24 | 0.95 ± 0.25 | **0.28 ± 0.03** | **1.6 ± 0.1** | 0.93 ± 0.36 | 0.85 ± 0.35 | 0.96 ± 0.38 | 0.96 ± 0.38 |
| 20:4n-6 (ARA) | **1.1 ± 0.1** | **1.8 ± 0.2** | 1.6 ± 0.2 | 1.4 ± 0.2 | 1.7 ± 0.4 | 1.3 ± 0.2 | 1.6 ± 0.4 | 1.4 ± 0.3 |
| 20:5n-3 (EPA) | **1.5 ± 0.4** | **2.0 ± 0.5** | **0.6 ± 0.1** | **2.9 ± 0.3** | 1.7 ± 0.7 | 1.6 ± 0.6 | 1.8 ± 0.7 | 1.9 ± 0.8 |
| ΣPUFA | **16 ± 1** | **18 ± 1** | 17 ± 1 | 17 ± 1 | 16 ± 2 | 16 ± 1 | 18 ± 2 | 17 ± 2 |
| ΣOmega-6 PUFA | 14 ± 1 | 15 ± 1 | **16 ± 1** | **13 ± 1** | 14 ± 2 | 14 ± 1 | 15 ± 2 | 14 ± 2 |
| ΣOmega-3 PUFA | **2.4 ± 0.6** | **3.1 ± 0.8** | **0.96 ± 0.11** | **4.5 ± 0.4** | 2.8 ± 1.1 | 2.5 ± 0.9 | 2.9 ± 1.1 | 2.9 ± 1.1 |
| ΣHUFA | **6.2 ± 0.5** | **8.0 ± 0.6** | **7.8 ± 0.6** | **6.4 ± 0.5** | 7.2 ± 1.0 | 6.5 ± 0.7 | 7.3 ± 1.1 | 7.3 ± 0.7 |
| Total FA | **51 ± 4** | **45 ± 3** | **45 ± 3** | **50 ± 4** | 47 ± 7 | 46 ± 5 | 51 ± 6 | 48 ± 5 |
| *Tetragnatha* spp. | | | | | | | | |
| 16:0 | **3.2 ± 0.4** | **4.8 ± 0.7** | 3.6 ± 0.5 | 3.8 ± 0.5 | 4.3 ± 0.9 | 3.5 ± 0.9 | 3.4 ± 0.7 | 3.7 ± 0.7 |
| 18:0 | **3.4 ± 0.4** | **4.1 ± 0.5** | 3.6 ± 0.4 | 3.6 ± 0.5 | 4.2 ± 0.7 | 3.3 ± 0.6 | 3.2 ± 0.4 | 4.0 ± 0.7 |
| ΣSAFA | **7.7 ± 0.8** | **10 ± 1** | 8.5 ± 1.0 | 8.5 ± 1.1 | 9.7 ± 1.6 | 7.9 ± 1.6 | 7.7 ± 1.2 | 8.9 ± 1.6 |
| 16:1n-7 | 0.92 ± 0.16 | 1.2 ± 0.3 | 0.91 ± 0.21 | 1.1 ± 0.2 | 1.2 ± 0.4 | 0.97 ± 0.30 | 0.88 ± 0.24 | 0.9 ± 0.2 |
| 18:1n-9 | **1.3 ± 0.2** | **1.7 ± 0.3** | **1.2 ± 0.3** | **1.6 ± 0.3** | 1.7 ± 0.4 | 1.4 ± 0.4 | 1.2 ± 0.3 | 1.5 ± 0.4 |
| ΣMUFA | 2.9 ± 0.4 | 3.3 ± 0.6 | **2.7 ± 0.5** | **3.3 ± 0.5** | 3.5 ± 0.9 | 2.9 ± 0.7 | 2.6 ± 0.5 | 3.1 ± 0.8 |
| 18:2n-6 | **3.2 ± 0.4** | **4.5 ± 0.8** | 3.6 ± 0.6 | 3.6 ± 0.6 | 4.3 ± 0.9 | 3.2 ± 0.8 | 3.1 ± 0.6 | 3.8 ± 0.9 |
| 18:3n-6 (GLA) | 0.58 ± 0.18 | 0.62 ± 0.36 | **1.1 ± 0.2** | **0.14 ± 0.05** | 0.78 ± 0.50 | 0.54 ± 0.23 | 0.49 ± 0.33 | 0.59 ± 0.35 |
| 18:3n-3 (ALA) | 0.21 ± 0.04 | 0.23 ± 0.08 | **0.14 ± 0.04** | **0.29 ± 0.05** | 0.26 ± 0.09 | 0.19 ± 0.07 | 0.19 ± 0.07 | 0.22 ± 0.07 |
| 20:4n-6 (ARA) | **0.57 ± 0.08** | **0.74 ± 0.15** | **0.72 ± 0.09** | **0.53 ± 0.10** | 0.74 ± 0.19 | 0.53 ± 0.11 | 0.53 ± 0.09 | 0.70 ± 0.17 |
| 20:5n-3 (EPA) | 0.91 ± 0.15 | 1.1 ± 0.4 | 0.86 ± 0.17 | 1.1 ± 0.2 | 1.2 ± 0.4 | 0.81 ± 0.21 | 0.81 ± 0.22 | 1.2 ± 0.4 |
| ΣPUFA | **5.6 ± 0.7** | **7.5 ± 1.2** | 6.6 ± 0.9 | 5.9 ± 0.9 | 7.5 ± 1.4 | 5.5 ± 1.1 | 5.3 ± 1.0 | 6.7 ± 1.6 |
| Σomega-6 PUFA | **4.5 ± 0.6** | **6.1 ± 1.1** | 5.6 ± 0.8 | 4.4 ± 0.6 | 6.0 ± 1.2 | 4.4 ± 0.9 | 4.3 ± 0.9 | 5.3 ± 1.3 |
| Σomega-3 PUFA | 1.2 ± 0.2 | 1.4 ± 0.4 | **1.1 ± 0.2** | **1.4 ± 0.3** | 1.5 ± 0.4 | 1.1 ± 0.3 | 1.1 ± 0.3 | 1.5 ± 0.4 |
| ΣHUFA | 2.4 ± 0.3 | 2.8 ± 0.6 | **3.0 ± 0.4** | **2.1 ± 0.4** | 3.0 ± 0.7 | 2.2 ± 0.4 | 2.1 ± 0.4 | 2.8 ± 0.7 |
| Total FA | **16 ± 2** | **21 ± 3** | 18 ± 2 | 18 ± 2 | 21 ± 4 | 16 ± 3 | 16 ± 2 | 19 ± 4 |

