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Research article

Chironomids regulate long-chain polyunsaturated fatty acid levels independent of lake nutrient or dissolved organic carbon concentrations

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Chironomids are keystone primary benthic consumers with semi-aquatic life cycles. They support aquatic and terrestrial consumers at higher trophic levels by conveying dietary nutrients, such as fatty acids. In this study, we combined field sampling and laboratory experiments to examine the effects of environmental parameters, including diet, on fatty acid composition and metabolism in chironomid larvae and imagines. Results from 53 lakes showed that lake size, depth, dissolved organic carbon (DOC) concentrations, and trophic state had only marginal effects on the content of long-chain polyunsaturated fatty acids (LC-PUFA) in chironomids. Compound-specific stable hydrogen isotope analyses confirmed that chironomids actively bioconvert dietary fatty acid precursors to LC-PUFA in all lake types, independent of nutrient or DOC concentrations. Moreover, fatty acid-specific stable carbon isotope data indicated that the diet of chironomids was subsidized, particularly in oligotrophic lakes in spring, by terrestrial C_{18} fatty acid precursors that were converted to LC-PUFA. Data from feeding experiments further confirmed that decreased dietary availability of LC-PUFA enhanced the conversion of dietary short-chain precursors to LC-PUFA. These results suggest that chironomids are PUFA regulators that can sustain LC-PUFA levels under varying environmental conditions. Furthermore, our results indicate that they bioconvert terrestrial low-quality material to high-quality resources, which, via chironomid emergence, support terrestrial food webs. Chironomids are abundant and widespread, and thus, the trophic transfer of LC-PUFA can have significant implications for the fitness and production of upper trophic level consumers in both aquatic and terrestrial ecosystems.

Keywords: bioconversion, *Chironomus*, compound-specific stable isotopes, dissolved organic carbon, essential fatty acids, eutrophication, trophic upgrading

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Introduction

Aquatic and terrestrial ecosystems are connected via reciprocal flows of dietary energy, materials, and organisms (Polis et al. 1997, Nakano and Murakami 2001, Loreau et al. 2003, Vanni et al. 2004). Whilst terrestrial subsidies can enhance aquatic production (Marcarelli et al. 2011), semi-aquatic insects, such as chironomids (Diptera: Chironomidae), are key in transferring essential nutrients from aquatic to terrestrial ecosystems. Such key nutrients include omega-3 (n-3) and omega-6 (n-6) long-chain polyunsaturated fatty acids (LC-PUFA) (Martin-Creuzburg et al. 2017, Scharnweber et al. 2020, Fehlinger et al. 2022, 2023, Bashinskiy et al. 2023). Chironomids have diversified and adapted to utilize almost every type of freshwater habitat and thrive even under harsh environmental conditions, such as dark, unproductive, anoxic and chemically contaminated waters (Nyman et al. 2005). In humic lakes, chironomids can contribute to > 50% of the benthic macroinvertebrate community both in numbers and biomass (Karima 2021, Kesti et al. 2022). In third-order streams they have been reported to account for up to 80% of insect secondary production (Berg and Hellenthal 1992). Chironomids are important dietary components for many fishes (Svenning et al. 2007, Hayden et al. 2015) and, upon emergence, for terrestrial consumers, such as riparian spiders (Mathieu-Resuge et al. 2022), bats (Hodkinson et al. 2001, Dreyer et al. 2012, Martin-Creuzburg et al. 2017), and birds (Twining et al. 2018). It has been shown that their emergence into terrestrial ecosystems can significantly increase the abundance of terrestrial arthropods and other higher-order consumers over multiple years (Entrekin et al. 2007, Hoekman et al. 2011). Therefore, chironomids are ideal model organisms to study cross-ecosystem fluxes of organic compounds (Gratton et al. 2008, Dreyer et al. 2015).

The cross-ecosystem trophic transfer of high quality resources is currently under increasing threat due to dramatic anthropogenic changes in aquatic ecosystems (Woodward et al. 2010, Loewen 2023, McFadden et al. 2023). LC-PUFA are predominantly produced by certain types of algae (Ballinger et al. 2006, Taipale et al. 2013) and most aquatic and terrestrial consumers at higher trophic levels cannot synthesize LC-PUFA de novo, but depend on dietary supply of these molecules for nervous tissues, somatic growth and reproduction (Pilecky et al. 2021b, Twining et al. 2022). Environmental parameters, such as temperature, and the concentrations of nutrients and dissolved organic carbon (DOC) in lakes affect the biomass and taxonomic composition of phytoplankton, and thus alter the primary synthesis of LC-PUFA (Watson et al. 1997, Rasconi et al. 2015, Strandberg et al. 2020a). Generally, increasing temperature and nutrient concentrations decrease the abundance of algal taxa that are most efficient in producing LC-PUFA (Galloway and Winder 2015, Rasconi et al. 2015, Ventelä et al. 2016), whereas the effects of increasing DOC concentrations on LC-PUFA production are complex and seem to be closely connected with temperature and nutrient availability (Strandberg et al. 2020a, Lau et al. 2021).

