



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

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ABSTRACT

Emergent aquatic insects serve as high-quality prey for terrestrial consumers. However, persistent contaminants, such as metals, can affect aquatic insect larvae development, potentially inducing a selection process favoring more tolerant individuals and populations. Whether this adaptation ultimately influences the quantity and quality of subsidy to terrestrial riparian ecosystems remains unclear. Here, we investigated the capacity of *Chironomus riparius* (Diptera: Chironomidae) populations to adapt to the essential metal copper (Cu). First, we assessed the initial sensitivity of *C. riparius* towards environmentally relevant Cu sediment contents (0–400 mg/kg dw) in combination with two basal food sources (Spirulina, low quality; TetraMin, high quality). After about six months of chronic exposure to Cu (100 mg/kg dw) across multiple overlapping generations, sensitivity of both naive and pre-exposed populations was re-assessed. Copper exposure mainly shaped the emergence quantity of *C. riparius*, resulting in dose-dependent decreases in adult emergence at 200 mg/kg dw and higher (up to about 90 % at 400 mg/kg dw). In contrast, food quality primarily determined emergence quality, approximated by the chironomid fatty acid profile. Unexpectedly, pre-exposed populations exhibited limited signs of adaptation. While emergence was higher in pre-exposed compared to naive populations (reduction in females by about 65 % and 85 %, respectively) at 300 mg/kg dw, the overall effect of exposure history on subsidy quantity and quality appeared to be superimposed by the Cu sediment contents used in the sensitivity tests and by food quality. While these findings require verification through extended chronic exposure phases, they imply sustained alterations of aquatic-terrestrial linkages.

1. 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 and Murakami, 2001) and scarce but physiologically important fatty acids (FA) (e.g., Shipley et al., 2024; Twining et al., 2019). In this context, 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, 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 (Schulz et al., 2015; 2024).

Changes in emergence can be induced by natural factors such as season and food quantity and quality (Goedkoop et al., 2007; Sabo and 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 and 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 benthic insect larvae (e.g., Arambourou et al., 2020; Paul et al., 2021; Pedrosa et al., 2017). Metal exposure can

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indeed impair the fitness of insect larvae (Timmermans et al., 1992), whereby the magnitude of effects depends, for example, on the food supply (i.e., high food availability may reduce toxic effects, depending on exposure levels; Haas et al., 2004). Ultimately, toxic effects are reflected in insect emergence, thereby modifying aquatic subsidy to terrestrial food webs (Kraus et al., 2014; Schmidt et al., 2013; Wesner et al., 2014). At the same time, pre-exposure to metals can change toxicokinetics (i.e., absorption, distribution, metabolism, and excretion) and toxicodynamics (i.e., effect on an organism) following a defined exposure in a metal specific manner (Del Ramo et al., 1993; Gao et al., 2021; Willuhn et al., 1996). Moreover, exposure over multiple generations can lead to physiological acclimation or genetic adaptation (Bundschuh et al., 2023; Morgan et al., 2007), which can be reflected in the organisms energy reserves (Canli, 2005; 2006). Regardless of the underlying process, such chronic (i.e., multigenerational) exposure may lead to more tolerant individuals (Canli, 2006) and populations (Newman and Clements, 2008; Stuhlbacher and Maltby, 1992; Tsui and Wang, 2005). The latter might be particularly fast in species with short generation times, such as some members of the family Daphniidae (Araujo et al., 2019) and Chironomidae (Pinder, 1986; Tokeshi, 1995). Indeed, chronic metal exposure has been shown to reduce chironomids' sensitivity (Miller and Hendricks, 1996; Postma and 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 indeed drives a selection process in populations that substantially alters their sensitivity and thus the quantitative and qualitative subsidy of terrestrial ecosystems remains unclear.

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 (sensitivity test 1; ST1) and after about six months of chronic exposure (sensitivity test 2; ST2) towards the same stressor. Food quality was assessed as an additional factor triggering differences in emergence, as it affects, for example, the larval and adult size and nutritional quality of adults (Goedkoop et al., 2007; Pietz et al., 2023b; Strandberg et al., 2020). In this context, we were particularly interested in understanding how potential adaptation to one stressor (Cu) might affect the larvae's response to an additional environmental change, here represented by a shift in food type. Spirulina and TetraMin served as food of differing quality during both sensitivity tests (ST), as evidenced by their respective FA profiles (e.g., higher levels of nutritionally valuable omega-3 PUFA in TetraMin; Goedkoop et al., 2007; Strandberg et al., 2020). The selection of *C. riparius* is motivated by the widespread occurrence of chironomids in freshwater habitats, the benthic larval lifestyle, and importance as prey for riparian terrestrial consumers (Armitage, 1995; Leeper and 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, and its worldwide detection in freshwater ecosystems (Feiler et al., 2013; Paul et al., 2021; Pedrosa et al., 2017). Moreover, Cu was shown to impair development and emergence of *C. riparius*, their FA profile and ultimately the growth of their terrestrial predators (i.e., spiders) consuming their adult life stages (Pietz et al., 2023a; 2023b). Stimulated by those earlier studies we characterized potential changes in the aquatic subsidy to terrestrial consumers in the present study, by assessing the number and weight of emergent adults as well as the time to emergence. The nutritional quality of emergent *C. riparius* was approximated using their FA profile.

