

Aquatic contaminants reduce the quantity and nutritional quality of aquatic subsidy to riparian consumers

by

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Declaration

I hereby declare that I independently conducted the work presented in this thesis entitled “Aquatic contaminants reduce the quantity and nutritional quality of aquatic subsidy to riparian consumers”. All used assistances are mentioned and involved contributors are either co-authors of or are acknowledged in the respective publication.

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

Landau, July 3rd, 2024

Place, date

Signature

The results presented in this cumulative thesis are documented in two peer-reviewed publications (**Appendix A1** and **A2**) and one manuscript that is currently under preparation (**Appendix A3**).

Since many researchers contributed to the work I present in this thesis (see **Appendix A4**), I will use the plural form when presenting and discussing these results.

Appendix A1

Pietz, S., Kainz, M. J., Schröder, H., Manfrin, A., Schäfer, R. B., Zubrod, J. P., & Bundschuh, M. (2023). Metal Exposure and Sex Shape the Fatty Acid Profile of Midges and Reduce the Aquatic Subsidy to Terrestrial Food Webs. *Environmental Science & Technology*, 57(2), 951-962.

Appendix A2

Pietz, S., Kolbenschlag, S., Röder, N., Roodt, A. P., Steinmetz, Z., Manfrin, A., Schwenk, K., Schulz, R., Schäfer, R. B., Zubrod, J. P., & Bundschuh, M. (2023). Subsidy quality affects common riparian web-building spiders: consequences of aquatic contamination and food resource. *Environmental Toxicology and Chemistry*. doi.org/10.1002/etc.5614.

Appendix A3

Pietz, S., Röder, N., Kolbenschlag, S., Schöndorfer, A., Schwenk, K., & Bundschuh, M. (*in preparation*). Effects of copper, food quality and exposure history on aquatic insect emergence: insights from a multigeneration study.

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1 ABSTRACT

Neighboring ecosystems are connected by fluxes of organic and inorganic matter. At the aquatic-terrestrial interface, researchers have primarily focused on the magnitude of resource fluxes, while largely disregarding differences in their nutritional quality for consumers in the recipient ecosystem. Aquatic resources, such as emergent aquatic insects, contain substantially higher levels of physiologically important long-chain polyunsaturated fatty acids (long-chain PUFA) than terrestrial resources. Since animals cannot typically biosynthesize long-chain PUFA in quantities sufficient to meet their physiological demands, they must obtain these compounds through their diet. Aquatic subsidy by emergent aquatic insects can therefore benefit the physiological condition and breeding success of riparian consumers such as birds and spiders. However, aquatic contaminants may disrupt aquatic insect emergence, altering the quantity and nutritional quality of aquatic subsidy to recipient terrestrial systems. Despite its importance to local consumers, effects of aquatic contaminants on this process are poorly studied and understood. The primary aim of this thesis is therefore to assess the effects of aquatic contaminants on aquatic-to-terrestrial subsidy, focusing on FA fluxes, and to determine whether shifts in subsidy quality affect riparian consumers. Effects were examined in a set of complex laboratory bioassays that also considered environmental factors (e.g., basal food resource) to increase environmental relevance.

Metals (i.e., cadmium, Cd and copper, Cu) substantially reduced aquatic insect emergence (up to 95%) and the flux of physiologically important long-chain PUFA (up to 80%) while being efficiently excreted during metamorphosis (reduction by ~90%). Given their relative scarcity in terrestrial ecosystems, local consumers may be particularly vulnerable to changes in the flux of physiologically important long-chain PUFA. Indeed, contaminant-induced alterations of aquatic subsidy quality can affect the physiological condition of riparian spiders, as suggested by a consistent decline in spider growth (40-50%), irrespective of the contaminant group (Cu, *Bacillus thuringiensis* var. *israelensis* (Bti) or a mixture of synthetic pesticides) to which their prey was exposed. Since spider neutral lipid FA contents were only reduced in two out of three contaminant treatments compared to the control (up to ~30%, Cu and Bti), this implies contaminant-dependent effect pathways modifying the transfer of energy within food webs at the aquatic-terrestrial interface. Furthermore, results from a multigeneration experiment point to a relatively constant reduction in aquatic insect emergence and thus sustained alteration in the aquatic-to-terrestrial subsidy. Finally, the basal resource quality and sex-specific differences in aquatic insects and terrestrial consumers may further complicate the assessment of contaminant-induced effects. This thesis therefore recommends to consider fluxes of long-chain PUFA to better understand and anticipate implications of aquatic contaminants on the aquatic-terrestrial meta-ecosystem and develop efficient measures to protect riparian systems and their communities.

2 INTRODUCTION

2.1 Background

On the landscape scale, ecosystems form mosaics connected by fluxes of nutrients, energy and organisms (Loreau et al. 2003; Richardson et al. 2010). Ecosystems can thus be affected by processes beyond their local boundaries (Holt 2008; Polis et al. 1997; Richardson et al. 2010). Resource fluxes subsidize the recipient system, supporting local productivity (Nakano & Murakami 2001; Polis et al. 1997) and food web stability (Barnes et al. 2018), which has been increasingly recognized around the early 2000s (e.g., Baxter et al. 2005; Henschel et al. 2001; Loreau et al. 2003; Nakano & Murakami 2001; Polis et al. 1997; Polis & Hurd 1996).

In this context, much of the knowledge has come from research at the interface of aquatic, particularly freshwater, and terrestrial systems, presumably due to distinct differences between the systems themselves and the organisms living in them (Richardson et al. 2010). Gravity-driven resource fluxes from terrestrial to freshwater systems consisting mainly of leaf litter and invertebrates, are typically larger than those in the opposite direction (Bartels et al. 2012; Richardson et al. 2010; Schindler & Smits 2017). Past research has thus focused on terrestrial-to-aquatic subsidies (Schindler & Smits 2017). However, it is becoming evident that resource quantity alone may not be an accurate indicator to judge the relevance of fluxes for the receiving ecosystem (Marcarelli et al. 2011; Twining et al. 2018b; 2019). In fact, the nutritional quality of resource fluxes can be even more important than their quantity (Twining et al. 2016b). This is because animals require elemental nutrients (e.g., nitrogen and phosphorous) and organic compounds (e.g., lipids and proteins), but ecosystems vary in their capacity to provide them (e.g., Hixson et al. 2015). The relative importance of a subsidy is thus a function of its availability in the recipient system as well as its spatial extent and temporal dynamics (Marczak & Richardson 2008; Clark & Hobson 2022).

At the aquatic-terrestrial interface, the availability of (at least) one group of organic compounds appears to differ strongly between the two systems: fatty acids (FA), more precisely long-chain polyunsaturated FA (long-chain PUFA; Hixson et al. 2015). In fact, long-chain PUFA, such as

arachidonic acid (ARA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3), are mainly synthesized by aquatic primary producers (Hixson et al. 2015; Twining et al. 2016a). Their precursors, linoleic acid (LIN, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3), respectively, and other less unsaturated FA are, in contrast, also produced by terrestrial primary producers (Hixson et al. 2015). This dichotomy tends to propagate along the aquatic and terrestrial food web. Consequently, aquatic insects differ in their biochemical composition compared to terrestrial insects, containing up to ten times higher levels of long-chain PUFA (Hixson et al. 2015). At the same time, long-chain PUFA are required by all animals, because they are involved in vital physiological functions, for example, serving as membrane components, or contributing to hormone regulation or immune response (Brett & Müller-Navarra 1997; Twining et al. 2016a). However, animals typically cannot biosynthesize long-chain PUFA (*de novo* or from precursors) in quantities sufficient to meet their physiological demands (Cook & McMaster 2002; Fritz et al. 2017; Twining et al. 2018a). They must therefore obtain these essential compounds through their diet (Twining et al. 2016a; 2018a). Aquatic-to-terrestrial subsidies provided by emergent aquatic insects are therefore considered to be of high importance for consumers in recipient terrestrial systems (Twining et al. 2019). Indeed, ARA (20:4n-6) and EPA (20:5n-3) have been shown to promote growth, breeding success and immune function in riparian consumers such as birds and spiders (Fritz et al. 2017; Twining et al. 2018b; 2019). However, anthropogenic activities may disrupt the quantity and nutritional quality of subsidy to recipient terrestrial systems and their local consumers.

Globally, many freshwater systems are impacted by anthropogenic activities in their catchment. Such activities include, among others, changes in agricultural practices, industrialization and urbanization (Ceballos et al. 2017; Millennium Ecosystem Assessment 2005; Maxwell et al. 2016; Tilman et al. 2001). They typically also lead to repeated or continuous exposure of aquatic organisms to a wide range of contaminants, such as metals, synthetic pesticides or biocontrol agents (Becker et al. 2018; Kumar et al. 2019; Malaj et al. 2014; Morrissey et al. 2015; Wolfram et al. 2018; Zubrod et al. 2019). This can, in turn, cause adverse effects on organisms' fitness and subsequent community turnover (Liber et al. 1996;

Liess et al. 2021; Liess & Schulz 1996; Wei et al. 2021). In case of aquatic insect larvae, effects may propagate to their terrestrial (adult) life stage – and may even be exacerbated by physiologically complex and energy demanding developmental steps (e.g., metamorphosis in holometabolous insects; Arrese & Soulages 2010; Hodin 2006; Schmidt et al. 2013) – with potential bottom-up or top-down effects in terrestrial food webs (Henschel et al. 2001; Kraus et al. 2014a; Paetzold et al. 2011; Schulz et al. 2015). Excluding changes in the community composition of emergent insects, effects of aquatic contaminants may include (I) shifts in the biomass flux (i.e., number and body weight of emerging adults), (II) its timing and (III) its nutritional quality (i.e., contaminant accumulation and retention as well as biochemical composition) (Kraus 2019). As contaminant groups have different propensities to affect aquatic insects or be retained in their bodies, the relative importance of those effect pathways differs substantially (Bundschuh et al. 2022; Kraus 2019; Kraus et al. 2014b). In addition, effects of aquatic contaminants may vary with sex, as emergent females and males differ in their nutritional quality (i.e., contaminant retention, Roodt et al. 2022; FA profile, Scharnweber et al. 2020) for terrestrial consumers.

Besides exposure to aquatic contaminants, anthropogenic activities may modify environmental factors (e.g., food resource) that shape the emergence of aquatic insects (Carey et al. 2012; Frouz et al. 2002; Goedkoop et al. 2007; Schindler & Vallentyne 2008). Eutrophication and increasing water temperatures due to excessive nutrient inputs and global climate change, respectively, affect basal aquatic resources, for example, by promoting cyanobacteria blooms (Carey et al. 2012; Maileht et al. 2013; Taipale et al. 2016; Paerl & Paul 2012). Since cyanobacteria contain no or only low levels of long-chain PUFA (Ahlgren et al. 1992; Strandberg et al. 2015) and suppress nutritionally more valuable primary producers (e.g., diatoms; Paerl & Paul 2012), this process lowers the content of these physiologically important compounds in the aquatic system (Taipale et al. 2016) and presumably their flux to adjacent terrestrial systems. Furthermore, effects of environmental factors can interact with those of contaminants, altering the response of aquatic organisms (Haas et al. 2004; Sokolova & Lannig 2008; Verheyen & Stoks 2019). Considering interactions between aquatic contaminants and

environmental factors may thus improve the understanding and prediction of their effects on insect emergence, specifically its nutritional quality, and the aquatic-terrestrial linkage in general.

2.2 Research motivation and objectives

Despite the fact that emergent aquatic insects provide adjacent terrestrial systems with physiologically important but locally scarce long-chain PUFA, the effects of aquatic contaminants on this process are poorly studied and understood. Previous research has mainly focused on the total biomass flux (Polis et al. 1997; Gratton & Vander Zanden 2009) and, more recently, on the flux of contaminants with emergent insects (Cetinić et al. 2021; Kraus et al. 2021a; 2021b; Previšić et al. 2021; Roodt et al. 2022). However, since terrestrial consumers rely on nutritionally valuable aquatic subsidy, ignoring potential shifts in the FA profiles of aquatic insects and the FA-flux to terrestrial systems may be misleading and hinder the assessment of contaminant-induced effects on aquatic-terrestrial linkages. Furthermore, the physiological consequences for terrestrial consumers, such as riparian birds and spiders, in response to altered subsidy quality are largely unexplored (but see Fritz et al. 2017; Ohler et al. 2023b; Twining et al. 2018b; 2019). In this context, it might be relevant to consider how contaminant groups differ in their effects on subsidy quality (see Kraus 2019). Moreover, repeated or continuous contaminant exposure has hardly been studied against the background of aquatic-terrestrial linkages, but may facilitate the understanding of chronic effects on the quantitative and qualitative subsidy of terrestrial ecosystems (Morgan et al. 2007).

The primary aim of this thesis is to assess whether aquatic contaminants affect the FA flux through emergent aquatic insects to adjacent terrestrial systems and whether shifts in subsidy quality propagate to terrestrial consumers. To increase the relevance of the laboratory bioassays, the effects of aquatic contaminants were combined with those of environmental factors to explore the following main research objectives (RO, see also Figure 1):

RO1: Effects of aquatic contaminants on aquatic-to-terrestrial subsidy – fluxes of contaminants and FA

Do contaminants affect the FA profile of aquatic insects and how do they alter the flux of FA to terrestrial systems? What is the relative importance of potential changes in FA fluxes compared to contaminant fluxes?

RO2: Effects of subsidy quality on terrestrial consumers

Can changes in subsidy quality affect riparian spiders? Do effects on subsidy quality and thus riparian spiders differ between contaminant groups?

RO3: Chronic effects on aquatic insect emergence

How does chronic contaminant exposure affect the sensitivity of aquatic insects and thus the quantitative and qualitative subsidy of terrestrial ecosystems?

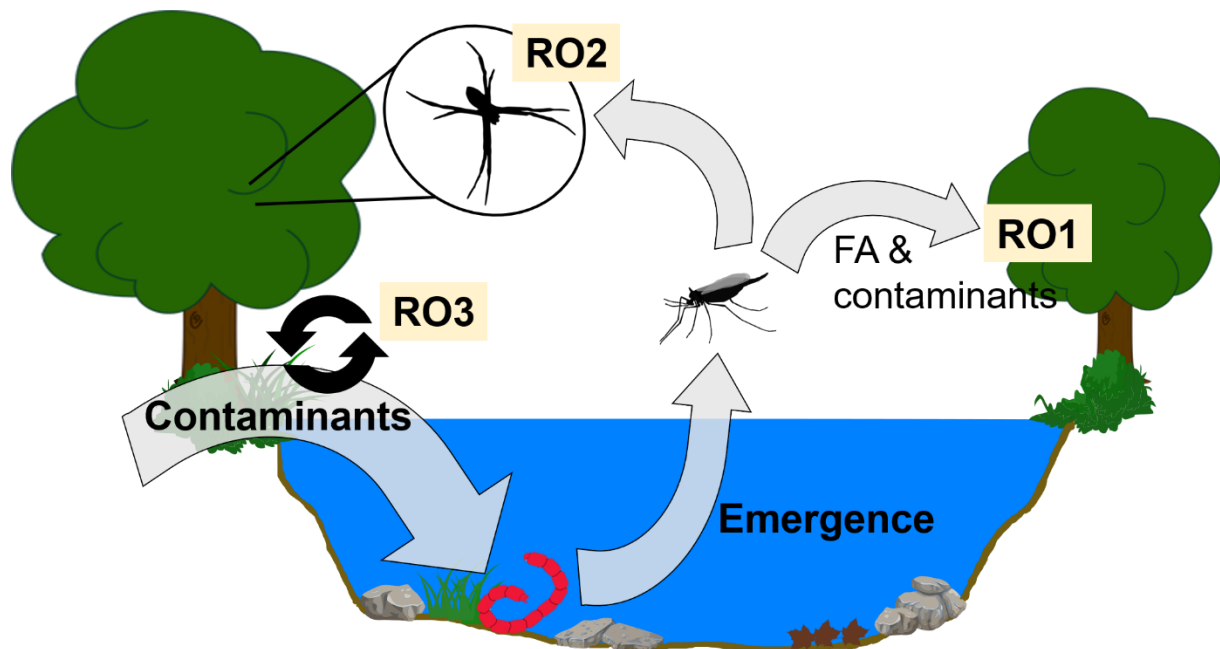


Figure 1: Conceptual overview of the three research objectives (RO1, RO2, & RO3) addressed in this thesis.

3 METHODOLOGY

3.1 Test species and aquatic contaminants

To answer the main research questions outlined above, the holometabolous aquatic insect *Chironomus riparius* (Diptera: Chironomidae; hereafter chironomids) was chosen as model species. The selection was motivated by their widespread occurrence in freshwater habitats (Cranston 1995) and the quantitative importance of chironomids for aquatic insect emergence and as prey for terrestrial consumers (Leeper & Taylor 1998; Martin-Creuzburg et al. 2017; Ohler et al. 2023a; Regester et al. 2008). Additionally, their larvae live in close association with the sediment, where metals and other contaminants can accumulate (Pedrosa et al. 2017).

Three model contaminant groups were used in this thesis: metals (cadmium, Cd and copper, Cu), the biocontrol agent *Bacillus thuringiensis* var. *israelensis* (Bti) and a mixture of synthetic pesticides (for details see **Appendix A2**). The selection of these groups was motivated by 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) while broadly varying in their propensity to affect chironomid emergence or be accumulated and retained during metamorphosis (Bundschuh et al. 2022; Kraus 2019). Moreover, these contaminant groups have either been shown to affect the lipid profile of aquatic invertebrates (Zubrod et al. 2011; 2015) or are known to compromise lipid membranes (Vachon et al. 2012).

3.2 RO1: Effects of aquatic contaminants on aquatic subsidy – fluxes of contaminants and FA

To investigate how metals affect different developmental stages of an aquatic insect, we exposed chironomid larvae to one of five sediment contents of the non-essential Cd and the essential Cu (0, 0.7, 2, 6, 18 mg Cd/kg dw or 0, 25, 50, 100, 200 mg Cu/kg dw) at a constant temperature of 20°C. We differentiated between non-essential and essential metals, as the latter are involved in physiological processes (Dow 2017), suggesting differences in accumulation and toxicity. Additionally, we assessed potential interactive effects between both metals (three sediment contents each) and periodically elevated water temperatures (20-

24°C), simulating heat waves and reflecting an aspect of climate change (Figure 2). Elevated water temperatures tend to enhance contaminant toxicity in aquatic ectotherms such as insects (Sokolova & Lannig 2008; Verheyen & Stoks 2019) and affect their biochemical composition (i.e., FA profile; Guschina & Harwood 2006; Parrish 2009).

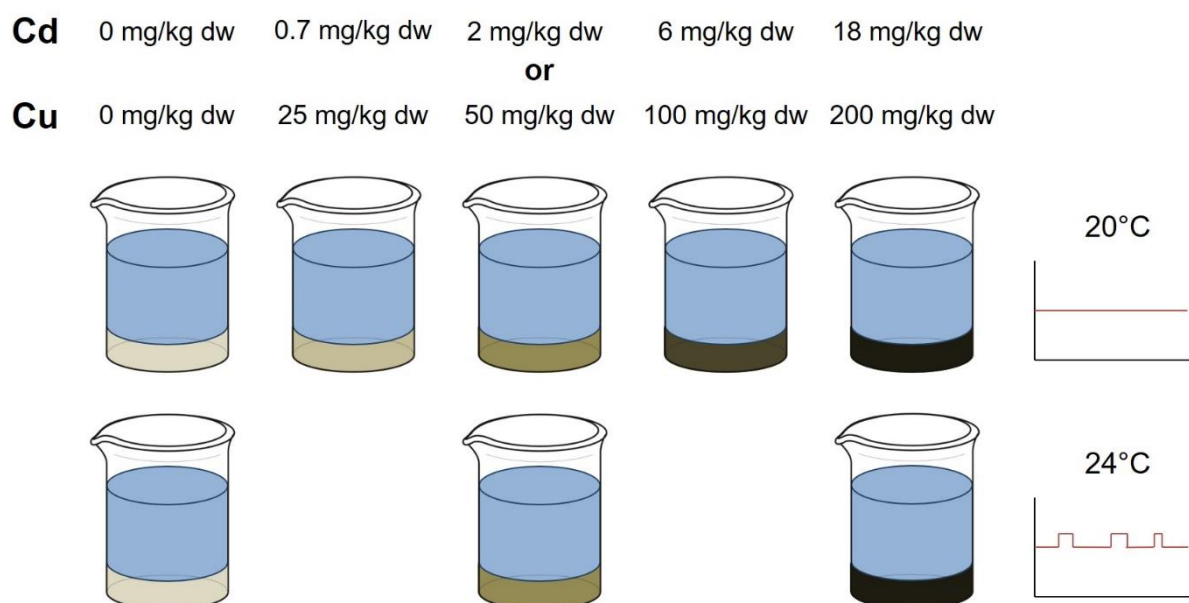


Figure 2: Scheme illustrating the eight treatments (i.e., combination of metal exposure and temperature regime) of each bioassay. Each treatment consisted of 25 replicates with chironomids (not shown) and two additional replicates without test organisms. Both bioassays lasted for 28 days. Figure taken from **Appendix A1**.

We sampled replicates from each bioassay at days seven, eleven, 15, 19 and 23 to investigate the effect of the treatment on chironomid development. In addition, emerging adults were collected daily, sorted by sex, counted and stored frozen. Furthermore, all chironomids were freeze-dried and weighed to the nearest 0.01 mg to assess treatment-related effects on their dry weight. We analyzed metal contents in larvae and adults from the control and the intermediate sediment metal content (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw) under both temperature regimes, to determine metal accumulation and metamorphic retention in chironomids. Samples were prepared by microwave-assisted digestion and quantified by inductively coupled plasma-triple-quadrupole mass spectrometry (ICP-QQQ-MS; Agilent 8900 series, Agilent Technology, Japan). Furthermore, we analysed FA in larvae and female and male adults from the control, the intermediate and the highest metal sediment content (i.e., 18 mg Cd/kg dw and 200 mg Cu/kg dw) under both temperature regimes to characterize their nutritional quality for consumers. For details see '3.5 Fatty acid analysis'.

3.3 RO2: Effects of subsidy quality on terrestrial consumers

We investigated the physiological consequences of an altered prey quality for riparian web-building spiders. For this, we first conducted a fully-factorial laboratory study assessing the effects of three aquatic contaminant groups (Cu, Bti, or a synthetic pesticide mixture) and food quality of the basal resource (Spirulina and TetraMin) on chironomids (Figure 3). Emerged adults were collected daily, sorted by sex, counted and stored frozen. Subsequently, adult spiders of the genus *Tetragnatha* (*Tetragnatha extensa* LINN. and *Tetragnatha montana* SIMON), were fed exclusively with emerged adults cultured under one of the eight treatments (i.e., combination of contaminant exposure and basal resource) of the chironomid laboratory study (Figure 3). We used *Tetragnatha* as model, web-building spider, because it is a common taxon in riparian areas that strongly depends on aquatic insects (Kato et al. 2004; Krell et al. 2015; Walters et al. 2018). Each spider was fed five thawed adult chironomids per week (two females and three males or vice versa) for a total of three weeks to allow direct comparison among treatments and to relate potential effects on spider physiology to prey nutritional quality rather than quantity. Ten spiders, kept in individual plastic terrariums, were allocated to each treatment considering spider species, sex and weight to minimize within treatment variability. Spiders were weighed to the nearest 0.1 mg at the start and end of the bioassay to determine their growth. At the end of the bioassay, spiders were euthanized in liquid nitrogen and stored frozen. We analysed the FA profiles of the basal resources as well as adult chironomids and spiders. We analyzed FA in basal resources, adult chironomids and spiders. For details see '3.5 Fatty acid analysis'.

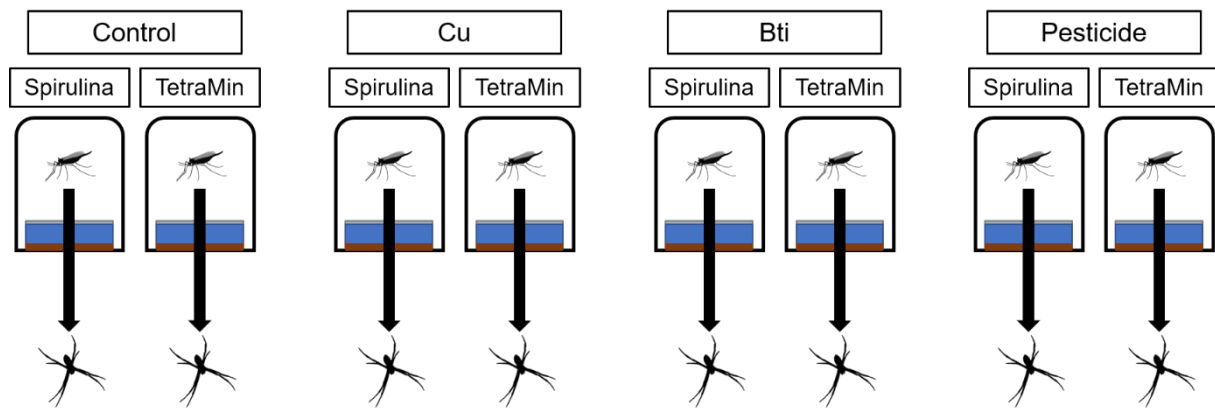


Figure 3: Scheme illustrating the study design. We conducted a fully-factorial laboratory study assessing the effects of three aquatic contaminant groups (Cu, Bti, or a synthetic pesticide mixture) and food quality of the basal resource (Spirulina and TetraMin) on chironomids. Adult spiders of the genus *Tetragnatha* were exclusively fed emerged adults cultured under one of the eight treatments.

3.4 RO3: Chronic effects on aquatic insect emergence

In order to investigate how chronic Cu exposure affects the quantitative and qualitative subsidy with emergent aquatic insect to adjacent terrestrial ecosystems, we conducted a multigeneration study. We assessed the sensitivity of chironomid populations to Cu before and after a chronic exposure (about six months) to the same stressor (Figure 4). For this, freshly hatched larvae originating from an in-house culture were used to concurrently start the chronic culture and the first sensitivity test (ST1). In the chronic culture, six independent chironomid populations were either reared in the absence of Cu (naïve) or at an environmentally relevant Cu sediment content of 100 mg/kg dw (pre-exposed) (Arambourou et al. 2020; Feiler et al. 2013; Steinmetz et al. 2017). We then used larvae from naïve and pre-exposed populations to conduct the second sensitivity test (ST2). To ensure truly independent replicates, each replicate of a treatment was created using larvae from one of the populations in the chronic culture.

Test conditions (e.g., temperature, day/night cycle, etc.), duration, and nominal Cu sediment contents (i.e., 0, 50, 100, 200, 300, and 400 mg/kg dw) were identical in both sensitivity tests and aimed to determine the potential effects of laboratory conditions (ST1) and exposure history (ST2) on the sensitivity of chironomids to Cu. Food quality of the basal resource offered to chironomid larvae was used as an additional factor triggering differences in sensitivity. While larvae were fed TetraMin in the in-house and chronic culture, they were fed either Spirulina or TetraMin in both sensitivity tests. Emerging adults were collected daily, sorted by sex, counted

and stored frozen. Freeze-dried adults were weighed to the nearest 0.01 mg and subsequently analysed for their FA profiles (see '3.5 Fatty acid analysis').

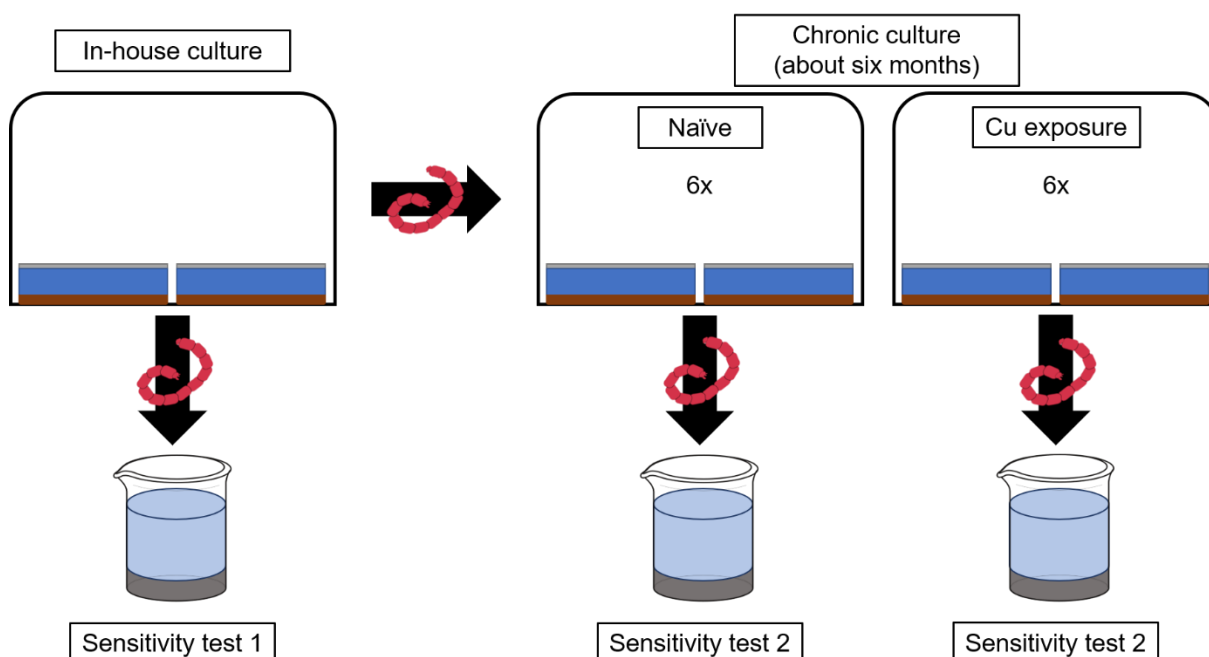


Figure 4: Scheme illustrating the study design. Larvae from the in-house chironomid culture were used to concurrently start the chronic culture and the first sensitivity test. In the chronic culture, six independent chironomid populations were reared in the absence (naïve) or presence (pre-exposed) of Cu (100 mg/kg dw) for about six months. The second sensitivity test was started with larvae originating from naïve or pre-exposed populations of the chronic culture. Each replicate was formed with larvae from one of the naïve or pre-exposed populations, making them truly independent.

3.5 Fatty acid analysis

To characterize the resource quality for riparian consumers, we analyzed total FA in basal resources and chironomids. For this, lipids from freeze-dried and weighed samples were extracted in a chloroform/methanol mixture (2:1; v:v; 5 mL). Lipid hydrolysis and subsequent methylation of FA to fatty acid methyl esters (FAME) was performed with 3N methanolic HCl. FAMES were extracted using isohexane, dried under a stream of nitrogen (at 40°C), redissolved in dichloromethane and stored frozen until measurement. We determined neutral lipid FA in riparian consumers (i.e., spiders), since they constitute an important energy storage in invertebrates (Azeez et al. 2014) and they are altered relatively fast by shifts in food quality compared to other groups (e.g., phospholipid FA; Iverson 2009; Koussoroplis et al. 2014). Lipids from freeze-dried and weighed samples were extracted in a chloroform/methanol/milli-Q-water (2:2:1.8; v:v:v; 8.7 mL) mixture. Neutral lipids were separated from glycolipids and phospholipids via solid-phase extraction by elution with 4 mL chloroform. Samples were dried

under a stream of nitrogen (at 40°C) and redissolved in dichloromethane. Lipid hydrolysis and subsequent methylation of FA to fatty acid methyl esters (FAME) was performed with trimethylsulfonium hydroxide. Samples were stored frozen until measurement. FAMES were quantified via gas chromatography with flame-ionization detection (GC-FID; Trace GC Ultra, Thermo Fisher Scientific, Bremen, Germany), irrespective of the sample preparation procedure. FA concentrations were normalized to the total volume and the dry weight of the sample ($\mu\text{g FA/mg dw}$). For further details see **Appendix A1-A3**.

4 RESULTS AND DISCUSSION

4.1 RO1: Effects of aquatic contaminants on aquatic subsidy – fluxes of contaminants and FA

4.1.1 Results

Both metals had negative effects on the development of chironomids, ultimately leading to a significant reduction in adult emergence by up to 95% (Cd) and 45% (Cu), respectively (Figure 5). Assuming an initial larval sex ratio of 1:1, female emergence tended to be more strongly reduced under Cu, but not Cd exposure [Appendix A1]. Elevated water temperatures had marginal effects on chironomids.

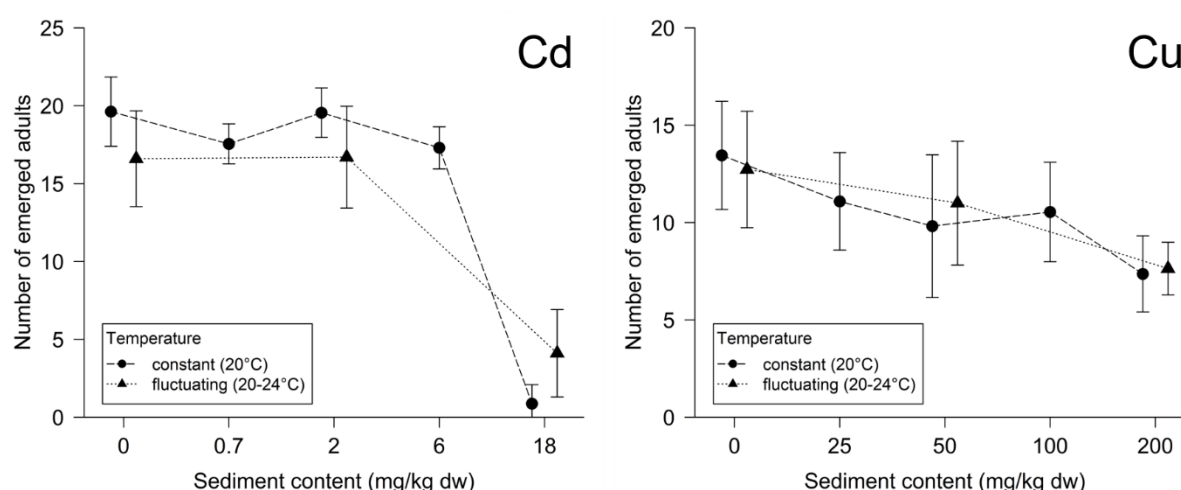


Figure 5: Mean ($\pm$ 95% CI) number of emerged adult chironomids exposed to 0, 0.7, 2, 6 and 18 mg Cd/kg dw and 0, 25, 50, 100 and 200 mg Cu/kg dw crossed with two temperature regimes (20°C; circles and 20-24°C; triangles) within the 28-day bioassay period. Lower confidence limits of the number of emerged adults below zero (18 mg/kg, 20°C) are of no biological meaning and were therefore set to zero. See Table S3 in **Appendix A1** for statistical evaluation. Figure taken from **Appendix A1**.