Chironomids rely on a combination of allochthonous, autochthonous, and methane-derived organic matter as food (Jones and Grey 2011, McCormick et al. 2021). Out of all the potential dietary sources of chironomids, only aquatic dietary organic matter contains LC-PUFA, while terrestrial organic matter exclusively contains the shorter-chained PUFA. However, there is laboratory evidence that chironomids can enzymatically convert dietary precursors, such as α-linolenic acid (ALA; 18:3n-3) to n-3 LC-PUFA or linoleic acid (LIN; 18:2n-6) to n-6 LC-PUFA (Strandberg et al. 2020b). Yet, it is unclear 1) to what extent LC-PUFA are bioaccumulated versus converted from dietary precursors in nature, and 2) how lake status (e.g. inorganic nutrients, DOC) affects the usage of different precursor sources, i.e. aquatic versus terrestrial. It has recently been shown that compound-specific stable isotope analysis of hydrogen can be employed to study fatty acid conversions in wild populations (Pilecky et al. 2022). During the conversion of C18 PUFA precursors to LC-PUFA, hydrogen atoms from the surrounding water are integrated into the newly formed LC-PUFA and that stable hydrogen isotope ratios can be used to distinguish between dietary acquisition of LC-PUFA and bioconversion of LC-PUFA from dietary C18 precursors (Pilecky et al. 2022). Distinguishing between chironomids as mere collectors of dietary PUFA or as regulators (i.e. trophic upgraders) of precursor PUFA is ecologically relevant because PUFA collectors are likely more susceptible to the predicted decline of PUFA production by algae due to environmental change than PUFA regulators, which might have capacity to mitigate the adverse changes in trophic chains. On the other hand, metabolic modifications of PUFA are susceptible to different stressors, such as high temperature and contaminant exposure (Strandberg et al. 2021, Pietz et al. 2023), which are assumed to increase in the future under global change scenarios. Thus, stressor-induced changes in fatty acid metabolism could decrease the availability of LC-PUFA to higher trophic level consumers (Kolbenschlag et al. 2023).

Based on the current knowledge, we assumed that chironomids are LC-PUFA regulators and therefore hypothesized that; 1) LC-PUFA composition and contents exported by chironomids are similar among lakes regardless of their sizes, trophic status or DOC concentrations; 2) chironomids convert dietary C18-PUFA to LC-PUFA independent of environmental and dietary conditions, and; 3) terrestrial fatty acids are used and upgraded by chironomids to LC-PUFA, particularly when autochthonous production is low (e.g. in oligotrophic lakes). We tested these hypotheses using a combination of field and controlled laboratory studies and by applying FA quantification as well as compound-specific stable isotope analyses to measure the PUFA composition and determine the origin of these molecules (Pilecky et al. 2022).

Material and methods

Field sampling

We selected 53 lakes in southern (n = 20) and eastern (n = 33) Finland that differed in surface area (0.4–36 000 ha), maximum depth (1.5–85 m), total nitrogen (TN; 169 to 1350 µg l^{-1}), total phosphorus (TP; 1.6–99 µg l^{-1}), and DOC concentrations (2.1–31.4 mg l^{-1}; Supporting information). We grouped the lakes into two size categories based on their surface area: small (< 50 ha; n = 32) and large lakes (> 50 ha; n = 21). Only the small lake category included waters with very high DOC concentrations (> 20 mg l^{-1}; Fig 1).

Chironomid larvae were collected in late May and July 2022 from littoral sediments using a kick-net (mesh size 500 µm); larvae were transferred to containers filled with lake water and kept on ice for 4 h to facilitate gut clearance, subsequently frozen, lyophilized, and stored at −20°C until further analysis. Emergent adults were caught at 16 selected lakes (Supporting information) in July 2022, using emergence traps (surface area: 0.36 m^2, covered with a 500 µm net). White collection containers installed on top of each of the pyramid-shaped traps facilitated the collection of trapped insects with a self-made aspirator every 3-days after deployment of the trap until a sufficient sample biomass was obtained (Fehlinger et al. 2022). Chironomids were separated under a microscope, stored at −80°C and subsequently freeze-dried. Chironomid taxa were pooled at family level to obtain sufficient sample mass for lipid analysis. Taxonomic identification was attempted post hoc on surplus freeze-dried material: most collected individuals could not be identified below the sub-family level of Chironominae, Tanypodinae and Orthocladiinae; some larval and adult specimens were well-enough preserved to additionally identify the genera *Chironomus* sp., *Polypedilum* sp., *Cladopelma* sp., *Tanytarsus* sp. and *Ablabesmyia* sp.

As representatives for the stable isotope composition of aquatic and terrestrial endmembers, we collected phytoplankton and terrestrial leaves from the shorelines in July, respectively. Phytoplankton samples (for logistical reasons only from 25 lakes, see Supporting information) were obtained by filtration of 1 litre of riparian lake water (Whatman 0.4 µm glass fiber filters). Fallen birch (*Betula* sp.) leaves (around eight lakes) were collected from the soil of the nearby riparian forests, freeze-dried, and homogenized using a blender.

Controlled feeding experiments

We performed two feeding experiments using a lab-reared *Chironomus riparius* culture (long-term culture maintained at the University of Eastern Finland, first established in 1991 from TNO Institute of Environmental Science, The Netherlands, and further supplemented in 2008 with organisms from University of Tübingen, Germany) to test the effect of three diets (i.e. high quality = TetraMin, low quality = *Spirulina*, terrestrial = nettle) and DOC concentrations of lake water on the chironomid fatty acid composition. 10-day experiments were conducted under continuous aeration in a temperature-controlled room at 20°C. Homogenized lake sediments, collected from Lake Höytiäinen (62°48′03.0″ N, 29°30′47.5″E), sieved through a 1 mm sieve to remove larger particles and animals, were used in all experiments and in the cultures. Sediments were transferred to beakers (400 ml), filled with artificial water (ratio 1:4; Ca + Mg hardness 0.5 mmol l^{-1}, pH 6.6), and allowed to settle. For labelling of ambient water, ^2H_2O (99.8%, Sigma) was added to a final concentration of 200 µl l^{-1}. This labelling yielded δ^2H values of the culture medium of 484.6‰ ± 16.2 compared to −89.8‰ ± 1.0 of the experimental water, i.e. resulting in a total isotopic enrichment of ~ 575‰. Prior to the start of the experiments, fresh egg clutches were collected and left to hatch in small beakers. Each treatment (in triplicates) contained 10 larvae. Larvae were fed 0.5 mg C ind^{-1} day^{-1} in all experiments.