In both STs, we hypothesized that (I) Cu exposure reduces the number of emergent individuals, adult weight, or delays emergence in a dose-dependent manner. It was further hypothesized that (II) chironomids would be more sensitive to Cu when fed the less nutritious food (i.e., Spirulina) compared to the more nutritious TetraMin. (III) Chronically copper-exposed populations were expected to be less sensitive to Cu compared to unexposed (naive) 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, based on previous findings (Pietz et al., 2023a), we hypothesized that (V) the transport (i.e., flux) of physiologically important PUFA by emergent adults would be primarily influenced by emergence success and thus decreases with increasing Cu sediment content.

2. Material and methods

2.1. Study design and in-house culture

The study comprised a chronic *C. riparius* culture and two STs. In the chronic culture, chironomids were reared in the absence (naive) or presence (pre-exposed) of Cu (100 mg/kg dry weight; dw) for about six months. The STs were conducted at the start and end of the chronic culture and lasted 35 days each. The procedure including the tested Cu sediment contents (0–400 mg/kg dw) were identical in both STs.

Laboratory cultures generally suffer from genetic impoverishment affecting chironomids' sensitivity (Nowak et al., 2007). To counteract such developments, *C. riparius* from our in-house laboratory culture were mixed with adult *C. riparius* of other institutions (i.e., BASF SE (Ludwigshafen, Germany), ECT Oekotoxikologie GmbH (Flörsheim, Germany), and SBiK-F/LOEWE-TBG (Frankfurt a.M., Germany)) in the four months prior to this study. No new individuals were introduced in the two months before the exposure, allowing the population to stabilize during that period. All laboratory cultures had previously been maintained under standard protocols (e.g., OECD guidelines). All cultures (i.e., in-house and chronic culture) and STs were maintained under the same conditions, that is, according to OECD guidelines (OECD, 2004, 2010) in a climate-controlled chamber at $20 \pm 1^\circ\text{C}$ with 65 % humidity, a light/dark cycle of 16/8 h, and under continuous aeration (i.e., few bubbles per second). SAM-5S medium (Borgmann, 1996) was used as culture and test medium and the pH measured in the overlying water of control replicates was always within the recommended range of 6–9 (OECD, 2004).

Prior to the start of chronic culture and ST1, freshly laid egg masses (84; < 24 h old) were removed from the in-house culture on four days between 21 and 28 April, temporarily stored in the refrigerator and then placed together in crystallization dishes filled with test medium. Crystallizing dishes were gently aerated (i.e., \leq one bubble per second) and a small amount of finely ground TetraMin (about a spatula tip; Tetra GmbH, Melle, Germany) was added as food for freshly hatched larvae (OECD, 2010). Hatched larvae were randomly and evenly allocated to the respective experimental units. ST2 was started with freshly hatched larvae originating from naive or pre-exposed populations of the chronic culture. For this, egg masses collected from each replicate of the chronic culture were sampled on three consecutive days (4 – 22 collected) and stored in SAM-5S medium until hatching. The procedures corresponded to those described above.

2.2. Chronic culture

Chironomus riparius larvae were reared for about six months in the absence or presence of Cu. Each exposure scenario (i.e., with or without Cu) comprised six replicates, each replicate consisted of a mesh cage ($50 \times 50 \times 35$ cm, 0.6 mm mesh size). Each mesh cage contained two sediment-water microcosms (glass vessels, about $32 \times 22 \times 7$ cm each), in which *C. riparius* larvae were cultured. The use of two microcosms per replicate provided a larger surface area for larval development, thereby supporting a sufficient population size and limiting stress due to competition for resources such as space. This setup allowed for clear separation of emerging adults by treatment and replicate, resulting in a total of twelve independent populations (i.e., six populations per exposure scenario). To achieve an environmentally relevant nominal Cu sediment content of 100 mg/kg dw (1.0–3100 mg/kg dw; Arambourou et al., 2020; Feiler et al., 2013; Steinmetz et al., 2017), a Cu ($\text{CuSO}_4 \times 5$

H₂O) solution, prepared according to Pietz et al. (2023a), was added to the artificial sediment (OECD, 2004, 2010) with reported background concentrations and spiking efficiency around 2 mg/kg dw and 100 %, respectively (Pietz et al., 2023a). Subsequently, 1.1 kg wet weight of the Cu-spiked or unspiked sediment was evenly distributed to each microcosm and carefully topped by test medium (2000 mL, Borgmann, 1996) to minimize sediment resuspension. All microcosms were prepared 16 days in advance, to ensure near-equilibrium conditions of Cu among 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). To facilitate their settlement, the aeration of the microcosms was suspended for 24 h after their introduction. Larvae were fed twice per week (Tuesday and Friday) with TetraMin at a rate of about 0.5 mg per larva and day (OECD, 2004). To counteract evaporation the water level was replenished in parallel to the feeding (OECD, 2004). Additionally, the test medium was renewed every two weeks to prevent deterioration in water quality (based on observations from preliminary experiments). The number of larvae was not assessed at any time during the chronic exposure period in any of the replicates as this procedure represents a substantial disturbance and stress to the larvae confounding our results. Therefore, larval numbers were assumed to be consistent with the initial density, based on the assumption that each chironomid typically requires approximately 2 cm² of sediment surface area for development (OECD, 2004). As we observed successful development and emergence of adults in all replicates, we can assume that population sizes remained viable throughout the experiment.