While larvae accumulated both metals, they were largely lost during metamorphosis (significant reduction by ~90%; Table 1). However, levels of Cd and Cu in adults were still 36-fold and 1.5-fold higher than in their respective control. Combining our data on adult emergence with their mean metal content, the flux of Cd was nevertheless lower than that of Cu [Appendix A1].

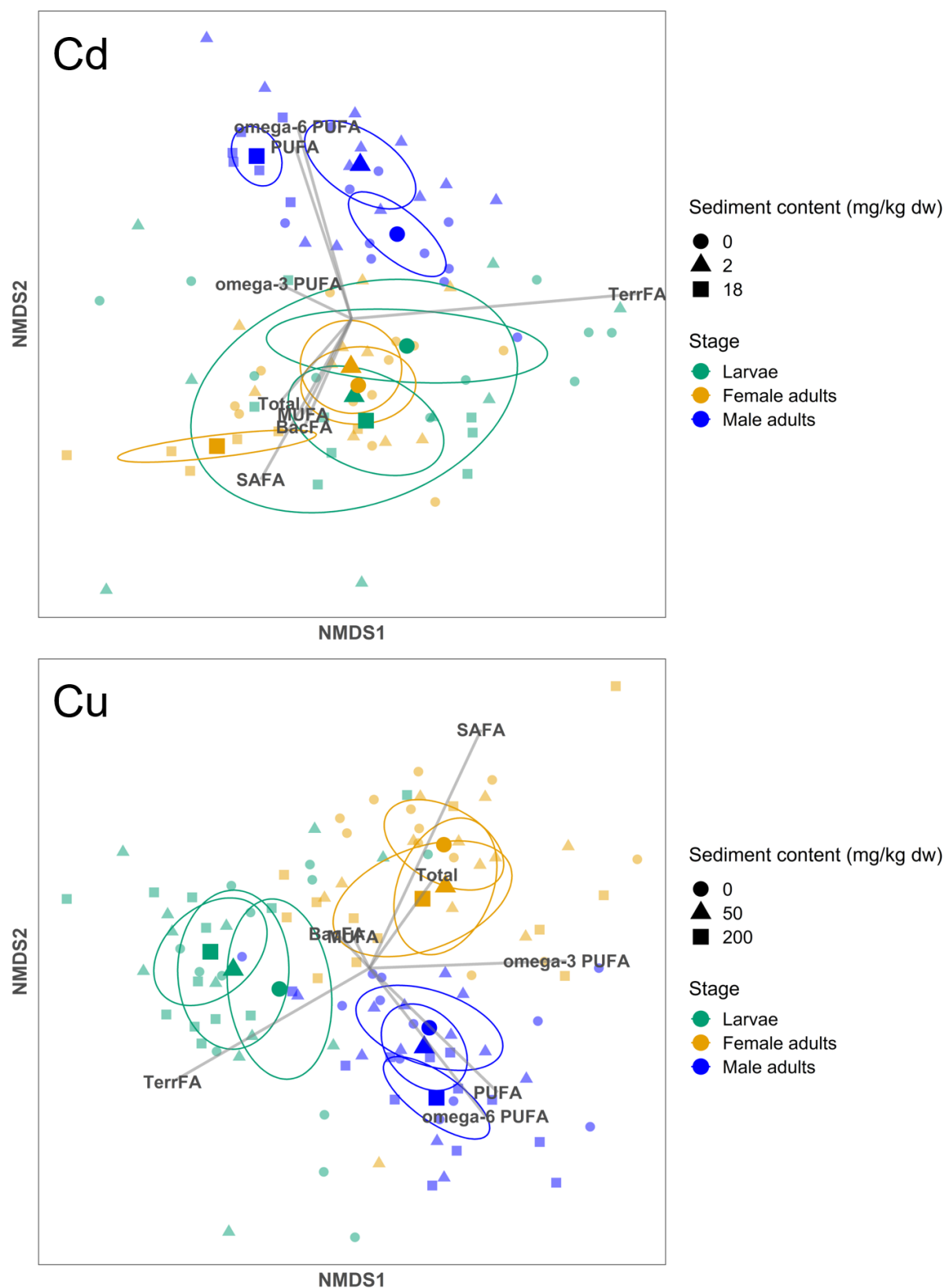


Figure 6: Non-metric multidimensional scaling (NMDS) plot for the FA composition ($\mu\text{g}/\text{mg dw}$) of chironomids from the Cd and Cu bioassay: Larvae (green), Female adults (yellow), Male adults (blue). Larvae were exposed to sediment metal contents of 0 (circle), 2 or 50 (triangle) or 18 or 200 (square) $\text{mg}/\text{kg dw}$. Centroids and 95% confidence ellipses are shown for each group. NMDS stress value was 0.145 (Cd) and 0.098 (Cu). SAFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, BacFA = Bacterial fatty acids, TerrFA = Terrestrial fatty acids, Total = Total fatty acids. See Table S3 in **Appendix A1** for statistical evaluation. Figure taken from **Appendix A1**.

Table 1: Mean ($\pm$ 95% CI) metal content in ng/mg dw of chironomid larvae and emerged adults from the control (0 mg/kg dw) and the intermediate sediment metal content (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw). Larvae: n = 9-12; Adults: n = 4. Table taken from **Appendix A1**.

Developmental stage	Cd		Cu	
	0 mg/kg	2 mg/kg	0 mg/kg	50 mg/kg
Larvae	0.61 ($\pm$ 0.10)	32.40 ($\pm$ 7.91)	12.77 ($\pm$ 1.37)	302.71 ($\pm$ 40.88)
Adults	0.14 ($\pm$ 0.05)	5.00 ($\pm$ 5.43)	15.22 ($\pm$ 1.82)	22.30 ($\pm$ 4.15)

Furthermore, the FA profile of chironomids was significantly altered during metamorphosis as a function of metal exposure and sex (Figure 6). Cd exposure significantly affected the FA composition in adults, with SAFA levels increasing by up to 35% in females and omega-3 PUFA levels increasing by up to 50% in males, compared to the respective control. Cu exposure resulted in a dose-dependent decrease of the FA content in larvae relative to the control (significant reduction by up to 30% for MUFA and PUFA). Adults were less negatively affected and even had slightly higher PUFA levels than control adults. Besides, and independent of treatment, adult females were predominately associated with SAFA and total FA, and adult males with PUFA. The flux of FA, including physiologically important PUFA, with emergent adults was reduced by up to 80% (Cd) and 55% (Cu), respectively [**Appendix A1**].

4.1.2 Discussion

Consistent with previous publications (e.g., Kraus et al. 2014a; Schmidt et al. 2013), both metals negatively affected the development and subsequent emergence of chironomids. Larvae accumulated metals (Table 1), which likely caused direct toxic effects, for example, by disrupting the mitochondrial function, thus interfering with organisms' energy supply (Sokolova 2004; Sokolova et al. 2012). At the same time, toxic effects likely induce detoxification and repair mechanisms, increasing the basal energy demand of exposed larvae (Sokolova et al. 2012; Sokolova & Lannig 2008). Since the larval developmental stage is followed by the physiologically complex and energetically demanding metamorphosis in holometabolous insects (Arrese & Soulages 2010; Hodin 2006), direct and indirect toxic effects are ultimately reflected in an impaired emergence (Schmidt et al. 2013). In contrast to some previous studies (reviewed by Sokolova & Lannig 2008), temperature fluctuation (i.e., heat waves) caused

limited additional effects, presumably due to temperature increases being within the acclimation zone of the test organism (Sweeney et al. 2018) and the use of short-term, recurrent instead of constantly increased temperatures (Sokolova & Lannig 2008).

Although we measured the metal body content of chironomids at only one exposure level (2 mg Cd/kg dw and 50 mg Cu/kg dw; Table 1), the emergence data indicate that metal body contents and thus larval mortality and energetic imbalances are likely to increase with increasing exposure levels. While the general trend in the data was similar for both metals, the relative bioaccumulation and metamorphic retention of the non-essential Cd seems to be greater than that of essential Cu in chironomids (Table 1, Krantzberg & Stokes 1989; Timmermans & Walker 1989). Nevertheless, both metals are lost during metamorphosis (Kraus et al. 2014b; Timmermans & Walker 1989), reducing the metal content in adults by about 85% (Cd) and 95% (Cu) compared to larvae. In addition, metal excretion appears to be more efficient at higher exposure levels (DeForest et al. 2007; Kraus et al. 2014b), indicating a relatively low availability for terrestrial relative to aquatic consumers. Accounting for the reduction in the flux of emergent chironomids to adjacent terrestrial systems further reduces the exposure risk for local consumers (e.g., Kraus et al. 2014a; 2021b).

Besides providing a potential route of exposure to metals, emergent aquatic insects are a source of FA, an equally important parameter for the physiological constitution of exposed organisms and their nutritional value for consumers. While both metals can alter the FA profile of chironomid larvae, their effect pattern appears to differ (e.g., the tendency for FA content to increase or decrease under Cd and Cu exposure, respectively), presumably due to different modes of action. Similar to the changes in the metal body content, metamorphosis also evokes cardinal changes in the FA profile of chironomids (Figure 6), resulting in a relative accumulation of FA (Borisova et al. 2016), in turn, increasing or buffering effects of Cd and Cu, respectively. Importantly, the accumulation efficiency was greatest for physiologically valuable PUFA, such as ARA and EPA (two- and six-fold increase, respectively), increasing their relative availability for consumers. Overall, the reduction in chironomid emergence, nevertheless, causes a

substantial decline in the total flux of FA to the terrestrial system by up to 80% (Cd) and 55% (Cu). This is particularly relevant, because chironomids oftentimes dominate aquatic insect communities (Martin-Creuzburg et al. 2017; Raitif et al. 2018; Stenroth et al. 2015). In this context, the sex-specific composition of insect emergence may also be important, as FA profiles of females and males differ markedly (see Figure 6; Scharnweber et al. 2020). Indeed, females are rich in SAFA and have a higher total FA content, whereas males are rich in PUFA (Figure 6). Yet, the higher body weight of females suggests that they can substantially contribute to fluxes of physiologically important PUFA **[Appendix A1]**. Sex-specific predation (Blanckenhorn et al. 2021) or sensitivity towards contaminants (Cavallaro et al. 2017; Sildanchandra & Crane 2000) may therefore affect the pool of FA available to terrestrial consumers. However, the physiological consequences of altered subsidy quality for riparian consumers remain unclear, but are addressed in the second research question.

4.2 RO2: Effects of subsidy quality on terrestrial consumers

4.2.1 Results

The basal resources offered to larvae differed significantly in their FA profiles and were the most and second most important predictor of the FA profile of adult chironomids (RDA1, Figure 7) and spiders (RDA2, Figure 8), respectively. Emergent chironomids and spiders both contained significantly higher levels of omega-6 PUFA, particularly gamma-linolenic acid (GLA, 18:3n-6; about 940% and 720% in chironomids and spiders, respectively), but significantly lower levels of omega-3 PUFA (about 80% and 25% in chironomids and spiders, respectively), such as 18:3n-3 (ALA) and 20:5n-3 (EPA), when the basal resource was Spirulina compared to TetraMin **[Appendix A2]**. Furthermore, sex was an important predictor for the FA profiles of adult chironomids (RDA2, Figure 7) and spiders (RDA1, Figure 8). The presence of aquatic contaminants during larval development had marginal effects on the FA profile of adult chironomids, but substantially reduced the FA content in spiders, particularly in Cu and Bti treatments **[Appendix A2]**. The content of the physiologically important PUFA, 20:4n-6 (ARA) and 20:5n-3 (EPA), decreased by about 30% compared to the control. Female spiders tended to be more strongly affected than male spiders.

Contaminant exposure during larval development near-significantly reduced chironomid emergence compared to the control, particularly in the presence of Cu (reduction by about 40%). We observed no significant effect of basal resource on adult emergence, although emergence decreased by about 15% when larvae were fed Spirulina compared to TetraMin **[Appendix A2]**. However, we offered each spider the same number of female and male adult chironomids at each feeding. While spider wet weights were similar across treatments at the beginning and end of the bioassay, their specific growth rate (SGR) was significantly reduced by 40%-50% relative to the control when chironomids were cultured in the presence of any of the three contaminant groups (Figure 9).

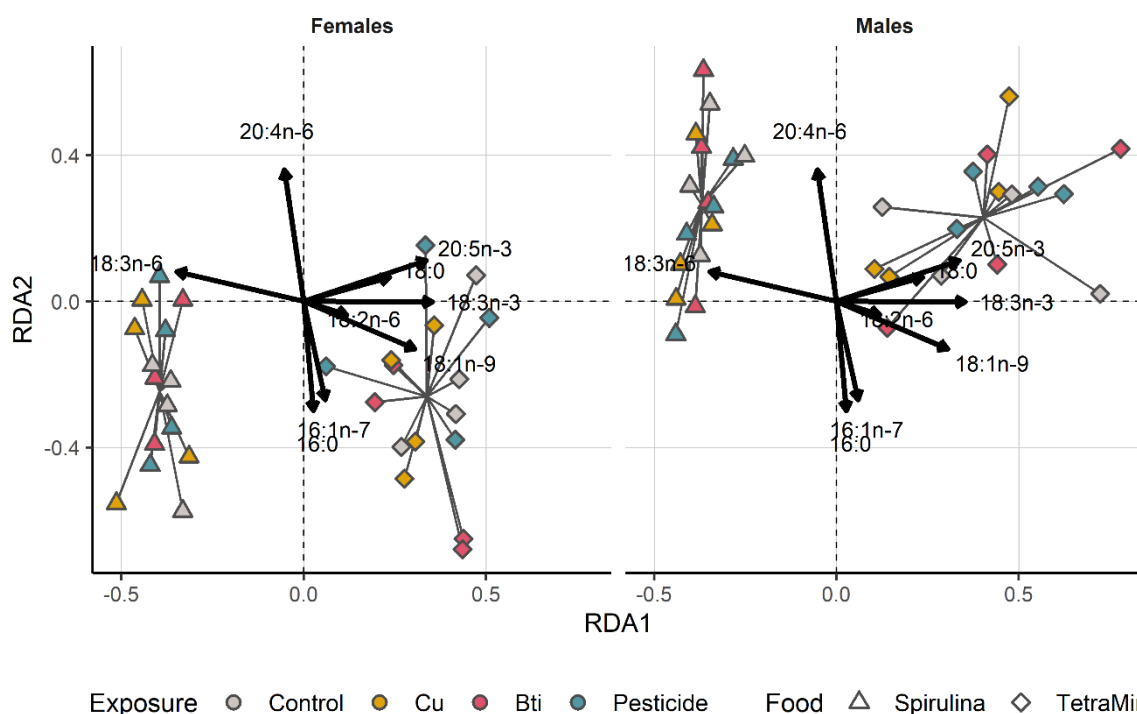


Figure 7: Redundancy analysis (RDA) using FA profiles ($\mu\text{g mg}^{-1} \text{ dw}$) of emergent chironomid females and males cultured under one of eight treatments (i.e., combination of contaminant exposure and basal resource). Basal resources are indicated by triangles (Spirulina) and diamonds (TetraMin®). Color represents larval exposure to control (grey), Cu (yellow), Bti (red), and synthetic pesticides (blue). Scores were calculated in the same ordination space but are separated between females and males in the ordination to avoid cluttering of the figure. Figure taken from **Appendix A2**.

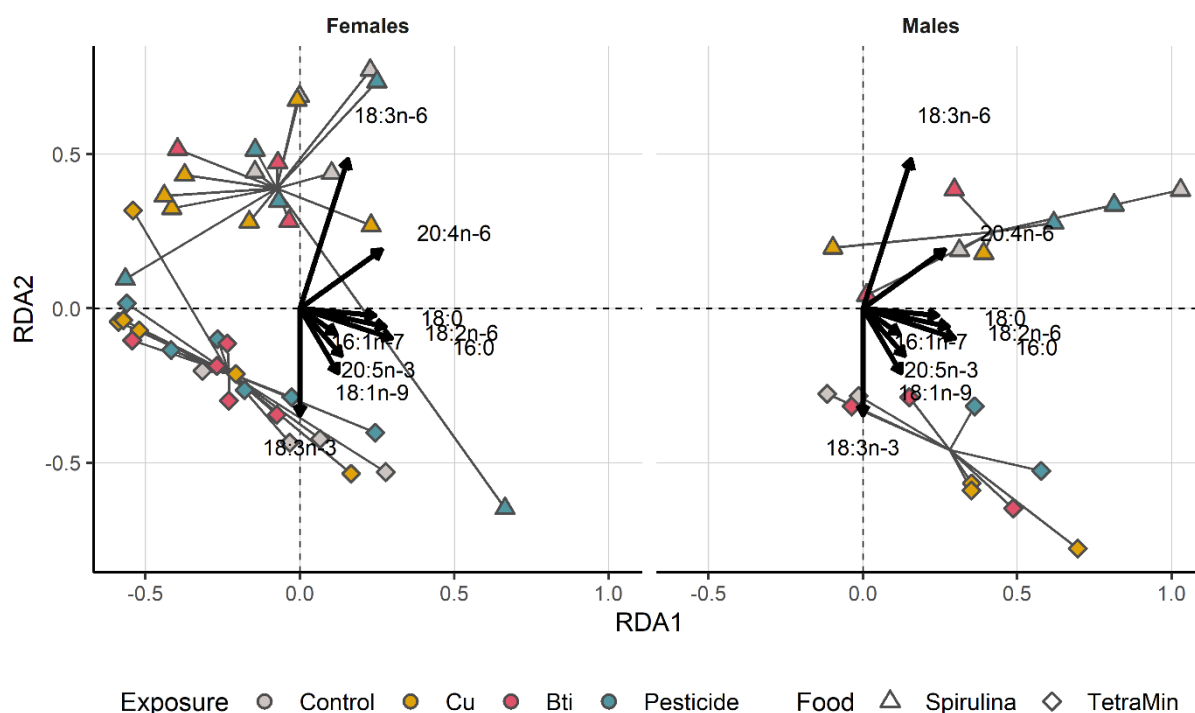


Figure 8: Redundancy analysis (RDA) using FA profiles ($\mu\text{g mg}^{-1} \text{ dw}$) of female and male spiders fed with adult chironomids cultured under one of eight treatments (i.e., combination of chemical stress and basal resource). Basal resources of larvae are indicated by triangles (Spirulina) and diamonds (TetraMin®). Color represents larval exposure to control (grey), Cu (yellow), Bti (red), and synthetic pesticides (blue). Scores were calculated in the same ordination space but are separated between females and males in the ordination to avoid cluttering of the figure. Figure taken from **Appendix A2**.

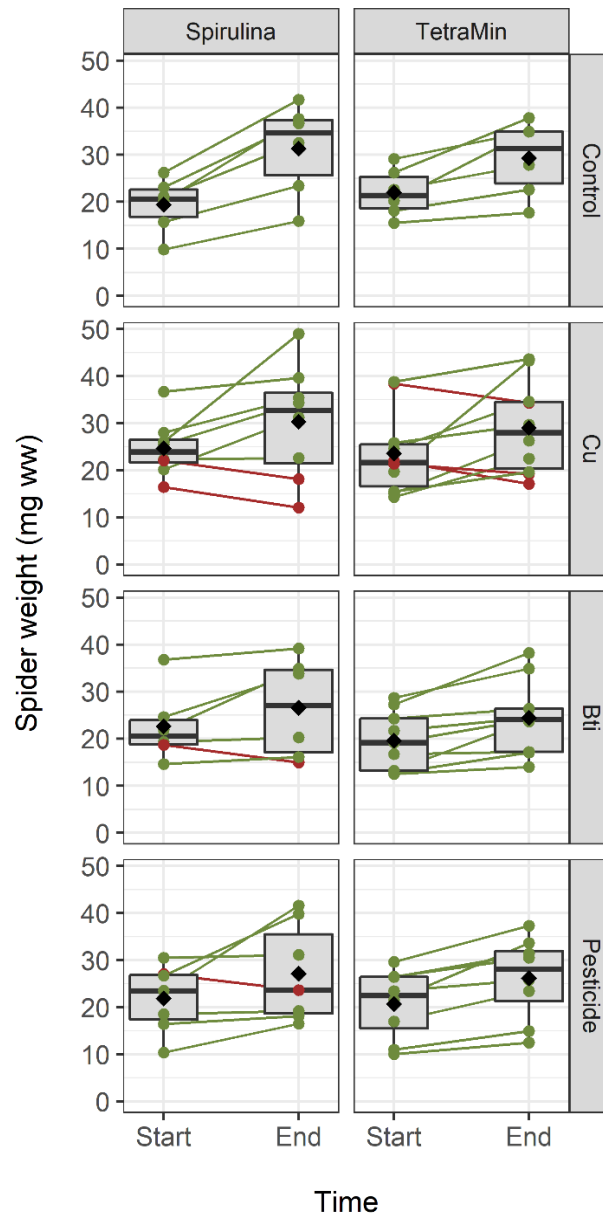


Figure 9: Spider wet weight (mg) at the start and end of the bioassay. Spiders were exclusively fed with adult chironomids cultured under one of eight treatments (i.e., combination of contaminant exposure and basal resource). Lines in boxes are medians, box ends are 25th and 75th percentiles. Whiskers 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. Figure taken from **Appendix A2**.

4.2.2 Discussion

In line with previous studies (Goedkoop et al. 2007; Ohler et al. 2023b; Strandberg et al. 2020), differences in the FA profile of the basal resources were reflected in chironomids, explaining most of the variation in their FA profiles. However, they had less effect on the FA profile of spiders, due to the capacity of both chironomids and spiders to modify dietary FA (Goedkoop et al. 2007; Mathieu-Resuge et al. 2021b; Strandberg et al. 2020). Since the modification of FA is likely more energetically demanding relative to their dietary uptake (Parrish 2009), the

extent to which both chironomids and spiders use this capacity may largely depend on the dietary availability of certain physiologically important FA (Goedkoop et al. 2007; Mathieu-Resuge et al. 2021b; Ohler et al. 2023b).

In contrast to food quality, the FA profile of chironomids remained largely unaffected by any contaminant group, whereas Cu and Bti treatments reduced the content of NLFA, particularly physiologically important 20:4n-6 (ARA) and 20:5n-3 (EPA), in spiders. We did not expect to find changes in the FA profile of spiders in the absence of such effects in its prey. This observation may be explained by the different FA pools that we assessed. The NLFA pool studied in spiders, constitutes an important energy storage in invertebrates (Azeez et al. 2014) and responds relatively fast to changes in food quality (Iverson 2009; Koussoroplis et al. 2014). The overall FA profile studied in chironomids, on the other hand, also contains more stable FA pools such as phospholipid FA (Iverson 2009; Koussoroplis et al. 2014). Since the relative availability of lipid groups may change following contaminant exposure (Arambourou et al. 2020) and FA can differ between lipid groups (Guschina & Harwood 2009), our results raise the question whether the total FA pool is an adequate indicator of FA available to consumers.

Besides, in chironomids, the 'minimal viable weight' required for metamorphosis (Mirth & Riddiford 2007; Nijhout 1975) and the relative accumulation of FA during this process (Borisova et al. 2016) **[Appendix A1]** may have masked potential contaminant-induced effects during the preceding larval development. For spiders, toxic effects through secondary (dietary) poisoning may have contributed to the decline in their NLFA content. In principle, this effect pathway appears to be more relevant for Cu and the pesticide mixture, because the mode of action of Bti is relatively specific to nematoceros dipterans (Becker 1997; Boisvert & Boisvert 2000). Indeed, Cu is known to induce lipid peroxidation (Gaetke & Chow 2003), and may thus directly or indirectly (e.g., through reallocation of NLFA; Koussoroplis et al. 2014) alter the NLFA profile of spiders. Such changes could impair immune function (Fritz et al. 2017) and may have contributed to the decline in their SGR (Marczak & Richardson 2008). However, the decline in spider SGR was similar in all contaminant treatments (reduction by 40%-50%

compared to the control), indicating that changes in spider NLFA profiles cannot solely explain these results. Most pesticides of the mixture were detected in adult chironomids (Roodt et al. 2022) at levels comparable to those reported from the field (Kraus et al. 2021a). Dietary poisoning of spiders may have adversely affected parameters of prey nutritional quality not investigated in this study (e.g., carbohydrates or proteins), or altered the energy allocation in spiders.

Importantly, the physiological changes in riparian spiders (i.e., decline in NLFA content and SGR) were likely triggered by an altered subsidy quality alone, as the chironomid biomass consumed by spiders was, due to our experimental design, fairly constant. However, aquatic contaminants may not only affect subsidy quality, but also its quantity (e.g., Kraus et al. 2014a) **[Appendix A2]**, further exacerbating adverse effects on spider physiology (i.e., growth and NLFA profile). This could, in turn, lead to potential implications in their population dynamics (Uno 2016), particularly because NLFA contents in female spiders were more strongly reduced than in males. Given their central position in riparian food webs, changes in spider density and nutritional quality could cascade onto lower and higher trophic levels in terrestrial systems, respectively.

4.3 RO3: Chronic effects on aquatic insect emergence

4.3.1 Results

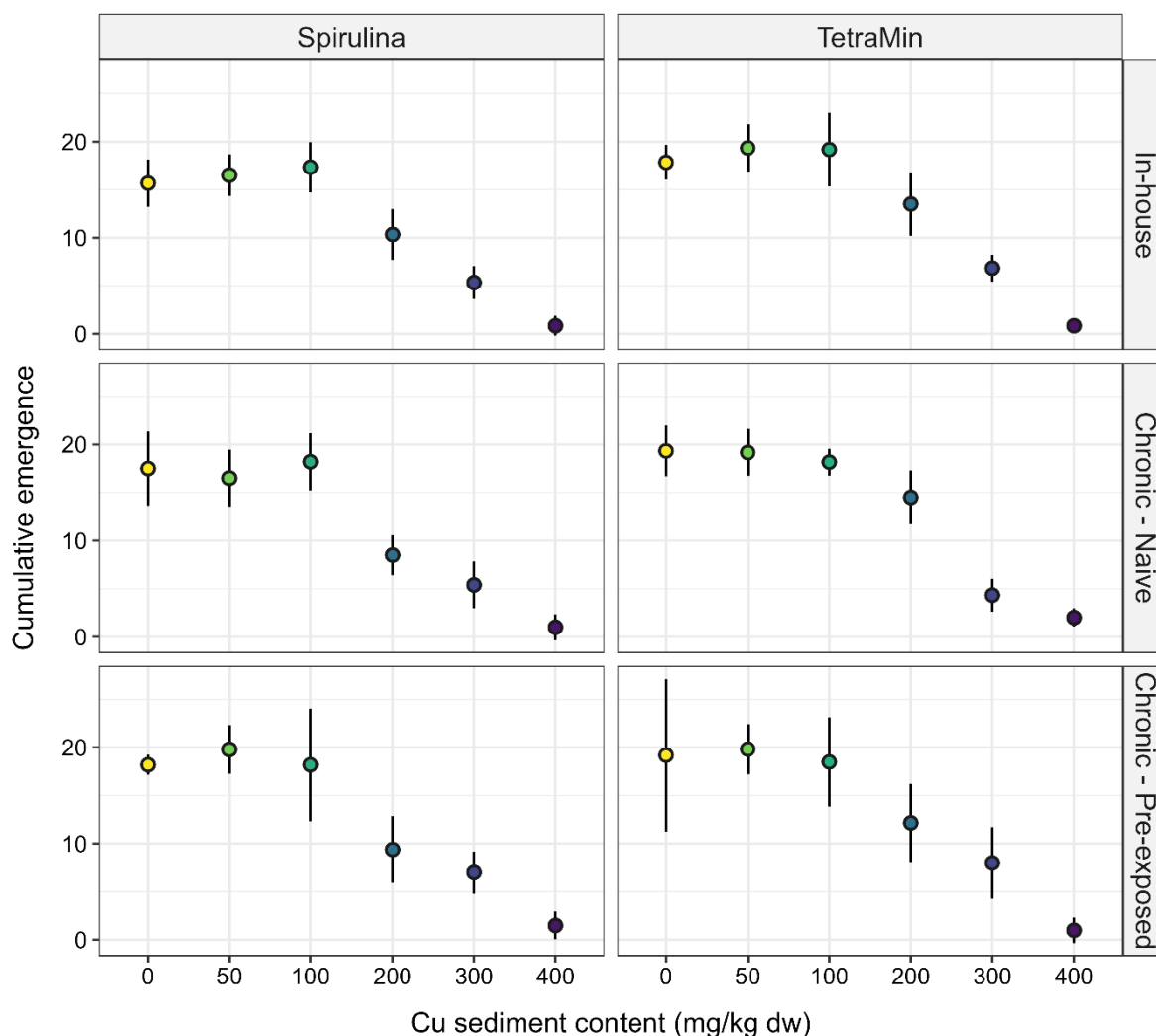


Figure 10: Mean ($\pm$ 95% CI) cumulative number of emerged adult chironomids at Cu sediment contents of 0, 50, 100, 200, 300 and 400 mg/kg dw (colour-coded for ease of comparison). Chironomid sensitivity was assessed for populations from the in-house culture (ST1; at test initiation) or reared in absence or presence of Cu for six months ('Chronic – Naive', 'Chronic – Pre-exposed', respectively) and larvae were fed one of two food sources ('Spirulina' or 'TetraMin'). Figure taken from **Appendix A3**.

Larval exposure to Cu significantly reduced chironomid emergence in a dose-dependent manner by about 40%, 65%, and 90% at Cu sediment contents of 200, 300, and 400 mg/kg dw, respectively (Figure 10). Furthermore, the time until 50% emergence was delayed by up to eleven days (400 mg/kg dw) and female and male dry weight were significantly increased at high Cu exposure levels (up to about 15%) compared to the control [**Appendix A3**]. At 200 mg/kg dw, emergence decreased significantly more in individuals fed Spirulina compared to those fed TetraMin (difference in effect size of about 20%, Figure 10). At 300 mg/kg dw, we

observed fewer emergence in individuals from naïve populations relative to pre-exposed populations (reduction by 85% and 65%, respectively, in female emergence, Figure 10).

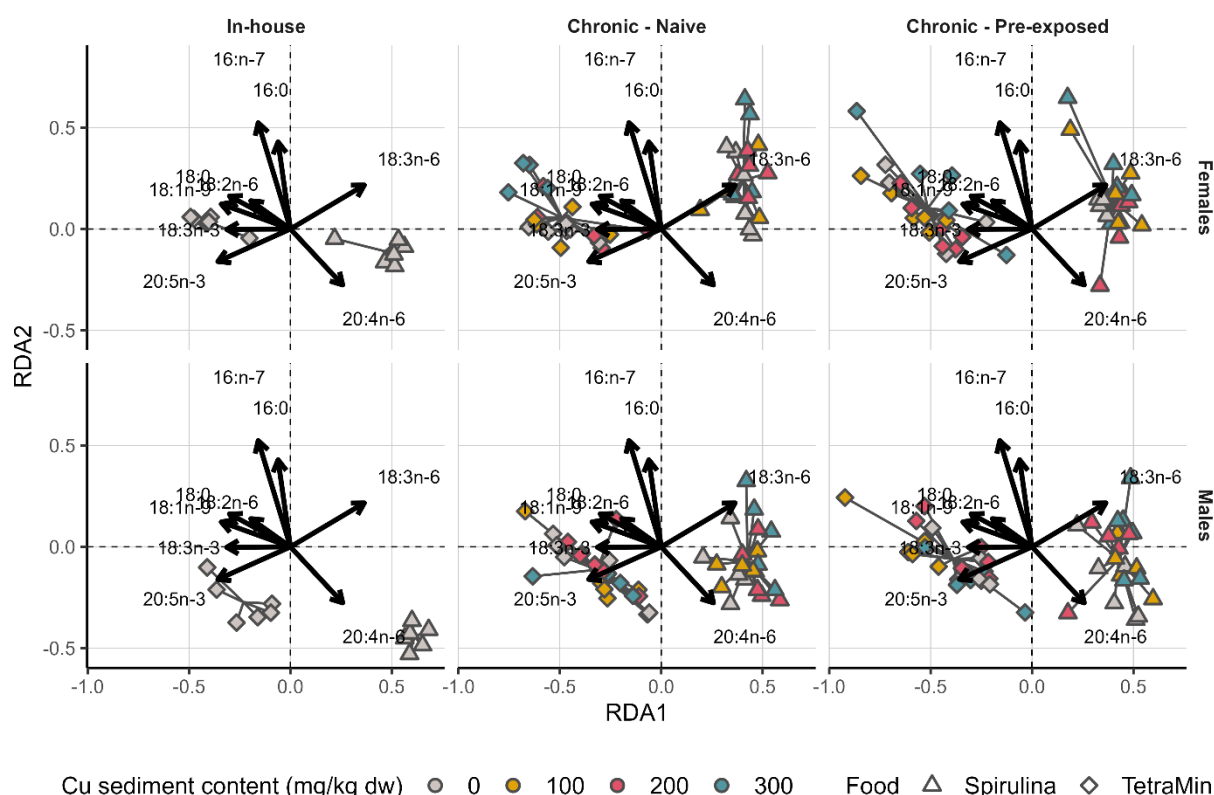


Figure 11: Redundancy analysis using FA profiles (micrograms per milligram dw) of *C. riparius* females and males from 16 treatments (i.e., combination of 'Cu sediment content', 'Food quality', and 'Exposure history') of ST2. Eight treatments were omitted because we did not expect any effects or the emergence was very low. Moreover, we analyzed female and male adults from the control treatments of ST1 (n = 6) to identify potential changes in the FA profiles due to the laboratory conditions. Copper sediment contents are represented by colour; 0 mg/kg dw (grey), 100 mg/kg dw (yellow), 200 mg/kg dw (red), and 300 mg/kg dw (blue). The food offered to *C. riparius* larvae is indicated by triangles (Spirulina) and diamonds (TetraMin). Scores were calculated in the same ordination space but separated between sex and the origin of larvae (i.e., 'In-house', ST1; 'Chronic – Naive' and 'Chronic – Pre-exposed, ST2) in the ordination to improve clarity of the figure. Figure taken from **Appendix A3**.