In the first experiment, larvae were raised on various combinations of commercial fish food and the cyanobacterium *Spirulina* sp.. While TetraMin was rich in LC-PUFA, *Spirulina* only contained linoleic acid (LIN) and γ-linoleic acid (GLA), and traces of α-linoleic acid (ALA) (Supporting information). In the second experiment, larvae were fed on either *Spirulina* or nettle powder supplied in filtered (0.4 µm) lake water

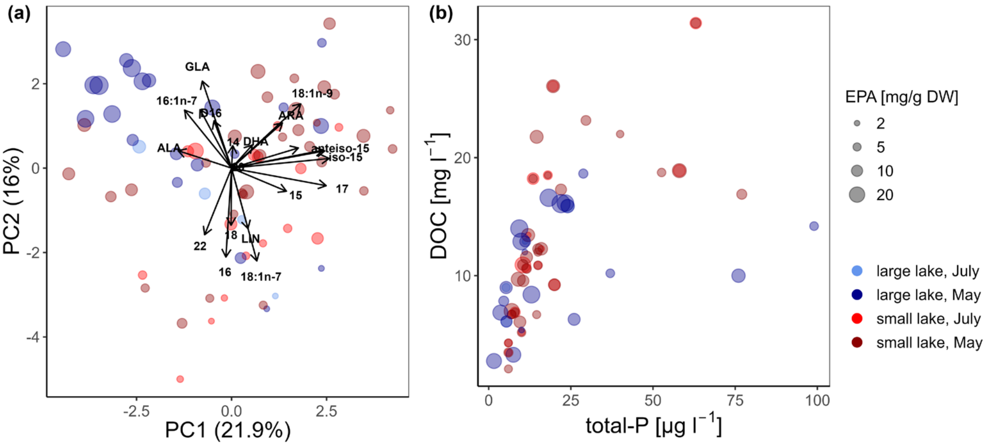

Figure 1. EPA mass fraction in chironomids in relation to (a) FA (%) profiles and (b) lake DOC/TP concentrations. Samples are marked according to lake size and sampling season. Additionally, the lake and season specific EPA content (µg/mg DW) are depicted with the size of the symbol (not included in the PCA ordination).

from the clear, oligotrophic Lake Kuorinka (62°36′47.2″N, 29°24′12.2″ E; TP = 1.6 μg l^{-1}, TN = 169 μg l^{-1}, DOC = 2.8 mg l^{-1}, $\delta^2H_{Water} = -65.5‰$) or from dark, eutrophic pond Rauanlampi (62°47′15.3″N, 29°30′24.5″E; TP = 58 μg l^{-1}, TN = 928 μg l^{-1}, DOC = 18.9 mg l^{-1}, $\delta^2H_{Water} = -87.8‰$).

Dissolved oxygen saturation, pH, and ammonium concentrations were monitored throughout the experiment and had no negative effects on larvae growth or survival. At the end of the experiment, the larvae were separated from the sediments, counted, stored at −80°C, lyophilized and weighed (0.6–2.6 mg dry weight) for subsequent lipid analysis.

Gas chromatography (GC) and isotope ratio mass spectrometry (IRMS)

Fatty acids were prepared from all samples according to Pilecky et al. (2023). Briefly, freeze-dried samples were homogenized, and lipids extracted using chloroform-methanol. For fatty acid methyl ester (FAME) formation, samples were incubated with sulfuric acid:methanol (1:100 V/V) for 16 h at 50°C. FAME were quantified using a gas chromatograph (TRACE GC ThermoFisher Scientific) equipped with an Agilent HP-88 column (100 m, 25 mm i.d., 0.2 μm film thickness, Agilent Technologies). FAME were identified and quantified by comparison of retention times to known reference standards (37-component FAME mix, and BAME mix, both SUPELCO; Sigma-Aldrich). FA are reported as μg mg^{-1} dry weight after applying a conversion factor for each individual FAME accounting for the mass fraction of the methyl group. Bacterial fatty acids (BFA) were calculated as the sum of the odd-chain saturated fatty acids 15:0 and 17:0, their iso- and anteiso-homologues, iso-16:0, as well as 9,10-methylene-16:0 and -18:0, and 18:1n-6 mass fractions.

The compound-specific stable isotope analysis of hydrogen (^2H) and carbon (^{13}C) were performed as reported elsewhere (Pilecky et al. 2021a, 2023), using a Thermo Trace 1310 GC, coupled by a ConFlo IV interface to a continuous-flow isotope-ratio mass spectrometer. Samples were run against certified Me-C20:0 stable isotope reference material for VPDB and VSMOW normalization (USGS70: $\delta^{13}C = -30.53‰$, $\delta^2H = -183.9‰$, USGS71: $\delta^{13}C = -10.5‰$, $\delta^2H = -4.9‰$ and USGS72: $\delta^{13}C = -1.54‰$, $\delta^2H = +348.3‰$). Weighted-average combined δ^2H values of mono-unsaturated fatty acids (MUFA = C14:1, C16:1, C18:1) were obtained by integrating over all isoforms (e.g. C18:1n-7 + C18:1n-9) because no clear baseline separation could be achieved.