The environmental conditions corresponded to those of the in-house culture. Naive and pre-exposed 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 naive and pre-exposed populations to start ST2.

2.3. Sensitivity tests

To determine the sensitivity of *C. riparius* to six environmentally relevant Cu sediment contents (up 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 naive 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, Spirulina (Bio-Nutra, BTG Berlin Trade GmbH, Germany) and TetraMin, which differ 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 each filled with 80 g wet weight sediment and 150 mL test medium. Twenty first instar larvae were added to each replicate, using a stereomicroscope and converted pipettes. For ST2, each replicate per treatment was formed with larvae from one of the naive or pre-exposed populations, making them truly independent. The larvae were fed every other day at a rate of approximately 0.5 mg per larva and day (OECD, 2004), regardless of the basal food source, to ensure that any potential effects were due to food quality rather than quantity. 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. Adults from all treatments of ST2 were freeze-dried (about 48 h, Christ ALPHA 1-2 LD plus), weighed to the nearest 0.01 mg and stored for FA analysis. Furthermore, adults from the control treatments of ST1 were freeze-dried (about 48 h, Christ ALPHA 1-2 LD plus), weighed to the nearest 0.01 mg and stored for FA analysis to assess potential effects of

the laboratory conditions.

2.4. Fatty acid analysis

Total FAs were quantified in female and male adults in 16 of 24 treatments of ST2 (n = 4-6, see Table S1) to investigate the effects of Cu sediment content, food quality and exposure history. Eight treatments were omitted because we did not observe meaningful effects on the emergence pattern (50 mg/kg dw) or the adult biomass was very low (400 mg/kg dw). Additionally, we analyzed female and male adults from the control treatments of ST1 (n = 6; see Table S1) 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 (µg FA/mL) 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 (µg FA/mg dw).

2.5. 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. Based on this criterion, a total of eight replicates (all from ST2, representing <4 % of all replicates) were excluded from further statistical analysis (Table S2). Outliers may have resulted from accidental handling errors, but given the low number of affected replicates, these appear to be isolated events.

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 interactions between Cu sediment content and food quality and between 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 Section 2.3). 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 interactions between Cu sediment content and food quality and between Cu sediment content and exposure history were implemented as explanatory variables. To investigate potential effects of the laboratory conditions on adult dry weight (dw) in the controls, we included larval origin as an explanatory variable. This factor distinguished between individuals originating from the mixed in-house culture used to perform ST1 and individuals from the chronic cultures used to perform ST2. 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* 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 and 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.

3. Results

3.1. Emergence success

Adult emergence in control treatments was comparable between ST1 and ST2 (Fig. 1). 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, Fig. 1), respectively. Spirulina had a significant negative effect on female emergence (reduction by about 20 %, $p = 0.043$, GLM, Fig. S1) and male emergence at 200 mg/kg dw (difference in effect sizes by about 45 %, $p = 0.021$, GLM, Fig. S1) compared to TetraMin.

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 (all $p < 0.001$, GLM, Fig. 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, Fig. 1), a pattern primarily driven by