Food quality (i.e., Spirulina vs. TetraMin) was the most important predictor of the chironomid FA profile, according to the RDA (RDA1, Figure 11). Individuals fed Spirulina contained higher levels of omega-6 PUFA, such as 20:4n-6 (ARA; about 120%) and its precursor 18:3n-6 (GLA, about 3200%), but lower levels of the omega-3 PUFA (about 85%), 20:5n-3 (EPA) and its precursor 18:3n-3 (ALA), MUFA (about 40%) and SFA (about 10%) [**Appendix A3**]. Sex, larval origin (i.e., larvae originating from the in-house culture, or from one of the chronic cultures) and Cu sediment content contributed to a lesser extent to the observed differences in the FA profiles [**Appendix A3**].

4.3.2 Discussion

Larval exposure to Cu likely offset the energy balance of individuals, interfering with their energy supply by disrupting mitochondrial function (Sokolova et al. 2012; Sokolova & Lannig 2008; Stohs & Bagchi 1995; Wallace & Starkov 2000) while at the same time increasing the basal energy demand due to detoxification and repair mechanisms (Calow 1991; Sibly & Calow 1989; Sokolova et al. 2012; Sokolova & Lannig 2008). Toxic effects ultimately translate to an impaired emergence, through reduced larval survival or failure to complete physiologically and energetically demanding subsequent developmental steps (e.g., metamorphosis in holometabolous insects such as chironomids; Schmidt et al. 2013, see also **Appendix A1**). While larvae may be able to efficiently regulate Cu body contents at lower sediment levels (up to 100 mg/kg dw), they may be less able to do so at higher exposure levels (≥ 200 mg/kg dw), explaining the dose-dependent decrease in emergence by about 40%, 65%, and 90%. Even if larvae were still able to cope with Cu-induced toxic effects, the proposed energetic imbalances may have caused a prolonged developmental time, reflected in a delayed emergence. This is, because certain developmental milestones (e.g., surpassing the 'minimal viable weight') must be reached to initiate metamorphosis (Mirth & Riddiford 2007; Nijhout 1975). In the context of reduced survival and prolonged developmental time of chironomids, surviving larvae had more resources available, as the amount of food was corrected for adult emergence but not larval survival, potentially contributing to the increase in adult dry weight.

Besides food availability, its nutritional quality affected how chironomids cope with Cu exposure. This may be due to Spirulina's lower capacity to provide certain nutrients (i.e., physiologically important PUFA; Strandberg et al. 2020), to meet the physiological requirements of chironomids compared to TetraMin. While chironomids are capable of modifying and preferentially retaining FA, this requires an energetic investment relative to the dietary uptake (Goedkoop et al. 2007; Ohler et al. 2023b; Parrish 2009; Strandberg et al. 2020; see also **Appendix A2**). Simultaneous exposure to Cu may thus lead to a failure to divert sufficient energy to modify dietary nutrients or counteract toxic effects of Cu, with adverse implications on adult emergence, as observed at 200 mg/kg dw.

Chironomids from pre-exposed populations were less sensitive to Cu than those from naïve populations at 300 mg/kg dw (reduction in females by about 65% and 85%, respectively), indicating an increased ability to cope with Cu-induced stress. While previous research provides evidence for both genetic adaptation (Miller & Hendricks 1996; Postma et al. 1995a; Postma et al. 1995b) and phenotypic plasticity (Marinković et al. 2012), utilizing population genomic methods to compare the genetic information of naïve and pre-exposed populations could clarify the underlying processes in this study. Regardless, exposure history had overall relatively little effect on chironomid Cu sensitivity, suggesting that it could be associated with a trade-off in individual fitness (Postma et al. 1995b). Furthermore, the experimental duration or the environmentally relevant Cu sediment content (100 mg/kg dw) may have not been sufficient to promote the formation or establishment of traits required for decreased sensitivity to Cu.

In line with previous findings, differences in the basal food sources (i.e., *Spirulina* or TetraMin) were reflected in chironomids (Strandberg et al. 2020, see also **Appendix A2**), and are therefore a strong predictor of the emergence quality. Similarly, differences in the FA profiles between females and males align with previous studies (Meier et al. 2000; Scharnweber et al. 2020, see also **Appendix A1**) and may be linked to distinct life history strategies for each sex (Servia et al. 2006). While Cu sediment content had little effect on the FA profile of chironomids, it appears to be the main cause of reductions in the transport of FA to adjacent terrestrial systems, due to the dose-dependent decrease in emergence success (see also **Appendix A1**). Accordingly, the exposure history of chironomids had only limited effects on their FA profile and overall minor implications for the transport of FA in general and physiologically important PUFA in particular.

While laboratory conditions may have influenced chironomid development to some degree (e.g., difference in the time until 50% emergence between ST1 and ST2), a general shift in chironomids' sensitivity in ST2 did not occur. We therefore expect that our results are biologically relevant and meaningful. Since the emergence of chironomids was mainly

influenced by the Cu content in the sediment, the results indicate a sustained reduction in emergence and thus aquatic-to-terrestrial subsidy at high exposure levels.

5 CONCLUSION AND OUTLOOK

This thesis illuminates the effects of aquatic contaminants on aquatic-to-terrestrial subsidy, focusing on changes in its nutritional quality. It explores these changes primarily in terms of the flux of FA, including physiologically important, but scarce long-chain PUFA, through emergent aquatic insects. It then demonstrates that changes in subsidy quality may affect riparian consumers with yet poorly understood consequences for local food webs.

Contaminants with a relatively high toxicity for aquatic insects at environmentally relevant levels and relatively low metamorphic retention (e.g., metals; Kraus 2019) may primarily affect the quality of aquatic subsidy to adjacent terrestrial systems by reducing the flux of beneficial nutrients such as FA **[Appendix A1]**. Fluxes of FA are reduced via two effect pathways: alteration of (I) the FA profile of aquatic insects and (II) emergent biomass. These findings suggest to consider fluxes of FA, particularly long-chain PUFA, to understand and anticipate implications of aquatic contaminants on aquatic-terrestrial linkages and specifically terrestrial consumers that depend on them **[Appendix A1]**. Contaminants that are more efficiently retained in aquatic insects may also increase (dietary) exposure of terrestrial consumers (Kraus 2019; Kraus et al. 2021a; Previšić et al. 2021; Roodt et al. 2022), with the relative importance of this process being driven by their direct toxic effects (i.e., reduction in the emergent biomass). This implies contaminant-dependent effect pathways modifying the transfer of energy within food webs at the aquatic-terrestrial interface (Kraus 2019) **[Appendix A2]**. However, these may lead to similar physiological consequences for terrestrial consumers, as suggested by a consistent decline in spider growth regardless of the aquatic contaminant group to which their prey was exposed. Importantly, effects on spider growth were likely based on changes in prey nutritional quality, not quantity **[Appendix A2]**, highlighting its importance for terrestrial consumers and supporting previous findings for riparian birds (Twining et al. 2016b; 2019). Moreover, reductions in spider growth may have been partly related to declines in their NLFA content (i.e., energy storage) **[Appendix A2]**. Aquatic contaminants could thus indirectly affect terrestrial food webs through lower spider density, fitness and nutritional quality (Fritz et al. 2017; Pekár 2012; Uno 2016). Understanding and anticipation of contaminant-

induced effects on aquatic subsidy and terrestrial food webs may be complicated by sex-specific differences in FA profiles as well as sensitivity to and load of contaminants (Cavallaro et al. 2017; Roodt et al. 2022; Scharnweber et al. 2020; Wesner et al. 2017) **[Appendix A1 and A2]**. Similarly, effects of environmental factors such as the basal resource quality may interact with those of aquatic contaminants, further modifying subsidy quantity and quality **[Appendix A2 and A3]**. Since aquatic contaminants may be persistent (e.g., metals) or repeatedly enter freshwater systems (e.g., Bti or synthetic pesticides), chronic exposure of aquatic insects is likely to occur. While this can lead to lower sensitivity of exposed organisms (Miller & Hendricks 1996; Postma & Davids 1995; Postma et al. 1995b), insect emergence and thus prey availability may, nevertheless, be strongly reduced **[Appendix A3]**. This points to a sustained alteration in the aquatic-terrestrial linkages which may ultimately re-shape riparian food webs (Henschel et al. 2001; Murakami & Nakano 2002; Poulin et al. 2010; Richardson et al. 2010; Recalde et al. 2020).

This thesis provides a novel perspective on the effects of aquatic contaminants at the aquatic-terrestrial interface. Its findings can therefore be used as a basis for further research on the topic. This may include but is not limited to the use of other insect and spider taxa (e.g., different orders of holo- and hemimetabolous insects) to verify the patterns observed in this thesis. Indeed, FA profiles as well as retention of and sensitivity to contaminants are known to differ between aquatic insect taxa (Martin-Creuzburg et al. 2017; Mathieu-Resuge et al. 2021a; Previšić et al. 2021; Schmidt et al. 2013). While chironomids typically dominate aquatic insects, particularly in anthropogenically affected freshwaters (Schmidt et al. 2013; Stenroth et al. 2015), this could nevertheless contribute to a deeper understanding of contaminant effects. By using female and male individuals it would also be possible to further examine the sex-specific differences with regard to nutritional quality and contaminant sensitivity observed in this thesis and other studies (e.g., Cavallaro et al. 2017; Cordellier et al. 2020; Roodt et al. 2022; Servia et al. 2006). It may also improve the understanding of species-specific requirements, as differences between females and males do not necessarily follow the same pattern **[Appendix A2]**. Moreover, effort should be put in the assessment of the underlying mechanisms with

which different groups of aquatic contaminants affect subsidy quality. In this context, defining 'quality' by two or more parameters (e.g., contaminant retention and beneficial nutrients/elements) and implementing aquatic-terrestrial model food chains may push the scientific understanding of how aquatic contaminants (or other stressors) affect the energy transfer at the aquatic-terrestrial interface. Furthermore, model food chains may provide a link between laboratory and field studies and may aid to explore further indirect effects in the terrestrial food web.

In light of the globally progressing anthropogenic impact on freshwater systems (e.g., climate change and increasing contaminant exposure and nutrient loading; Bernhardt et al. 2017; Millennium Ecosystem Assessment 2005; Paerl & Paul 2012), impacts on the aquatic-terrestrial meta-ecosystem (Loreau et al. 2003) may become more severe in the future. In this context, effects may be increasingly driven by a reduction in the flux of physiologically important long-chain PUFA with emergent aquatic insects, for example, due to increasing pesticide toxicity to aquatic invertebrates (Schulz et al. 2021) and shifts in aquatic primary producer composition reducing the availability of these compounds at the bottom of the food web (Paerl & Paul 2012; Taipale et al. 2016). Understanding and anticipating those changes is therefore a first necessary step to develop efficient measures to protect riparian systems and their communities.

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7 APPENDICES

Appendix A1 and **A2** represent the latest versions submitted to the respective journal, **Appendix A3** is the latest version of the manuscript in preparation.

Appendix A1

Metal Exposure and Sex Shape the Fatty Acid Profile of Midges and Reduce the Aquatic Subsidy to Terrestrial Food Webs

Pietz, Kainz, Schröder, Manfrin, Schäfer, Zubrod, & Bundschuh

Appendix A2

Subsidy quality affects common riparian web-building spiders: consequences of aquatic contamination and food resource

Pietz, Kolbenschlag, Röder, Roodt, Steinmetz, Manfrin, Schwenk, Schulz, Schäfer, Zubrod, & Bundschuh

Appendix A3

Effects of copper, food quality and exposure history on aquatic insect emergence: insights from a multigeneration study (*in preparation*)

Pietz, Röder, Kolbenschlag, Schöndorfer, Schwenk, & Bundschuh

7.1 Appendix A1

Metal Exposure and Sex Shape the Fatty Acid Profile of Midges and Reduce the Aquatic Subsidy to Terrestrial Food Webs

Environmental Science & Technology

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Abstract

Aquatic micropollutants can be transported to terrestrial systems and their consumers by emergent aquatic insects. However, micropollutants, such as metals, may also affect the flux of physiologically important polyunsaturated fatty acids (PUFA). As certain PUFA have been linked to physiological fitness and breeding success of terrestrial consumers, reduced fluxes from aquatic systems could affect terrestrial populations and food webs. We chronically exposed larvae of the aquatic insect *Chironomus riparius* to a range of environmentally relevant sediment contents of cadmium (Cd) or copper (Cu) in a 28-day microcosm study. Since elevated water temperatures can enhance metals' toxic effects, we used two temperature regimes, a control and periodically elevated temperatures (heat waves) reflecting an aspect of climate change. Cd and Cu significantly reduced adult emergence by up to 95% and 45%, respectively, while elevated temperatures had negligible effects. Both metal contents were strongly reduced (~90%) during metamorphosis. Furthermore, the chironomid FA profile was significantly altered during metamorphosis with the factors sex and metal exposure being relevant predictors. Consequently, fluxes of physiologically important PUFA by emergent adults were reduced by up to ~80%. Our results suggest that considering fluxes of physiologically important compounds, such as PUFA, by emergent aquatic insects is important to understand the implications of aquatic micropollutants on aquatic-terrestrial meta-ecosystems.

Key words: aquatic-terrestrial linkage, riparian consumers, PUFA, aquatic insects, *Chironomus riparius*, copper, cadmium

Synopsis: Aquatic micropollutants (i.e., metals) reduce the flux of physiologically important fatty acids from aquatic to terrestrial food webs through adult aquatic insects, while metals are largely lost during metamorphosis.

Introduction

Ecosystems are connected through fluxes of nutrients, energy, and organisms¹. While the quantity of organic carbon fluxes is generally higher from terrestrial to freshwater systems, nutritional quality is considered higher for fluxes in the opposite direction mainly through the emergence of aquatic insects. Indeed, aquatic insects contain several physiologically important fatty acids (FA), which are typically not synthesized by terrestrial invertebrates. Consequently, aquatic insects constitute a central nutritional component in aquatic and particularly terrestrial food webs^{2,3}. In fact, emergent aquatic insects are important prey for terrestrial consumers such as birds and spiders providing them with dietary energy⁴ and essential polyunsaturated fatty acids (PUFA)^{5,6}, such as arachidonic acid (ARA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3), which benefits their physiological condition^{6,7}. However, stressors may disrupt nutritional fluxes to recipient terrestrial consumers.

Anthropogenic activities disturb aquatic ecosystems with micropollutants (i.e., pollutants of anthropogenic origin that occur and cause toxic effects at low concentrations) playing a considerable role⁸⁻¹⁰. Persistent micropollutants, such as metals, might be of particular concern, because they accumulate in sediments¹¹ from where they can be taken up by benthic organisms, including aquatic insect larvae¹². This, in turn, may decrease fitness of these organisms¹³⁻¹⁵. This decrease in fitness of aquatic insect larvae may propagate to their terrestrial life stage with potential alterations of adjacent terrestrial food webs (e.g., alterations in predation pattern)^{16,17}. According to a recently proposed heuristic model¹⁸, effects of aquatic micropollutants may propagate to terrestrial systems via two pathways: (I) changes in the biomass-related flux (i.e., number and body mass of emerging adults) and its timing and (II) shifts in nutritional quality (i.e., biochemical composition and micropollutant accumulation). While the accumulation of micropollutants and their effects on emergence have been increasingly studied in recent years¹⁹⁻²³, effects on the biochemical composition, such as nutritionally valuable PUFA, have been largely ignored²⁴. Since certain PUFA have been linked to somatic growth, body condition, immune responses, and breeding success in terrestrial

consumers^{6,7,25}, ignoring these parameters may lead to incorrect conclusions and hinder the assessment of effects to consumers and riparian food webs. Sex-specific differences in the FA profile of emergent aquatic insects²⁶, due to different life cycles of males and females²⁷, may further improve our understanding and thus prediction of micropollutant-induced effects.

Besides micropollutants, environmental factors such as water temperature affect the development and emergence of aquatic insects²⁸. Furthermore, metals can interact with water temperature²⁹ potentially changing their effects on food webs in general and on aquatic-to-terrestrial subsidies specifically. Elevated water temperatures, which are projected as a consequence of global climate change, tend to enhance metals' toxic effects in aquatic ectotherms²⁹. While interactions have been primarily studied under scenarios with a constantly elevated water temperature²⁹, heat waves, projected to increase as well³⁰, have been studied less frequently.

Here we test how a non-essential (cadmium, Cd) and an essential (copper, Cu) metal in combination with short-term, recurrent temperature increases (heat waves) affect different developmental stages of an aquatic insect. A distinction between non-essential and essential metals was made since the latter is needed for physiological processes (e.g., metal-containing enzymes³¹), suggesting differences in accumulation and toxicity. Moreover, Cd and Cu are important environmental contaminants (i.e., classified as toxic and priority pollutants³²), motivating their selection for this study. *Chironomus riparius* (Diptera: Chironomidae) was chosen as model aquatic insect, because of the larval benthic way of life³³ and widespread occurrence of chironomids in freshwater habitats³⁴. In addition, chironomids are common and abundant emergent aquatic insects and an important prey for many terrestrial consumers³⁵. To provide a comprehensive assessment of the interactive effects between metal exposure and temperature increases, we examined survival and body mass of larvae, number and body mass of emerging adults and their time of emergence. Those data were combined with metal accumulation and metamorphic retention as well as FA profile (i.e., composition and content) in insect larvae and adults.

We hypothesized that metal exposure would reduce the number of emerging adults, body mass at emergence or prolong the development or the time to emergence. Elevated temperatures were expected to enhance toxic effects of both metals. With regard to the nutritional quality, we expected metals to be bioaccumulated in larvae but lost during metamorphosis. Furthermore, metal exposure, metamorphosis (i.e., larva or adult) and sex were hypothesized to affect the FA composition and content in *C. riparius*. Finally, we hypothesized that metal exposure would affect fluxes of FA while those of metals themselves would be strongly reduced compared to their availability before metamorphosis.

Methods

Test organisms

Chironomus riparius larvae used in this study were obtained from an in-house culture maintained according to the OECD Guideline 218³⁶. Larvae were reared in crystallizing dishes (6x18x26 cm) with fine quartz sand as substrate at 20±1°C, a light/dark cycle of 16/8 hours and under continuous aeration. The culture medium (Elendt "M4"³⁷ was renewed once a week and larvae were fed *ad libitum* with ground fish flake food (TetraMin®, Tetra GmbH, Melle, Germany). Two crystallizing dishes were placed in a mesh cage (48x39x32 cm) to allow swarming, copulation and deposition of egg masses by adults. Four days prior to the start of each bioassay, 20 egg masses were placed in three separate crystallizing dishes (700 mL) filled with 500 mL culture medium. Crystallizing dishes were gently aerated and a small amount of finely ground fish flakes was provided as food source for freshly hatched larvae³⁸. At the start of the experiment, eighteen first instar larvae (24 to 48 hours old) were carefully and randomly allocated to replicates using glass pipettes. We introduced a total of 3600 larvae (450 per treatment), assuming a sex ratio of 1:1³⁶, although some deviation from this assumption for individual replicates may occur.

Sediment preparation, spiking and aging

Sediment was formulated and spiked with Cd ($\text{CdCl}_2 \times \text{H}_2\text{O}$) or Cu ($\text{CuSO}_4 \times 5\text{H}_2\text{O}$) according to OECD Guideline 218³⁶. Briefly, artificial sediment was composed of 75 % quartz sand, 20 % kaolinite, 5 % moss peat, and calcium carbonate (0.05 – 0.1 %). One day prior to the sediment spiking, the respective amount of all constituents (10.5, 2.8, 0.7 and 0.014 kg) was thoroughly homogenized by hand for 30 min. Afterwards, the required amounts (1.8 kg or 3.4 kg) of artificial sediment for each treatment were spiked in plastic buckets by addition of 500 mL of Cd or Cu solution and subsequent thorough mixing. Deionized water (500 mL) served as control. All stock solutions were prepared according to the sediment dry weight (dw) and resulted in five nominal contents of 0 mg/kg, 0.7 mg/kg, 2 mg/kg, 6 mg/kg and 18 mg/kg for Cd and 0 mg/kg, 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg for Cu. These environmentally relevant metal contents were chosen based on field data (measured contents range from 0.3 to 33 mg Cd/kg dw and 1.0 to 3100 mg Cu/kg dw^{11,39}) and published effect thresholds for *C. riparius* (e.g., Marinković et al. 2011⁴⁰).

The spiked sediment (~80 g wet weight) was evenly distributed to the test vessels (250-mL glass beakers) leading to a sediment layer of 1.5 cm. Subsequently, approximately 6 cm of overlaying water (i.e., 200 mL of SAM-5S medium⁴¹) was added ensuring a volumetric ratio of sediment to overlying water phase of 1:4⁴¹. SAM-5S medium was applied instead of the culture medium (i.e., Elendt “M4”) since the latter contains the metal complexing agent EDTA making it inadequate for the present study. All test vessels were prepared 31 (Cd) and 32 (Cu) days prior to the addition of chironomids assuming equilibrium conditions between environmental media^{36,42} at the start of the bioassay (i.e., introduction of larvae). Test vessels were maintained at $20 \pm 1^\circ\text{C}$ with a light/dark cycle of 16/8 hours, a light intensity between 500 and 1000 lux and under gentle aeration (i.e., one to a few bubbles per second) during the equilibration period.

Bioassays

Two 28-day bioassays, following a 3×2 factorial test design, were set up, one for Cd and one for Cu: chironomids being exposed to three metal contents (0, 2, 18 mg Cd/kg or 0, 50, 200 mg Cu/kg) at a constant temperature of 20°C or under short-term water temperature increase

to 24°C. For the latter, the ambient temperature was increased by 4°C three times for two to three days each (Figure 1). The magnitude of temperature simulates a mild heat wave scenario⁴³. Furthermore, we included two additional metal contents (i.e., 0.7, 6 mg Cd/kg or 25, 100 mg Cu/kg) under the constant temperature regime to facilitate the establishment of dose-response relationships (Figure 1).

Each treatment (i.e., combination of metal content and temperature regime) consisted of 25 replicates with test organisms and two additional replicates without test organisms used for sediment metal analysis. Test conditions largely followed the equilibration period, while aeration was provided after the initial 24 hours to facilitate the settlement of larvae during the first day. Larvae were fed every second day with ~0.3 mg ground fish flake food (TetraMin®, Tetra GmbH, Melle, Germany) for the first 10 days and ~0.6 mg per larvae and day afterwards³⁶.

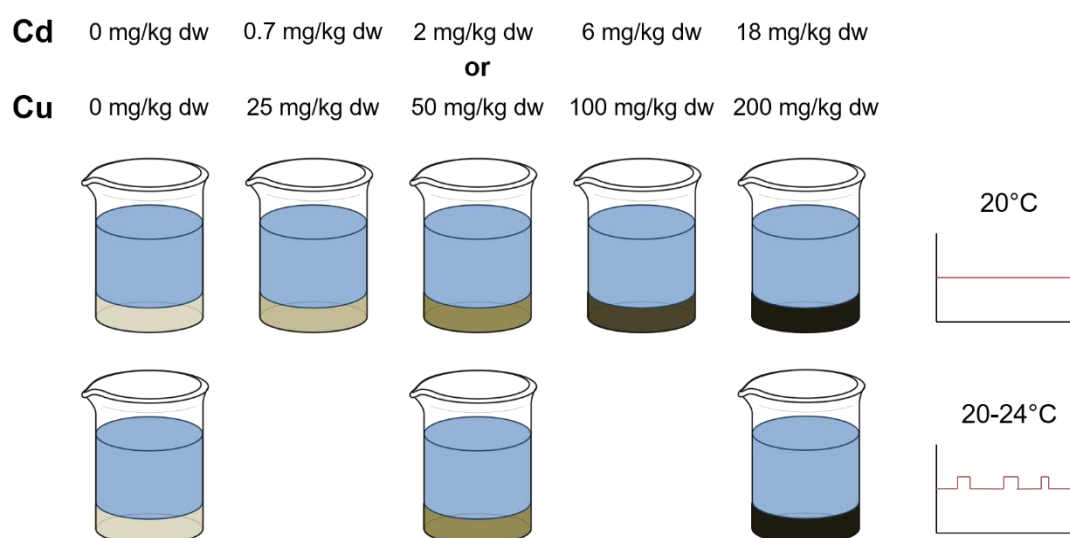


Figure 7: Scheme illustrating the eight treatments (i.e., combination of metal exposure and temperature regime) of each bioassay. Each treatment consisted of 25 replicates with *C. riparius* (not shown) and two additional replicates without test organisms. Both bioassays lasted for 28 days.

To investigate the effects of treatments on the development of *C. riparius*, replicates were removed from each bioassay at Day 7 (n = 4-5), Day 11 (n = 2-4), Day 15 (n = 2-3), Day 19 (n = 2) and Day 23 (n = 1-2). The difference in replication was due to the anticipated larval body mass at each time point, ensuring the availability of sufficient biomass for subsequent analyses. Larvae of each replicate were kept in freshly prepared medium for 24 h so that

metals and metalloids would desorb from organisms' surfaces and the organisms purge their gut (cf. Schaller et al. 2011⁴⁴). To avoid cannibalism a small amount of ground fish flake food was added. After 24 hours, larvae were flash-frozen and stored at -20°C. The number of emerged adults (males and females) was checked twice a day (morning and evening). Emerging adults were sorted by sex (males being identified by their plumose antennae³⁶), flash frozen and stored at -20°C. Shortly after the end of each bioassay, all chironomids were freeze-dried (Christ ALPHA 1-2 LD plus) and stored in a desiccator for at least 15 minutes prior to being weighed to the nearest 0.01 mg (Mettler Toledo XA DualRange) to assess treatment-related effects on larval and adult dry mass.

Metal analysis

Test organisms

Metal contents were analysed in larvae and emerged adults from the control and the intermediate metal content (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw) under both temperature regimes. Microwave (MARS 6; CEM, Germany) assisted digestion of the samples was performed in 10 mL reaction vessels (MARSPRESS PFA; CEM, Germany) with 0.5 mL redistilled (DST-1000; Savillex, USA) nitric acid (HNO₃, 65% w/w, for analysis, Merck GmbH, Germany) at 180°C with 15 min ramp time and 30 min hold time. The digestions were filled up to 10 mL with ultrapure water (ELGA LabWater, Germany), transferred to 15 mL centrifuge tubes (VWR catalyst Laboratory Services, USA, cleaned in 1.3% nitric acid for >24 h rinsed with ultrapure water) and stored at 4°C until analyses. Quantification of Cd and Cu was performed by inductively coupled plasma-triple-quadrupole mass spectrometry (ICP-QQQ-MS; Agilent 8900 series, Agilent Technology, Japan). The elements were measured by application of a collision cell with O₂ (30% O₂ content) or He (gas flow 4.6 mL/min) as cell gas. The four certified reference materials SPS-SW1, SPS-SW2 (both Spectrapure Standards AG, Norway), NW-TM-15.3 and NW-TM.24.4 (both Environment and Climate Change Canada, CRM Sales, Canada) and 3 internal standards (⁷⁴Ge, ¹⁰³Rh, ¹⁸⁵Re) were included in the measurements for validation purposes. The recoveries of the certified contents were in the

range of $\pm 10\%$. The maximum accepted variation of the internal standards was 20% from the initial value. Details on the measurements are given in Table S1.

Sediment

Since sediment metal contents remain fairly constant over time⁴⁵, we analysed samples of each sediment metal contents from the start of the bioassays. Sediments were dried at 105°C for 48 h, ground, homogenised and weighed to 0.5 (± 0.1) g in a digestion vessel. Microwave (MARS Xpress; CEM, Germany) assisted digestion of samples was performed in 5 mL aqua regia (3 HNO₃ + HCl, 65% and 32% supra-pure assay, respectively, Carl Roth, Germany) at 175°C with 15 min ramp time and 60 min hold time. The digestions were 1:20 diluted with ultrapure water (ELGA LabWater, Germany) and stored at 4°C until analysis. Quantification of Cd and Cu was performed by inductively coupled plasma optical emission spectroscopy (ICP-OES; 720, Agilent, Germany) at a wavelength of 214.439 nm and 327.395 nm, respectively. The relative spiking efficiency for Cd and Cu ranged from 75% to 104% and 96% to 111%, respectively (Table S2). Although measured contents deviated more than 20% from the nominal value at the highest Cd content (18 mg/kg dw), spiking was reasonably accurate justifying the use of nominal contents throughout the manuscript.

Fatty acid analysis

We analysed FA in larvae as well as female and male adults (called 'Stage' in the following) of *C. riparius* from the control, the intermediate (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw) and the highest metal contents (i.e., 18 mg Cd/kg dw and 200 mg Cu/kg dw) under both temperature regimes. FA analysis was performed according to Fink (2013)⁴⁶ with slight modifications as described by Konschak et al. (2020)⁴⁷. Briefly, lipids from freeze-dried samples (approx. 3-5 mg dry weight) were extracted in 5 mL of a chloroform/methanol mixture (2:1) overnight at -20°C. Samples were dried under a stream of nitrogen and FAs were subsequently transesterified to fatty acid methyl esters (FAMES) with 3N methanolic HCl (Sigma-Aldrich, St. Louis, US). FAMES were liquid-liquid extracted using isohexane and subsequently analysed via gas chromatography with flame-ionization detection (GC-FID; Trace GC Ultra, Thermo

Fisher Scientific, Bremen, Germany) using a SupelcoTM SP-2560 capillary GC column (100m, 0.25mm i.d., 0.20 µm film thickness) and helium (1 mL/min) as carrier gas. For each sample, FAME were identified based on retention times using FAME standards (37-component FAME Mix, Supelco 47885-U; Bacterial Acid Methyl Ester Mix, Supelco 47080-U) and quantified using external standard calibration. FA contents of each sample were blank corrected, extrapolated to the total volume and normalized to the dry weight of the sample (i.e., µg FA/mg dw).

Data analysis

Information on statistical tests (e.g., *p*-values and test statistics) for each endpoint are provided in Table S3. To analyse whether larval development and adult emergence differed between treatments, we used null hypothesis significance testing (NHST). Effects of our explanatory variables (i.e., 'Content' and 'Temperature') and their interaction on the number and body mass of surviving larvae as well as number and body mass of emergent adults were evaluated by implementation of two-way analysis of variance (ANOVA). Data properties (i.e., normality and homogeneity of variance) were checked by visual inspection of model residuals. For non-parametric data, data were rank-transformed before performing a two-way ANOVA and the test of Brunner-Dette-Munk⁴⁸ was used if the assumption of homoscedasticity was violated. In case of significant multi-level explanatory variables or interactions one-way ANOVA followed by Student's *t*-test was used if assumptions were met. If assumptions were not met, Wilcoxon rank sum test or Welch's *t*-test were used. Bonferroni correction for multiple comparisons was used if more than two factor levels were tested. To assess whether the time to emergence of *C. riparius* adults differed among treatments, we ran non-linear regression analysis using the emergence data as response and treatment as explanatory variable. For this, several dose-response models (Weibull, log-normal and log-logistic models) were fitted to the cumulative emergence data, expressed as percent relative to the total number of emerged individuals at the end of each bioassay. The best fitting model (mostly Weibull models) was selected based on the lowest Akaike's Information Criterion score. Finally, the time by which 50% of larvae had emerged was derived from the best-fitting model for each treatment and subsequently

compared across treatments (cf. Bundschuh et al. 2019⁴⁹). To examine the quantity of emerging *C. riparius* adults and their time to emergence, only replicates that were kept until the end of the 28-day bioassay period were included in the analysis (n = 8-11).

Metal contents were compared between chironomids of the same developmental stage (i.e., larva or adult) cultured in the presence and absence of metals and between developmental stages cultured under the same conditions (i.e., control or metal exposure) using NHST as described above. Metal flux by emergent adults was estimated by multiplication of the mean metal content (ng/mg dw) with their mean dry mass (in mg) and the mean emergence.

Multivariate data (i.e., FA) were standardized to the maximum of each individual FA or FA group (i.e., Saturated FA, SAFA; Monounsaturated FA, MUFA; etc.) to reduce the discriminatory power of dominant FA or FA groups. To visualise the variation in *C. riparius* FA composition, we applied non-metric multidimensional scaling (NMDS) with Euclidean distances. Permutational multivariate analysis of variance (PERMANOVA⁵⁰) on Euclidean distances from standardized data was used to test the effects of 'Content', 'Temperature' and 'Stage' (i.e., larvae, female adults, male adults) on the variation of *C. riparius* FA composition. The assumption of homogeneous within-group dispersion was fulfilled in the Cu bioassay, but violated in the Cd bioassay as dispersion was higher for larvae than for female and male adults. Both the unknown proportion of the two sexes and the instar of larvae may have contributed to the higher dispersion in this group. However, PERMANOVA is relatively insensitive to heterogeneity of dispersion in balanced designs⁵¹ as in this case. In addition, the visualisation of the variation in *C. riparius* FA composition, indicates that differences mainly exist between female and male adults and different Cd contents within both groups. The results of the PERMANOVA can therefore be primarily attributed to location rather than dispersion effects. A similarity percentages routine (SIMPER) was performed to identify FA groups that contributed most to the observed differences in the statistically significant explanatory variables. Differences in the FA content ($\mu\text{g}/\text{mg dw}$) between chironomids exposed to different metal levels were evaluated using univariate analyses, as described above for other endpoints.

To estimate the flux of FA, mean FA content in $\mu\text{g}/\text{mg}$ dw was calculated for female and male adults in the control, the intermediate and highest metal content and multiplied with the mean body mass (in mg) and the mean emergence from the respective group.

All statistical analyses and figures were performed with R Version 4.1.0 for Windows⁵². The significance level α was set at 0.05. Effect sizes (in percent) were calculated by normalizing the mean of the respective endpoint of each treatment to the control's mean.