For δ^2H analysis of pond and experimental waters, three replicates of 20 ml were filtered (0.4 μm) before isotope analysis using a L2130-I with IAEA-604 (+799.0‰) and IAEA VSMOW2 (0.0‰) as bracketing standards, using the techniques described elsewhere (Coplen and Wassenaar 2015).

Data analysis

Data analyses were performed and plots produced in R (ver. 4.2.2, www.r-project.org), using the packages 'rstatix', 'ggplot2', 'ggpubr', 'lme4' and 'corrplot', and with PRIMER ver. 6.1.15. with PERMANOVA+ add on ver. 1.0.5. Data were tested for normal distribution using the Kolmogorov-Smirnov test. Data were log-transformed to meet the assumption of normally distributed FA mass fractions, total phosphorus (TP), total nitrogen (TN) and dissolved organic carbon (DOC) concentrations. Relative data (%) were arcsine-square root transformed.

Paired samples t-tests were used for group comparison when appropriate (e.g. FA content of larvae and adults from the same lakes), while ANOVA was applied for multiple group comparison with Tukey's HSD post hoc test for comparison of pairwise means. The Pearson method was used for correlation analysis. Distance based linear models were fitted to investigate the effects of lake size category, sampling season, DOC, and TP concentrations on the fatty acid profiles of chironomids. Euclidean distances of arcsine square root transformed fatty acid data were used as the resemblance matrix. Lake size category and sampling season (May or July) were used as factors, and DOC and TP concentrations as variables. We conducted stepwise backward model selection and used AIC as criterion for the most parsimonious model.

Isotope data were summarized as the mean carbon or hydrogen isotope δ value ± SD. The kinetic isotope fractionation factor (α given with 95% CI) represents the slope in the regression equation between source (e.g. ambient water) and product (i.e. FA) isotope values and the trophic isotope fractionation factor (TIF) represents the intercept (i.e. offset in δ values ± SD) between consumer and diet for the same molecule.

To assess the contribution of terrestrial fatty acids to chironomids, Bayesian mixing models were applied using the 'simmR' package (Parnell et al. 2013, Pilecky et al. 2024a) using $\delta^{13}C$ values of ALA and 22:0 from phytoplankton and leaves as aquatic and terrestrial source endmembers, respectively, while accounting for trophic isotope fractionation factors as determined under controlled conditions (Supporting information).

Results

Effect of environmental predictors on Chironomidae FA profiles and δ2H/δ13C values

Distance-based linear modelling showed that 13.8% of the variation in fatty acid profiles in Chironomidae could be explained by the selected environmental predictors (Table 1). The most important predictor was lake size category, which explained 6.5% of the variation. After considering the effect of lake size, sampling month explained 4.5% of the remaining variation. The proportions of 14:0, 18:4n-3 and EPA were generally higher in large lakes, and higher in May than in July. The proportions of fatty acids typically associated with bacterial or terrestrial origin (e.g. 17:0 and 22:0, respectively) were greater in small lakes and in July. After lake size and sampling month had been considered, TP explained 2.8% of the residual variation in the chironomid fatty acid profiles.

Although lake size explained a small proportion of the variation in fatty acid proportions of chironomids, neither EPA nor ARA mass fractions or the EPA/ARA ratio were associated with the environmental variables TP, TN, DOC, lake size or depth. However, EPA mass fractions were statistically significantly lower in July ($p < 0.001$), while ARA mass fractions did not differ between months ($p = 0.067$). Larvae had lower mass fractions of EPA ($p = 0.017$) than adults (Supporting information).

No significant differences in fatty acid specific δ^2H or δ^{13}C values were found between adults and larvae (Supporting information), except for higher $\delta^2H_{18:0}$ values of imagines; $\Delta = 21.5$‰ [95% CI = 10.7; 32.3], paired t-test, $p = 0.005$). Subsequently, the isotope data for both imagines and larvae were combined. In the lake samples, chironomid δ^2H_{EPA} values correlated negatively with EPA mass fractions ($R = -0.72$, $p < 0.001$) and positively with some BFA (e.g. iso-16:0; $R = 0.66$, $p < 0.001$), and LIN ($R = 0.61$, $p < 0.001$). The δ^2H_{ARA} values were negatively correlated with the ARA mass fractions ($R = -0.68$, $p < 0.001$) and mass fraction of 22:0 ($R = -0.4$, $p = 0.004$).

The δ^2H values of water as well as those of saturated and monounsaturated FA in chironomids were approximately ~ 10‰ higher in July compared to May (paired t-test, $p < 0.05$). Lake water δ^2H values correlated highly with SFA and MUFA of chironomids (e.g. $\delta^2H_{16:0}$ ($\alpha_{16:0} = 1.07$ [0.70; 1.44], $R = 0.55$, $p < 0.001$) as well as δ^2H_{ARA} ($R = 0.42$, $p < 0.001$) and δ^2H_{EPA} ($R = 0.39$, $p < 0.001$), but not with δ^2H_{LIN}, δ^2H_{GLA}, δ^2H_{ALA}, or δ^2H_{SDA} values (Fig. 2A–C). The α values steadily increased with fatty acid chain-length and position in the enzymatic bioconversion chain for both n-3 PUFA ($\alpha_{ALA} = 0.29$ [−0.16; 0.75], $\alpha_{SDA} = 0.74$ [−0.30; 1.79], $\alpha_{EPA} = 1.64$ [0.64; 2.26]) and n-6 PUFA ($\alpha_{LIN} = 0.28$ [−0.08; 0.64], $\alpha_{GLA} = 1.36$ [0.08; 2.64], $\alpha_{ARA} = 1.98$ [0.98; 2.98]). Correlations of fatty acid specific stable isotope values with environmental factors can be seen in Fig. 2D–E and the Supporting information.