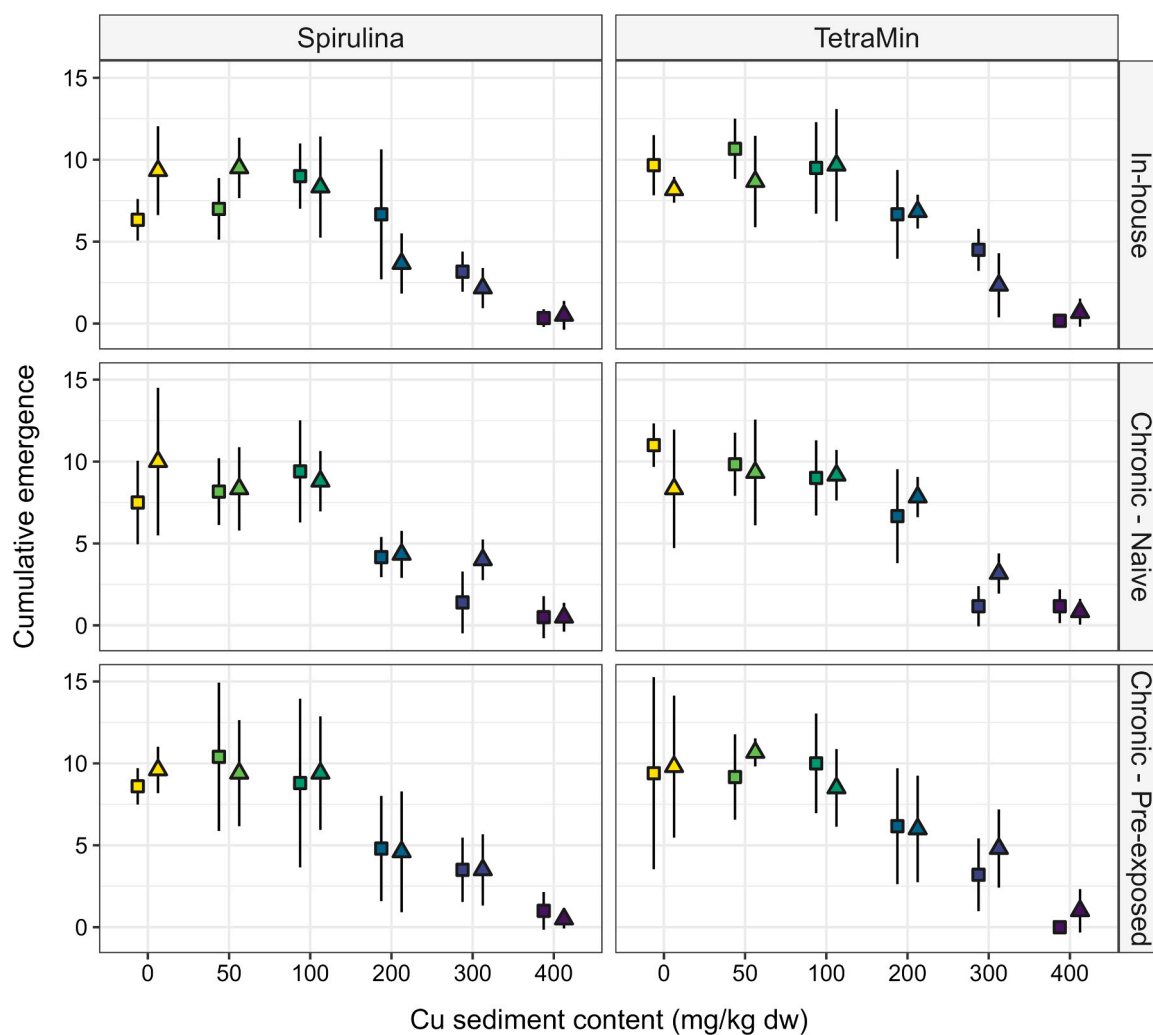


Fig. 1. Mean (\pm 95 % CI; $n = 5-6$) cumulative number of emerged *C. riparius* at Cu sediment contents of 0, 50, 100, 200, 300 and 400 mg/kg dw (colour-coded for ease of comparison). The sensitivity was assessed for populations at test initiation (‘In-house’, ST1) or reared in absence or presence of Cu for six months (‘Chronic – Naive’, ‘Chronic – Pre-exposed’, respectively, ST2) and larvae were fed one of two food sources (‘Spirulina’ or ‘TetraMin’).

males (Fig. S1). In females, we observed a non-significant tendency toward lower emergence (about 15 %) when fed with Spirulina compared to TetraMin ($p = 0.079$, GLM, Fig. S1). At 300 mg/kg dw, the number of emergent adults significantly varied based on the exposure history ($p = 0.038$, GLM, Fig. 1), with female emergence being reduced by about 85 % and 65 % in naive and pre-exposed populations, respectively (Fig. S1).

3.2. Time to 50 % emergence

Control treatments showed comparable EmT50 values in ST1 and ST2 (Fig. 2, Table S3). In ST1, the EmT50 differed significantly from the control at all Cu exposure levels except 100 mg/kg. However, EmT50 increased substantially only at a Cu sediment content of 200 mg/kg dw or higher, with delays up to 13 days at 400 mg/kg dw (Fig. 2, Table S3). While males generally emerged about three days earlier than females, the difference was almost absent or reversed at 400 mg/kg dw (Fig. 2, Table S3). The food quality did not affect EmT50 (Fig. 2, Table S3).

In ST2, individuals emerged significantly later at 400 mg/kg dw (up to about eleven days; Fig. 2, Table S3). Males generally emerged about three days before females, though EmT50 became more similar at the two highest Cu sediment levels (Fig. 2, Table S3). Spirulina significantly delayed the EmT50 by about one day with effects being most pronounced at Cu sediment contents up to 100 mg/kg dw (Fig. 2, Table S3). Individuals from pre-exposed populations emerged significantly but only slightly later than those from naive populations (about 0.5 days; Fig. 2, Table S3).

3.3. Adult dry weight

Emergent males were about 50 % lighter than females (Fig. 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, Fig. 3). Female dw was significantly increased by about 15 % at 300 mg/kg dw compared to the control ($p < 0.001$, LM, Fig. 3). Positive effects induced by Cu on the dw