Results

C. riparius development and emergence

Larvae. The percentage of larvae recovered from the sediment as well as their body mass were significantly lower (up to 46% and 76%, respectively), at the highest Cd content (18 mg/kg dw), whereas the temperature regime exhibited no significant influence on both endpoints (Tables S3, S4 and S5). Cu also had a significant negative effect on the percentage of recovered larvae (up to 20% at 200 mg/kg dw) and body mass was up to 32% reduced (200 mg Cu/kg dw), though this was not statistically significant (Tables S3, S4 and S5).

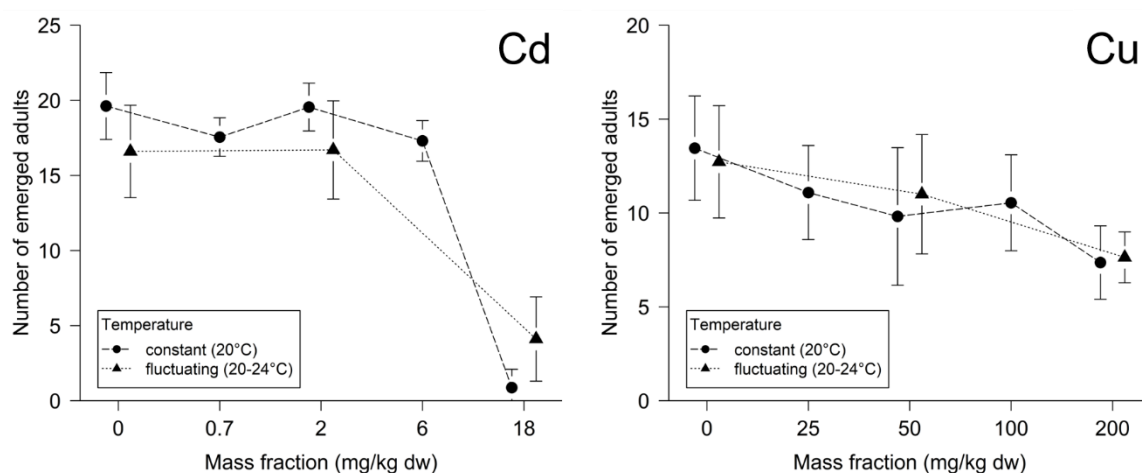


Figure 8: Mean ($\pm$ 95% CI) number of emerged *C. riparius* adults exposed to 0, 0.7, 2, 6 and 18 mg Cd/kg dw and 0, 25, 50, 100 and 200 mg Cu/kg dw crossed with two temperature regimes (20°C; circles and 20-24°C; triangles) within the 28-day bioassay period. Lower confidence limits of the number of emerged adults below zero (18 mg/kg, 20°C) are of no biological meaning and were therefore set to zero. See Table S3 for statistical evaluation.

Adults. The number of emerged *C. riparius* adults was significantly reduced by 95% at a Cd content of 18 mg/kg dw and constant temperature (Figures 2 and S1, Table S3). At lower Cd

contents the number of emerged adults was similar across treatments. Temperature fluctuation partly alleviated this effect, although adult emergence varied considerably (Figure 2). Furthermore, the median time until 50% emergence was delayed by up to ~7 days and male and female emergence occurred simultaneously at 18 mg Cd/kg dw (Table S6), whereas at lower contents the time difference was only one to two days with males emerging before females (2 and 6 mg Cd/kg dw; Tables S3 and S6). In addition, larval exposure to 18 mg Cd/kg dw resulted in a significant increase in male body mass of 24% compared to the control (Tables S3 and S7). A similar albeit statistically non-significant increase (13%) was observed for females. At lower Cd contents the adult body mass was similar across treatments. The highest Cu content (200 mg/kg dw) resulted in a statistically significant reduced number of emerged chironomids (up to 45%, Figure 2, Table S3). Assuming an initial larval sex ratio of 1:1, female emergence was more strongly reduced than that of males at all Cu contents (Figure S2, Table S3). Neither Cu nor temperature regime affected the time to emergence (Tables S3 and S6). We observed a reduction in male body mass under temperature fluctuation only in presence of Cu (Tables S3 and S7). Female body mass was significantly reduced by Cu (up to 13% at 200 mg/kg dw) and temperature fluctuation (up to 12% at 50 mg/kg dw; Tables S3 and S7).

Table 1: Mean ($\pm$ 95% CI) metal content in ng/mg dw of *C. riparius* larvae and emerged adults from the control (0 mg/kg dw) and the intermediate sediment metal content (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw). Larvae: n = 9-12; Adults: n = 4.

Developmental stage	Cd		Cu	
	0 mg/kg	2 mg/kg	0 mg/kg	50 mg/kg
Larvae	0.61 ($\pm$ 0.10)	32.40 ($\pm$ 7.91)	12.77 ($\pm$ 1.37)	302.71 ($\pm$ 40.88)
Adults	0.14 ($\pm$ 0.05)	5.00 ($\pm$ 5.43)	15.22 ($\pm$ 1.82)	22.30 ($\pm$ 4.15)

***C. riparius* nutritional quality**

Larvae reared in metal-spiked sediments accumulated metals, resulting in mean body contents of 32 ng Cd/mg dw and 303 ng Cu/mg dw and thus a significant 53-fold and 23-fold increase, respectively, compared to the control (Tables 1 and S3). Mean metal body contents of *C. riparius* adults were 5 ng Cd/mg dw and 22 ng Cu/mg dw and thus significantly lower relative to larval life stages (85% and 93%, respectively; Tables 1 and S3). Nonetheless, adult metal

contents were still 36-fold and 1.5-fold higher than the control for Cd and Cu, respectively (Tables 1 and S3).

Cd had a significant effect on the chironomid FA composition, with increases in PUFA and SAFA being the strongest contributors to observed differences according to SIMPER analysis (Figure 3, Tables S3 and S8). At 18 mg Cd/kg dw, we observed an increase in the content of SAFA in adult females and omega-3 PUFA in adult males by up to 36% and 53%, respectively, compared to the control (Table 2). The FA content in larvae exposed to Cu was always lower compared to the control and effects tended to increase in a dose-dependent manner (Table 2). Reductions were statistically significant at 200 mg Cu/kg dw and as high as 33% (MUFA) and 30% (PUFA) (Tables 2 and S3). By contrast, adults were less negatively affected and even had slightly higher levels of PUFA (females and males 7% and 15%, respectively) than control adults (not significant; Table 2). In both bioassays, the content of the physiologically most important PUFA (e.g., ARA and EPA) and their precursors, linoleic acid (LIN, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3) were similarly affected as the group of PUFA (Table S9). Overall, LIN contributed by far the most to the PUFA content, while DHA was only found at trace levels (Table S9). Temperature regime did not affect FA composition and content.

Furthermore, and independent of the bioassay, the FA composition differed with 'Stage' (i.e., larvae, adult females, adult males; Table S3). While larvae were positively correlated with SAFA, MUFA and bacterial FA (BacFA), male and female adults were predominately associated with PUFA, particularly omega-6 PUFA, as well as SAFA and total FA, respectively (Figure 3). Accordingly, the FA content was always highest in females (Table 2).

Fluxes of metals and FA to the terrestrial system

Combining our data on the mean metal content in *C. riparius* adults with the total emergence biomass, the Cd flux was increased 35-fold compared to the control, while the Cu flux was similar to the control (Table S10). Overall, the Cd flux was nevertheless lower than that of Cu (Table S10). The flux of FA as mass per individual was increased (~50% in females and 70-

80% in males; Table S11) at the highest Cd content but strongly reduced (~80%; Table S11) when accounting for the total number of emergent adults. At 200 mg Cu/kg dw, the FA flux as mass per adult female or male tended to be reduced (up to ~25%, SAFA; 200 mg/kg dw) compared to the control (Table S11), except for PUFA. Accounting for the total number of emergent adults, the total FA flux (including PUFA) was reduced by ~30% and ~55% at the intermediate and highest Cu sediment content, respectively (Table S11).

Table 2: Mean ($\pm$ 95% CI) FA content in $\mu\text{g}/\text{mg}$ dw of *C. riparius* larvae and emerged females and males from the control (0 mg/kg dw), the intermediate sediment metal content (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw) and the highest sediment metal content (i.e., 18 mg Cd/kg dw and 200 mg Cu /kg dw). FA contents that differ significantly from the respective control are printed in bold. SAFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, BacFA = Bacterial fatty acids, TerrFA = Terrestrial fatty acids, Total = Total fatty acids.

Cd bioassay									
FA group	Larvae			Female adults			Male adults		
	0	2	18	0	2	18	0	2	18
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
SAFAs	7.38	9.31	9.34	11.38	10.92	15.45	6.59	7.13	8.58
	($\pm$ 2.32)	($\pm$ 4.71)	($\pm$ 2.15)	($\pm$ 1.35)	($\pm$ 2.83)	($\pm$ 2.27)	($\pm$ 1.25)	($\pm$ 0.97)	($\pm$ 0.85)
MUFAs	7.68	8.41	8.45	8.42	6.92	10.94	5.71	6.12	7.73
	($\pm$ 1.90)	($\pm$ 4.71)	($\pm$ 1.56)	($\pm$ 1.12)	($\pm$ 1.68)	($\pm$ 2.29)	($\pm$ 0.95)	($\pm$ 1.02)	($\pm$ 0.75)
PUFAs	2.53	2.67	2.42	2.71	2.62	3.51	3.43	4.43	5.05
	($\pm$ 0.74)	($\pm$ 0.76)	($\pm$ 0.55)	($\pm$ 0.43)	($\pm$ 0.65)	($\pm$ 0.60)	($\pm$ 0.71)	($\pm$ 0.51)	($\pm$ 0.55)
omega-3	0.36	0.42	0.45	0.41	0.37	0.44	0.38	0.42	0.58
	($\pm$ 0.13)	($\pm$ 0.19)	($\pm$ 0.07)	($\pm$ 0.09)	($\pm$ 0.09)	($\pm$ 0.20)	($\pm$ 0.06)	($\pm$ 0.08)	($\pm$ 0.11)
omega-6	2.17	2.25	1.97	2.31	2.25	3.06	3.05	4.01	4.46
	($\pm$ 0.66)	($\pm$ 0.66)	($\pm$ 0.55)	($\pm$ 0.39)	($\pm$ 0.56)	($\pm$ 0.57)	($\pm$ 0.68)	($\pm$ 0.44)	($\pm$ 0.64)
Total FAs	17.88	20.72	20.75	22.74	20.74	30.35	15.86	17.83	21.69
	($\pm$ 4.50)	($\pm$ 7.02)	($\pm$ 3.20)	($\pm$ 2.68)	($\pm$ 4.70)	($\pm$ 4.95)	($\pm$ 2.63)	($\pm$ 2.17)	($\pm$ 1.73)
Cu bioassay									
FA group	Larvae			Female adults			Male adults		
	0	50	200	0	50	200	0	50	200
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
SAFAs	4.56	3.89	3.18	11.15	10.58	9.41	6.81	6.60	5.69
	($\pm$ 1.65)	($\pm$ 1.45)	($\pm$ 1.65)	($\pm$ 0.97)	($\pm$ 2.06)	($\pm$ 2.00)	($\pm$ 1.22)	($\pm$ 1.13)	($\pm$ 0.66)
MUFAs	5.43	4.11	3.64	6.86	6.88	6.41	5.51	5.82	5.33
	($\pm$ 1.28)	($\pm$ 1.03)	($\pm$ 1.01)	($\pm$ 0.57)	($\pm$ 0.73)	($\pm$ 1.57)	($\pm$ 1.10)	($\pm$ 0.63)	($\pm$ 0.80)
PUFAs	1.94	1.55	1.36	3.06	3.28	3.28	3.98	4.14	4.59
	($\pm$ 0.39)	($\pm$ 0.39)	($\pm$ 0.34)	($\pm$ 0.68)	($\pm$ 0.52)	($\pm$ 0.76)	($\pm$ 0.65)	($\pm$ 0.50)	($\pm$ 0.67)
omega-3	0.29	0.24	0.20	0.63	0.57	0.50	0.65	0.50	0.57
	($\pm$ 0.10)	($\pm$ 0.10)	($\pm$ 0.10)	($\pm$ 0.21)	($\pm$ 0.14)	($\pm$ 0.13)	($\pm$ 0.18)	($\pm$ 0.10)	($\pm$ 0.12)
omega-6	1.65	1.31	1.16	2.42	2.71	2.78	3.33	3.64	4.02
	($\pm$ 0.35)	($\pm$ 0.32)	($\pm$ 0.27)	($\pm$ 0.50)	($\pm$ 0.44)	($\pm$ 0.66)	($\pm$ 0.53)	($\pm$ 0.47)	($\pm$ 0.61)
Total FAs	12.27	9.80	8.35	21.47	21.16	19.48	16.59	16.83	15.91
	($\pm$ 2.77)	($\pm$ 2.58)	($\pm$ 2.78)	($\pm$ 1.69)	($\pm$ 2.37)	($\pm$ 4.07)	($\pm$ 2.67)	($\pm$ 1.78)	($\pm$ 1.72)

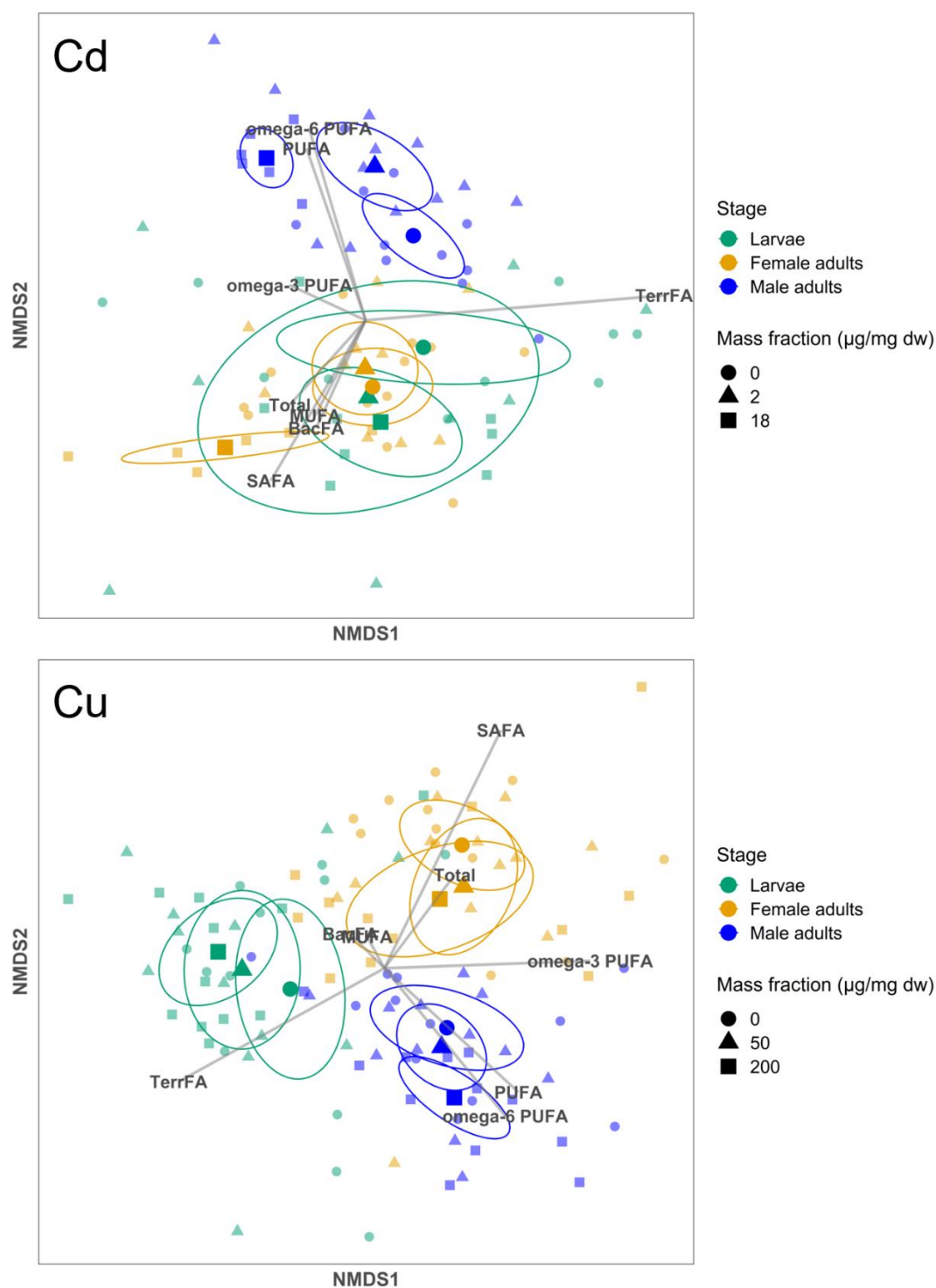


Figure 9: Non-metric multidimensional scaling (NMDS) plot for the FA composition ($\mu\text{g/mg dw}$) of *C. riparius* from the Cd and Cu bioassay: Larvae (green), Female adults (yellow), Male adults (blue). Larvae were exposed to sediment metal contents of 0 (circle), 2 or 50 (triangle) or 18 or 200 (square) mg/kg dw . Centroids and 95% confidence ellipses are shown for each group. NMDS stress value was 0.145 (Cd) and 0.098 (Cu). SAFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, BacFA = Bacterial fatty acids, TerrFA = Terrestrial fatty acids, Total = Total fatty acids. See Table S3 for statistical evaluation.

Discussion

C. riparius development and emergence

In line with our hypothesis, we observed negative effects of both metals on the larval development and either the number of emerging adults, body mass at emergence or a prolonged time until emergence. Sediment metal exposure likely increased the basal energy demand, due to cellular detoxification or repair mechanisms^{29,53}. This is partly supported by our FA analysis, as larval FA content was significantly reduced in the presence of Cu (Table 2; not for Cd though). Moreover, metals affect energy supply, for example, by interfering with mitochondrial ATP production^{53,54}. Even though larvae may have been partly able to compensate for this energetic imbalance, these effects may have had implications in physiologically complex and energetically demanding processes such as metamorphosis^{55,56} reflected by impairments in emergence. Since emergence of aquatic insects is a function of larval survival and physiological fitness^{57,58}, endpoints related to adult emergence were more sensitive than those related to larval development: larval survival, for instance, was reduced by up to 46% and 20% (Table S4), while emergence was reduced by 95% and 45% at the highest Cd and Cu content, respectively (Figure 2).

Furthermore, Cd substantially delayed adult emergence and aligned emergence times of males and females (i.e., offset protandry) at the highest content (Table S6). This, in turn, may have negative consequences for reproduction due to reduced mating success and outbreeding^{34,59}. Besides, the prolonged developmental period in combination with increased larval mortality observed at this Cd content likely drove the increase in adult body mass (Table S7). This may be explained by reduced competition for food and space for survivors^{60,61} since the amount of available food was independent from the number of surviving organisms over the course of the experiment and larval interactions (e.g., competition for resources) may have been reduced⁶¹.

Short-term, recurrent temperature increases, mimicking heat waves, caused limited additional effects on the larval development of *C. riparius* contradicting our hypothesis and some earlier studies with aquatic ectotherms (as reviewed by Sokolova & Lannig 2008²⁹). This may be related to the fact that most studies targeting the response of aquatic ectotherms to a temperature rise have been employing constantly increased values instead of fluctuations²⁹. Moreover, the temperature increase employed in the present study appeared to be within the thermal acclimation zone of this species⁶². In the present study, temperature fluctuation reduced female body mass independent of Cu (Table S7), a pattern that may be explained by a tendency for smaller sizes at higher temperatures²⁸. Male body mass was reduced by temperature fluctuation only in the presence of Cu, which may be related to synergistic effects leading to, for example, higher metal bioavailability and impaired mitochondrial function²⁹. The lack of an effect in combination with Cd may be explained by temperature increases inducing the production of chaperon heat shock proteins, which was shown to reduce Cd toxicity in *Artemia*⁶³. In support of Pestana et al. (2016)⁶³, adult emergence under fluctuating temperature and at the highest Cd level began five days earlier compared to the constant temperature regime. These observations point to a faster development²⁸ of tolerant individuals or avoidance of unfavorable environmental conditions⁶² ultimately leading to a higher adult emergence compared to the constant temperature regime, albeit still at a very low level compared to the control (-75%; Figure 2).

***C. riparius* nutritional quality**

Consistent with our hypothesis, metals were accumulated by larvae but largely lost during metamorphosis, although Cd was more readily accumulated and retained. Larvae reared at 2 mg Cd/kg or 50 mg Cu/kg accumulated mean metal contents of 32 ng Cd/mg dw and 303 ng Cu/mg dw, however, the relative bioaccumulation of the non-essential Cd was much greater compared to the essential Cu (~53-fold for Cd, ~23-fold for Cu, Table 1). This is in accordance with previous findings highlighting the ability of chironomid larvae to regulate Cu but not Cd accumulation⁶⁴. A similar trend was observed in adults, with metal contents being 36-fold (Cd)

and 1.5-fold (Cu) higher than in the control (Table 1), suggesting incomplete elimination⁶⁵, though contents varied considerably for Cd. Besides, both metals are lost during metamorphosis, reducing metal contents in adults by 85% (Cd) and 93% (Cu) relative to their larval stage, which is in line with previous studies^{19,65}.

Besides the accumulation and metamorphic retention of metals, FA are an additional and equally important parameter characterising the physiological constitution of exposed organisms and their nutritional value for consumers. As expected, metamorphosis, adult sex and metal exposure affected the FA profile of *C. riparius*, although effects of Cd and Cu differed. In line with the accumulation of Cu in larval bodies and the adverse effects on their development, larval FA content was reduced in a dose-dependent manner (Table 2). Likely, energy-demanding cellular protection and detoxification mechanisms were fuelled by the break-down of energy reserves, such as lipids^{29,66}. Moreover, the accumulation of reactive oxygen species (ROS), a common mechanism under Cu exposure⁶⁷, may have caused lipid peroxidation^{66–68}. In contrast to Cu, FA content in Cd-exposed larvae was comparable to or higher than (~25% for SAFA and omega-3 PUFA, not significant) the control (Table 2). Similarly, Arambourou et al. (2020)³⁹ observed positive effects of metal exposure on important components of storage and membrane lipids, which are generally characterized by a high share of SAFA and PUFA, respectively, following metal exposure of *C. riparius*. These results were explained by a reduced energy expenditure through decreased respiration rates and protection mechanisms against lipid peroxidation that involve the redistribution of PUFA into storage lipids^{68,39}. At the same time, Cd seems to be a potent disruptor of cellular bioenergetic processes⁶⁶ and may exert endocrine disruptive effects⁶⁹ altering cellular metabolism. These include changes in the lipid metabolism and adipogenetic processes^{70,71}, as documented for zebrafish⁷², potentially contributing to increases in FA levels observed in this study.

After metamorphosis, a relative accumulation of FA was observed increasing or buffering the effect of Cd and Cu, respectively, pointing towards a potentially unique capability of chironomids^{73,74}. The accumulation efficiency in *C. riparius* was greatest for PUFA (Table 2).

The content of physiologically important ARA and EPA was increased up to a factor of two and six, respectively, compared to larvae (Table S9). As documented for mosquitos^{73,75}, both PUFA are closely associated with the ability to fly, suggesting that the enrichment in ARA and EPA may enable or benefit chironomid swarming and thus mating success³⁴. Additionally, increased PUFA levels likely benefit terrestrial consumers by increasing the availability of physiologically important FA²⁵.

Fluxes of metals and FA to the terrestrial system

In line with our hypothesis, metal exposure can be expected to affect fluxes of FA by emergent aquatic insects. Metal fluxes varied for Cd and Cu, but were relatively low compared to the availability before metamorphosis. At the respective intermediate sediment metal content, total flux of Cu (214 ng) was comparable to the control (194 ng), while total flux of Cd (92 ng) was substantially higher compared to the control (3 ng; Table S10). Furthermore, as mentioned above, mean Cd contents in adults varied strongly (Table 2). At higher metal exposure, metal excretion appears to be more efficient^{19,76}, which in combination with a reduced flux of insect biomass (Figure 2) to adjacent terrestrial systems reduces the exposure risk for local consumers^{17,20}.

At the same time, the drastic reductions in aquatic insect emergence at environmentally relevant metal levels¹¹ cause a decline in the total FA flux to the terrestrial system by ~80% (Cd; Tables S11 and S12). While the overall fluxes are certainly important to understand a more general impact of stressors, considering sex-specific properties could be relevant particularly in situations of sex-specific predation⁷⁷ or sensitivity (Figure S2). In fact, FA profiles of adult *C. riparius* females and males differed markedly, supporting findings for another chironomid²⁶ and a mayfly species⁷⁸. Due to different life cycles^{27,79}, females are predominately associated with SAFA and have a higher total FA content while males are characterised by higher PUFA levels (Figure 3, Table 2), indicating, for example, higher requirements in terms of their flying ability. Nonetheless, the higher female body mass suggests a substantial contribution to PUFA fluxes to the terrestrial system (Tables S11 and S12). Consequently, the

higher sensitivity of females to Cu drives the observed reduction in FA fluxes (~50%) at field relevant levels (Figure S2, Tables S11 and S12). Since aquatic insect communities are oftentimes dominated by chironomids^{5,80,81}, they are likely important drivers of micropollutant-induced effects across aquatic-terrestrial ecosystems.

In more general terms, metals and micropollutants with similar properties (i.e., moderate to high toxicity at environmentally relevant levels and low to moderate metamorphic retention) may therefore primarily affect terrestrial consumers by changes in the flux of critical nutrients such as FA. Micropollutants that are (more) efficiently retained during metamorphosis, however, may not only change the FA profile but also increase exposure of terrestrial consumers to these micropollutants⁸². How changes in the nutritious quality (micropollutants vs beneficial nutrients & elements) interact in their implications for terrestrial consumers remains unclear particularly when considering the sex specific differences of emergent aquatic insects regarding their FA profile, load of^{23,83} and sensitivity to micropollutants.

Overall, both metals strongly reduced the number of emerging adults (up to 95%) with females being more susceptible than males. Crossing metal exposure with short-term, recurrent temperature increases caused limited additional stress. As both metals were accumulated by larvae but mostly lost (~90%) during metamorphosis, metal-induced effects on the nutritional quality of emerging aquatic insects are dominated by alterations in their biochemical composition: our study presents the first evidence that the FA profile of aquatic insects is affected by (I) the exposure to micropollutants (non-essential and essential metals), (II) metamorphosis, and (III) sex. Accordingly, FA flux to terrestrial consumers may be influenced via two effect pathways: direct alterations of the FA profile as well as (sex-specific) alterations in the prey availability. Given that several physiologically important PUFA are mostly synthesized in the aquatic environment², terrestrial consumers appear to be particularly vulnerable to such changes. Therefore, our findings highlight the need to consider the biochemical composition of aquatic insects and changes therein to better understand and

anticipate implications of aquatic micropollutants on riparian terrestrial consumers and more generally on aquatic-terrestrial meta-ecosystems¹.

Supporting Information. Details on ICP-QQQ-MS measurements, Nominal and measured metal contents, Details on statistical analysis, Mean percentage of larvae recovered from each treatment, Mean body mass of larvae, Median time until 50% adult emergence, Mean adult body mass, SIMPER analysis on FA groups, Mean FA content, Estimate of the metal and FA flux, Mean number of emergent females and males

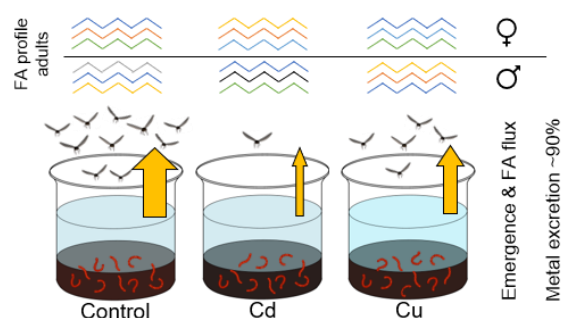
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Graphical Abstract



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Supporting material for Appendix A1

Table S1: Details on ICP-QQQ-MS measurements.

Certified reference materials	¹¹⁴ Cd [O ₂]			⁶³ Cu [He]		
	Reference value (µg/L)	Measured concentration (µg/L)	Recovery (%)	Reference value (µg/L)	Measured concentration (µg/L)	Recovery (%)
SPS SW 1	0.50	0.498	99.6	20.00	20.166	100.8
	0.50	0.521	104.2	20.00	18.268	91.3
	0.50	0.525	105.0	20.00	18.113	90.6
SPS SW 2	2.50	2.381	95.2	100.00	100.216	100.2
	2.50	2.431	97.2	100.00	92.579	92.6
	2.50	2.466	98.6	100.00	91.168	91.2
TM15.3	13.10	12.800	97.7	17.20	17.181	99.9
	13.10	13.033	99.5	17.20	16.059	93.4
	13.10	12.936	98.7	17.20	15.898	92.4
TM24.4	3.98	4.073	102.3	6.37	6.260	98.3
	3.98	4.305	108.2	6.37	5.875	92.2
	3.98	4.270	107.3	6.37	5.736	90.0

Table S2: Nominal and measured ($\pm$ 95% CI) metal contents (mg/kg dw) in sediments and relative spiking efficiency ($\pm$ 95% CI; in %) of the Cd and Cu bioassay. Measured contents that deviate more than 20% from the respective nominal value are printed in bold. Data are based on measurements of three technical replicates per metal content.

Bioassay	Content (mg/kg dw)		Relative spiking efficiency (%)
	Nominal	Measured ($\pm$ 95% CI)	
Cd	0	0.136 (0.113 – 0.159)	-
	0.7	0.621 (0.593 – 0.649)	88.71 (84.71 – 92.71)
	2	2.075 (1.884 – 2.266)	103.75 (94.20 – 113.30)
	6	5.378 (4.486 – 6.270)	89.63 (74.77 – 104.50)
	18	13.431 (10.591 – 16.271)	74.62 (58.84 – 90.39)
Cu	0	2.052 (1.723 – 2.381)	-
	25	25.452 (23.603 – 27.301)	101.81 (94.41 – 109.20)
	50	48.221 (41.417 – 55.025)	96.44 (82.83 – 110.05)
	100	110.683 (99.166 – 122.200)	110.68 (99.17 – 122.20)
	200	201.312 (186.290 – 216.334)	100.66 (93.15 – 108.17)

Table S3: Statistical analysis for all investigated endpoints.