Estimation of source contribution to chironomid diet and fatty acid conversion

Chl-*a*, DOC, TP, and TN concentrations did not affect the EPA content of phytoplankton samples, which ranged from 1.5 to 5%. No statistically significant differences between potential terrestrial (birch leaves) and aquatic (algae) diet sources were observed for any fatty acid specific δ^2H value (Fig. 3A). However, a significant (~ 5‰) difference in $\delta^{13}C_{ALA}$ and $\delta^{13}C_{LIN}$ values as well as an inverse 6‰ difference in $\delta^{13}C_{22:0}$ values between phytoplankton and leaves from across these study sites was detected (Fig. 3B), which allowed the use of fatty acid-specific stable carbon analysis to estimate diet source contributions in different environments. In a mixing model, these fatty acid-specific carbon stable isotope values were used as terrestrial and aquatic endmembers to assess their respective contribution to chironomids. After accounting for trophic isotope fractionation (Supporting information), the Bayesian mixing model revealed that chironomid larvae retained more terrestrial fatty acids shortly after ice off in May than in July. Furthermore, a clear trend in terrestrial source use from oligotrophic (> 75% in May) to eutrophic lakes (< 25%) was observed, while a seasonal trend was less pronounced (Fig. 3C). There were no significant differences in trophic isotope fractionation factors between different diet sources (Supporting information) that could confound the source contributions in the mixing models and result on large confidence intervals in the model results (Fig. 3).

Laboratory evidence of *Chironomus* PUFA conversion

In the experiment 1, diet sources influenced the FA profiles and mass fractions of LC-PUFA in *Chironomus* (Supporting information). The EPA mass fraction ranged, depending on the diet, from ~ 0.5 mg g dw^{-1} to 2.4 mg g^{-1} dw. The TetraMin diet resulted in the highest EPA mass fractions of *Chironomus*. On pure *Spirulina* diet (lacking EPA), *Chironomus* contained ~ 0.5 mg g dw^{-1} of EPA which was completely obtained via conversion. The mass fractions of converted EPA steadily decreased with increasing dietary supplementation of EPA to a minimum between 2–3 mg EPA day^{-1} ind^{-1}. The increasing dietary *Spirulina*:Tetramin gradient resulted in higher bioconversion of n-3 PUFA than of n-6 PUFA (Fig 4). ARA showed no significant difference among treatment levels (avg.: 312‰ ± 21, Fig. 4A). The ARA mass fractions in *Chironomus* increased with increasing provision of *Spirulina*, although *Spirulina* did not contain any ARA (Supporting information). Also, the δ^2H values of SAFA and MUFA (Supporting information) were consistently higher in *Chironomus* than in their diets.

Table 1. Marginal test results and the best model (AIC = −234.97) from the distanced-based linear model of chironomid fatty acid profiles, using sampling season, lake area and the concentrations of TP and DOC as explanatory variables.

Marginal test	SS(trace)	Pseudo-F	p-value	Prop. explained		
Variable						
Season	0.16234	4.432	0.001	0.060		
Lake area	0.17574	4.8234	0.001	0.065		
TP	0.086	2.2817	0.03	0.032		
DOC	0.056	1.4773	0.159	0.021		
Step-wise test	**SS(trace)**	**Pseudo-F**	**p-value**	**Prop. explained**	**Cumulative**	**Res.df**
Lake area	0.176	4.823	0.001	0.065	0.065	69
Season	0.121	3.427	0.003	0.045	0.110	68
TP	0.076	2.188	0.028	0.028	0.138	67