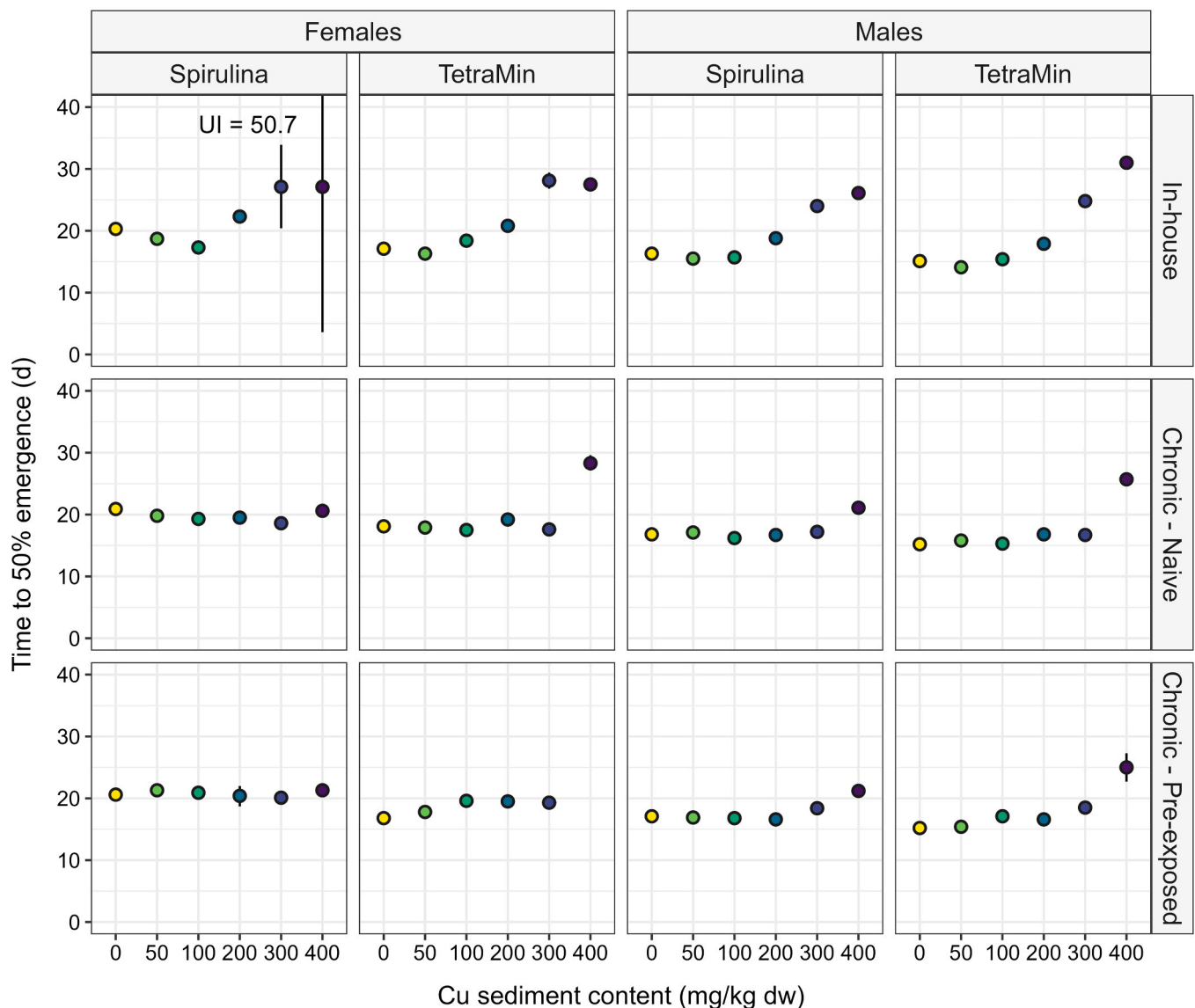


Fig. 2. Time to 50 % emergence (EmT50, \pm 95 % CI; in days; $n = 0-6$) of *C. riparius* females and males. Chironomids originated from one of three cultures ('In-house', ST1; 'Chronic - Naive' and 'Chronic - Pre-exposed', ST2), were fed either Spirulina or TetraMin, and exposed to one of six Cu sediment contents, that is 0, 50, 100, 200, 300 and 400 mg/kg dw (colour-coded for ease of comparison). The 95 % confidence intervals were mostly very small (see Table S2) and could not be visually represented, as they are obscured by the symbols representing the means. 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.