Bioassay	Endpoint	Factor	d.f.1	SS / d.f.2	R ²	F-value	p-value	Test
Cd	Larvae – number	Con	43188	2		30.272	< 0.001	Two-way ANOVA
		Temp	711	1		0.997	0.321	
		Con:Temp	833	2		0.584	0.560	
		Residuals	57066	80				
	Larvae – weight	Con	1.983	75.679		30.489	< 0.001	Brunner- Dette-Munk test
		Temp	1.000	75.679		0.128	0.722	
		Con:Temp	1.983	75.679		0.162	0.849	
		Residuals						
	Emergence – total	Con	1.974	35.264		57.244	< 0.001	Brunner- Dette-Munk test
		Temp	1.000	35.264		3.380	0.074	
		Con:Temp	1.974	35.264		3.515	0.041	
		Residuals						
	Emergence – females	Con	1.853	33.236		53.883	< 0.001	Brunner- Dette-Munk test
		Temp	1.000	33.236		3.278	0.079	
		Con:Temp	1.853	33.236		5.219	0.012	
		Residuals						
	Emergence – males	Con	1.775	34.322		42.723	< 0.001	Brunner- Dette-Munk test
		Temp	1.000	34.322		0.029	0.866	
		Con:Temp	1.775	34.322		1.572	0.223	
		Residuals						
	Time – total	Con					sig.	CI-testing
		Temp					sig.	
	Time – females	Con					sig.	CI-testing
		Temp					sig.	
	Time – males	Con					sig.	CI-testing
		Temp					sig.	
	Weight – females	Con	1.763	9.601		2.546	0.133	Brunner- Dette-Munk test
		Temp	1.000	9.601		1.670	0.227	
		Con:Temp	1.763	9.601		2.524	0.135	
		Residuals						
	Weight – males	Con	2	0.294		23.862	< 0.001	Two-way ANOVA
		Temp	1	0.002		0.300	0.586	
		Con:Temp	2	0.001		0.085	0.919	
		Residuals	65	0.400				
	Metal accumulation	Larvae					< 0.001	Welsh's <i>t</i>
		Adults					0.065	Welsh's <i>t</i>
		Larvae – Adults (exposed)					< 0.001	Welsh's <i>t</i>
	FA composition (FA groups)	Con	2	1.293	0.073	4.287	0.001	PERMANOVA
		Temp	1	0.122	0.007	0.806	0.503	
		Stage	2	4.804	0.270	15.924	0.001	
		Con:Temp	2	0.124	0.007	0.411	0.915	
		Con:Stage	4	0.714	0.040	1.183	0.279	
		Temp:Stage	2	0.518	0.029	1.718	0.118	
		Con:Temp:Sta ge	4	0.432	0.024	0.716	0.757	
		Residuals	65	9.805	0.550			
		Total	82	17.812	1.000			
	Total content	FA	Con	2	307.74	5.479	0.006	Three-way ANOVA
		Temp	1	0.46	0.017	0.898		
		Stage	2	502.34	8.944	< 0.001		
		Con:Temp	2	87.44	1.557	0.218		
		Con:Stage	4	183.08	1.630	0.177		
		Temp:Stage	2	80.93	1.441	0.244		
		Con:Temp:Sta ge	4	42.57	0.379	0.823		
		Residuals	67	1881.47				
		SAFA content	Con	2	73.05	3.962	0.024	Three-way ANOVA
		Temp	1	0.39	0.043	0.837		
		Stage	2	361.30	19.597	< 0.001		
		Con:Temp	2	43.01	2.333	0.105		
		Con:Stage	4	42.62	1.156	0.338		
		Temp:Stage	2	15.94	0.865	0.426		
		Con:Temp:Sta ge	4	12.84	0.348	0.845		
		Residuals	67	617.63				
	MUFA content	Con	2	40.946		4.721	0.012	Three-way ANOVA
		Temp	1	1.065		0.246	0.622	

		Stage	2	70.823		8.166	< 0.001	
		Con:Temp	2	2.795		0.322	0.726	
		Con:Stage	4	33.474		1.930	0.116	
		Temp:Stage	2	8.429		0.972	0.384	
		Con:Temp:Stage	4	8.470		0.448	0.744	
		Residuals	67	290.550				
	PUFA content	Con	2	5.741		3.911	0.025	Three-way ANOVA
		Temp	1	0.020		0.028	0.868	
		Stage	2	42.331		28.838	< 0.001	
		Con:Temp	2	1.017		0.693	0.504	
		Con:Stage	4	7.759		2.643	0.041	
		Temp:Stage	2	3.755		2.558	0.085	
		Con:Temp:Stage	4	2.659		0.906	0.466	
		Residuals	67	49.175				
Cu	Larvae – number	Con	3078.9	2		6.280	0.003	Two-way ANOVA
		Temp	1030.5	1		4.204	0.044	
		Con:Temp	670.3	2		1.367	0.261	
		Residuals	17649.7	72				
	Larvae – weight	Con	1.981	67.617		1.985	0.146	Brunner-Dette-Munk test
		Temp	1.000	67.617		0.439	0.510	
		Con:Temp	1.981	67.617		0.038	0.962	
		Residuals						
	Emergence – total	Con	2	344.03		10.162	< 0.001	Two-way ANOVA
		Temp	1	0.97		0.057	0.812	
		Con:Temp	2	10.03		0.296	0.745	
		Residuals	60	1015.64				
	Emergence – females	Con	2	114.394		11.169	< 0.001	Two-way ANOVA
		Temp	1	3.409		0.666	0.418	
		Con:Temp	2	3.909		0.382	0.684	
		Residuals	60	307.273				
	Emergence – males	Con	2	68.58		3.712	0.030	Two-way ANOVA
		Temp	1	0.74		0.080	0.778	
		Con:Temp	2	1.48		0.080	0.923	
		Residuals	60	554.18				
	Time – total	Con					n.s.	CI-testing
		Temp					n.s.	
	Time – females	Con					n.s.	CI-testing
		Temp					n.s.	
	Time – males	Con					n.s.	CI-testing
		Temp					n.s.	
	Weight – females	Con	2	0.245		3.496	0.036	Two-way ANOVA
		Temp	1	0.262		7.503	0.008	
		Con:Temp	2	0.015		0.209	0.812	
		Residuals	62	2.169				
	Weight - males	Con	2	0.018		1.345	0.266	Two-way ANOVA
		Temp	1	0.034		5.116	0.026	
		Con:Temp	2	0.05073		3.807	0.026	
		Residuals	82	0.54642				
	Metal accumulation	Larvae					< 0.001	Welsh's <i>t</i>
		Adults					0.007	Welsh's <i>t</i>
		Larvae – Adults (exposed)					< 0.001	Welsh's <i>t</i>
	FA composition (FA groups)	Con	2	0.600	0.019	1.799	0.114	PERMANOVA
		Temp	1	0.132	0.004	0.792	0.445	
		Stage	2	13.944	0.448	41.819	0.001	
		Con:Temp	2	0.249	0.008	0.746	0.547	
		Con:Stage	4	0.527	0.017	0.790	0.630	
		Temp:Stage	2	0.220	0.007	0.659	0.631	
		Con:Temp:Stage	4	0.466	0.015	0.698	0.688	
		Residuals	90	15.004	0.482			
	Total content	Total	107	31.141	1.000			
		Con	2	88.26		2.589	0.081	Three-way ANOVA
		Temp	1	1.64		0.096	0.757	
		Stage	2	2033.75		59.652	< 0.001	
		Con:Temp	2	32.19		0.915	0.404	
		Con:Stage	4	38.68		0.567	0.687	
		Temp:Stage	2	1.29		0.038	0.963	

	Con:Temp:Stage	4	78.15	1.146	0.340	
	Residuals	90	1534.22			
SAFA content	Con	2	37.19	3.496	0.035	Three-way ANOVA
	Temp	1	1.79	0.336	0.563	
	Stage	2	774.77	72.825	< 0.001	
	Con:Temp	2	16.33	1.535	0.221	
	Con:Stage	4	1.68	0.079	0.989	
	Temp:Stage	2	3.70	0.348	0.707	
	Con:Temp:Stage	4	42.39	1.992	0.102	
	Residuals	90	478.74			
MUFA content	Con	2	11.845	2.160	0.121	Three-way ANOVA
	Temp	1	0.893	0.326	0.570	
	Stage	2	97.573	17.792	< 0.001	
	Con:Temp	2	2.832	0.516	0.598	
	Con:Stage	4	11.953	1.090	0.366	
	Temp:Stage	2	0.119	0.022	0.979	
	Con:Temp:Stage	4	2.195	0.200	0.938	
	Residuals	90	246.783			
PUFA content	Con	2	0.175	0.108	0.898	Three-way ANOVA
	Temp	1	1.510	1.866	0.175	
	Stage	2	125.457	77.508	< 0.001	
	Con:Temp	2	0.460	0.284	0.754	
	Con:Stage	4	4.742	1.465	0.220	
	Temp:Stage	2	1.561	0.964	0.385	
	Con:Temp:Stage	4	1.246	0.385	0.819	
	Residuals	90	72.839			

Con = Content, Temp = Temperature, sig. = significant, n.s. = not significant

Table S4: Mean ($\pm$ 95% CI) percentage of larvae recovered from each treatment on days seven, eleven, 15, 19 and 23 of the Cd and Cu bioassay. Eighteen larvae were originally defined as 100%, but this was adjusted as adults began to emerge. Negative CI are not displayed as they are of no biological meaning. Percentages of larvae recovered from the sediment that differ significantly are printed in bold.

Bioassay	Temp.	Con (mg/kg)	Day 7 (n = 4-5)	Day 11 (n = 2-4)	Day 15 (n = 2-3)	Day 19 (n = 2)	Day 23 (n = 1-2)	Average (n = 12-16)
Cd	20°C	0	103.3 (90.80 – 115.9)	111.1 (98.6 – 123.6)	100.0 (100.0 – 100.0)	99.1 (0.0 – 341.7)	0.0 (-)	97.1 (79.7 – 114.5)
		0.7	105.6 (85.5 – 107.8)	105.6 (93.1 – 118.1)	131.3 (0.0 – 528.3)	98.4 (0.0 – 259.8)	0.0 (0.0 – 0.0)	94.0 (70.5 – 117.5)
		2	107.8 (87.9 – 127.7)	116.7 (109.5 – 123.9)	94.8 (0.0 – 240.4)	120.0 (0.0 – 374.1)	100.0 (-)	109.7 (100.7 – 118.6)
		6	100.0 (88.1 – 112.0)	105.6 (83.9 – 127.2)	99.4 (0.0 – 345.6)	108.3 (0.0 – 426.0)	40.0 (-)	98.4 (85.2 – 111.6)
		18	18.9 (0.0 – 46.3)	50.0 (13.2 – 86.8)	61.1 (37.2 – 85.0)	69.4 (0.0 – 316.5)	108.8 (0.0 – 370.4)	52.1 (33.6 – 70.7)
	20-24°C	0	94.4 (80.7 – 108.2)	105.6 (79.5 – 131.6)	88.1 (27.6 – 148.6)	90.0 (0.0 – 725.3)	33.3 (-)	91.7 (75.2 – 108.2)
		2	101.1 (93.6 – 108.7)	101.4 (92.9 – 109.9)	80.4 (0.0 – 408.9)	80.4 (0.0 – 408.9)	-	95.3 (85.0 – 105.6)
		18	25.6 (11.3 – 39.9)	47.2 (7.4 – 87.1)	74.1 (58.1 – 90.0)	86.1 (0.0 – 192.0)	68.6 (43.7 – 93.5)	53.0 (38.9 – 67.2)
Cu	20°C	0	92.2 (73.0 – 111.5)	108.3 (2.5 – 214.2)	111.1 (111.1 – 111.1)	100.0 (0.0 – 269.4)	88.8 (0.0 – 524.2)	98.3 (86.3 – 110.3)
		25	93.3 (75.5 – 111.2)	108.3 (73.0 – 143.6)	100.0 (29.4 – 170.6)	93.7 (0.0 – 193.6)	87.5 (87.5 – 87.5)	94.3 (86.8 – 101.8)
		50	98.9 (93.1 – 104.7)	102.8 (67.5 – 138.1)	88.9 (88.9 – 88.9)	105.6 (35.0 – 176.2)	84.0 (75.8 – 92.1)	96.7 (91.6 – 101.8)
		100	94.4 (80.7 – 108.2)	91.7 (56.4 – 127.0)	100.0 (0.0 – 382.4)	117.2 (0.0 – 674.9)	96.3 (0.0 – 525.1)	98.7 (82.6 – 114.8)
		200	85.6 (71.3 – 99.9)	105.6 (35.0 – 176.2)	86.1 (50.8 – 121.4)	95.5 (37.7 – 153.2)	84.9 (0.0 – 347.1)	90.1 (81.9 – 98.4)
	20-24°C	0	102.2 (90.7 – 113.8)	100.0 (29.4 – 170.6)	94.3 (92.2 – 96.4)	90.8 (48.8 – 132.9)	64.3 (0.0 – 155.1)	93.1 (84.0 – 102.2)
		50	93.3 (84.3 – 102.3)	108.3 (73.0 – 143.6)	91.7 (56.4 – 127.0)	93.8 (14.3 – 173.2)	92.6 (0.0 – 345.3)	95.3 (88.6 – 102.1)
		200	84.4 (69.5 – 99.4)	100.0 (0.0 – 241.2)	65.7 (53.2 – 78.1)	70.6 (0.0 – 220.1)	39.4 (0.0 – 116.4)	74.9 (61.8 – 88.0)

Temp. = Temperature, Con = Content

Table S5: Mean ($\pm$ 95% CI) body mass in mg dw of larvae from each treatment on days seven, eleven, 15, 19 and 23 of the Cd and Cu bioassay. Negative CI are not displayed as they are of no biological meaning. Body masses that differ significantly are printed in bold.

Bioassay	Temp.	Con (mg/kg)	Day 7 (n = 4-5)	Day 11 (n = 2-4)	Day 15 (n = 2-3)	Day 19 (n = 2)	Day 23 (n = 0-2)	Average (n = 13-16)
Cd	20°C	0	0.28 (0.23 – 0.33)	0.95 (0.74 – 1.16)	1.43 (0.50 – 2.36)	2.03 (0.86 – 3.21)	1.09 (-)	0.94 (0.58 – 1.31)
		0.7	0.30 (0.25 – 0.35)	0.90 (0.63 – 1.18)	1.24 (0.00 – 5.08)	1.88 (0.00 – 10.03)	-	0.87 (0.48 – 1.26)
		2	0.27 (0.22 – 0.32)	0.83 (0.74 – 0.92)	1.52 (0.86 – 2.18)	2.17 (0.03 – 4.31)	1.12 (-)	0.94 (0.55 – 1.34)
		6	0.24 (0.17 – 0.30)	0.77 (0.53 – 1.00)	1.35 (0.40 – 2.31)	1.77 (0.00 – 6.53)	1.97 (0.00 – 13.28)	0.96 (0.53 – 1.39)
		18	0.07 (0.02 – 0.12)	0.14 (0.05 – 0.23)	0.11 (0.00 – 0.34)	0.31 (0.00 – 2.57)	0.80 (0.00 – 3.23)	0.23 (0.08 – 0.37)
		0	0.36 (0.28 – 0.43)	0.90 (0.42 – 1.38)	1.62 (0.00 – 6.02)	2.20 (0.00 – 4.61)	2.74 (-)	1.12 (0.64 – 1.61)
	20-24°C	2	0.31 (0.23 – 0.38)	0.87 (0.66 – 1.09)	1.55 (0.00 – 8.73)	1.73 (0.89 – 2.58)	-	0.89 (0.51 – 1.27)
		18	0.04 (0.01 – 0.08)	0.07 (0.01 – 0.14)	0.16 (0.06 – 0.26)	0.50 (0.00 – 4.96)	1.46 (0.00 – 11.22)	0.31 (0.00 – 0.61)
Cu	20°C	0	0.19 (0.16 – 0.22)	0.46 (0.00 – 0.99)	0.88 (0.00 – 2.47)	1.12 (0.00 – 2.48)	1.74 (0.00 – 5.46)	0.72 (0.36 – 1.08)
		25	0.22 (0.20 – 0.25)	0.57 (0.00 – 1.64)	0.74 (0.29 – 1.18)	1.14 (0.00 – 2.37)	1.87 (0.41 – 3.34)	0.75 (0.39 – 1.11)
		50	0.21 (0.14 – 0.28)	0.58 (0.00 – 1.21)	0.80 (0.00 – 1.21)	1.30 (0.00 – 3.02)	1.65 (1.00 – 2.31)	0.75 (0.41 – 1.09)
		100	0.23 (0.18 – 0.27)	0.67 (0.00 – 1.77)	0.85 (0.00 – 3.71)	1.07 (0.00 – 4.37)	1.44 (0.00 – 3.61)	0.71 (0.41 – 1.00)
		200	0.10 (0.08 – 0.12)	0.41 (0.00 – 1.19)	0.55 (0.00 – 1.34)	1.00 (0.00 – 5.35)	0.99 (0.22 – 1.76)	0.49 (0.24 – 0.74)
		0	0.28 (0.23 – 0.34)	0.52 (0.00 – 1.64)	0.88 (0.00 – 1.94)	0.86 (0.65 – 1.08)	1.37 (0.00 – 4.57)	0.67 (0.42 – 0.92)
	20-24°C	50	0.31 (0.19 – 0.44)	0.65 (0.54 – 0.76)	0.79 (0.00 – 1.60)	1.36 (1.16 – 1.57)	1.72 (1.11 – 2.33)	0.82 (0.49 – 1.15)
		200	0.12 (0.09 – 0.16)	0.35 (0.00 – 1.23)	0.83 (0.00 – 2.61)	1.00 (0.00 – 2.36)	1.50 (0.00 – 3.23)	0.61 (0.29 – 0.97)

Temp. = Temperature, Con = Content

Table S6: Median ($\pm$ 95% CI) time until 50% emergence of *C. riparius* (female, male and total adults) from each treatment (i.e., combination of temperature regime and metal content) of the Cd and Cu bioassay. Times until 50% emergence that differ significantly are printed in bold.

Bioassay	Temperature	Content	Females	Males	Total
Cd	20°C	0 mg/kg	21.9 (21.4 – 22.4)	16.7 (16.3 – 17.1)	19.4 (18.3 – 20.5)
		0.7 mg/kg	22.3 (21.3 – 23.3)	16.9 (16.4 – 17.5)	19.5 (17.9 – 21.1)
		2 mg/kg	22.4 (22.0 – 22.9)	16.7 (16.5 – 16.9)	19.5 (18.5 – 20.5)
		6 mg/kg	23.2 (22.7 – 23.7)	17.3 (17.0 – 17.6)	20.0 (18.1 – 22.0)
		18 mg/kg	26.6 (20.8 – 32.4)	26.2 (25.8 – 26.7)	26.9 (20.0 – 33.7)
	20-24°C	0 mg/kg	22.8 (22.1 – 23.5)	19.3 (18.8 – 19.8)	20.9 (20.2 – 21.7)
		2 mg/kg	21.6 (21.1 – 22.2)	16.8 (16.5 – 17.2)	19.3 (18.5 – 20.1)
		18 mg/kg	25.2 (24.5 – 25.8)	25.5 (23.7 – 27.4)	25.4 (24.3 – 26.5)
Cu	20°C	0 mg/kg	25.0 (18.6 – 31.3)	21.2 (18.4 – 24.1)	23.1 (13.9 – 32.4)
		25 mg/kg	25.5 (15.2 – 35.8)	22.3 (17.4 – 27.1)	23.3 (15.5 – 31.1)
		50 mg/kg	25.5 (15.5 – 35.4)	22.8 (20.9 – 24.7)	23.6 (19.3 – 28.0)
		100 mg/kg	25.2 (0.0 – 54.6)	22.1 (11.3 – 32.8)	23.4 (12.6 – 34.2)
		200 mg/kg	24.3 (22.7 – 25.9)	21.7 (16.4 – 27.0)	23.0 (12.3 – 33.7)
	20-24°C	0 mg/kg	24.4 (23.8 – 25.0)	21.0 (17.6 – 24.3)	22.7 (12.2 – 33.2)
		50 mg/kg	24.3 (20.5 – 28.2)	20.9 (19.4 – 22.4)	22.3 (21.1 – 23.5)
		200 mg/kg	25.1 (23.3 – 26.9)	21.1 (18.9 – 23.2)	22.8 (20.4 – 25.2)

Table S7: Mean ($\pm$ 95% CI) dry body mass in mg of females and males from each treatment (i.e., combination of temperature regime and metal content) of the Cd and Cu bioassay. Body masses that differ significantly are printed in bold.

Bioassay	Temperature	Content	Female adults	Male adults
Cd	20°C	0 mg/kg	1.46 (1.32 – 1.60)	0.61 (0.58 – 0.65)
		0.7 mg/kg	1.51 (1.31 – 1.72)	0.62 (0.58 – 0.66)
		2 mg/kg	1.53 (1.41 – 1.64)	0.57 (0.53 – 0.62)
		6 mg/kg	1.36 (1.24 – 1.48)	0.58 (0.55 – 0.61)
		18 mg/kg	1.65 (0.49 – 2.81)	0.76 (0.54 – 0.97)
	20-24°C	0 mg/kg	1.58 (1.50 – 1.65)	0.61 (0.57 – 0.64)
		2 mg/kg	1.44 (1.33 – 1.55)	0.56 (0.51 – 0.60)
		18 mg/kg	1.77 (1.64 – 1.89)	0.75 (0.66 – 0.85)
Cu	20°C	0 mg/kg	1.42 (1.31 – 1.54)	0.56 (0.52 – 0.60)
		25 mg/kg	1.43 (1.28 – 1.59)	0.59 (0.55 – 0.63)
		50 mg/kg	1.38 (1.22 – 1.53)	0.58 (0.55 – 0.62)
		100 mg/kg	1.29 (1.15 – 1.43)	0.57 (0.52 – 0.63)
		200 mg/kg	1.30 (1.17 – 1.44)	0.58 (0.52 – 0.64)
	20-24°C	0 mg/kg	1.34 (1.26 – 1.42)	0.58 (0.53 – 0.64)
		50 mg/kg	1.22 (1.08 – 1.36)	0.51 (0.48 – 0.55)
		200 mg/kg	1.17 (1.05 – 1.28)	0.50 (0.46 – 0.54)

Table S8: FA groups driving the dissimilarity (cumulative contribution ~0.7) after SIMPER analysis on FA groups standardized to the maximum of each group. SIMPER analysis was conducted for predictor variables ('Stage' or 'Content') that had a statistically significant effect (PERMANOVA) on the FA composition of *C. riparius* (see Table S3 for statistical evaluation). average = contribution to dissimilarity between group a and b; sd = standard deviation of contribution; ratio = ratio between average and sd; av = average abundance for groups a and b, respectively; consum = cumulative contribution (shown until ~0.7).

Bioassay	Predictor	Contrast	FA groups	average	sd	ratio	ava	avb	consum
Cd	Stage	Larvae – Males	omega-6 PUFA	0.042	0.024	1.721	0.388	0.694	0.193
			PUFA	0.039	0.024	1.642	0.414	0.694	0.373
			omega-3 PUFA	0.027	0.022	1.222	0.510	0.558	0.499
			BacFA	0.026	0.019	1.367	0.550	0.379	0.621
			MUFA	0.022	0.014	1.521	0.555	0.438	0.721
		Larvae – Females	SAFA	0.031	0.021	1.464	0.413	0.600	0.164
			omega-3 PUFA	0.027	0.021	1.263	0.510	0.520	0.305
			Total	0.026	0.020	1.313	0.531	0.658	0.440
			BacFA	0.024	0.015	1.524	0.550	0.489	0.564
			MUFA	0.022	0.016	1.382	0.555	0.589	0.679
		Males – Females	omega-6 PUFA	0.031	0.019	1.647	0.694	0.459	0.170
			SAFA	0.030	0.016	1.864	0.356	0.600	0.332
			PUFA	0.029	0.018	1.585	0.694	0.478	0.489
			Total	0.022	0.015	1.445	0.494	0.658	0.609
			omega-3 PUFA	0.022	0.017	1.327	0.558	0.520	0.729
	Content	0 mg/kg – 2 mg/kg	omega-6 PUFA	0.027	0.021	1.325	0.469	0.550	0.152
			PUFA	0.026	0.020	1.312	0.484	0.560	0.295
			omega-3 PUFA	0.025	0.020	1.250	0.491	0.519	0.434
			SAFA	0.024	0.018	1.302	0.420	0.448	0.567
			Total	0.021	0.016	1.322	0.522	0.546	0.681
		0 mg/kg – 18 mg/kg	omega-6 PUFA	0.028	0.021	1.352	0.469	0.554	0.149
			PUFA	0.026	0.020	1.336	0.484	0.576	0.291
			omega-3 PUFA	0.026	0.022	1.204	0.491	0.611	0.430
			SAFA	0.024	0.018	1.313	0.420	0.528	0.557
			BacFA	0.023	0.017	1.370	0.447	0.568	0.681
		2 mg/kg – 18 mg/kg	omega-6 PUFA	0.027	0.020	1.345	0.550	0.554	0.149
			PUFA	0.026	0.019	1.343	0.560	0.576	0.289
			omega-3 PUFA	0.025	0.020	1.257	0.519	0.611	0.425
			SAFA	0.024	0.018	1.311	0.448	0.528	0.557
			BacFA	0.024	0.017	1.417	0.428	0.568	0.686
Cu	Stage	Larvae – Males	omega-6 PUFA	0.065	0.029	2.195	0.252	0.672	0.202
			PUFA	0.064	0.029	2.220	0.257	0.674	0.402
			omega-3 PUFA	0.040	0.025	1.573	0.174	0.413	0.525
			Total	0.036	0.023	1.582	0.324	0.526	0.637
			SAFA	0.031	0.018	1.712	0.233	0.382	0.734
		Larvae – Females	SAFA	0.061	0.030	2.078	0.233	0.624	0.189
			Total	0.053	0.028	1.860	0.324	0.662	0.351
			PUFA	0.039	0.025	1.554	0.257	0.510	0.471
			MUFA	0.038	0.027	1.420	0.391	0.598	0.588
			omega-3 PUFA	0.038	0.025	1.490	0.174	0.409	0.704
		Males – Females	SAFA	0.032	0.017	1.885	0.382	0.624	0.170
			omega-6 PUFA	0.029	0.019	1.502	0.672	0.484	0.326
			PUFA	0.028	0.019	1.464	0.674	0.510	0.474
			omega-3 PUFA	0.022	0.018	1.243	0.413	0.409	0.591
			Total	0.021	0.015	1.470	0.526	0.662	0.706

Table S9: Mean ($\pm$ 95% CI) content in $\mu\text{g}/\text{mg}$ dw of certain physiologically important FA of *C. riparius* larvae and emerged females and males from the control (0 mg/kg dw), the intermediate sediment metal content (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw) and the highest sediment metal content (i.e., 18 mg Cd/kg dw and 200 mg Cu/kg dw) in sediments. LIN = Linoleic acid, ALA = alpha-Linolenic acid, ARA = Arachidonic acid, EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid.

Cd bioassay									
FA	Larvae			Female adults			Male adults		
	0 mg/kg	2 mg/kg	18 mg/kg	0 mg/kg	2 mg/kg	18 mg/kg	0 mg/kg	2 mg/kg	18 mg/kg
LIN	2.11 ($\pm$ 0.65)	2.19 ($\pm$ 0.66)	1.90 ($\pm$ 0.56)	2.26 ($\pm$ 0.38)	2.20 ($\pm$ 0.55)	2.99 ($\pm$ 0.57)	2.98 ($\pm$ 0.68)	3.94 ($\pm$ 0.42)	4.37 ($\pm$ 0.67)
ALA	0.09 ($\pm$ 0.03)	0.12 ($\pm$ 0.08)	0.09 ($\pm$ 0.03)	0.08 ($\pm$ 0.02)	0.08 ($\pm$ 0.02)	0.09 ($\pm$ 0.04)	0.10 ($\pm$ 0.02)	0.13 ($\pm$ 0.03)	0.16 ($\pm$ 0.04)
ARA	0.02 ($\pm$ 0.01)	0.02 ($\pm$ 0.01)	0.02 ($\pm$ 0.01)	0.01 ($\pm$ 0.00)	0.01 ($\pm$ 0.01)	0.02 ($\pm$ 0.01)	0.03 ($\pm$ 0.01)	0.04 ($\pm$ 0.02)	0.05 ($\pm$ 0.01)
EPA	0.06 ($\pm$ 0.02)	0.06 ($\pm$ 0.04)	0.06 ($\pm$ 0.02)	0.05 ($\pm$ 0.02)	0.05 ($\pm$ 0.02)	0.05 ($\pm$ 0.03)	0.12 ($\pm$ 0.03)	0.13 ($\pm$ 0.04)	0.19 ($\pm$ 0.05)
DHA	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -
Cu bioassay									
FA	Larvae			Females			Males		
	0 mg/kg	50 mg/kg	200 mg/kg	0 mg/kg	50 mg/kg	200 mg/kg	0 mg/kg	50 mg/kg	200 mg/kg
LIN	1.60 ($\pm$ 0.35)	1.28 ($\pm$ 0.32)	1.12 ($\pm$ 0.26)	2.37 ($\pm$ 0.50)	2.64 ($\pm$ 0.43)	2.72 ($\pm$ 0.66)	3.23 ($\pm$ 0.53)	3.54 ($\pm$ 0.46)	3.93 ($\pm$ 0.61)
ALA	0.10 ($\pm$ 0.05)	0.08 ($\pm$ 0.04)	0.06 ($\pm$ 0.03)	0.29 ($\pm$ 0.16)	0.21 ($\pm$ 0.10)	0.19 ($\pm$ 0.09)	0.33 ($\pm$ 0.14)	0.19 ($\pm$ 0.07)	0.26 ($\pm$ 0.07)
ARA	0.02 ($\pm$ 0.01)	0.01 ($\pm$ 0.01)	0.02 ($\pm$ 0.02)	0.02 ($\pm$ 0.01)	0.02 ($\pm$ 0.01)	0.02 ($\pm$ 0.01)	0.04 ($\pm$ 0.01)	0.05 ($\pm$ 0.01)	0.05 ($\pm$ 0.02)
EPA	0.05 ($\pm$ 0.01)	0.04 ($\pm$ 0.01)	0.03 ($\pm$ 0.01)	0.08 ($\pm$ 0.03)	0.08 ($\pm$ 0.02)	0.08 ($\pm$ 0.03)	0.15 ($\pm$ 0.05)	0.15 ($\pm$ 0.04)	0.18 ($\pm$ 0.06)
DHA	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -	< 0.01 -

Estimate of the metal flux with *C. riparius*. Mean metal contents (in ng/mg dw) from Table 1 were multiplied with the mean dry mass (in mg) of these groups to estimate the metal flux (in ng) per individual. Multiplication of this value with the observed mean larval survival (Table S4) and adult emergence (Figure 2) gave an estimate of the total metal flux (in ng).

Table S10: Metal flux (in ng; per individual and total) with *C. riparius* larvae and emerging adults from the control (0 mg/kg dw) and the intermediate metal content (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw) in sediments.

Bioassay	Developmental stage	Content	Metal flux	
			per individual (ng)	Total (ng)
Cd	Larvae	0 mg/kg	0.63	10.70
		2 mg/kg	29.71	550.92
	Adults	0 mg/kg	0.15	2.64
		2 mg/kg	5.11	92.24
Cu	Larvae	0 mg/kg	8.86	152.54
		50 mg/kg	237.17	4098.65
	Adults	0 mg/kg	14.81	193.92
		50 mg/kg	20.55	213.89

Estimate of the FA flux with *C. riparius*. Mean FA content (in µg/mg dw) for the most relevant FA groups from Table 2 were multiplied with the mean dry mass (in mg) of either female or male adults to estimate the FA flux (in µg) per individual. Multiplication of this value with the observed adult emergence (Figures S1 and S2) gave an estimate of the total FA flux (in µg).

Table S81: FA flux (in µg; per individual and total) with emergent *C. riparius* females and males from the control (0 mg/kg dw), the intermediate metal content (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw) and the highest metal content (i.e., 18 mg Cd/kg dw and 200 mg Cu /kg dw) in sediments. SAFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, Total = Total fatty acids.

Bioassay	Stage	Content	FA flux							
			per individual (µg)				Total (µg)			
			SAFA	MUFA	PUFA	Total	SAFA	MUFA	PUFA	Total
Cd	Female adults	0 mg/kg	17.40	12.88	4.15	34.77	153.68	113.80	36.65	307.17
		2 mg/kg	16.17	10.25	3.88	30.70	149.77	94.92	35.98	284.38
		18 mg/kg	26.74	18.93	6.07	52.52	29.88	21.16	6.78	58.70
	Male adults	0 mg/kg	4.02	3.49	2.09	9.69	36.64	31.76	19.08	88.26
		2 mg/kg	4.02	3.45	2.50	10.06	35.32	30.36	21.96	88.40
		18 mg/kg	6.47	5.83	3.81	16.37	9.52	8.58	5.60	24.07
Cu	Female adults	0 mg/kg	15.36	9.45	4.21	29.57	87.95	54.10	24.10	169.33
		50 mg/kg	13.71	8.92	4.25	27.43	50.47	32.85	15.64	100.97
		200 mg/kg	11.62	7.92	4.05	24.07	29.59	20.16	10.32	61.26
	Male adults	0 mg/kg	3.88	3.14	2.27	9.46	28.60	23.13	16.70	69.67
		50 mg/kg	3.60	3.18	2.26	9.19	24.25	21.38	15.22	61.85
		200 mg/kg	3.05	2.85	2.46	8.52	15.11	14.14	12.18	42.23

Table S9: Flux (in µg; per individual and total) of certain physiologically important FA with emergent *C. riparius* females and males from the control (0 mg/kg dw), the intermediate sediment metal content (i.e., 2 mg Cd/kg dw and 50 mg Cu/kg dw) and the highest sediment metal content (i.e., 18 mg Cd/kg dw and 200 mg Cu /kg dw) in sediments. LIN = Linoleic acid, ALA = alpha-Linolenic acid, ARA = Arachidonic acid, EPA = Eicosapentaenoic acid.

Bioassay	Stage	Content	FA flux							
			per individual (µg)				Total (µg)			
			LIN	ALA	ARA	EPA	LIN	ALA	ARA	EPA
Cd	Female adults	0 mg/kg	3.45	0.13	0.02	0.08	30.52	1.11	0.14	0.71
		2 mg/kg	3.25	0.12	0.02	0.08	30.10	1.10	0.19	0.71
		18 mg/kg	5.18	0.16	0.03	0.09	5.79	0.18	0.03	0.10
	Male adults	0 mg/kg	1.82	0.06	0.02	0.07	16.60	0.57	0.15	0.67
		2 mg/kg	2.22	0.07	0.02	0.07	19.54	0.65	0.19	0.65
		18 mg/kg	3.29	0.12	0.04	0.14	4.84	0.18	0.06	0.21
Cu	Female adults	0 mg/kg	3.27	0.40	0.03	0.11	18.73	2.29	0.16	0.62
		50 mg/kg	3.42	0.28	0.03	0.10	12.60	1.02	0.12	0.38
		200 mg/kg	3.37	0.23	0.03	0.10	8.57	0.59	0.08	0.25
	Male adults	0 mg/kg	1.84	0.19	0.02	0.09	13.54	1.38	0.18	0.64
		50 mg/kg	1.93	0.11	0.03	0.08	13.00	0.71	0.17	0.56
		200 mg/kg	2.11	0.14	0.03	0.10	10.43	0.68	0.14	0.49

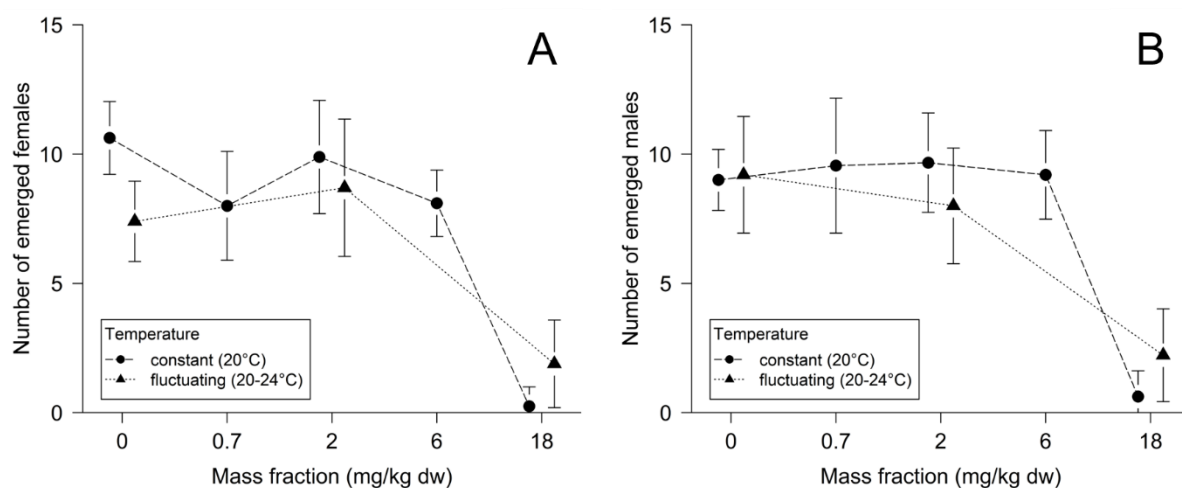


Figure S1: Mean ($\pm$ 95% CI) number of emerged *C. riparius* females (A) and males (B) exposed to 0, 0.7, 2, 6 and 18 mg Cd/kg dw crossed with two temperature regimes (20°C; circles and 20-24°C; triangles) within the 28-day bioassay period. See Table S3 for statistical evaluation.