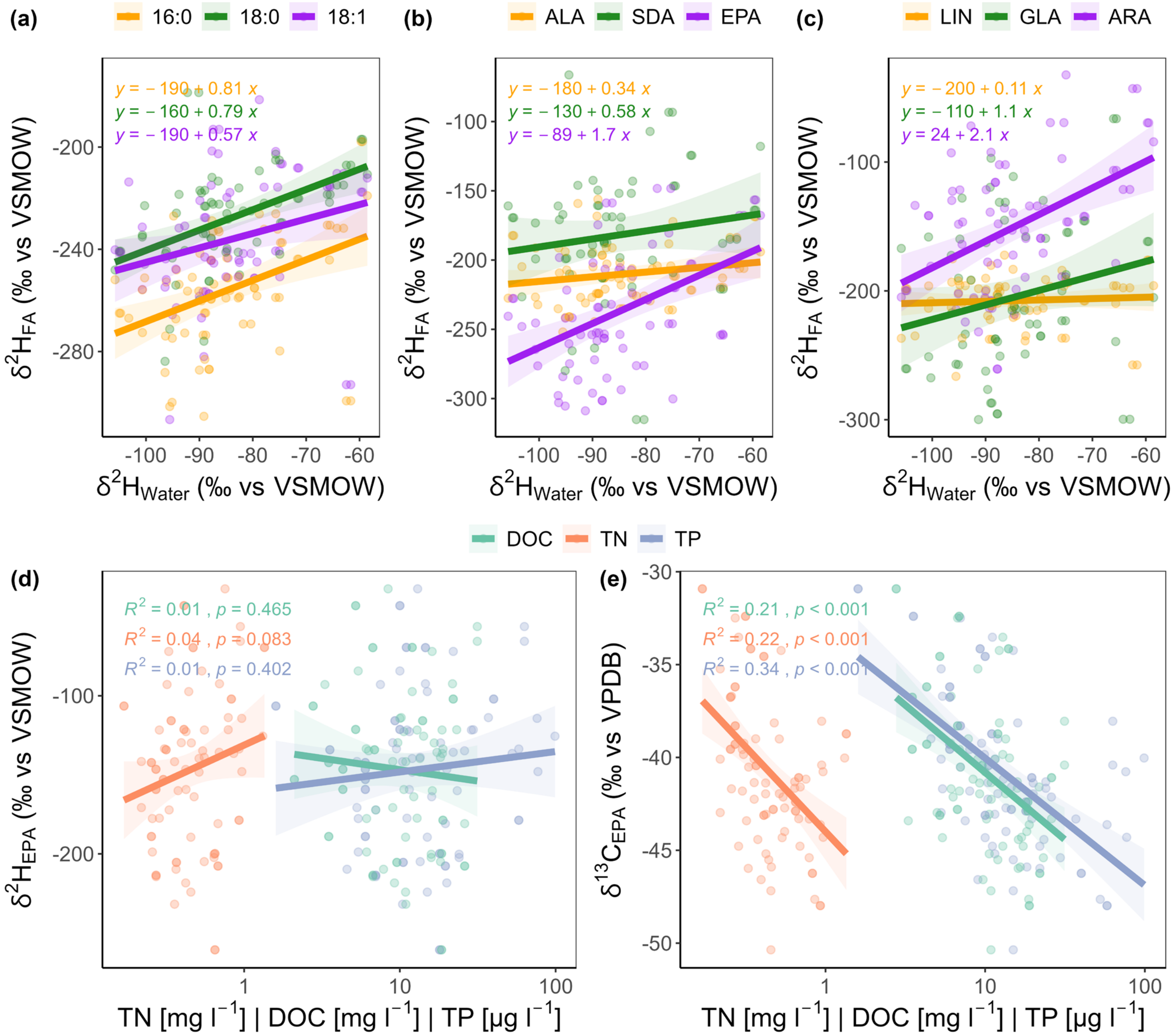

Figure 2. Kinetic isotope fractionation in chironomid fatty acids (upper panel) and the correlation of δ^2H and δ^{13}C values of EPA with environmental factors (lower panel). (a) The kinetic isotope fractionation factor represents the slope in the regression equation between source (i.e. ambient water) and product (i.e. FA) isotope values and the trophic isotope fractionation factor represents the intercept (i.e. offset in δ values ± SD) between consumer and diet for the same molecule. δ^2H values of saturated and monounsaturated fatty acids, which can be de novo synthesized by consumers, correlated with δ^2H values of ambient water. In case of (b) n-3 PUFA and (c) n-6 PUFA the α-values increased in the putative products (EPA/ARA) compared to their essential precursors (ALA/LIN) suggesting high bioconversion rates. Environmental parameters (d) did not highly correlate with δ^2H_{EPA} values, however, (e) correlated with $\delta^{13}C_{EPA}$ values. This suggests that they had no influence on the bioconversion process performed by the chironomids, however, influenced the source of the precursors.

In the experiment 2, larvae feeding on *Spirulina* or nettle powder, or when kept in low or high DOC water, integrated ^{2}H from water at similar rates into most of their FA, but differences were found for ALA and ARA, which had higher δ^2H values on nettle diet (503‰ ± 58) compared to *Spirulina* diet (293 ‰ ± 18). Furthermore, $\delta^2H_{20:0}$ values were statistically significantly different between larvae kept in the high DOC lake water from Lake Rauanlampi (526‰ ± 33) compared to the low DOC lake water from Lake Kuorinka (408‰ ± 20). Diet treatment showed no effect on $\delta^2H_{20:0}$ values (Fig. 4B).

Discussion

This study suggests that allochthonous resources of poor dietary quality, in terms of low LC-PUFA content, are upgraded by chironomids through bioconversion and subsequently supplied, via emerging adults, to consumers in terrestrial ecosystems. The fatty acid specific hydrogen stable isotope data of both lake and laboratory samples indicated that chironomids consistently convert fatty acids. These metabolic processes probably contribute to maintaining the physiological LC-PUFA requirements despite environmental variations, which suggests