Fatty acids that jointly account for ≥70% of the total content of the respective fatty acid group or are physiologically important are shown. Fatty acid contents that differ significantly are printed in bold.
ALA = alpha-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; PUFA = polyunsaturated fatty acid; FA = fatty acid; Bti = *Bacillus thuringiensis* var. *israelensis*; SAFA = saturated fatty acid (i.e., three or more double bonds); MUFA = monounsaturated fatty acid; GLA = gamma-linolenic acid; HUFA = highly unsaturated fatty acid.

SAFA, and PUFA contents in females decreased by up to 30%, 30%, and 35%, respectively, compared to the control (Supporting Information, Figure S4).

## DISCUSSION

Contaminant exposure and food quality affected the FA profiles of organisms in our model food chain, but their relative importance varied with the trophic level. In fact, the effect of contaminant exposure was overridden by food quality when assessing the FA profile of emergent chironomids, whereas the impact of contaminant exposure was visible at the level of their terrestrial consumer, that is, spiders. Moreover, sex was an important discriminating factor for the FA profiles of chironomids and spiders, pointing to sex-specific life-history strategies. Although the emergent biomass of chironomids was affected by contaminant exposure, biomass consumption by spiders remained unaffected by our experimental design (i.e., spiders were offered a constant amount of prey). Hence, changes in the predators' (i.e., spider) FA profile are likely triggered by changes in prey (i.e., chironomid) quality and not quantity.