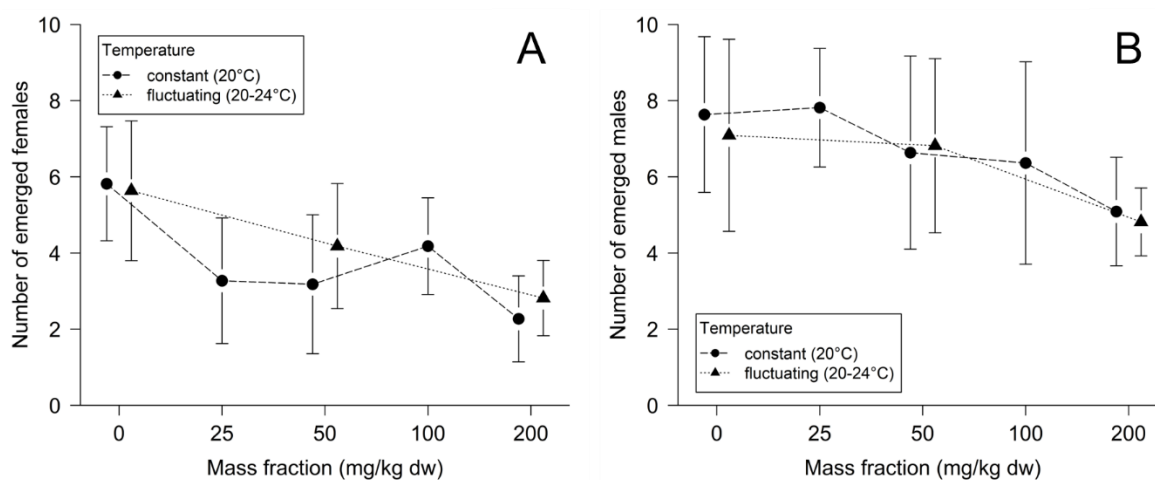


Figure S2: Mean ($\pm$ 95% CI) number of emerged *C. riparius* females (A) and males (B) exposed to 0, 25, 50, 100 and 200 mg Cu /kg dw crossed with two temperature regimes (20°C; circles and 20-24°C; triangles) within the 28-day bioassay period. See Table S3 for statistical evaluation.

7.2 Appendix A2

Subsidy quality affects common riparian web-building spiders: consequences of aquatic contamination and food resource

Environmental Toxicology & Chemistry

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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 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 FA, such as 20:4n-6 (ARA) and 20:5n-3 (EPA), in spiders by about 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, as biomass consumption by spiders was, due to 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.

Keywords: fatty acids, emergent aquatic insects, *Tetragnatha*, aquatic-terrestrial linkage, pesticides, copper, *Bacillus thuringiensis* var. *israelensis*

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 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 fatty acids (PUFA) 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 et al. 2018b; Twining et al. 2019). Since 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 et al. 2018a). At the aquatic-terrestrial interface, FA profiles of aquatic primary producers strongly differ from those of their terrestrial counterpart (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 ten times higher levels of long-chain PUFA 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 et al. 2021b), the physiological consequences for terrestrial consumers (such as riparian spiders) remain unclear. The goal of this study was to explicitly investigate this linkage through a two-step process. First, we conducted a fully-factorial laboratory study testing the importance of the 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 by using Spirulina or 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 due to 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). Additionally, 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 nematoceros 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 et al. 2023a; 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 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 (about 32 x 22 x 7 cm) containing a 2-cm layer of artificial sediment (see OECD (2004)) topped by a 4-cm column of test medium (SAM-5S, Borgmann (1996)). Each microcosm was placed in a mesh cage (38 x 23 x 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; Table S1) paired with two basal resources, i.e., *Spirulina* (*Arthrospira platensis*, Bionutra, BTG Berlin Trade GmbH, Berlin, Germany) and TetraMin® (Tetra GmbH, Melle, Germany). Cu 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 (dw; 1.0 to 3100 mg kg⁻¹ dw; (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±8 mg kg⁻¹ dw and are presented in 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 ITU L⁻¹) was prepared from the product VectoBac WDG (Valent BioSciences, Illinois, USA) 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 Table S1 (for details see Roodt et al. (2022)).

These stressors in aquatic systems were paired with two basal resources (i.e., Spirulina or 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. As 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 $20\pm1^{\circ}\text{C}$ with 70% relative humidity, a light/dark cycle of 16/8 hours and under 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., $\leq$ one bubble per second) aerated and 400 freshly hatched larvae ($\leq$ 2 days old) were carefully and randomly allocated to each microcosm, resulting in about 2 cm² surface area per larva. Aeration was suspended for 24 hours 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 (ww), 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 the genus *Tetragnatha* spp. were collected at the Eußerthal Ecosystem Research Station (EERES; $49^{\circ}15'14''\text{N}$, $7^{\circ}57'42''\text{E}$), Germany, between 14th and 16th of May 2020. Spiders were sampled by hand in close proximity ($\leq$ 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 x 16.5 x 7 cm). Spiders were maintained in a climatic chamber with a light/dark cycle of 16/8 hours, at 21±1°C with 70% relative humidity throughout the acclimation phase (one week) and the subsequent bioassay period of three 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, ten 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. Additionally, ten 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 three 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 five minutes, 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).

Additionally, 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. Afterwards, spiders were euthanized in liquid nitrogen and stored at -80°C until FA analysis.

Fatty acid analysis

We analyzed the FA profile of both basal resources (Spirulina and TetraMin®), as well as adult chironomids (females and males) and adult spiders (species, females and males) from each of the eight treatments. To characterize the resource quality for spiders, total FA 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 dw and 3-6 mg dw 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, Solna, Sweden) 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 FA to fatty acid methyl esters (FAME) was achieved with 3N methanolic HCl (Sigma-Aldrich, St. Louis, US) 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), since they constitute an important energy storage in invertebrates (Azeez et al. 2014) and their composition and content is altered relatively fast, compared to phospholipid FA, 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 dw) 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 pre-conditioned (4 mL chloroform) polar modified polystyrene/divinylbenzene copolymer cartridges (Chromabond® easy polypropylene columns, Macherey-Nagel, Düren, Germany). 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 FA to FAME was achieved with trimethylsulfonium hydroxide (Sigma-Aldrich, St. Louis, US-MO).

FAMES were analyzed via gas chromatography with flame-ionization detection (GC-FID; Trace GC Ultra, Thermo Fisher Scientific, Bremen, Germany) using a Restek FAMEWAX capillary GC column (30 m x 0.25 mm, 0.25 µm film thickness) and helium (1.4 mL min⁻¹) as carrier gas. For each sample, FAMES were identified based on different retention times using FAME standards (37-component FAME Mix, Supelco CRM47885) and quantified (µg FA mL⁻¹) using external standard calibration. In addition, the FAME of the internal standard (Methyl D-35 Octadecanoate, Larodan, Solna, Sweden) 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. FA concentrations 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 (µg FA mg⁻¹ dw).

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 (LM) using 'Exposure', 'Food' and their interaction as well as 'Sex' as explanatory variables. The specific growth rate (SGR) for each spider was calculated as:

$$SGR = \frac{(\ln(ww_t) - \ln(ww_0)) * 100}{t} \quad (1)$$

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 a linear model was appropriate (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 $\geq 70\%$ 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 ($\mu\text{g mg}^{-1} \text{ dw}$) among different treatments were assessed using null hypothesis significance testing (NHST). 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 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., percent difference between the mean of each treatment to the control's mean) as a measure for the relative importance of an effect.

Results

Basal resources

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

Chironomid emergence and nutritional quality

Contaminant exposure (i.e., Cu, Bti and the pesticide mixture) during aquatic life stages near-significantly reduced adult emergence compared to the control, a pattern driven by the ~40% decrease in presence of Cu ($p = 0.054$, GLM, Figure 1, Table S3). Basal resource did not significantly affect adult emergence, though the Spirulina treatment exhibited a ~15% lower emergence relative to TetraMin[®] (Figure S1, Table S3). The dry weight of emerged chironomids differed significantly between sexes, with males being ~50% lighter than females ($p < 0.001$, LM, Figure S2, Table S3). We observed a tendency towards 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 (Figure S2, 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 Correspondence Analysis, Table S3), explaining 34% and 13% of the total FA variation (69% and 27% of the constrained variation), respectively. RDA1 reflected differences in the FA 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., $\geq$ three double bonds) levels by 25% and 21%, respectively ($p < 0.001$, Two-way ANOVA, Tables 1 and S3). In contrast, total FA, MUFA and omega-3 PUFA content in adults were significantly lower (30%-80%) when larvae were fed with *Spirulina* compared to TetraMin® ($p < 0.05$, Two-way ANOVA and Brunner-Dette-Munk test, Tables 1 and S3). 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 a significantly higher total FA, SAFA and MUFA content than males ($p < 0.05$, t-tests, Tables 1 and 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, Tables 1 and S3).

The presence of contaminants during chironomid development had marginal effects on the FA profile of adults. The content of total FA, SAFA, MUFA and PUFA and individual FA were comparable to the control. Only the content 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 about 15% lower in male than female spiders, but similar among treatments (Figure S3, 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 about 45% lower in males than in females.

The FA content of spiders fed during the bioassay was significantly higher (50-130%) than individuals preserved at test initiation (Tables S4 and S6). The two spider species did not differ in their FA profile (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, 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 FA (e.g., 25% more 20:5n-3, EPA) and all FA groups (up to ~35% for SAFA, $p < 0.001$, Wilcoxon rank sum test and omega-6 PUFA, $p = 0.003$, t-test) (Figure 4, Tables 1 and S4). RDA2 reflected the differences in the spider FA profile based on the basal resource offered to the consumed chironomids, i.e., *Spirulina* or TetraMin® (Figure 4). Spiders had significantly higher levels of omega-6 PUFA (~25%, 0.023, Two-way ANOVA, Table S4), such as 18:3n-6 (GLA, ~720%) and 20:4n-3 (ARA, ~35%), when chironomids were fed with *Spirulina* relative to those fed with TetraMin®, while they had significantly lower levels of omega-3 PUFA (~25%, $p = 0.008$, Brunner-Dette-Munk test, 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 (Table S4). Total FA and PUFA content decreased near significantly ($p = 0.078$, Brunner-Dette-Munk test, $p = 0.057$, Two-way ANOVA) by about 20% and 30%, respectively, and the HUFA content decreased significantly ($p = 0.034$, Brunner-Dette-Munk

test) by about 30%, when larvae were cultured in the presence of Bti or Cu. Similarly, ARA and EPA contents were about 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, SAFA and PUFA content in females decreased by up to 30%, 30% and 35%, respectively, compared to the control (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, i.e., 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 due to our experimental design (i.e., spiders were offered a constant amount of prey). Hence, changes in the predators (i.e., spider) FA profile is 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 et al. 2023b; Strandberg et al. 2020). The capability to modify or preferentially retain certain dietary FA was

stronger when chironomids were fed with *Spirulina*, which could be explained by a greater discrepancy between chironomids' physiological demands and supply in this basal resource (Goedkoop et al. 2007). This observation may be interpreted as an adaptation supporting survival and sustaining populations of non-selectively feeding organisms, such as chironomids, on poor-quality food (Goedkoop et al. 2007; Twining et al. 2021a). Thereby, chironomids increase the availability of physiologically important PUFAs to consumers. This is supported by the increase in FA with a higher number of double bonds expressed in higher relative proportions of physiologically important PUFA 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 a recent laboratory (Bordalo et al. 2020) and semi-field (Kolbensschlag et al. 2023) study. This combined evidence supports previous findings that insect metamorphosis is triggered by certain size requirements (Nijhout 1975; Mirth & Riddiford 2007). 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 PUFA, such as ARA and EPA, were most affected with a reduction in their content by about 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 FA; 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 nematoceros 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)) 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. Additionally, 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 about 40%-50% less compared to those individuals fed prey cultured under control conditions, while this effect size is subject to considerable variability. Since 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 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 dw and 3-102 ng g⁻¹ dw in females and males, respectively (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 (Callow 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 FA (Mathieu-Resuge et al. 2021; Ohler et al. 2023b), as indicated by the lower relative importance of the FA profile of the basal resource for the FA profile of spiders (Figure TetraRDA). 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 also 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. Cu reduced chironomid emergence by ~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. Bti exposure concentration 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 (48h EC50 for *Daphnia magna* and 96h EC50 for *Chironomus riparius*) which and decreased over time (TWAC 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 non-significant effect in chironomid emergence. In fact, Spirulina reduced emergence by ~15% compared to TetraMin®. This observation may be linked to a higher energetic requirement of chironomids to modify FA 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 also exhibit nutritional differences other than those measured in this 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 matches previous studies focusing on insects (Meier et al. 2000; Pietz et al. 2023; Scharnweber et al. 2020), but may also occur in spiders, presumably due to 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 (this study, (Pietz et al. 2023)) as 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 the 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.

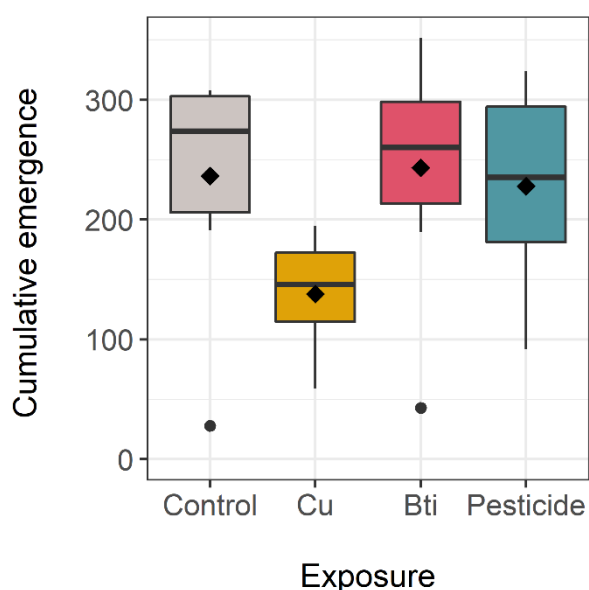


Figure 1: Cumulative emergence of chironomid adults under different exposure conditions aggregated across basal resources. Lines in boxes are medians, box ends are 25th and 75th percentiles. Whiskers and black dots show data points outside the lower and upper quartiles. Black diamonds show the mean cumulative emergence.

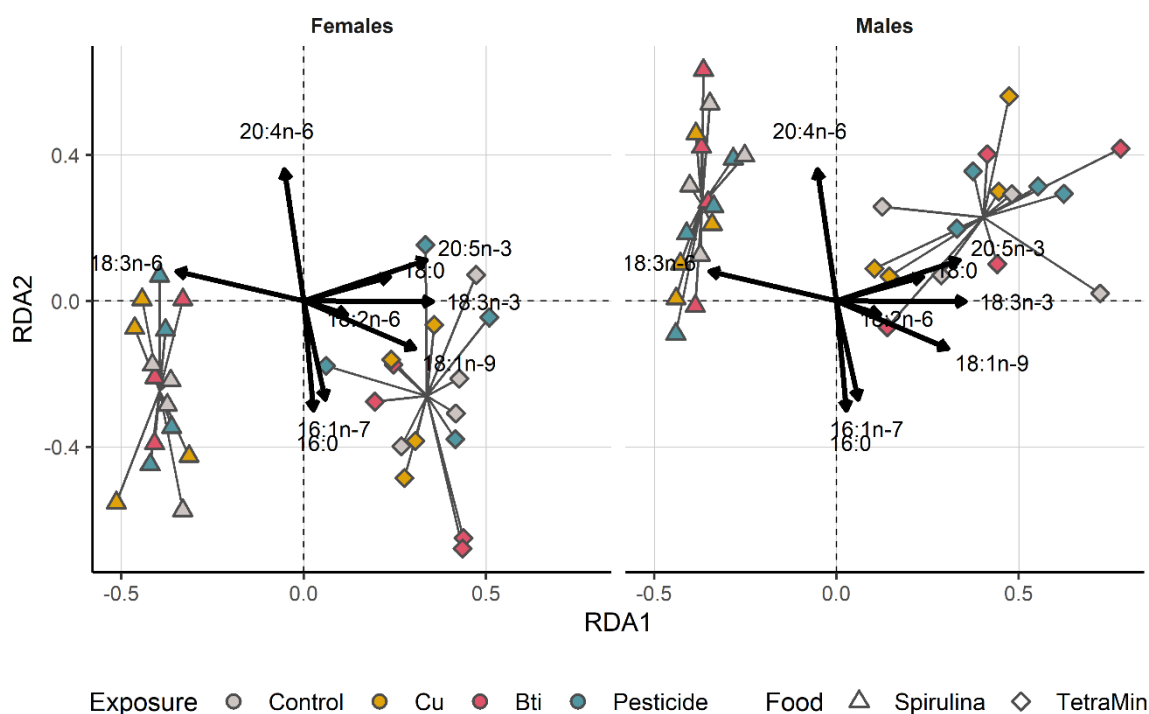


Figure 2: Redundancy analysis (RDA) using FA profiles ($\mu\text{g mg}^{-1} \text{ dw}$) of emergent chironomid females and males cultured under one of eight treatments (i.e., combination of contaminant exposure and basal resource). Basal resources are indicated by triangles (Spirulina) and diamonds (TetraMin®). Color represents larval exposure to control (grey), Cu (yellow), Bti (red), and synthetic pesticides (blue). Scores were calculated in the same ordination space but are separated between females and males in the ordination to avoid cluttering of the figure.

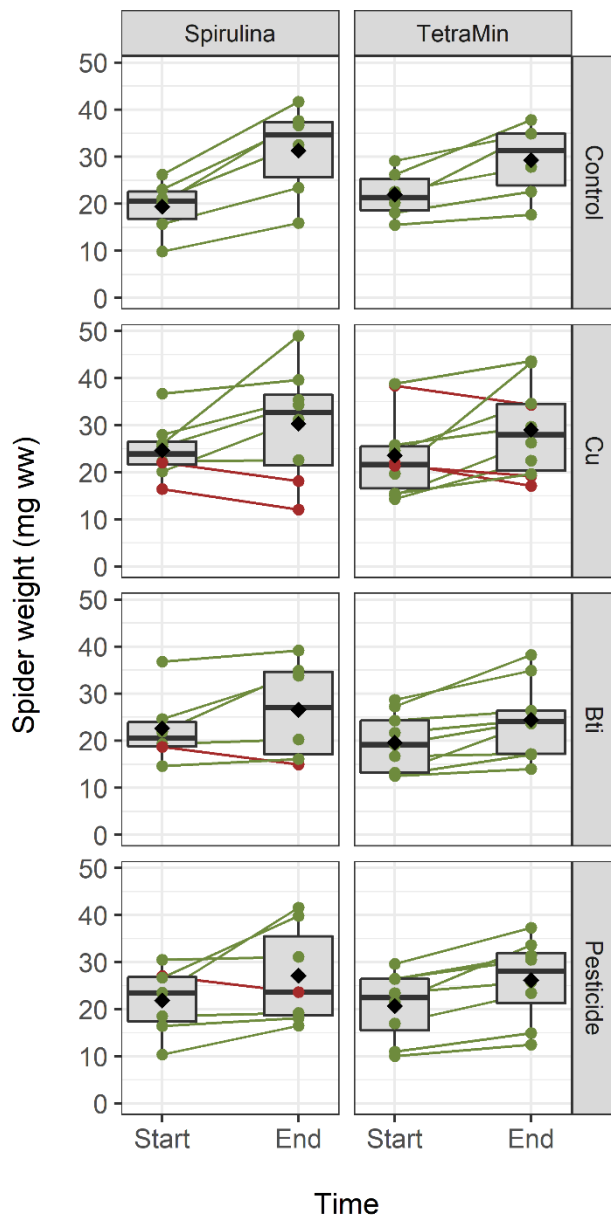


Figure 3: Spider wet weight (mg) at the start and end of the bioassay. Spiders were exclusively fed with adult chironomids cultured under one of eight treatments (i.e., combination of contaminant exposure and basal resource). Lines in boxes are medians, box ends are 25th and 75th percentiles. Whiskers 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.

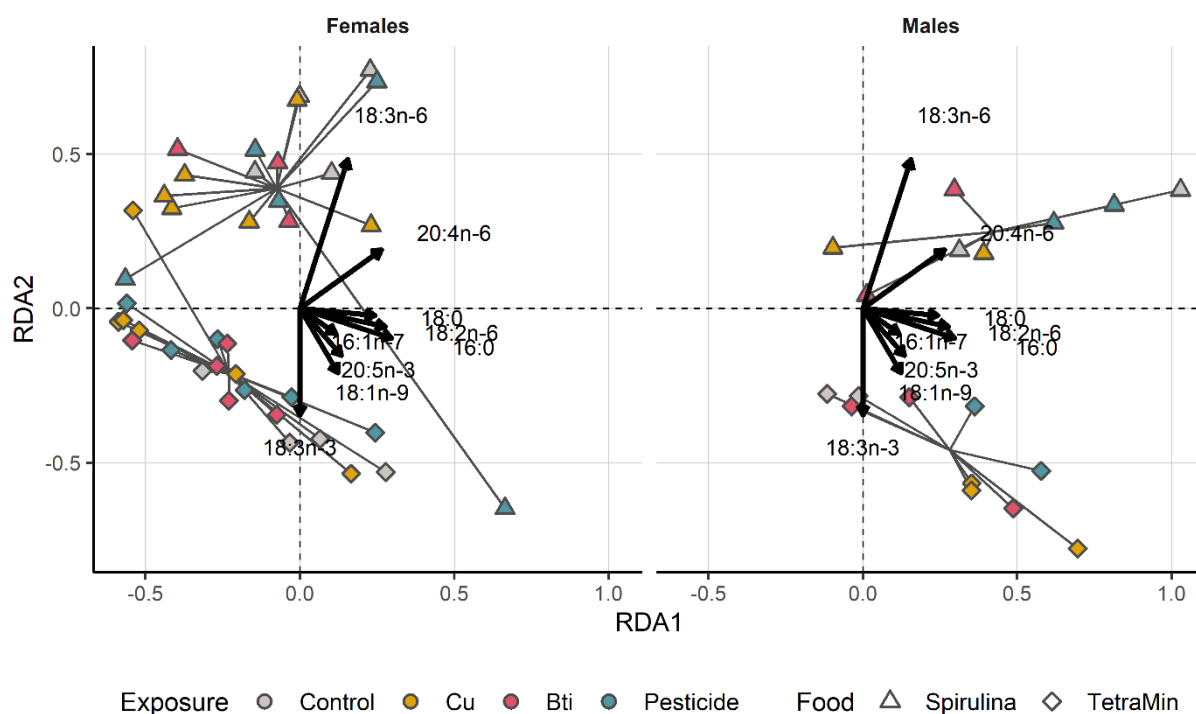


Figure 4: Redundancy analysis (RDA) using FA profiles ($\mu\text{g mg}^{-1} \text{ dw}$) of female and male spiders fed with adult chironomids cultured under one of eight treatments (i.e., combination of chemical stress and basal resource). Basal resources of larvae are indicated by triangles (Spirulina) and diamonds (TetraMin®). Color represents larval exposure to control (grey), Cu (yellow), Bti (red), and synthetic pesticides (blue). Scores were calculated in the same ordination space but are separated between females and males in the ordination to avoid cluttering of the figure.

Table 1: Mean ($\pm$ 95% CI) FA content in $\mu\text{g mg}^{-1}$ dw of adult chironomids and *spiders* by sex, food and contaminant exposure. FAs that jointly account for $\geq 70\%$ of the total content of the respective FA group or are physiologically important are shown. FA contents that differ significantly are printed in bold. GLA = gamma-Linolenic acid, ALA = alpha-Linolenic acid, ARA = Arachidonic acid, EPA = Eicosapentaenoic acid, SAFA = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid, HUFA = Highly unsaturated fatty acids (i.e., $\geq$ three double bonds)

<i>Chironomus riparius</i>								
FA (group)	Sex		Food			Exposure		
	Females	Males	Spirulina	TetraMin®	Control	Cu	Bti	Pesticide
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
<i>Tetraghatha spp.</i>								
FA (group)	Sex		Food			Exposure		
	Females	Males	Spirulina	TetraMin®	Control	Cu	Bti	Pesticide
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

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Supporting material for Appendix A2

Pesticide concentrations. Details on the analysis of pesticide concentrations in aqueous medium as well as method validation and quality assurance are given in Roodt et al. (2022).

Table S10: Information on fungicides and herbicides and their concentrations (initial and TWAC) in the pesticide mixture.

Class	Formulation product	Active substance (label concentration)	Initial concentration ($\mu\text{g L}^{-1}$)	TWAC* ($\mu\text{g L}^{-1}$)
Fungicide	Ortiva®	Azoxystrobin (250 g/L)	20.00	9.05
Fungicide	Cantus®	Boscalid (500 g/kg)	35.10	16.32
Fungicide	Vegas®	Cyflufenamid (51.3 g/L)	17.50	8.75
Fungicide	Luna Experience®	Fluopyram (200 g/L)	19.30	8.03
Fungicide	Pyrus®	Pyrimethanil (400 g/L)	25.50	12.40
Fungicide	Luna Experience	Tebuconazole (200 g/L)	18.30	7.23
Fungicide	Flint®	Trifloxystrobin (500 g/kg)	23.40	22.71
Herbicide	-	Napropamide**	35	15.90
Herbicide	Kerb Flo®	Propyzamide (400 g/L)	26.40	10.60

*TWAC = Time weighted average concentration; medium concentrations were drawn on days 0, 14, and 20

**Napropamide was used as pure substance

Cu sediment content. Sediments were dried at 105°C for 48 h, ground, homogenized and weighed to 0.5 ($\pm$ 0.1) g in a digestion vessel. Microwave (MARS Xpress; CEM, Germany) assisted digestion of samples was performed in 5 mL aqua regia (3 HNO₃ + HCl, 65% and 32% supra-pure assay, respectively, Carl Roth, Germany) at 175°C with 15 min ramp time and 60 min hold time. The digestions were 1:40 diluted with ultrapure water (ELGA LabWater, Germany) and stored at 4°C until analysis. Quantification of Cu was performed by inductively coupled plasma optical emission spectroscopy (ICP-OES; 720, Agilent, Germany) at a wavelength of 327.395 nm.

Table S11: Mean ($\pm$ SD) Cu sediment content based on measurements of four sediment samples from replicates (two for each food quality) of the *C. riparius* the culture taken at the end of the bioassay.

	Food				Mean $\pm$ SD
	Spirulina	Spirulina	TetraMin®	TetraMin®	
Content (mg kg ⁻¹ dw)	164.25	149.39	142.14	150.16	151.48 $\pm$ 8.01

Table S12: Statistical analysis for investigated endpoints in adult chironomids. d.f. = degree of freedom; SS = sum of squares, LR Chisq = likelihood ratio chi square

Endpoint	Factor	d.f.1	SS / d.f.2 / Variance	F-value / LR Chisq	p-value	Test
Emergence	Food	1		1.120	0.290	Generalized linear model
	Exposure	3		7.657	0.054	
	Food:Exposure	3		2.175	0.537	
	Residuals					
Dry weight	Food	1	0.053	3.689	0.060	General linear model
	Exposure	3	0.041	0.957	0.420	
	Sex	1	11.915	829.875	< 0.001	
	Food:Exposure	3	0.029	0.666	0.576	
	Residuals	55	0.790			
RDA model	Model	5	10.771	10.936	0.001	Detrended Correspondence Analysis
	Residuals	57	11.229			
RDA axes	RDA1	1	7.398	37.555	0.001	Detrended Correspondence Analysis
	RDA2	1	2.897	14.705	0.001	
	RDA3	1	0.246	1.251	0.779	
	RDA4	1	0.131	0.665	0.949	
	RDA5	1	0.099	0.504	0.863	
	Residual	57	11.229			
Total content	FA					Two-way ANOVA
	Food	1	471	4.303	0.043	
	Exposure	3	177	0.538	0.658	
	Food:Exposure	3	94	0.288	0.834	
	Residuals	55	6020			
SAFA content	Sex	61			0.032	Student's t Two-way ANOVA
	Food	1	69.2	2.743	0.103	
	Exposure	3	25.0	0.329	0.804	
	Food:Exposure	3	15.0	0.198	0.897	
	Residuals	55	1388.7			
MUFA content	Sex	61			< 0.001	Student's t Two-way ANOVA
	Food	1	152.6	12.421	< 0.001	
	Exposure	3	15.6	0.424	0.737	
	Food:Exposure	3	6.2	0.168	0.917	
	Residuals	55	675.5			
PUFA content	Sex	61			0.001	Student's t Two-way ANOVA
	Food	1	1.1	0.101	0.751	
	Exposure	3	27.7	0.889	0.453	
	Food:Exposure	3	19.9	0.637	0.594	
	Residuals	55	571.8			
omega-6 PUFA content	Sex	61			0.044	Student's t Two-way ANOVA
	Food	1	170.5	21.406	< 0.001	
	Exposure	3	21.1	0.883	0.456	
	Food:Exposure	3	33.3	1.394	0.254	
	Residuals	55	438.1			
omega3 PUFA content	Sex	61			0.248	Student's t Brunner-Dette-Munk
	Food	1.000	51.556	180.029	< 0.001	
	Exposure	2.951	51.556	0.233	0.870	
	Food:Exposure	2.951	51.556	1.308	0.282	
	Residuals					
HUFA content	Sex				0.034	Wilcoxon rank sum Two-way ANOVA
	Food	1	30.42	12.975	< 0.001	
	Exposure	3	6.91	0.983	0.407	
	Food:Exposure	3	2.01	0.286	0.835	
	Residuals	55	128.94			
	Sex	61			< 0.001	Student's t

Table S13: Statistical analysis for investigated endpoints in spiders. d.f. = degree of freedom; SS = sum of squares

Endpoint	Factor	d.f.1	SS / d.f.2 / Variance	F-value	p-value	Test
Specific growth rate	Intercept	1	0.824	0.717	0.401	General linear model
	Food	1	4.138	3.603	0.063	
	Exposure	3	11.846	3.438	0.024	
	BiomassConsumed	1	5.682	4.947	0.031	
	Food:Exposure	3	5.520	1.602	0.200	
	Residuals	51	58.577			
RDA model	Model	5	6.714	4.480	0.001	Detrended Correspondence Analysis
	Residuals	51	15.286			
RDA axes	RDA1	1	3.482	11.619	0.001	Detrended Correspondence Analysis
	RDA2	1	2.433	8.117	0.001	
	RDA3	1	0.610	2.034	0.408	
	RDA4	1	0.125	0.418	0.989	
	RDA5	1	0.064	0.214	0.984	
	Residual	51	15.286			
Total FA content	Food	1.000	46.051	0.004	0.950	Brunner-Dette-Munk
	Exposure	2.797	46.051	2.464	0.078	
	Food:Exposure	2.797	46.051	0.454	0.703	
	Residuals					
	Sex	58			0.002	
	Species				0.871	
SAFA content	Time	18.638			< 0.001	Wilcoxon rank sum Welch Brunner-Dette-Munk
	Food	1.000	45.809	0.005	0.945	
	Exposure	2.804	45.809	1.836	0.157	
	Food:Exposure	2.804	45.809	0.440	0.712	
	Residuals					
	Sex				< 0.001	
MUFA content	Species				0.558	Wilcoxon rank sum Wilcoxon rank sum Welch Brunner-Dette-Munk
	Time	13.622			0.004	
	Food	1.000	43.886	4.526	0.039	
	Exposure	2.905	43.886	1.104	0.356	
	Food:Exposure	2.905	43.886	0.439	0.720	
	Residuals					
PUFA content	Sex				0.105	Wilcoxon rank sum Wilcoxon rank sum Welch Two-way ANOVA
	Species				0.502	
	Time	32.292			< 0.001	
	Food	1	9.02	1.645	0.205	
	Exposure	3	43.95	2.670	0.057	
	Food:Exposure	3	5.22	0.317	0.813	
omega-6 PUFA content	Residuals	52	285.27			Student's t Student's t Welch Two-way ANOVA
	Sex	58			0.005	
	Species	68			0.521	
	Time	32.507			< 0.001	
	Food	1	19.78	5.515	0.023	
	Exposure	3	26.16	2.432	0.075	
omega3 PUFA content	Food:Exposure	3	7.73	0.719	0.545	Student's t Student's t Welch Brunner-Dette-Munk
	Residuals	52	186.48			
	Sex	58			0.003	
	Species	68			0.455	
	Time	41.543			< 0.001	
	Food	1.000	39.663	7.819	0.008	
HUFA content	Exposure	2.876	39.663	1.663	0.192	Brunner-Dette-Munk Brunner-Dette-Munk
	Food:Exposure	2.876	39.663	1.729	0.178	
	Residuals					
	Sex	58			0.265	
	Species				0.722	
	Time	1	12.61	2.95	0.110	
HUFA content	Food	1	10.38	11.377	0.001	Brunner-Dette-Munk Brunner-Dette-Munk
	Exposure	3	8.50	3.105	0.034	
	Food:Exposure	3	0.09	0.032	0.992	
	Residuals	52	47.45			
	Sex	58			0.121	
	Species	68			0.721	
	Time	27.985			< 0.001	Welch

Table S14: Mean ($\pm$ 95% CI) FA content in $\mu\text{g mg}^{-1}$ dw of Spirulina and TetraMin[®]. FA that jointly account for $\geq 70\%$ of the total content of the respective FA group or are physiologically important are shown. FA contents that differ significantly are printed in bold. GLA = gamma-Linolenic acid, ALA = alpha-Linolenic acid, ARA = Arachidonic acid, EPA = Eicosapentaenoic acid, SAFA = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid, HUFA = Highly unsaturated fatty acids (i.e., $\geq$ three double bonds)

FA (group)	Food		<i>p</i> -value	Test
	Spirulina	TetraMin [®]		
16:0	26.65$\pm$14.10	7.38$\pm$0.96	< 0.001	Brunner-Dette-Munk
18:0	0.73$\pm$0.47	3.69$\pm$0.54	< 0.001	Student's t
Σ SAFA	27.99$\pm$14.73	13.35$\pm$1.68	0.008	Brunner-Dette-Munk
16:1n-7	2.16 $\pm$ 1.16	2.26 $\pm$ 0.34	0.824	Welch
18:1n-9	0.67$\pm$0.36	4.31$\pm$0.58	< 0.001	Student's t
Σ MUFA	2.96$\pm$1.52	10.92$\pm$1.51	< 0.001	Welch
18:2n-6	5.32 $\pm$ 2.96	3.76 $\pm$ 0.51	0.219	Welch
18:3n-6 (GLA)	8.72$\pm$4.77	0.05$\pm$0.01	< 0.001	Brunner-Dette-Munk
18:3n-3 (ALA)	0.03$\pm$0.01	1.27$\pm$0.16	< 0.001	Brunner-Dette-Munk
20:4n-6 (ARA)	0.06$\pm$0.04	0.18$\pm$0.06	0.004	Student's t
20:5n-3 (EPA)	0.06$\pm$0.06	1.47$\pm$0.21	< 0.001	Welch
Σ PUFA	14.51 $\pm$ 7.95	8.54 $\pm$ 1.23	0.106	Welch
Σ omega-6 PUFA	14.34$\pm$7.89	4.26$\pm$0.62	< 0.001	Brunner-Dette-Munk
Σ omega-3 PUFA	0.16$\pm$0.10	4.28$\pm$0.61	< 0.001	Brunner-Dette-Munk
Σ HUFA	9.04$\pm$4.90	4.58$\pm$0.66	0.021	Brunner-Dette-Munk
Total FA	45.45 $\pm$ 24.20	32.82 $\pm$ 4.39	0.254	Brunner-Dette-Munk

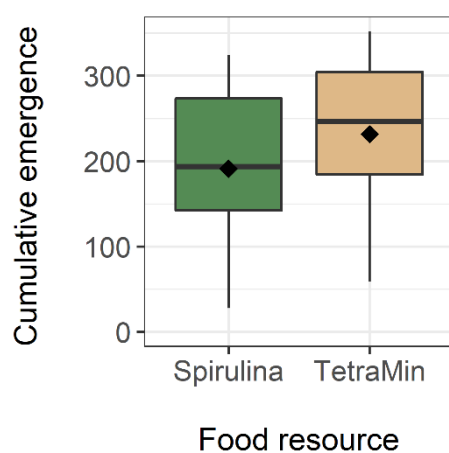


Figure S3: Cumulative emergence of chironomids fed with Spirulina or TetraMin[®] aggregated across contaminant exposure scenarios. Lines in boxes are medians, box ends are 25th and 75th percentiles. Whiskers and black dots show data points outside the lower and upper quartiles. Black diamonds show the mean cumulative emergence.