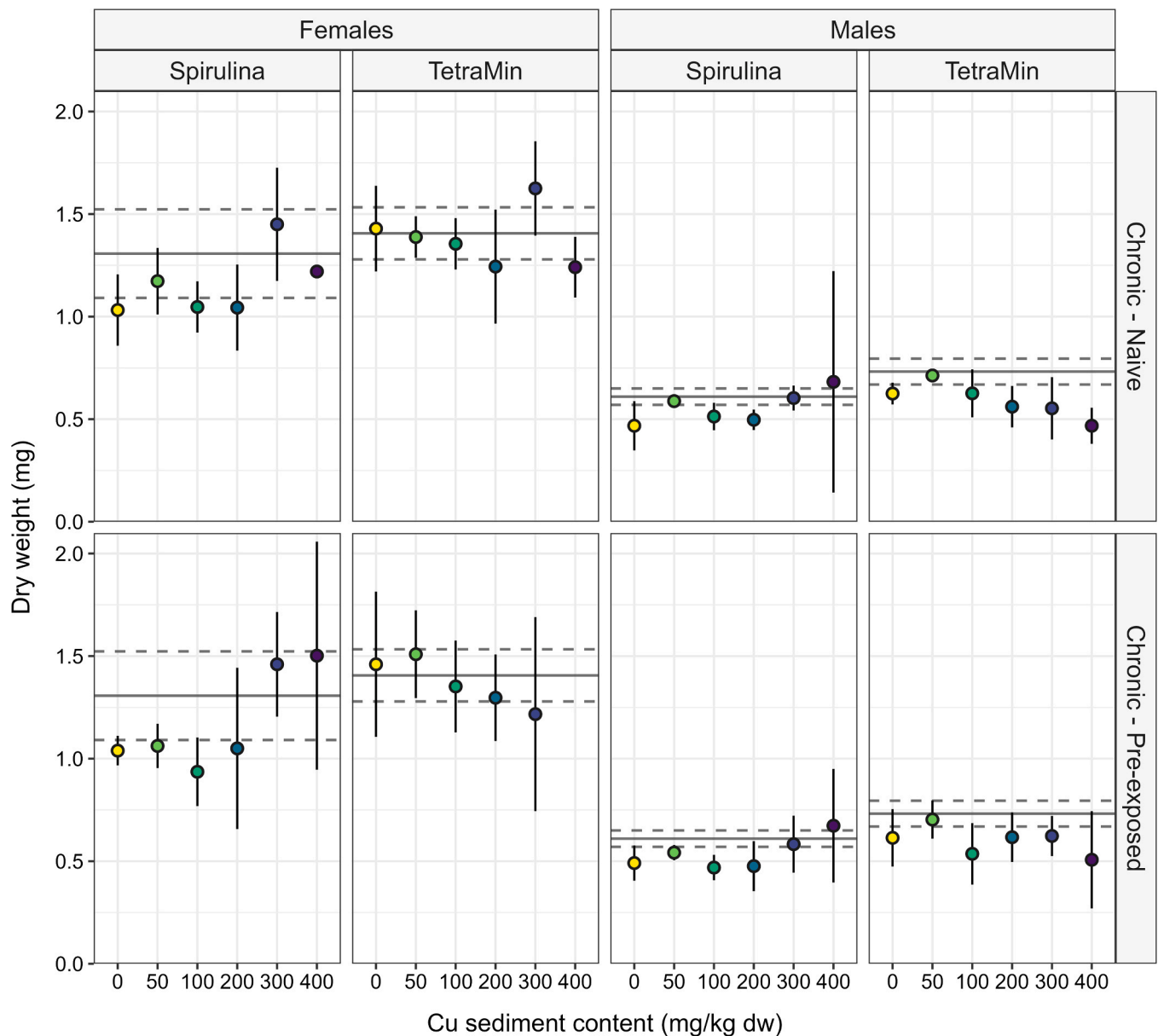


Fig. 3. Mean (\pm 95 % CI; $n = 1-6$) dw (mg) of *C. riparius* females and males from all treatments (i.e., combination of ‘Cu sediment content’, ‘Food quality’, and ‘Exposure history’) of ST2. Chironomid larvae were exposed to Cu sediment contents of 0, 50, 100, 200, 300 and 400 mg/kg dw (colour-coded for ease of comparison). Chironomids originated from one of two cultures (‘Chronic - Naive’ and ‘Chronic - Pre-exposed’) and larvae were fed one of two basal food sources (‘Spirulina’ or ‘TetraMin’). Additionally, the dw of females and males from the control treatments of ST1 were assessed to identify potential changes due to the laboratory conditions – mean \pm 95 % CI are indicated by the solid and dashed horizontal lines, respectively.

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 (Fig. 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, Fig. 3). Overall, adult dw was similar for individuals originating from naive and pre-exposed populations ($p = 0.829$ and $p = 0.877$ for females and males, respectively).

3.4. 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 (Fig. 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 profile in individuals fed Spirulina or TetraMin (food quality; Fig. 4). Individuals fed Spirulina contained substantially higher contents of 20:4n-6 (ARA; about 120 %), its precursor 18:3n-6 (GLA, about 30-fold more) and, thus, omega-6 PUFA (about 20 %), compared to individuals fed TetraMin (Table S4). In contrast, Spirulina-fed 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 (Fig. 4). Females contained high levels of 16:0, 18:0, 16:1n-7, 18:1n-9 and thus SFA and MUFA (Table S4). While females 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 %) resulting in a