### Effects of contaminants and food on prey quality and spiders

In line with our hypothesis, food quality explained most of the variation in the FA profile of chironomids. In fact, the differences between Spirulina and TetraMin, which served as food, were partially reflected in the FA profile of adult chironomids, while also suggesting that chironomids have the capacity to modify dietary FA (Goedkoop et al., 2007; Ohler, Schreiner, Martin-Creuzburg, & Schäfer, et al., 2023; Strandberg et al., 2020). The capability to modify or preferentially retain certain dietary FAs was stronger when chironomids were fed with Spirulina, which could be explained by a greater
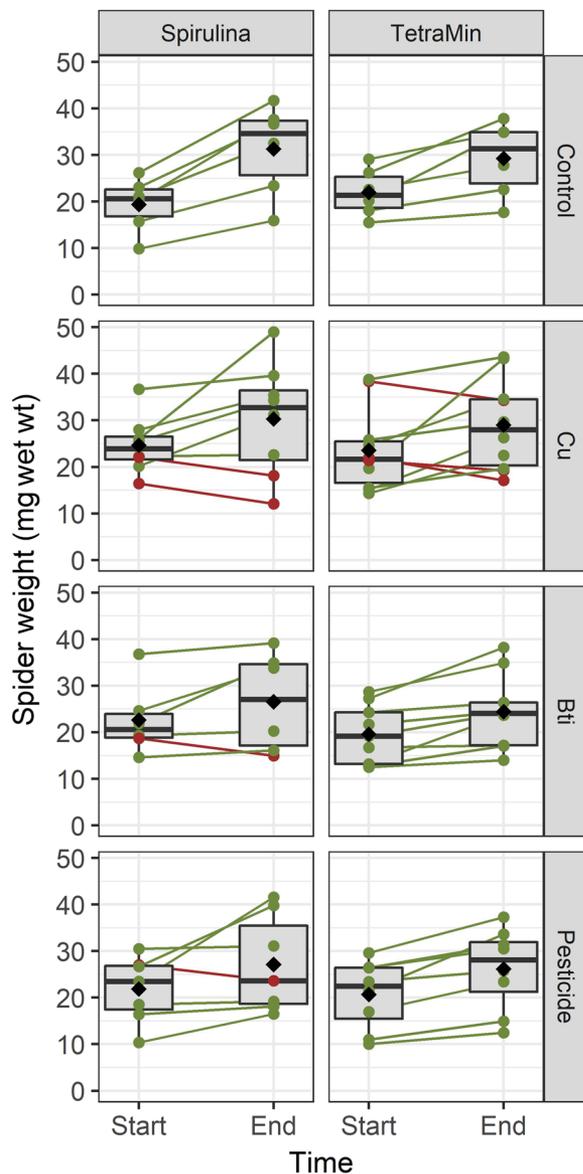
**FIGURE 3:** Spider wet weight at the start and end of the bioassay. Spiders were exclusively fed with adult chironomids cultured under one of eight treatments (i.e., combination of contaminant exposure and basal resource). Lines in boxes are medians; box ends are 25th and 75th percentiles. Whiskers and black dots show data points outside the lower and upper quartiles. Black diamonds show the mean spider wet weight, and dots represent individual data points. Lines connect data points of the same spider (i.e., same replicate). Green dots and lines indicate an increase in weight; red dots and lines, a decrease. Bti = *Bacillus thuringiensis* var. *israelensis*.

important PUFAs such as ARA (~30-fold, Spirulina) and EPA (~10-fold, Spirulina) and some precursors (e.g., 18:2n-6). In contrast, proportions of DHA remained constant or declined (~30-fold, TetraMin) in adult chironomids relative to their basal resource (Strandberg et al., 2020), indicating a low physiological relevance of DHA in chironomids.

In contrast to food quality and our hypothesis, contaminant exposure had marginal effects on the FA profile of adult chironomids. This observation may be explained by the relative accumulation of FAs during chironomid metamorphosis (Borisova et al., 2016; Pietz et al., 2023), potentially masking contaminant-induced negative effects that may occur during larval development. Alternatively, contaminant exposure may have been too weak to cause meaningful implications in adult chironomid FA profiles. For example, Bti did not affect the lipid content of chironomids in recent laboratory (Bordalo et al., 2020) and semifield (Kolbenschlag et al., 2023) studies. This combined evidence supports previous findings that insect metamorphosis is triggered by certain size requirements (Mirth & Riddiford, 2007; Nijhout, 1975). Surpassing the "minimum viable weight" ensures that the larva has accumulated sufficient energy reserves to survive the energy-demanding process of metamorphosis (Mirth & Riddiford, 2007). Consequently, the potential effects of our contaminants may be more likely manifested in terms of a prolonged developmental time or failure to emerge.