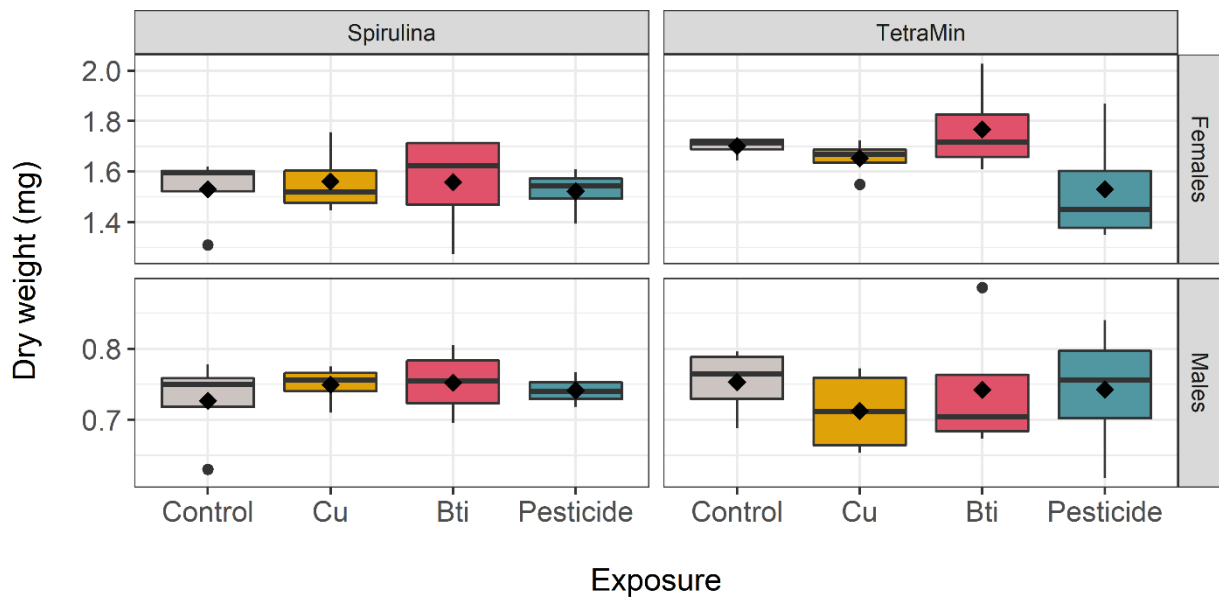


Figure S4: Dry weight (mg) of female and male chironomids when larvae were cultured under one of eight treatments (i.e., combination of contaminant exposure and basal resource). Lines in boxes are medians, box ends are 25th and 75th percentiles. Whiskers and black dots show data points outside the lower and upper quartiles. Black diamonds show the mean cumulative emergence.

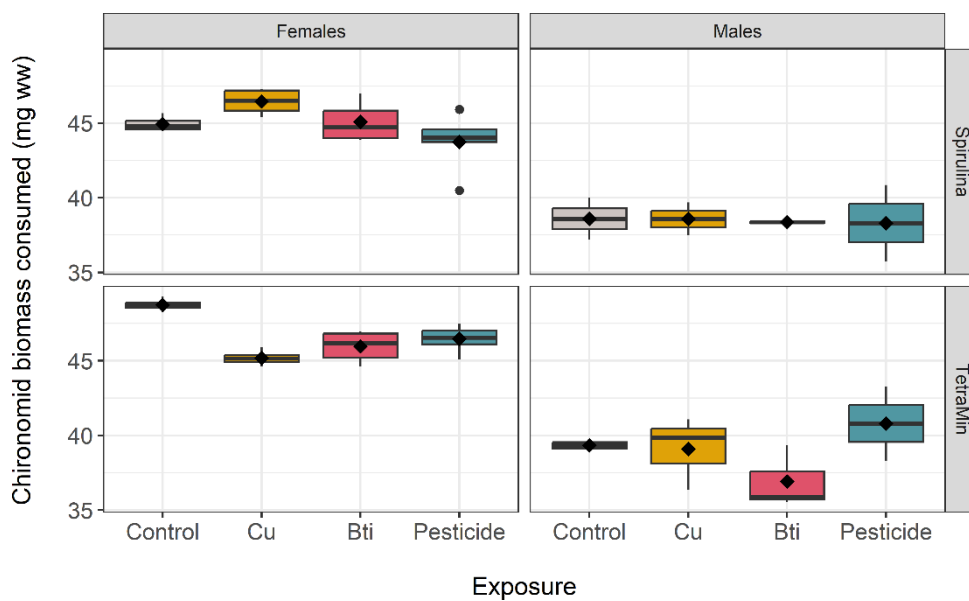


Figure S5: Total chironomid biomass (mg ww) consumed by female and male spiders. Spiders were exclusively fed with adult chironomids cultured under one of eight treatments (i.e., combination of contaminant exposure and basal resource, i.e., Spirulina and TetraMin®). Lines in boxes are medians, box ends are 25th and 75th percentiles. Whiskers and black dots show data points outside the lower and upper quartiles. Black diamonds show the mean consumed biomass.

Table S15: Mean ($\pm$ 95% CI) content in $\mu\text{g mg}^{-1}$ dw of FA groups in spiders from the start and end of the bioassay. FA contents that differ significantly are printed in bold. SAFA = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid, HUFA = Highly unsaturated fatty acids (i.e., $\geq$ three double bonds)

FA (group)	Spider bioassay	
	Start	End
Σ SAFA	5.66$\pm$1.67	8.49$\pm$0.72
Σ MUFA	1.33$\pm$0.38	3.01$\pm$0.34
Σ PUFA	2.54$\pm$0.69	6.21$\pm$0.62
Σ omega-6 PUFA	1.66$\pm$0.48	4.96$\pm$0.52
Σ omega-3 PUFA	0.87 $\pm$ 0.24	1.25 $\pm$ 0.17
Σ HUFA	1.25$\pm$0.33	2.50$\pm$0.27
Total FA	9.53$\pm$2.64	17.71$\pm$1.59

Table S16: Mean ($\pm$ 95% CI) content in $\mu\text{g mg}^{-1}$ dw of FA groups in both *Tetragnatha* species (*T. extensa* and *T. montana*) used in the bioassay. SAFA = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid, HUFA = Highly unsaturated fatty acids (i.e., $\geq$ three double bonds)

FA (group)	Species	
	<i>T. extensa</i>	<i>T. montana</i>
Σ SAFA	8.47 $\pm$ 1.33	7.86 $\pm$ 0.80
Σ MUFA	2.66 $\pm$ 0.55	2.84 $\pm$ 0.43
Σ PUFA	5.95 $\pm$ 1.23	5.53 $\pm$ 0.71
Σ omega-6 PUFA	4.75 $\pm$ 1.09	4.33 $\pm$ 0.57
Σ omega-3 PUFA	1.20 $\pm$ 0.22	1.20 $\pm$ 0.21
Σ HUFA	2.38 $\pm$ 0.52	2.29 $\pm$ 0.29
Total FA	17.08 $\pm$ 3.01	16.23 $\pm$ 1.82

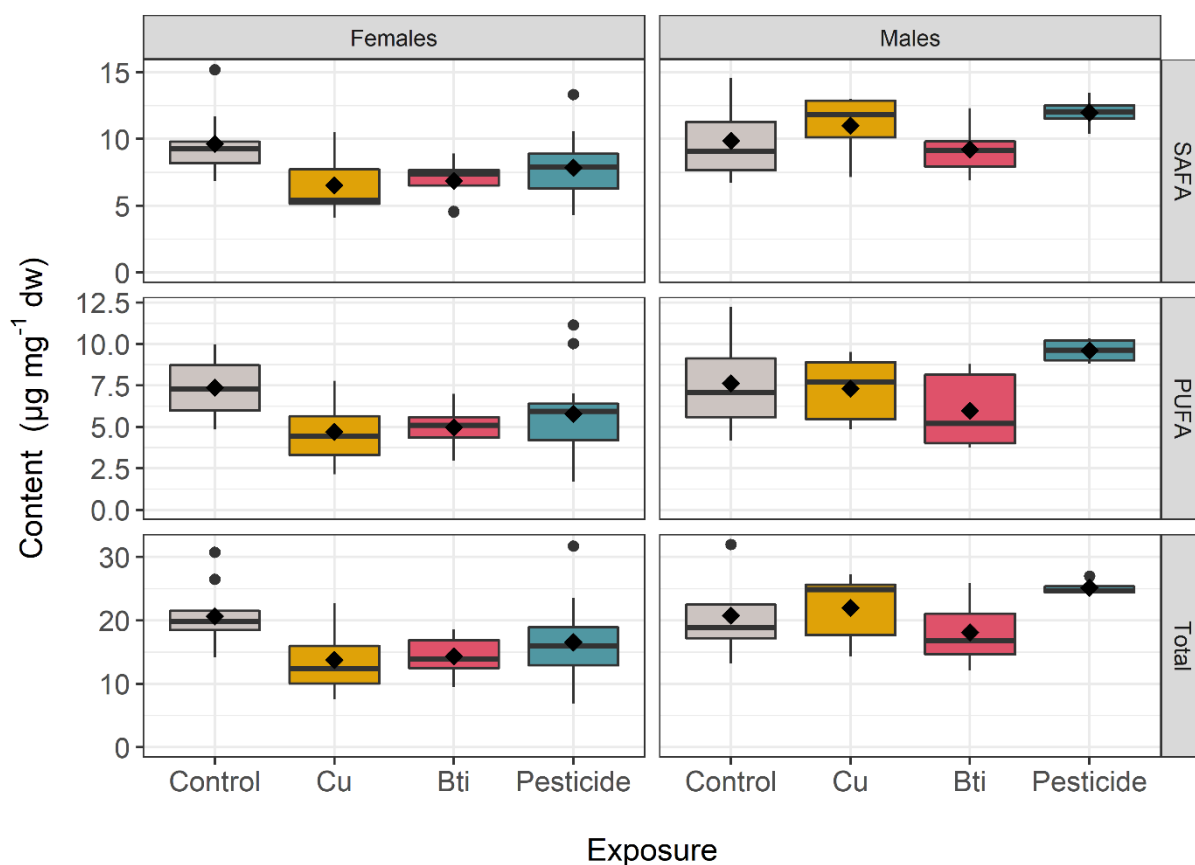


Figure S6: SAFA, PUFA and total FA content ($\mu\text{g mg}^{-1}\text{ dw}$) of female and male spiders fed with adult chironomids cultured under different exposure conditions. Lines in boxes are medians, box ends are 25th and 75th percentiles. Whiskers and black dots show data points outside the lower and upper quartiles. Black diamonds show the mean FA content.

Pesticide contents in adult chironomids. Details on the analysis of pesticide contents in adult chironomids are given in Roodt et al. (2022).

Table S17: Information on pesticide contents ($\text{ng g}^{-1}\text{ dw}$) in adult chironomids.

Active substance (label concentration)	Pesticide contents ($\text{ng g}^{-1}\text{ dw}$) in adult chironomids	
	Live females	Live males
Azoxystrobin	54.1±14.7	73.9±11.1
Boscalid	97.3±36.8	21.1±10.7
Cyflufenamid	7.3±2.7	3.2±1.8
Fluopyram	11.5±3.5	8.2±3.5
Pyrimethanil	ND	ND
Tebuconazole	<LOQ	11.7±7.5
Trifloxystrobin	18.2±4.6	102.4±25.6
Napropamide	3.2±0.5	<LOQ
Propyzamide	1331.8±237.3	<LOQ

References:

Roodt, A. P.; Röder, N.; Pietz, S.; Kolbensschlag, S.; Manfrin, A.; Schwenk, K.; Bundschuh, M.; and Schulz, R. (2022). Emerging Midges Transport Pesticides from Aquatic to Terrestrial Ecosystems: Importance of Compound- and Organism-Specific Parameters. *Environmental Science & Technology*, 56(9), 5478–5488.

7.3 Appendix A3

**Effects of copper, food quality and exposure history on aquatic insect emergence:
insights from a multigeneration study (working title)**

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Introduction

Reciprocal fluxes of organisms connect aquatic and terrestrial ecosystems through the transfer of energy and nutrients (Loreau et al. 2003). These fluxes subsidize recipient ecosystems by supporting local productivity (Polis et al. 1997) and increasing food web stability (Barnes et al. 2018). Emergent aquatic insects, for instance, serve as high-quality prey for terrestrial consumers such as bats, birds and spiders, by providing energy (Bartels et al. 2012; Baxter et al. 2005; Nakano & Murakami 2001) and scarce but physiologically important fatty acids (FA) (e.g., Twining et al. 2019). In particular, certain polyunsaturated FA (PUFA), such as arachidonic acid (20:4n-6, ARA) and eicosapentaenoic acid (20:5n-3, EPA), have been shown to promote immune response, breeding success and growth in terrestrial consumers (Fritz et al. 2017; Twining et al. 2018; Twining et al. 2019). Changes in the pattern (i.e., biomass and timing) and quality (e.g., FA profile) of aquatic emergence may therefore affect terrestrial consumers and cascade bottom-up and top-down in terrestrial food webs (reviewed in Schulz et al. 2015).

Changes in emergence can be induced by natural factors such as season and food quantity and quality (Goedkoop et al. 2007; Sabo & Power 2002) or by anthropogenic factors that modify aquatic ecosystems. Mining, industry and agriculture are, for example, important sources of a wide range of contaminants to aquatic ecosystems (Kumar et al. 2019; Stehle & Schulz 2015; Wolfram et al. 2018). Due to their persistence, metals accumulate in sediments (e.g., Feiler et al. 2013) leading to chronic exposure of aquatic insect larvae inhabiting them (e.g., Arambourou et al. 2020; Paul et al. 2021; Pedrosa et al. 2017). Metal exposure can indeed impair the fitness of insect larvae (Timmermans et al. 1992) which is reflected in their emergence, modifying aquatic subsidy to terrestrial food webs (Kraus et al. 2014; Schmidt et al. 2013; Wesner et al. 2014). At the same time, exposure over multiple generations can lead to physiological acclimation or genetic adaptation (Morgan et al. 2007). Regardless of the underlying process such chronic (i.e., multigenerational) exposure may thus lead to more tolerant populations (Newman & Clements 2008) and might be particularly fast in species with

short generation times, such as some members of the family Chironomidae (Pinder 1986; Tokeshi 1995). Indeed, chronic metal exposure has been shown to reduce chironomids' sensitivity (Miller & Hendricks 1996; Postma & Davids 1995; Postma et al. 1995b), which could, in turn, positively reflect on prey availability for riparian consumers and thus the flux of physiologically important nutrients over time. However, whether chronic exposure drives a selection process in populations that substantially alters their sensitivity and thus the quantitative and qualitative subsidy of terrestrial ecosystems remains unclear.

With the aim to address this knowledge gap, we assessed the sensitivity of populations of the holometabolous aquatic insect *Chironomus riparius* (Diptera: Chironomidae) when exposed to the essential metal copper (Cu), both before and after about six months of chronic exposure towards this stressor. Food quality was assessed as an additional factor triggering differences in sensitivity. The selection of *C. riparius* is motivated by the widespread occurrence of chironomids in freshwater habitats, the benthic lifestyle of the larvae, and importance as prey for riparian terrestrial consumers (Armitage 1995; Leeper & Taylor 1998; Martin-Creuzburg et al. 2017; Ohler et al. 2023a; Pedrosa et al. 2017). Copper was chosen based on its wide application as fungicide, particularly in vineyards (Roßberg 2009), and its worldwide detection in freshwater ecosystems (Feiler et al. 2013; Paul et al. 2021; Pedrosa et al. 2017). Finally, Spirulina and TetraMin served as food of differing quality during the sensitivity tests. The difference in quality is supported by their respective FA profiles (Goedkoop et al. 2007; Strandberg et al. 2020). To characterize potential changes in the aquatic subsidy to terrestrial consumers, we assessed the number and weight of emergent adults as well as the time to emergence. The nutritional quality of emergent *C. riparius* was approximated based on their FA profile.

In both sensitivity tests (ST), we hypothesized that (I) Cu exposure reduces the number of emergent adults, their weight at emergence or delays emergence in a dose-dependent manner. It was further hypothesized that (II) the less nutritious food (i.e., Spirulina) enhances Cu toxicity compared to the more nutritious TetraMin. (III) Copper-exposed populations were

expected to be less sensitive to Cu compared to unexposed (naïve) populations in the second sensitivity test (ST2). (IV) The FA profile in adult chironomids was expected to differ mainly depending on the basal food source (Spirulina vs. TetraMin). Yet, we hypothesized that (V) the transport of physiologically important PUFA by emergent adults would be primarily influenced by emergence success and would decrease with increasing Cu sediment content.

Material and methods

Study design and in-house culture

The study comprised a chronic *C. riparius* culture and two STs. The chronic culture and the first sensitivity test (ST1) were started in parallel with larvae originating from an in-house culture. The in-house culture was maintained according to OECD guidelines (OECD 2004; OECD 2010) and kept in a climate-controlled chamber at $20\pm1^{\circ}\text{C}$ with 65% humidity, a light/dark cycle of 16/8 hours and under continuous aeration (i.e., few bubbles per second). Laboratory cultures generally suffer from genetic impoverishment affecting chironomids' sensitivity (Nowak et al. 2007). The refreshment with egg ropes from laboratory cultures of other institutions (i.e., BASF SE (Ludwigshafen, Germany), ECT Oekotoxicology GmbH (Flörsheim, Germany) and SBiK-F/LOEWE-TBG (Frankfurt a.M., Germany)) and the maintenance of a large population size aimed to create a more robust and adaptable population.

Prior to the start of the chronic culture and ST1, freshly laid egg masses (< 24 h) were removed from the in-house culture and placed in crystallizing dishes filled with test medium (SAM-5S, Borgmann 1996). Crystallizing dishes were gently (i.e., $\leq$ one bubble per second) aerated and a small amount of finely ground TetraMin (about a spatula tip) was added as food for freshly hatched larvae (OECD 2010). While chironomids were exposed for 35 days to one of six Cu sediment contents (0-400 mg/kg dw) as part of ST1, they were reared in the absence (naïve) or presence (pre-exposed) of Cu (100 mg/kg dw) for about six months in the chronic culture.

ST2 was started with freshly hatched larvae originating from naïve or pre-exposed populations of the chronic culture. The procedures and tested Cu sediment contents corresponded to those of ST1.

Chronic culture

Chironomus riparius larvae were reared in sediment-water microcosms for about six months in the absence or presence of Cu (Figure 1). Both exposure scenarios had six replicates, each consisting of two microcosms (glass vessel, about 32x22x7 cm each) placed in a mesh cage (50x50x35, 0.6 mm mesh size) to keep emergent adults separated by treatment and replicate, creating a total of twelve independent populations. To achieve an environmentally relevant nominal Cu sediment content of 100 mg/kg dry weight (dw; 1.0 to 3100 mg/kg dw; Arambourou et al. 2020; Feiler et al. 2013; Steinmetz et al. 2017), a Cu solution prepared according to Pietz et al. (2023a) was added to the artificial sediment (OECD 2004; OECD 2010). Subsequently, 1,100 g wet weight of the Cu-spiked or unspiked sediment was evenly distributed to each microcosm and carefully topped by test medium (2,000 mL, Borgmann 1996) to disturb the sediment as little as possible. All microcosms were prepared 16 days in advance, to ensure near-equilibrium conditions of Cu between environmental media before introducing larvae (OECD 2004; Simpson et al. 2004).

Each population was started by introducing about 400 randomly chosen first instar larvae (OECD 2004). Aeration was suspended for 24 h to facilitate their settlement. Larvae were fed twice per week with TetraMin at a rate of about 0.5 mg per larva and day. To counteract evaporation the water level was replenished in parallel to the feeding. Additionally, the test medium was renewed every two weeks to prevent deterioration in water quality. The environmental conditions corresponded to those of the in-house culture. Populations were evenly distributed within the climate-controlled chamber. At the end of the culture period, freshly laid egg ropes (<24 h) were collected from naïve and pre-exposed populations and used to start ST2.

Sensitivity tests

To determine the sensitivity of *C. riparius* to six environmentally relevant Cu sediment contents (0 to 400 mg/kg dw), two toxicity tests were conducted, each lasting 35 days. While larvae from the in-house culture were used in ST1, larvae from naïve and pre-exposed populations of the chronic culture were used in ST2 about six months later. The sediment preparation and test conditions were identical for both STs and followed the procedure employed for the chronic culture.

Both sensitivity tests took advantage of increasing Cu sediment contents (i.e., 0, 50, 100, 200, 300 and 400 mg/kg dw). Each Cu sediment content was paired with two basal food sources (i.e., Spirulina and TetraMin) of differing quality in terms of their FA profile (Strandberg et al. 2020). Each treatment (i.e., combination of Cu sediment content, food quality and larval origin) consisted of six 250-mL glass beakers filled with 80 g wet weight sediment and 150 mL test medium. Twenty first instar larvae were added to each replicate. For ST2, each replicate per treatment was formed with larvae from one of the naïve or pre-exposed populations, making them truly independent. Larvae were fed every second day at a rate of about 0.5 mg per larva per day, independent of the basal food source. Additionally, the water level was replenished to counteract evaporation. Each replicate was covered by mesh tents (0.6 mm mesh size) to keep emerged adults separated. Adults were collected daily, separated by sex, counted, and stored at -80 °C. Freeze-dried adults from all treatments of ST2 were weighed to the nearest 0.01 mg. Furthermore, adults from the control treatments of ST1 were freeze-dried and weighed to the nearest 0.01 mg to assess potential effects of the laboratory conditions on the dw and fatty acid profile ultimately supporting the interpretation of effects induced by the specifically tested factors.

Fatty acid analysis

Total FAs were quantified in female and male adults in 16 of 24 treatments of ST2 (n = 4-6) to investigate the effects of 'Cu sediment content', 'Food quality' and 'Exposure history'. Eight treatments were omitted because we did not expect any effects or the emergence was very

low. Additionally, we analyzed female and male adults from the control treatments of ST1 (n = 6) to identify potential changes in the FA profiles due to the laboratory conditions.

The FA analysis was performed according to the procedure described in Pietz et al. (2023b). Briefly, lipids were extracted from freeze-dried and weighed samples (0.4 – 5.7 mg dw) in a 5 ml chloroform/methanol mixture (2:1; v:v). As internal standard, a triacylglycerol with three deuterated 18:0 FAs (Tristearin-D105; Larodan) (Konschak et al. 2020) was added and the samples were stored at –20 °C overnight. The next day, samples were dried under a stream of nitrogen at 40 °C and FA were converted to fatty acid methyl esters (FAMES) using 3 N methanolic HCl (Sigma-Aldrich) at 70 °C for 20 min. Subsequently, FAMES were extracted using isohexane, dried under a stream of nitrogen at 40 °C, and dissolved in 100 µl of dichloromethane. 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. Using FAME standards (37-component FAME Mix; Supelco CRM47885), FAMES from each sample were identified based on retention times 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 to assess the recovery rate and hence adjust FA concentrations. Furthermore, FA concentrations were adjusted by blank correction and the corrected FA concentrations were normalized to the total volume and dw of the sample (microgram FA per milligram dry wt).

Data analysis

Prior to statistical analysis, the data were checked for outliers, that is, the number of emergent adults in a replicate being outside the 95% CI. Deviations could be related to the inadvertent addition of eggs or mating of early emergent adults. A total of eight replicates (all from ST2, <4% of all replicates) were excluded from further statistical analysis.

We assessed differences in the number of emergent adults using a generalized linear model (GLM) with poisson distribution. For ST1, 'Cu sediment content' and 'Food quality' as well as their interaction were implemented as explanatory variables. For ST2, 'Cu sediment content', 'Food quality' and 'Exposure history' as well as the interaction between 'Cu sediment content' and 'Food quality' and 'Cu sediment content' and 'Exposure history' were implemented as explanatory variables. The adult dw was assessed in all treatments of ST2 and the control of ST1 (see 'Sensitivity tests'). Differences in the dw of adult females and males were assessed using linear models (LM). For ST2, 'Cu sediment content', 'Food quality' and 'Exposure history' as well as the interaction between 'Cu sediment content' and 'Food quality' and 'Cu sediment content' and 'Exposure history' were implemented as explanatory variables. To investigate potential effects of the laboratory conditions on the adult dw in the controls, 'Larval origin' (i.e., larvae originating from the in-house culture, or from one of the chronic cultures) was implemented as explanatory variable. Visual inspection of model residuals was used to check model assumptions.

The emergence time was assessed using the EmT50 (time to 50% emergence) for all individuals as well as for females and males separately. For this, we ran non-linear regression analysis on the emergence data, expressed as percent of the cumulative emergence at the end of each ST, to identify the time by which 50% ($\pm$ 95% CI) of *C. riparius* larvae had emerged. Several log-normal, log-logistic and Weibull models were fitted to the data using the package "drc" (Ritz et al. 2015) and the best model fit (mostly Weibull models) was determined based on the lowest Akaike's Information Criterion score. Obtained EmT50 values were compared using CI-testing (cf. Bundschuh et al. 2019).

We used redundancy analysis (RDA) to evaluate the effect of 'Cu sediment content', 'Food quality', 'Larval origin', and 'Sex' on the FA profile of chironomids. The gradient lengths determined by detrended correspondence analysis were always below one standard deviation, suggesting a monotonic response and the appropriate use of a linear model (Braak & Prentice 1988). Data were scaled (i.e., standardized to unit variance) and a permutation test was used

to assess the significance of the RDA model and canonical axes. Scores were calculated in the same ordination space but separated between 'Sex' and 'Larval origin' in the ordination to improve clarity. Similarly, we included only FAs that constitute a large proportion ($\geq 25\%$) of a FA group (i.e., saturated fatty acids - SFA, monounsaturated fatty acids - MUFA, PUFA), are physiologically important, or serve as precursor to a physiologically important FA.

Statistical analyses and data visualization were performed with R Version 4.3.2 for Windows (R Core Team 2021). The significance level α was set at 0.05. In addition, effect sizes (i.e., percentage of difference between the mean of each treatment and the control's mean) were used to interpret the data.

Results

Emergence success

In ST1, adult emergence significantly decreased by about 30%, 65%, and 95% at Cu sediment levels of 200, 300, and 400 mg/kg dw ($p = 0.011$, $p < 0.001$, $p < 0.001$, GLM, Figure 1), respectively. In ST2, adult emergence significantly decreased in a dose-dependent manner by about 40%, 65%, and 90% at Cu sediment contents of 200, 300, and 400 mg/kg dw, respectively (always $p < 0.001$, GLM, Figure 1). At 200 mg/kg dw, emergence was significantly more reduced if individuals were fed *Spirulina* compared to TetraMin (difference in effect sizes by about 20%, $p = 0.043$, GLM, Figure 1), a pattern primarily driven by males. In females, we observed a non-significant tendency toward lower emergence (about 15%) in the *Spirulina* compared to the TetraMin treatment ($p = 0.079$, GLM). At 300 mg/kg dw, the number of emergent adults significantly varied based on the exposure history ($p = 0.038$, GLM, Figure 2), with female emergence being reduced by about 85% and 65% in naïve and pre-exposed populations, respectively.

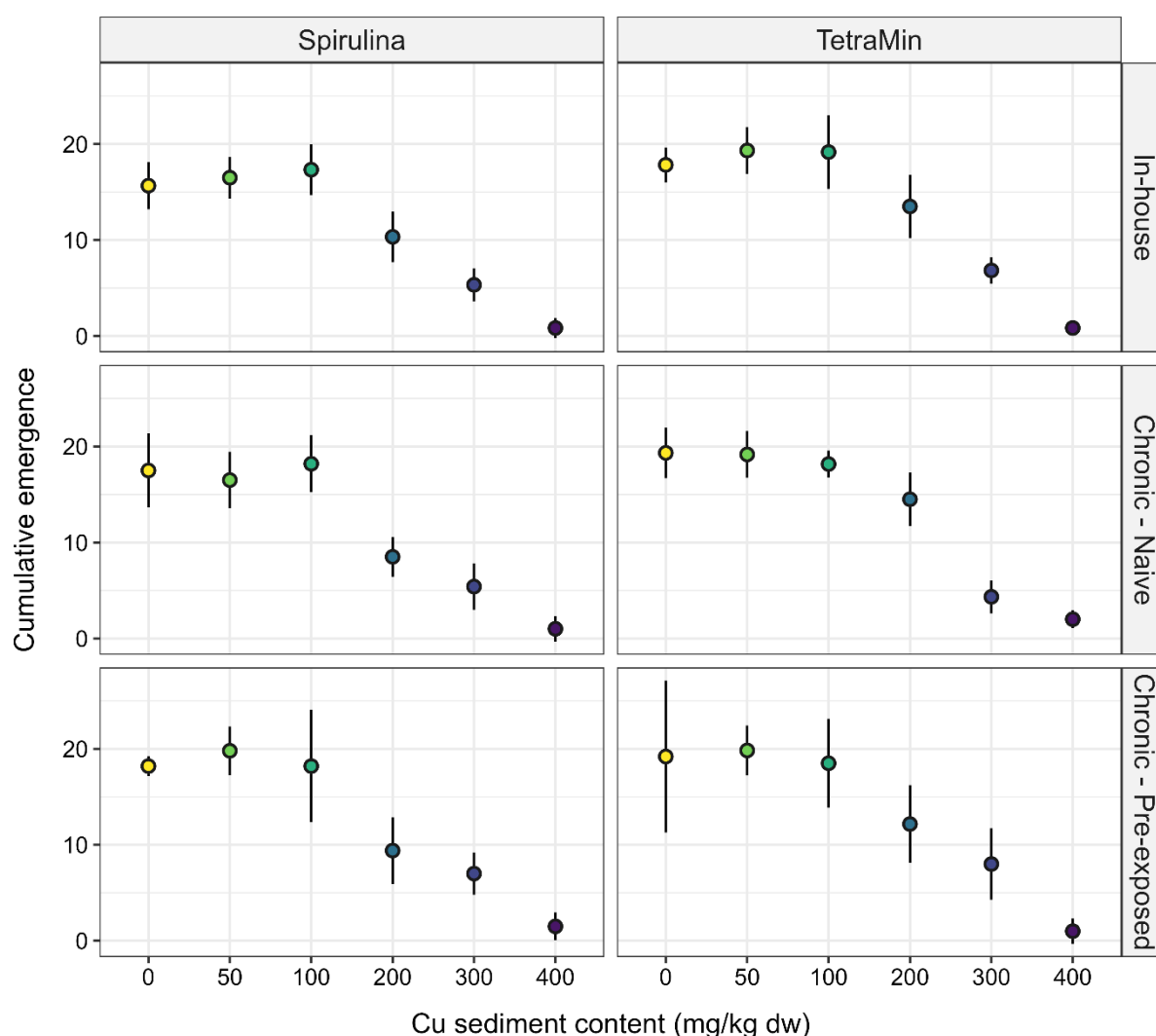


Figure 1: Mean ($\pm$ 95% CI) cumulative number of emerged *C. riparius* at Cu sediment contents of 0, 50, 100, 200, 300 and 400 mg/kg dw (colour-coded for ease of comparison). *Chironomus riparius* sensitivity was assessed for populations at test initiation ('In-house', ST1) or reared in absence or presence of Cu for six months ('Chronic – Naive', 'Chronic – Pre-exposed', respectively, ST2) and larvae were fed one of two food sources ('Spirulina' or 'TetraMin').

Time to 50% emergence

In ST1, the EmT50 differed significantly from the control at all Cu exposure levels, but only substantially increased at a Cu sediment content of 200 mg/kg dw or higher, with delays reaching up to 13 days at 400 mg/kg dw (Figure 2). While males generally emerged about three days earlier than females, the difference was almost absent or reversed at 400 mg/kg dw (Figure 2). The food quality did not affect the EmT50 (Figure 2). In ST2, adults emerged

significantly earlier at 50 and 100 mg/kg dw (up to about one day), whereas they emerged significantly later at 400 mg/kg dw (up to about eleven days; Figure 2). Males generally emerged about three days before females, though EmT50 became more similar at the two highest Cu sediment levels (Figure 2). Spirulina significantly delayed the EmT50 by about one day with effects being most pronounced at Cu sediment contents up to 100 mg/kg dw (Figure 2). Adults from pre-exposed populations emerged significantly but only slightly later than adults from naïve populations (about 0.5 days; Figure 2).