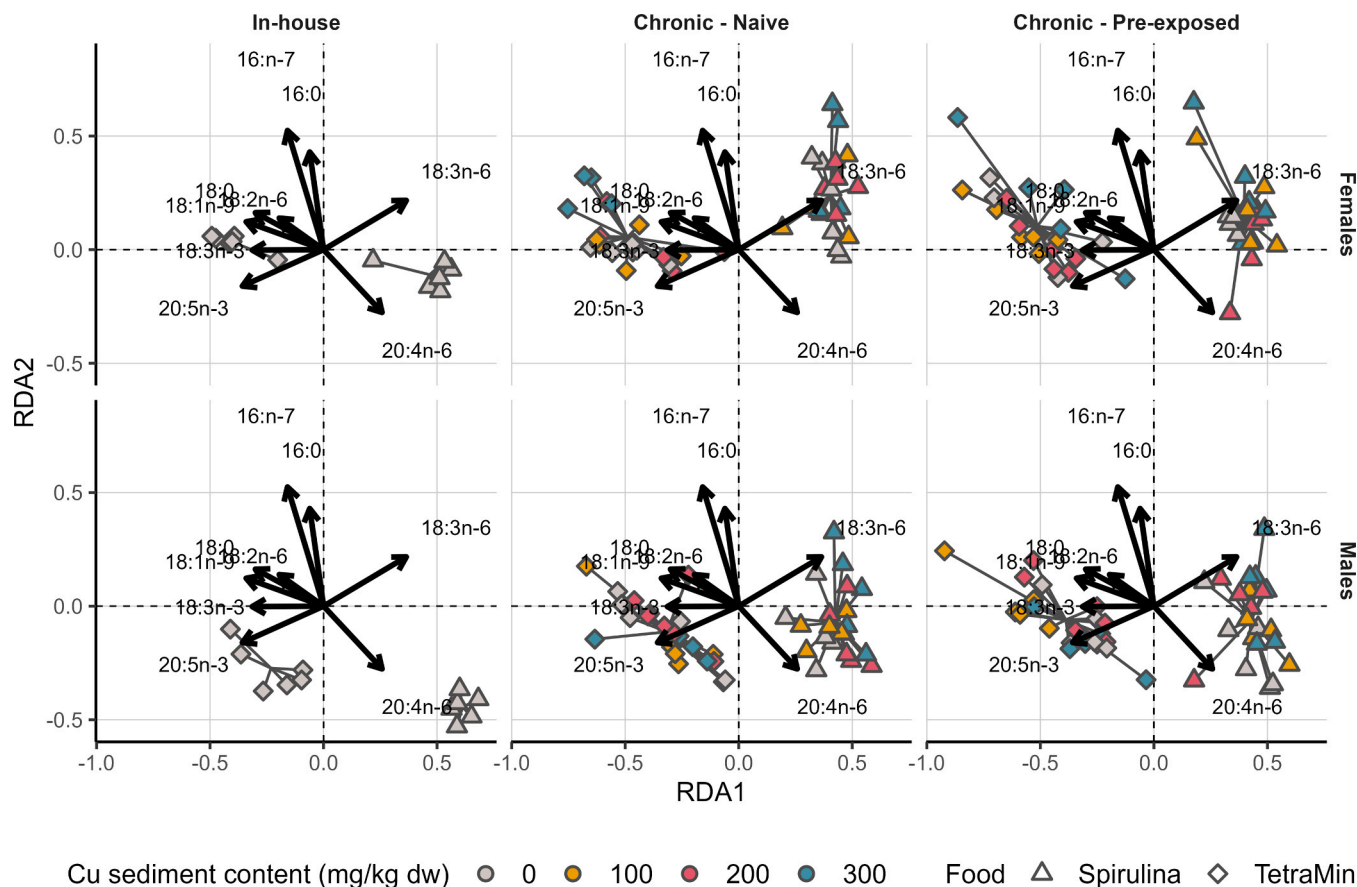


Fig. 4. Redundancy analysis using FA profiles (micrograms per milligram dw; $n = 4-6$) of *C. riparius* females and males from 16 treatments (i.e., combination of Cu sediment content, food quality, and exposure history) of ST2. Eight treatments were omitted because we did not observe meaningful effects on the emergence pattern or the adult biomass was very low. Moreover, we analyzed female and male adults from the control treatments of ST1 ($n = 6$; see Table S1) 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.

similar content of omega-3 and omega-6 PUFA (Table S4). 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 (Table S4). The FA levels in individuals from naive 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 (Table S4). 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/kg dw (Fig. 4). At this Cu content, individuals were richer 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 (Table S4). 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%).

4. Discussion

4.1. Emergence: number, time and dry weight

4.1.1. Cu sediment content

In line with our hypothesis, Cu reduced the number of emergent adults. Differences compared to the control were observed at Cu sediment contents of 200 mg/kg dw and higher. 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 and Calow, 1989; Sokolova and 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 and Lannig, 2008; Sokolova et al., 2012; Stohs and Bagchi, 1995; Wallace and Starkov, 2000). Since the larval developmental stage in holometabolous aquatic insects is followed by the energetically demanding metamorphosis (Arrese and Soulagés, 2010), such toxic effects can impair emergence. In fact, 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 (Fig. 1). At lower sediment contents (50 and 100 mg/kg dw), larvae seemingly coped with or benefited from Cu exposure, possibly by controlling Cu body burdens (see Morgan et al., 2007), at levels needed for physiological processes (e.g., component of enzymes, Flemming and Trevors, 1989).

Given that holometabolous insects require a certain amount of energy to survive metamorphosis (Mirth and Riddiford, 2007) and Cu exposure likely affected energy storage, emergence was delayed by up to eleven days at 400 mg/kg dw, an observation consistent with our hypothesis. Additionally, differences in the emergence times between females and males (i.e., protandry, Armitage, 1995), as observed in the control treatments of both STs, tended to become negligible or even

reverse (i.e., females emerging before males, Fig. 2) at this Cu sediment content. This could influence reproduction, as males with reduced physiological fitness may benefit from a more synchronized emergence pattern. However, the reduced total emergence may limit encounters between females and males and mating may thus be reduced (Armitage, 1995). Besides, the increase in larval mortality with increasing Cu sediment levels may have contributed to the increase in adult dw (Fig. 3), contradicting our hypothesis. This may be assumed as the amount of added food was corrected for emergence but not for larval mortality suggesting a higher food availability per surviving larva in combination with lower competition (Kraus and Vonesh, 2012; Postma et al., 1994).

4.1.2. 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 certain FAs (Goedkoop et al., 2007; Ohler et al., 2023b; Pietz et al., 2023b; Strandberg et al., 2020), this process 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 %, Fig. 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 the observed sublethal effects (i.e., delayed emergence when larvae were fed Spirulina compared to TetraMin; Fig. 2). Furthermore, the dw of females and males fed Spirulina decreased relative to those fed TetraMin (about 15 % and 10 %, respectively; Fig. 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 in dipterans 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 may not be directly attributed to differences in food availability. While Spirulina has lower levels of physiologically important omega-3 PUFAs, there are other differences between the basal food sources, such as the higher protein content in Spirulina (Table S5), that may have contributed to these results. Moreover, the food sources may release different qualities of dissolved organic carbon, which could have affected Cu-bioavailability through their differential ability for complexation (Crémazy et al., 2022).