While contaminant exposure was not a contributing factor to FA-profile differences in chironomids, it was for spiders. It was unexpected to find changes in the FA profile at a higher trophic level, despite the absence of such effects on its prey. Moreover, physiologically important PUFAs, such as ARA and EPA, were most affected, with a reduction in their content by approximately 30% in Cu and Bti treatments compared to the control. A potential explanation for this observation could be related to the differences in FA pools that we examined. For spiders, we specifically assessed the NLFA profile, which is the FA pool that more quickly responds to changes in food quality, whereas we assessed the overall FA profile (including more stable FA pools such as phospholipid FAs; Iverson, 2009; Koussoroplis et al., 2014) in chironomids. Indeed, Arambourou et al. (2020) observed substantial differences in the lipid profile of chironomid larvae following exposure to metal-contaminated sediments, while their total lipid quantity remained constant. This indicates that the overall FA pool may be a suboptimal proxy of the FA available to a consumer or predator—a hypothesis that requires further validation.

Toxic effects through secondary (dietary) poisoning may also explain the observed decrease in NLFAs of spiders. While this effect pathway may be less relevant for Bti with a relatively specific mode of action against nematocerous dipterans (Becker, 1997; Boisvert & Boisvert, 2000), it may be more relevant for Cu and the pesticide mixture. Indeed, Cu induces lipid peroxidation (Gaetke & Chow, 2003), which may directly or indirectly change the PUFA content of storage lipids (i.e., NLFAs). A potential underlying indirect mechanism may be related to the oxidation of membrane lipids (such as phospholipid FAs; Bailey et al., 2015; Gaetke & Chow, 2003)
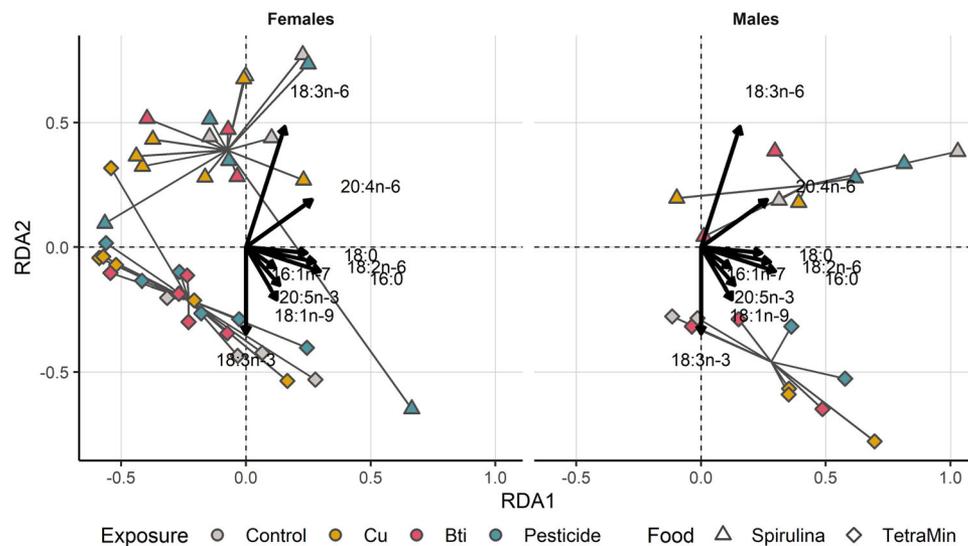
discrepancy between chironomids' physiological demands and supply of this basal resource (Goedkoop et al., 2007). This observation may be interpreted as an adaptation supporting survival and sustaining populations of nonselectively feeding organisms, such as chironomids, on poor-quality food (Goedkoop et al., 2007; Twining, Bernhardt, et al., 2021). Thereby, chironomids increase the availability of physiologically important PUFAs to consumers. This is supported by the increase in FAs with a higher number of double bonds expressed in higher relative proportions of physiologically