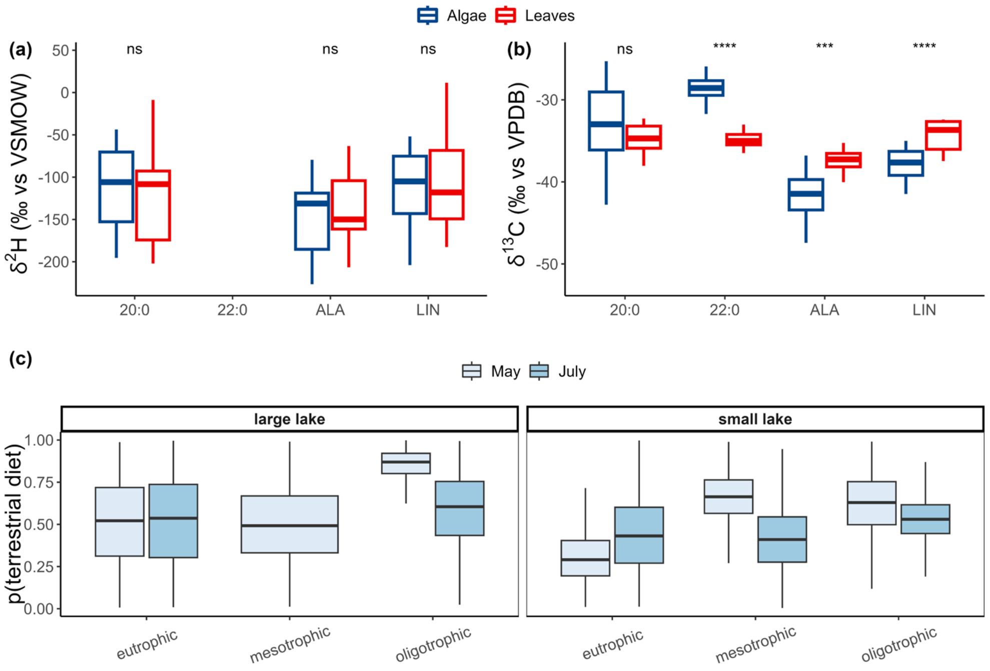

Figure 3. Differences in compound specific isotopic composition between terrestrial and aquatic source endmembers reveal different fatty acid contributions to chironomid diet. (a) While δ^2H values showed no significant differences, (b) a significant discrimination in $\delta^{13}C_{22:0}$, $\delta^{13}C_{ALA}$ and $\delta^{13}C_{LIN}$ values could be observed. (c) Using $\delta^{13}C_{22:0}$ and $\delta^{13}C_{ALA}$, a Bayesian mixing model was used to assess the contribution of terrestrial diet in lakes of different trophic states and DOC concentrations.

a high degree of flexibility on a molecular level of this insect group. Decreased dietary availability of EPA increased the overall bioconversion of dietary precursors, namely ALA, to EPA, indicating that dietary availability of LC-PUFA is an important regulator for the PUFA metabolism in chironomids. Furthermore, EPA is retained during emergence and imagines rely on fatty acids obtained during larval stages. The findings support the hypothesis that chironomids are fatty acid regulators, and further imply that chironomids are, also due to their abundance and high biomasses, important trophic upgraders

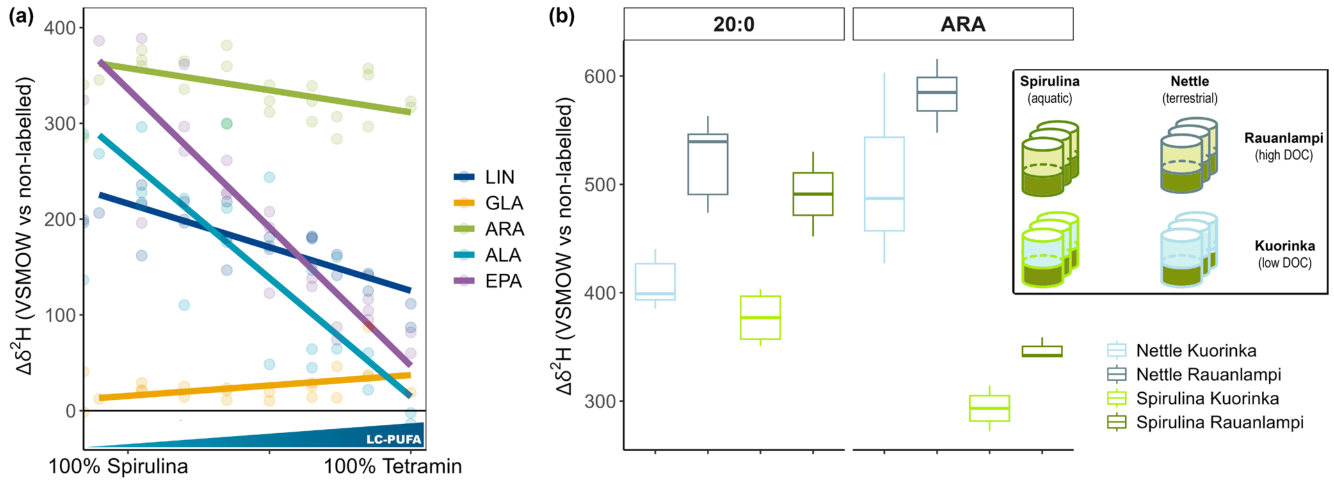

Figure 4. Scheme of the laboratory experiments and most important results. (a) Larvae were fed an increasing ratio of TetraMin (containing LC-PUFA) relative to *Spirulina* (contains only traces of n-3 PUFA, but high amounts of LIN and GLA), while keeping the total amount of C ind^{-1} day^{-1} constant. While almost no integration of deuterium (indicating metabolism) into ALA and EPA could be seen when fed only on TetraMin, increasing δ^2H values were observed when diet was continuously replaced by *Spirulina*, indicating ongoing bioconversion (Supporting information). High integration of deuterium was also observed for the n-6 PUFA LIN and ARA at all treatment levels, but only at low dietary GLA mass fractions (high % of TetraMin) slightly elevated consumer δ^2H values could be observed. (b) For evaluation of the influence of DOC and terrestrial vs. aquatic carbon on the isotopic values, larvae were either fed with *Spirulina* or nettle powder and kept in filtered water from a high DOC or low DOC lake. DOC only influenced the $\delta^2H_{20:0}$ values, while larvae fed *Spirulina* had lower integration of ^{2}H from ambient water into ARA, probably due to different n-3 to n-6 PUFA ratios of the diets.

among the cross-ecosystem vectors. This is of great ecological relevance, particularly in lakes presenting low dietary LC-PUFA availability, such as cyanobacteria-dominated lakes, or oligotrophic lakes with high terrestrial input (Calderini et al. 2023).