4.1.3. Exposure history

Adaptation has been highlighted as a cause for decreased sensitivity in metal-exposed (field) populations (Miller and Hendricks, 1996; Postma et al., 1995a; 1995b). As hypothesized, emergence was higher in pre-exposed compared to naive populations at 300 mg/kg dw (Fig. 1). These results suggest that pre-exposed populations have an enhanced ability to cope with Cu-induced stress compared to naive populations – for example through the ability to regulate metal levels to minimize negative effects or cellular detoxification and repair mechanisms. However, in our study, the effect of exposure history appeared to be superimposed by the effect of Cu sediment content used in the STs and by food quality. Increased tolerance to Cu was only observed at high exposure levels, suggesting a potential trade-off with individual fitness (Postma et al., 1995b). Similar findings were reported by Kolbenschlager

et al. (2024), where chronic exposure to the biocontrol agent *Bacillus thuringiensis* var. *israelensis* resulted in only limited adaptive responses in *C. riparius*. Their results also highlighted the strong modulating effects of food quality as well as exposure timing and dosage. Additionally, the adaptive capacity may depend on the initial presence of traits required for increased tolerance, and the experimental duration may not have been sufficient to promote their development or establishment. Based on population genomic analyses, Röder et al., (submitted) found weak genome-wide signals of selection under Cu exposure. Nucleotide diversity was significantly reduced and signals of selection in biological pathways related to detoxification and DNA repair mechanisms were detected, indicating early-stage adaptation. In our study, we selected a moderate, environmentally relevant Cu sediment content (100 mg/kg dw) for the chronic exposure phase, yet this selective pressure may have been insufficient, at least over the exposure duration tested. Higher exposure levels, as reported for some heavily contaminated sites (e.g., Pedrosa et al., 2017), could have intensified selection but may have limited the informative value of the findings for less contaminated sites. Additionally, higher Cu sediment contents during the chronic exposure phase would have increased the risk of population decline or extinction. It is likely that laboratory conditions may have affected the physiological conditions (i.e., developmental time, dw and FA profile) of naive and pre-exposed populations to some extent. This may be a common phenomenon in chronic population-level laboratory studies caused by genetic impoverishment (Nowak et al., 2007) and by directional selection (Pfenninger and Foucault, 2020). However, the data do not support a general shift in chironomid sensitivity (e.g., similar pattern in emergence success in ST1 and ST2).

4.2. 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 predominantly 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 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 and Chow, 2003), may have caused lipid peroxidation (Bailey et al., 2015; Gaetke and Chow, 2003; Lannig et al., 2006), contributing to the decrease in highly unsaturated FAs. However, 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. Since emergent individuals have met the physiological requirements to achieve key developmental milestones (e.g., metamorphosis in holometabolous insects; Mirth and Riddiford, 2007), the effects of contaminants may be more pronounced in other endpoints, such as developmental time and emergence success. Following this reasoning, the effects of exposure history on the FA profile of the chironomids were also limited with the data suggesting that chronic Cu exposure does not lead to substantial changes in the nutritional quality

of emerging chironomids. However, due to the lower sensitivity of pre-exposed compared to naive individuals at 300 mg/kg dw, the availability of FAs in adjacent terrestrial systems might slightly increase, albeit at an overall low level (reduction in emergence by about 65 %, Fig. 1).

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, a lower quality basal food source in aquatic ecosystems (as simulated by *Spirulina* in this study) can reduce the availability of FAs, particularly physiologically important omega-3 PUFA, in adjacent terrestrial systems. In this context, changes may occur even at exposure levels where Cu or other contaminants do not directly affect insect emergence – provided that the composition of aquatic primary producers, which serve as the basal food source, is altered, for example through eutrophication (Konschak et al., 2021; Paerl and 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).

5. Conclusion

Copper sediment contents and food quality primarily shaped emergence quantity and quality of *C. riparius*, respectively. Pre-exposed *C. riparius* populations showed only limited signs of adaptation. However, laboratory conditions may have affected the physiological conditions (i.e., developmental time, dw and FA profile) of naive and pre-exposed populations to some extent. To investigate if our observations are robust and of biological relevance, future studies should include extended chronic exposure phases. In addition, anthropogenic stressors and potential adaptive responses may not only affect single populations but also interspecific interactions (e.g., among aquatic insects) and thus community dynamics. To capture these complexities, more integrative test systems are needed that reflect both direct and indirect effects within aquatic ecosystems and across aquatic–terrestrial boundaries. If confirmed, our results suggest sustained alterations of aquatic–terrestrial linkages, that may ultimately re-shape riparian food webs.

CRedit authorship contribution statement

Mirco Bundschuh: Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Klaus Schwenk:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Nina Röder:** Writing – review & editing, Investigation, Conceptualization. **Sebastian Pietz:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Agnes Schöndorfer:** Investigation. **Sara Kolbenschlager:** Writing – review & editing, Investigation, Conceptualization.

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Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118893.

Data availability

Data are shared after publication on zenodo. Emergence data from multi-generation study on *Chironomus riparius* [Data set].

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