**FIGURE 4:** Redundancy analysis using fatty acid profiles (micrograms per milligram dry wt) of female and male spiders fed with adult chironomids cultured under one of eight treatments (i.e., combination of chemical stress and basal resource). Basal resources of larvae are indicated by triangles (Spirulina) and diamonds (TetraMin). Color represents larval exposure to control (gray), Cu (yellow), Bti (red), and synthetic (blue) pesticides. Scores were calculated in the same ordination space but are separated between females and males in the ordination to avoid cluttering of the figure. RDA = redundancy analysis; Bti = *Bacillus thuringiensis* var. *israelensis*.

affecting membrane functionality (Dobretsov et al., 1977; Garcia et al., 2005; Hulbert & Else, 2000; Koussoroplis et al., 2014), which may be compensated by the reallocation of NLFAs to phospholipid FAs (Koussoroplis et al., 2014). Irrespective of the underlying mechanism, reduced levels of PUFAs in spiders may adversely affect their immune function (see, for wolf spider, Fritz et al., 2017), potentially increasing vulnerability to environmental pressures including parasites. In addition, the reduction in spiders' storage lipids (i.e., NLFAs) may have contributed to the observed decline in their growth (Marczak & Richardson, 2008).

Spiders fed with chironomids exposed to any of the contaminants grew approximately 40%–50% less compared to those individuals fed prey cultured under control conditions, while this effect size is subject to considerable variability. Because the total chironomid biomass consumed by spiders was similar in all treatments, the observed differences in spider growth are most likely a function of chironomids' nutritional quality for spiders. Considering that the effect on spider growth was similar for all contaminant treatments and that differences in the spiders' NLFA profiles were only observed for Cu and Bti, this parameter can only partly explain our results. However, the pesticide mixture may have influenced other parameters describing the nutritional quality for predators such as proteins and carbohydrates. Moreover, most of the pesticides in this mixture were also detected in adult chironomids (Roodt et al., 2022). While metamorphosis tended to lower pesticide levels, these levels still ranged between 3–1332 ng g$^{-1}$ dry weight and 3–102 ng g$^{-1}$ dry weight in females and males, respectively (Supporting Information, Table S8). Those contents are, moreover, comparable to those reported from the field (up to 572 ng g$^{-1}$ dry mass; Kraus et al., 2021), supporting the assumption of secondary poisoning of riparian consumers.

Secondary poisoning of spiders may require higher energetic investments for detoxification (Calow, 1991; Maltby, 1999), potentially altering energy allocation, which is ultimately reflected in reduced SGR. Although the underlying mechanisms leading to shifts in spider growth remain speculative, the present study clearly points to stressor-dependent effect pathways modifying the transfer of energy within food webs across ecosystem boundaries.

Changes in the energy transfer can be further amplified by the nutritional quality of (basal) resources. Similar to chironomids, spiders of the genus *Tetragnatha* have the capacity to modify dietary FAs (Mathieu-Resuge et al., 2021; Ohler, Schreiner, Martin-Creuzburg, & Schäfer, 2023), as indicated by the lower relative importance of the FA profile of the basal resource for the FA profile of spiders (Figure 4). However, the extent to which spiders use this ability depends largely on the dietary availability of certain physiologically important FAs because the modification of FAs is considered more energetically costly relative to their dietary uptake (Parrish, 2009). Nutritionally less valuable resources in combination with direct and indirect effects induced by aquatic contaminants may modify spiders' energy balance, reflected in an altered energy allocation and lower specific growth rate. In addition to food quality, food availability affects spider growth, with more resources generally promoting higher growth rates (Marczak & Richardson, 2008).

## Chironomid emergence

We observed stressor-dependent changes in the emergence of chironomids. Copper reduced chironomid emergence by approximately 40%, an effect consistent with our

hypothesis. Such an alteration in the emergence pattern can per se affect riparian spiders (Kraus et al., 2014) and might exacerbate observed reductions in spider growth and their PUFA content under constant prey availability. Similar effects were absent from the other treatments, which probably is a function of their lower intensity. Exposure concentration to Bti was relatively low (i.e., 10% of the recommended field rate) to avoid excessive mortality of early instars, which are considered particularly sensitive (Kästel et al., 2017), but was still expected to cause effects on chironomid development over the entire study duration. Similarly, the ecotoxicological potential of the entire pesticide mixture had an initial toxic unit of 0.15 (48-h median effect concentration [EC50] for *Daphnia magna* and 96-h EC50 for *C. riparius*), which decreased over time (time-weighted average concentration toxic unit of 0.08), making strong effects on chironomid development unlikely (Lewis et al., 2016; Roodt et al., 2022).