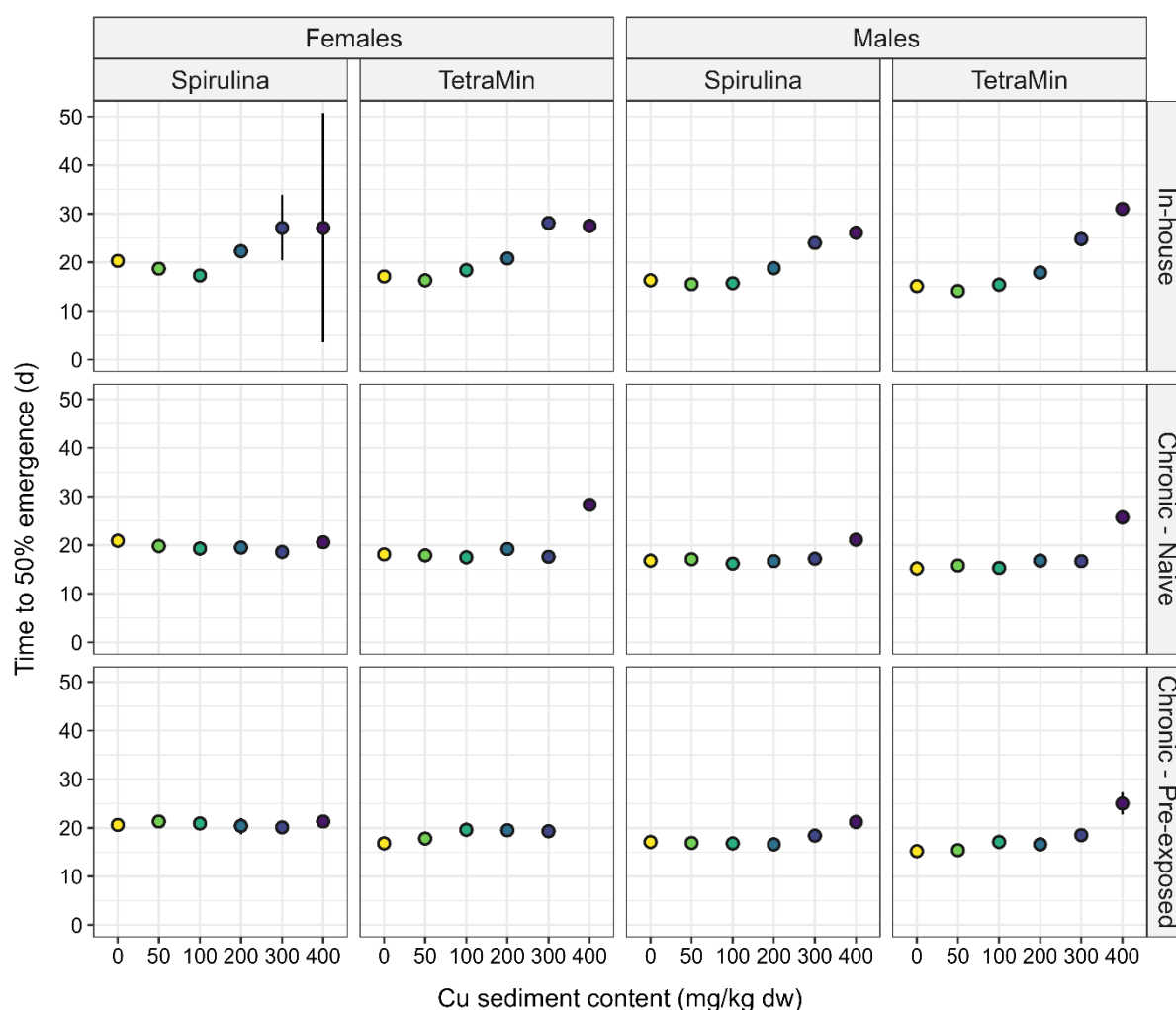


Figure 2: Time to 50% emergence (EmT50, $\pm$ 95% CI) of *C. riparius* females and males. Chironomids originated from one of three cultures ('In-house', ST1; 'Chronic – Naive' and 'Chronic – Pre-exposed, ST2), were fed either Spirulina or TetraMin, and exposed to one of six Cu sediment contents, that is 0, 50, 100, 200, 300 and 400 mg/kg dw (colour-coded for ease of comparison). 95% CIs were mostly very small and could not be displayed. Due to the high mortality, no EmT50 could be calculated for females from the 'Chronic -Pre-exposed' population fed with TetraMin at 400 mg Cu/kg dw.

Dry weight at emergence

Emergent males were about 50% lighter than females (Figure 3). In ST2, male adult dw significantly increased by about 15%, 7%, and 3%, at 50, 300, and 400 mg/kg dw, respectively, compared to the control ($p = 0.030$, $p = 0.037$, and $p = 0.003$, LM, Figure 3). Female dw was significantly increased by about 15% at 300 mg/kg dw compared to the control ($p < 0.001$, LM, Figure 3). Positive effects induced by Cu on the dw were significantly more pronounced in adult females (at 300 mg/kg dw, $p < 0.001$, LM) and males (at 300 and 400 mg/kg dw, $p = 0.008$, $p < 0.001$, LM) when larvae were fed Spirulina relative to TetraMin (Figure 3). However, the dw of females and males was generally significantly lower (about 15% and 10%, respectively) when larvae were fed Spirulina relative to TetraMin ($p < 0.001$ for both sexes, LM, Figure 3). Overall, adult dw was similar for individuals originating from naïve and pre-exposed populations.

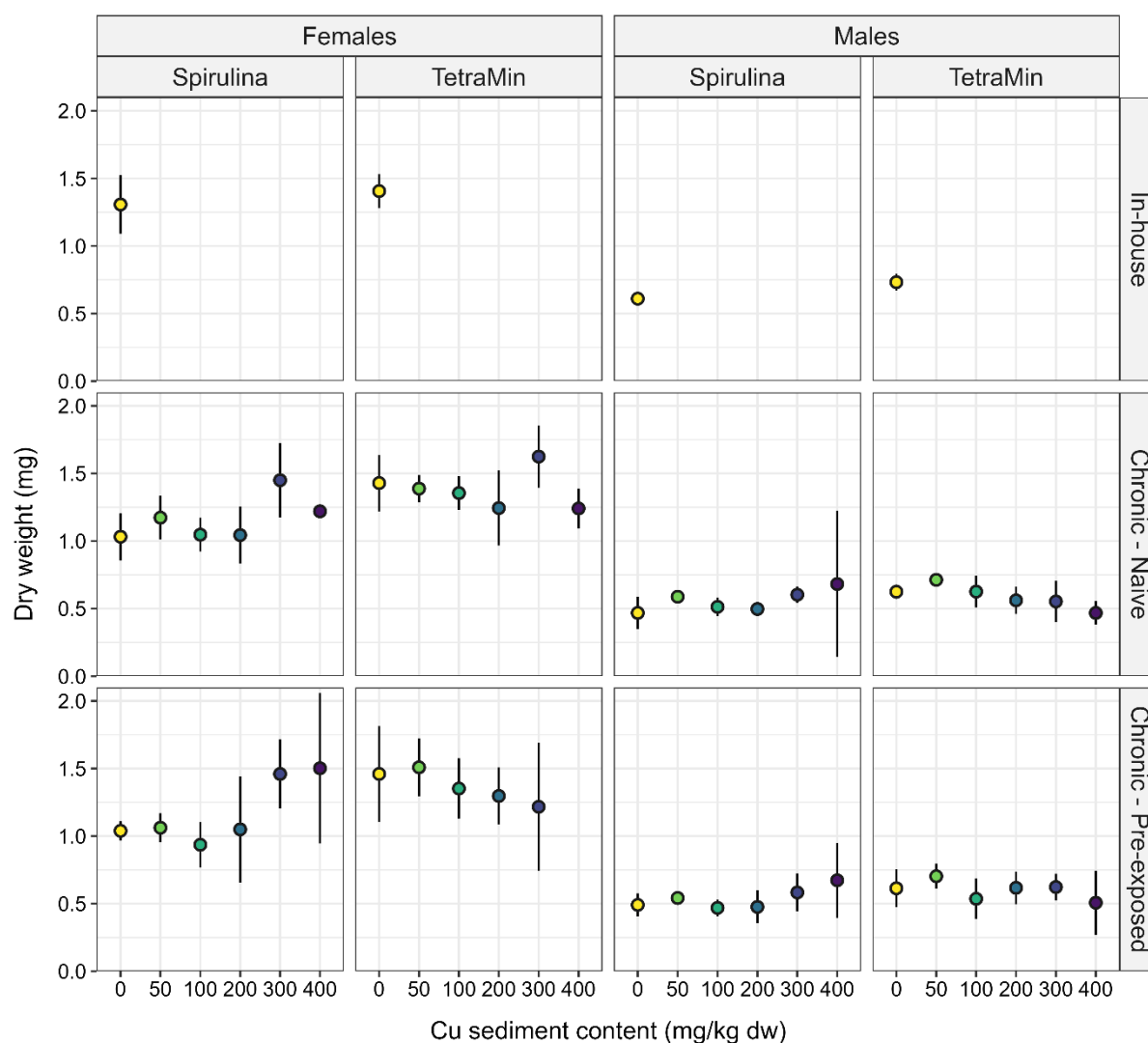


Figure 3: Mean ($\pm$ 95% CI) dw (mg) of *C. riparius* females and males from all treatments (i.e., combination of 'Cu sediment content', 'Food quality', and 'Exposure history') of ST2. Female and male dw from the control treatments of ST1 were assessed to identify potential changes due to the laboratory conditions. Chironomid larvae were exposed to Cu sediment contents of 0, 50, 100, 200, 300 and 400 mg/kg dw (colour-coded for ease of comparison). Chironomids originated from one of three cultures ('In-house', ST1; 'Chronic – Naive' and 'Chronic – Pre-exposed', ST2) and larvae were fed one of two basal food sources ('Spirulina' or 'TetraMin').

Emergence quality

According to the RDA, 'Food quality' was the most important predictor of the chironomid FA profile, while 'Sex', 'Larval origin', and 'Cu sediment content' contributed to the observed differences to a lesser extent (Figure 4). The first three RDA axes were significant ($p = 0.001$,

Detrended correspondence analysis), explaining 43%, 8%, and 2% of the total variation in the data (79%, 15%, and 3% of the constrained variation), respectively.

Axis RDA1 reflected differences in the FA profil in individuals fed Spirulina or TetraMin ('Food quality'; Figure 5). Individuals fed Spirulina contained substantially higher contents of 20:4n-6 (ARA; about 120%), its precursor 18:3n-6 (GLA, about 3200%) and, thus, omega-6 PUFA (about 20%), compared to individuals fed TetraMin. In contrast, those individuals contained lower levels of the omega-3 PUFA (about 85%), 20:5n-3 (EPA) and its precursor 18:3n-3 (ALA), MUFA (about 40%) and SFA (about 10%). Axis RDA2 was most strongly influenced by 'Sex', followed by 'Larval origin' (Figure 5). Females contained high levels of 16:0, 18:0, 16:1n-7, 18:1n-9 and thus SFA and MUFA. While they were also rich in 18:3n-6 and 18:3n-3, males contained higher levels in the physiologically important 20:4n-6 (ARA; about 35%) and 20:5n-3 (EPA; about 10%) and thus a similar content of omega-3 and omega-6 PUFA. Individuals from the control treatments of either population in ST2 contained more 16:0, 18:0, 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-6 (GLA), 18:3n-3 (ALA), and 20:5n-3 (EPA) (about 10-45%), but less 20:4n-6 (about 50%) compared to individuals from the control treatments in ST1. The FA levels in individuals from naïve and pre-exposed populations were relatively similar though 18:3n-3 (ALA) and 20:4n-6 (ARA) levels tended to be higher (about 10% and 15%, respectively) in pre-exposed populations. Besides 'Larval origin', axis RDA3 was most strongly influenced by 'Cu sediment content' and separated the FA profiles between individuals from the control and those exposed to 300 mg Cu/kg dw (Figure 5). At this Cu content, individuals were rich in 16:0, 16:1n-7, 18:1n-9, 18:2n-6 (by about 15%-35%) and thus had higher SFA (about 25%) and MUFA (about 25%) contents than individuals from the control. However, they contained less 20:4n-6 (ARA) as well as 20:5n-3 (EPA) and its precursor 18:3n-3 (ALA) (about 7%-15%).

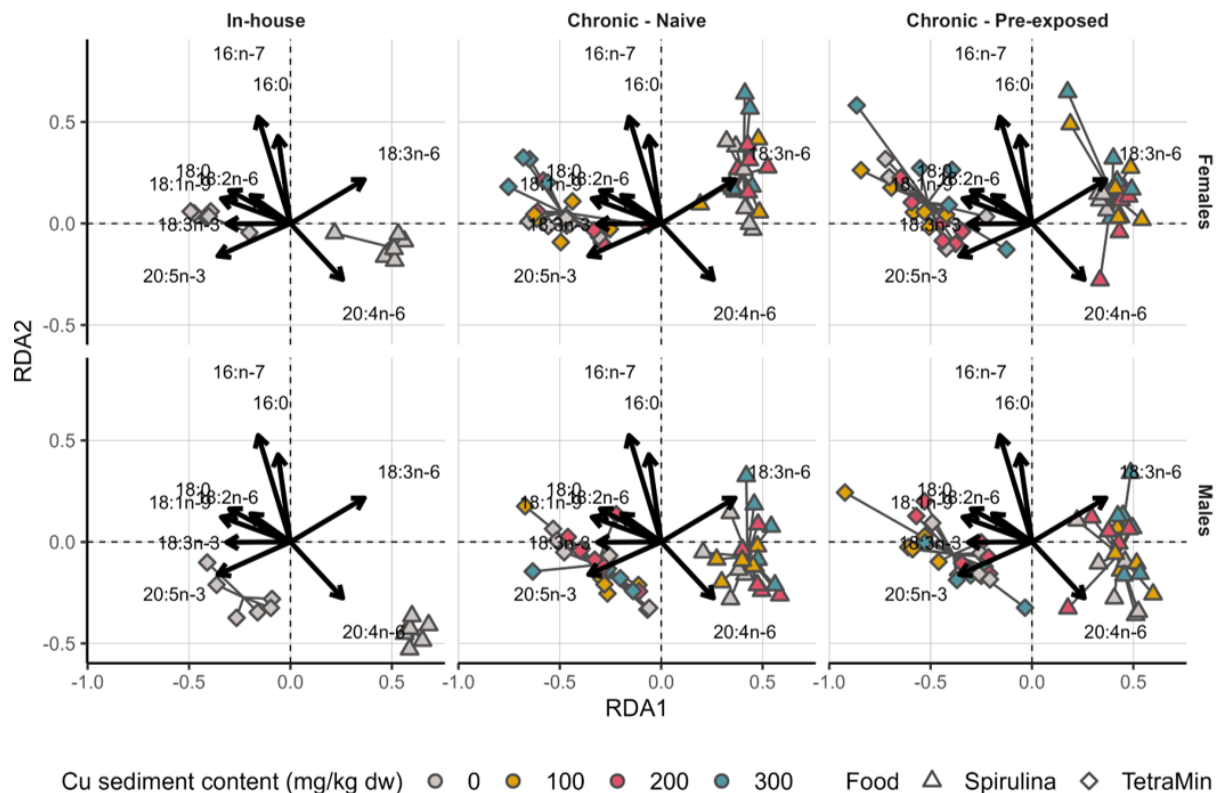


Figure 4: Redundancy analysis using FA profiles (micrograms per milligram dw) of *C. riparius* females and males from 16 treatments (i.e., combination of 'Cu sediment content', 'Food quality', and 'Exposure history') of ST2. Eight treatments were omitted because we did not expect any effects or the emergence was very low. Moreover, we analyzed female and male adults from the control treatments of ST1 (n = 6) to identify potential changes in the FA profiles due to the laboratory conditions. Copper sediment contents are represented by colour; 0 mg/kg dw (grey), 100 mg/kg dw (yellow), 200 mg/kg dw (red), and 300 mg/kg dw (blue). The food offered to *C. riparius* larvae is indicated by triangles (Spirulina) and diamonds (TetraMin). Scores were calculated in the same ordination space but separated between sex and the origin of larvae (i.e., 'In-house', ST1; 'Chronic – Naive' and 'Chronic – Pre-exposed, ST2) in the ordination to improve clarity of the figure.

Discussion

Emergence: number, time and dry weight

Cu sediment content

In line with our hypothesis, Cu reduced the number of emergent adults. Differences compared to the control were observed starting from Cu sediment contents of 200 mg/kg dw. Larval Cu exposure likely caused direct toxic effects and induced cellular detoxification (e.g., synthesis of metallothioneins and antioxidants) and repair mechanisms leading to a higher basal energy

demand (Calow 1991; Sibly & Calow 1989; Sokolova & Lannig 2008; Sokolova et al. 2012). At the same time, metals can also interfere with organisms' energy supply by disrupting the mitochondrial function, thus, offsetting their energy balance (Sokolova & Lannig 2008; Sokolova et al. 2012; Stohs & Bagchi 1995; Wallace & Starkov 2000). Since the larval developmental stage in holometabolous aquatic insects is followed by the energetically demanding metamorphosis (Arrese & Soulages 2010), toxic effects ultimately translate to an impaired emergence. Both larval mortality and energetic imbalances are likely to increase with increasing Cu sediment contents, explaining the dose-dependent decrease in emergence by about 40%, 65%, and 90% at 200 mg/kg dw, 300 mg/kg dw and 400 mg/kg dw, respectively (Figure 1). At lower sediment contents (50 and 100 mg/kg dw), larvae seemed able to cope with or benefit from Cu exposure, possibly by minimizing Cu body burdens or bioavailable fractions (see Morgan et al. 2007), and the need for Cu in certain physiological processes (e.g., component of enzymes, Flemming & Trevors 1989), respectively.

Given that insects require a certain amount of energy to survive metamorphosis (Mirth & Riddiford 2007) and Cu exposure likely affected energy storage, emergence was delayed by up to eleven days at 400 mg/kg dw, consistent with our hypothesis. Additionally, differences in the emergence times between females and males (i.e., protandry, Armitage 1995) tended to become negligible or even reverse (i.e., females emerging before males, Figure 2) at this Cu sediment content. This could influence mating success (Armitage 1995), particularly in combination with the reduced total emergence. The tendency for a prolonged larval development at high Cu sediment contents in combination with the substantial reduction in emergence may have contributed to the increase in adult dw (Figure 3), contradicting our hypothesis. This may be assumed as the amount of added food was corrected for emergence but not for mortality suggesting a higher food availability per surviving larva in combination with lower competition (Kraus & Vonesh 2012; Postma et al. 1994).

Food quality

Besides food availability, its nutritional quality affected *C. riparius* sensitivity towards Cu, an observation in support of our hypothesis. This observation may be related to a larger discrepancy between nutrients provided by Spirulina and the physiological demands of *C. riparius* compared to TetraMin (Goedkoop et al. 2007). For example, Spirulina contains low levels of the physiologically important omega-3 PUFA 20:5n-3 (EPA) and its precursor 18:3n-3 (ALA) (Pietz et al. 2023b; Strandberg et al. 2020). While *C. riparius* has the ability to modify and preferentially retain FA (Goedkoop et al. 2007; Ohler et al. 2023b; Pietz et al. 2023b; Strandberg et al. 2020) this requires an energetic investment relative to a dietary uptake (Parrish 2009) and is thus less favorable. Consequently, failure to divert sufficient energy to counteract toxic effects of Cu or to upgrade dietary nutrients to meet physiological demands may have contributed to a lower emergence success, as observed at 200 mg/kg dw (reduction by about 20%, Figure 1). Although the proposed energetic investments did not affect emergence success at lower Cu sediment contents (0-100 mg/kg dw), they may have contributed to prolonged larval development and thus delayed emergence (Figure 2). Furthermore, the dw of females and males fed Spirulina decreased relative to those fed TetraMin (about 15% and 10%, respectively; Figure 3). This result is generally in agreement with previous findings investigating different food types (Goedkoop et al. 2007) and suggests potential implications for reproduction, as female weight correlates with fecundity (Rodrigues et al. 2015; Sibley et al. 2001). However, contrary to our hypothesis and the results discussed above, adult dw increased more strongly when larvae were fed Spirulina compared to TetraMin at high Cu exposure levels. Since emergence success was similar in both food types, this observation cannot be attributed to differences in food availability. While Spirulina has lower levels of physiologically important omega-3 PUFAs, both basal food sources differ in more than just this aspect. Differences in other nutrients, such as the higher protein content in Spirulina, may have contributed to these results. A generally more efficient utilization of low-quality resources or altered energy allocation is, however, unlikely as both chironomid populations were exclusively fed TetraMin, providing no selective pressure unlike the simulated chronic Cu exposure.

Exposure history

Consistent with our hypothesis, emergence was higher in pre-exposed compared to naïve populations at 300 mg/kg dw (reduction in females by about 65% and 85%, respectively, Figure 1), indicating differences in their ability to cope with Cu-induced stress. Genetic adaptation has been highlighted as a cause for decreased sensitivity in metal-exposed (field) populations (Miller & Hendricks 1996; Postma et al. 1995a; 1995b), but phenotypic plasticity may be another explanation (Marinković et al. 2012). Implementing population genomic methods to compare the genetic information in naïve and pre-exposed populations could provide clarity on the underlying processes. Regardless, the effect of exposure history appeared to be superimposed by the effect of Cu sediment content and food quality. Increased tolerance to Cu was only observed at high exposure levels, suggesting that it may come with a trade-off in individual fitness (Postma et al. 1995b). Additionally, sensitivity may depend on the initial presence of traits required for increased tolerance, and the experimental duration may not have been sufficient to promote their formation or establishment. Alternatively, based on the results of both STs, the selective pressure from chronic Cu exposure (100 mg/kg dw) may have been too weak. However, higher exposure levels reduce environmental relevance, which was considered undesirable.

Emergence quality and potential food web effects

In line with our hypothesis, food quality was the most important predictor of chironomids' FA profile. As a consequence of the differences in the basal food sources (i.e., *Spirulina* and TetraMin; Pietz et al. 2023b; Strandberg et al. 2020), individuals fed *Spirulina* contained higher levels of omega-6 PUFA (about 20%), including 20:4n-6 (ARA), but lower levels of all other FA groups, particularly omega-3 PUFA (about 85%), including 20:5n-3 (EPA), which are predominately synthesized by aquatic primary producers (Hixson et al. 2015; Twining et al. 2016). Similarly, differences in the FA profiles between sexes are consistent with previous findings (Meier et al. 2000; Pietz et al. 2023a; Scharnweber et al. 2020) and may be attributed to sex-specific life history strategies. Sexual dimorphism in morphology and energy allocation

have been documented previously (Servia et al. 2006). For instance, elevated levels of 20:4n-6 (ARA) and 20:5n-3 (EPA) in males may enhance flight ability (Dadd 1980; Sushchik et al. 2013), thereby improving swarming and mating (Armitage 1995). In contrast, females, who require higher energy reserves (e.g., for egg production), exhibited higher levels of less unsaturated FAs, typically associated with storage lipids.

In contrast to the emergence success, the Cu sediment content had relatively little effect on the FA profile. While SFA and MUFA tended to be higher at 300 mg/kg dw compared to the control, an opposite trend was observed for physiologically important PUFA, such as 20:4n-6 (ARA) and 20:5n-3 (EPA). The formation of reactive oxygen species (ROS), commonly observed under Cu exposure (Gaetke & Chow 2003), may have caused lipid peroxidation (Bailey et al. 2015; Gaetke & Chow 2003; Lannig et al. 2006), contributing to the decrease in highly unsaturated FAs. In contrast, the relative accumulation of FAs during the metamorphosis of chironomids (Borisova et al. 2016; Sushchik et al. 2013) might have partially counteracted Cu-induced negative effects. In addition, certain developmental milestones must be reached to initiate metamorphosis (Mirth & Riddiford 2007), indicating that emergent individuals had previously attained a minimum level of physiological fitness. Consequently, the effects of contaminants may be more likely to manifest in other endpoints, such as developmental time and emergence success. Accordingly, the effects of the exposure history on the FA profile of the chironomids were limited, although we did observe slightly elevated levels of 18:3n-3 (ALA) and 20:4n-6 (ARA). Nonetheless, the data rather suggest that chronic Cu exposure does not lead to a substantial improvement in the nutritional quality of emerging chironomids. However, due to the lower sensitivity of pre-exposed compared to naïve individuals at 300 mg/kg bw, the availability of FAs in adjacent terrestrial systems might slightly increase at this exposure level, albeit at an overall low level (reduction in emergence by 65%, Figure 2).

In line with our expectation (hypothesis V), increasing Cu sediment contents (≥ 200 mg/kg dw) cause a substantial reduction in the transport of FAs, including physiologically important PUFA, through the dose-dependent decrease in emergence success. This result is consistent with

previous findings by Pietz et al. (2023a) who observed reductions in the FA flux by up to 80% and 50% at field relevant levels of cadmium and Cu, respectively. Additionally, the quality of the basal food source can affect the transport of FA with emergent chironomids from aquatic to terrestrial systems both by alterations of the FA profile and emergence success. Therefore, lower quality food (i.e., *Spirulina*) can reduce the availability of FAs, particularly physiologically important omega-3 PUFA, in adjacent terrestrial systems. In this context, changes may be expected even at exposure levels where no direct effects on insect emergence are observed, if the aquatic primary producer composition is altered (Konschak et al. 2021; Paerl & Paul 2012; Taipale et al. 2016). Regardless of the underlying mechanism, reductions in the transport of PUFA with emergent chironomids, and aquatic insects in general, can adversely affect riparian terrestrial consumers, such as spiders, birds or bats through the food chain (Fritz et al. 2017; Fukui et al. 2006; Kato et al. 2003; Paetzold et al. 2011; Twining et al. 2018; 2019). Owing to their central position in riparian food webs, changes in the physiological fitness and reproduction may cascade onto lower and higher trophic levels (Bartels et al. 2012; Schulz et al. 2015).

Conclusion

Copper sediment contents and food quality primarily shaped the emergence quantity and quality of *C. riparius*, respectively. Pre-exposed *C. riparius* populations showed only limited signs of adaptation and thus a similar sensitivity to Cu exposure compared to naïve populations. However, laboratory conditions may have affected the physiological condition (i.e., developmental time, dw and FA profile) of naïve and pre-exposed populations to some degree. This may be a common phenomenon in chronic laboratory studies caused by genetic impoverishment generally observed in chironomid laboratory cultures. Yet, a drastic loss of genetic diversity in naïve and pre-exposed populations is rather unlikely because egg ropes from other laboratories were introduced in the months prior to the study with the intention to increase genetic diversity. Besides, the reproductive isolation lasted about six months (i.e.,

about eight generations; assuming a full life-cycle of 20-25 days, at 20°C; Marinković et al. 2012) but a decline in genetic diversity was previously observed only after about ten generations (Nowak et al. 2007). Accordingly, a general shift in chironomids' sensitivity in ST2 is not supported by the data (e.g., similar pattern in emergence success in ST1 and ST2). Therefore, we expect our observations to be robust and of biological relevance. This implies sustained alterations of aquatic-terrestrial linkages. Given the quantitative importance of chironomids for aquatic emergence and as prey for many terrestrial consumers (Leeper & Taylor 1998; Martin-Creuzburg et al. 2017; Ohler et al. 2023a; Regester et al. 2008) these alterations may ultimately re-shape riparian food webs (Henschel et al. 2001; Murakami & Nakano 2002; Poulin et al. 2010; Richardson et al. 2010; Recalde et al. 2020). The potential of populations to adapt to chronic exposure to contaminants, or other environmental pressures, is therefore important to assess the impact on the whole aquatic-terrestrial meta-ecosystem.

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7.4 Appendix A4

Author contributions

Metal exposure and sex shape the fatty acid profile of midges and reduce the aquatic subsidy to terrestrial food webs

Sebastian Pietz, Martin J. Kainz, Henning Schröder, Alessandro Manfrin, Ralf B. Schäfer, Jochen P. Zubrod, Mirco Bundschuh

Sebastian Pietz:	Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing
Martin J. Kainz:	Investigation, Formal analysis, Writing – review & editing
Henning Schröder:	Investigation, Writing – review & editing
Alessandro Manfrin:	Formal analysis, Writing – review & editing
Ralf B. Schäfer:	Formal analysis, Writing – review & editing
Jochen P. Zubrod:	Conceptualization, Methodology, Formal analysis, Writing – review & editing
Mirco Bundschuh:	Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing

Subsidy quality affects common riparian web-building spiders: consequences of aquatic contamination and food resource

Sebastian Pietz, Sara Kolbenschlag, Nina Röder, Alexis P. Roodt, Zacharias Steinmetz, Alessandro Manfrin, Klaus Schwenk, Ralf Schulz, Ralf B. Schäfer, Jochen P. Zubrod, Mirco Bundschuh

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

Effects of copper, food quality and exposure history on aquatic insect emergence: insights from a multigeneration study

Sebastian Pietz, Nina Röder, Sara Kolbensschlag, Agnes Schöndorfer, Klaus Schwenk, Mirco Bundschuh

Sebastian Pietz: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing

Nina Röder: Conceptualization, Methodology, Investigation

Sara Kolbensschlag: Conceptualization, Methodology, Investigation

Agnes Schöndorfer: Investigation

Klaus Schwenk: Conceptualization, Methodology

Mirco Bundschuh: Conceptualization, Methodology, Supervision, Writing – review & editing

7.5 Appendix A5

Curriculum vitae

Name: Sebastian Pietz
Citizenship: German



Education

10/2019 – present	PhD student at the iES Landau, RPTU Kaiserslautern-Landau DFG Research Training Group 2360 – “SystemLink” Title: Aquatic contaminants reduce the quantity and nutritional quality of aquatic subsidy to riparian consumers Reviewer: Jun.-Prof. Dr. Mirco Bundschuh, Prof. Dr. Ralf B. Schäfer
10/2016 – 09/2019	Master degree in Ecotoxicology (M. Sc.) at the University of Koblenz-Landau Master thesis: “Effects of heavy metals and temperature fluctuation on the development and emergence of <i>Chironomus riparius</i> in spiked-sediment bioassays” Reviewer: Jun.-Prof. Dr. Mirco Bundschuh; Dr. Jochen P. Zubrod
10/2012 – 09/2016	Bachelor degree in Environmental Sciences (B. Sc.) at the University of Koblenz-Landau Bachelor thesis: “Implications of Thiacloprid on <i>Gammarus fossarum</i>: a 21-day study to determine the relevance of different exposure pathways” Reviewer: Jun.-Prof. Dr. Mirco Bundschuh; Prof. Dr. Ralf Schulz
03/2012	Higher education entrance qualification (“Abitur”), Kurfürst Balduin Gymnasium Münstermaifeld

Professional experience

10/2019 – present	Research associate at the RPTU Kaiserslautern-Landau
02/2017 – 09/2019	Student assistant, working groups 'Ecotoxicology & Environment' & 'Functional Aquatic Ecotoxicology' Conducting / assisting with (laboratory) experiments Maintenance of animal and fungal cultures Conference organisation (SETAC GLB Meeting 2019)
06/2017 – 08/2017	Internship at Innovative Environmental Services (IES) Ltd., Witterswil, Switzerland
07/2016 – 09/2016	Internship at the University of Sydney, Faculty of Agriculture and Environment, Sydney, Australia (as part of the DAAD Rise Scholarship)
08/2014 – 09/2014	Internship at Bilsa Biological Station, Jatun Sacha Foundation, Ecuador

Peer-reviewed publications

Pietz, S., Kolbenschlag, S., Röder, N., Roodt, A. P., Steinmetz, Z., Manfrin, A., Schwenk, K., Schulz, R., Schäfer, R. B., Zubrod, J. P., & Bundschuh, M. (2023). Subsidy quality affects common riparian web-building spiders: consequences of aquatic contamination and food resource. *Environmental Toxicology and Chemistry*. doi.org/10.1002/etc.5614.

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Bundschuh, M., **Pietz, S.**, Roodt, A. P., & Kraus, J. M. (2022). Contaminant fluxes across ecosystems mediated by aquatic insects. *Current Opinion in Insect Science*, 100885.

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Konschak, M., Zubrod, J. P., Baudy, P., Fink, P., **Pietz, S.**, Duque, T., Bakanov, N., Schulz, R., & Bundschuh, M. (2021). Mixture effects of a fungicide and an antibiotic: Assessment and prediction using a decomposer-detritivore system. *Aquatic Toxicology*, 232, 105762.

Englert, D., Zubrod, J. P., **Pietz, S.**, Stefani, S., Krauss, M., Schulz, R., & Bundschuh, M. (2017). Relative importance of dietary uptake and waterborne exposure for a leaf-shredding amphipod exposed to thiacloprid-contaminated leaves. *Scientific reports*, 7(1), 16182.

Conference contributions (presenting author)

11/2022	SFE2 / GfÖ / EES Joint Meeting, International Conference on Ecological Sciences, Metz, France Onsite platform presentation “Sex-specific responses to aquatic micropollutants affect the fatty acid transport via hemi- and holometabolous aquatic insects”
08/2022	36th Congress of the Society of Limnology, Berlin, Germany Onsite platform presentation “Effects of aquatic micropollutants on the fatty acid transport via hemi- and holometabolous aquatic insects”
05/2022	32nd Annual Meeting of the Society of Environmental Toxicology and Chemistry, Copenhagen, Denmark Onsite poster presentation “Fatty acid profiles of <i>Chironomus riparius</i> : effects of developmental stage and heavy metal exposure”

05/2021	31st Annual Meeting of the Society of Environmental Toxicology and Chemistry, Virtuall Conference Online poster presentation “Trophic upgrading: Is <i>Chironomus riparius</i> able to compensate adverse effects of aquatic stressors for riparian consumers?”
05/2020	30th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Virtuall Conference Online platform presentation “Transcending borders? Effects of heavy metals on the development and emergence of <i>Chironomus riparius</i> in spiked-sediment bioassays”
02/2019	8th Young Environmental Scientists Meeting, Society of Environmental Toxicology and Chemistry, Ghent, Belgium. Onsite platform presentation “Effects of an antimicrobial mixture on the leaf-shredding amphipod <i>Gammarus fossarum</i> under different exposure pathways”