Besides the contaminant exposure, the quality of food had a meaningful but statistically nonsignificant effect on chironomid emergence. In fact, Spirulina reduced emergence by approximately 15% compared to TetraMin. This observation may be linked to a higher energetic requirement of chironomids to modify FAs from dietary sources in the case of Spirulina relative to TetraMin (as discussed above and see Parrish, 2009). In combination with an energetically demanding metamorphosis (Arrese & Soulages, 2010; Hodin, 2006), this may have ultimately limited emergence and is supported by a decrease (on average 7%) in the dry weight of emergent females. However, the dry weight of males was similar between treatments, and the biological relevance of this effect remains uncertain. In addition, it should be noted that the basal resources may exhibit nutritional differences other than those measured in the present study, which could affect emergence success.

### Sex-related differences

Consistent with our hypothesis, sex was an important discriminating factor for the FA profiles of chironomids and spiders, explaining 13% and 16% of the total variation, respectively. The differences in the FA profiles of females and males match previous studies focusing on insects (Meier et al., 2000; Pietz et al., 2023; Scharnweber et al., 2020) but may also occur in spiders, presumably because of sex-specific life-history strategies. Indeed, sexual dimorphism in terms of morphology (i.e., females being larger than males) and reproductive investment strategies (i.e., energy storage in females and mating success in males) seem to be similar in both groups (Cordellier et al., 2020; Servia et al., 2006). For example, male chironomids contain high PUFA levels (present study; Pietz et al., 2023) because these are suggested to be associated with their flight ability (Dadd, 1980; Sushchik et al., 2013), ultimately promoting swarming and mating success (Armitage, 1995). Females, on the other hand, contain higher levels of total FAs and less unsaturated FAs, which are typically associated with storage lipids. However, differences in the FA profiles between females and males did not follow the same pattern in spiders, suggesting species-specific

requirements. Yet, sex-specific differences may have contributed to the stronger reduction in NLFA contents of female spiders observed in the present study. One explanation for this effect could be related to food consumption, which was higher for female than male spiders and may thus be a function of body size.

## CONCLUSION

While earlier studies have focused on total biomass flux as a descriptor for contaminant-induced changes in the aquatic subsidy for terrestrial food webs, the present study highlights the relevance of nutritional quality. As a consequence, studying such aquatic-to-terrestrial model food chains will push the scientific understanding of stress-induced changes in meta-ecosystems. Our study, however, also highlights that characterizing nutritional quality with only one parameter (such as FAs) may fall short and should be supported by additional parameters (e.g., proteins, carbohydrates, and contaminant retention) complementing the picture. Nonetheless, the data presented in our study, in particular the reductions in energy storage and growth of spiders, point to potential implications in their population dynamics (e.g., reduced egg production), as suggested by Uno (2016). Owing to their central position in riparian food webs, alterations in the density and nutritional quality of spiders could, in turn, cause top-down (i.e., shift in predation pattern) and bottom-up (i.e., provisioning of nutrients and energy for higher trophic levels) directed effects in terrestrial systems.

*Author Contributions Statement*—**Sebastian Pietz:** Conceptualization; Methodology; Investigation; Formal analysis; Visualization; Writing—original draft; Writing—review & editing. **Sara Kolbenschlag, Nina Röder, Alexis P. Roodt:** Methodology; Investigation; Writing—review & editing. **Zacharias Steinmetz:** Investigation; Formal analysis; Writing—review & editing. **Alessandro Manfrin, Ralf B. Schäfer:** Formal analysis; Writing—review & editing. **Klaus Schwenk, Ralf Schulz:** Methodology; Writing—review & editing. **Jochen P. Zubrod:** Conceptualization; Methodology; Writing—review & editing. **Mirco Bundschuh:** Conceptualization; Methodology; Supervision; Writing—review & editing.

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*Data Availability Statement*—Data not available in the Supporting Information are available from the corresponding authors (mirco.bundschuh@rptu.de, sebastian.pietz@rptu.de).

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