Fatty acid specific carbon isotope data indicated that season, lake trophic state and surface area were related to the dependence of chironomid populations on lipids from terrestrial resources. Generally, the relative utilization of terrestrial lipid resources seemed to decrease from May to July in oligotrophic to mesotrophic lakes. These shifts in the retention of terrestrial vs. aquatic lipid resources may be linked to the overall availability of allochthonous versus autochthonous organic matter. This is in accordance with previous results, in which relatively high terrestrial contributions to chironomid diet have been observed in oligotrophic lakes with low primary production (Belle et al. 2017, 2018), in contrast to oligochaetes and *Asellus aquaticus*, which exclusively retained periphyton carbon (Vesterinen et al. 2022).

In a previous study, the concentrations of DOC, TP and TN significantly affected the LC-PUFA content of chironomids collected from boreal lakes (Vesterinen et al. 2022). Similarly, Kesti et al. (2022) found that the abundance of ALA and EPA in chironomids was significantly higher in oligotrophic clear-water lakes than in lakes with high DOC content. Contrastingly, our results suggest that lake trophic state or DOC concentrations do not substantially affect the dietary availability of EPA and ARA to consumers at higher trophic levels. However, in all studies, both the sample size, as well as the geographic range were clearly smaller than in the current study and results might have been strongly affected by individual lakes.

Mass fractions of EPA and ARA in chironomids correlated negatively with their δ^2H_{EPA} and δ^2H_{ARA} values, respectively. This indicates that lower dietary supply triggers the bioconversion of precursors to EPA and ARA in chironomids collected from different lakes. Furthermore, the high correlations of δ^2H_{Water} and $\delta^2H_{16:0}$, the latter FA being the primary FA synthesis product in all animals, with δ^2H_{EPA} and δ^2H_{ARA} values in chironomids strongly indicate significant n-3 and n-6 LC-PUFA bioconversion from ALA and LIN to EPA and ARA, respectively, in all lakes. Parmar et al. (2022) recently found that EPA mass fractions in emerging chironomids span from very low to very high in temperate lakes and cover the same range as the EPA mass fractions from all other aquatic insect species. The LC-PUFA regulating capacities of chironomids might provide this taxon with an advantage to thrive in environments which are drastically limiting for other species, e.g. by limited PUFA availability. The result of this unique combination of features are high abundances and biomasses with high dietary quality that can have significant effects on the species richness and population densities at higher aquatic trophic levels and in associated terrestrial habitats (Hoekman et al. 2011).

The laboratory experiment further confirmed that diet sources affected the PUFA bioconversion pathway in chironomids. Decreased dietary availability of EPA increased the bioconversion of precursors to EPA in *Chironomus riparius*. This is in accordance with previous studies showing that the Δ5-/Δ6-desaturase activity is regulated by dietary intake of LC-PUFA (Li et al. 2005, Gonzalez-Soto and Mutch 2021). The TetraMin diet, corresponding to high dietary intake of EPA, suppressed the n-3 PUFA bioconversion pathway. The bioconversion of EPA was initiated at *Spirulina*: TetraMin ratios > 2, which equaled an EPA supplementation of < 2.2 μg^{-1} day ind^{-1}, similar to a previous study (Goedkoop et al. 2007) and resulted in conversion of C18 precursors up to a mass fraction of 0.5 mg g^{-1} dw in chironomids. Conversion processes of n-3 and n-6 PUFA require the same enzymes, but n-3 PUFA have a higher affinity to desaturases than n-6 PUFA (Monroig and Kabeya 2018). The abundance of different precursors, as well as the composition of dietary macronutrients, i.e. lipids, carbohydrates, and proteins, influence the enzyme affinities of Δ5-/Δ6-desaturases and elongases to fatty acid precursors (Gonzalez-Soto and Mutch 2021). Contrary to EPA, none of the experimental diets contained any considerable amounts of ARA and *C. riparius* had to rely on bioconversion of precursors to ARA throughout the diet gradient; i.e. the δ^2H_{ARA} values were high in all the treatments, indicating that a large proportion of ARA originated from the bioconversion pathway. The higher ARA levels in chironomids fed with pure *Spirulina* probably result from the bioconversion of dietary 18:3n-6 (Strandberg et al. 2020b, Supporting information). TetraMin does not contain any tracable amounts of 18:3n-6 and the main precursors for ARA in TetraMin was likely 18:2n-6 (Strandberg et al. 2020b, Supporting information). Thus, the precursor of ARA changed from 18:3n-6 to 18:2n-6 along the *Spirulina*: Tetramin gradient, resulting in decreasing ARA levels in *C. riparius* because of a more limited conversion of 18:2n-6 to ARA. The bioconversion of 18:3n-6 to ARA is more efficient because it does not require Δ6-desaturase, which is considered the rate limiting step in the bioconversion of 18:2n-6 to ARA (Cook and McMaster 2002). The δ^2H_{ARA} value in *C. riparius* in TetraMin diet treatment is partly confounded by direct dietary intake of ARA as TetraMin contains some ARA.

Previous studies have indicated that environmental factors, such as temperature and contaminants, may influence the bioconversion of PUFA and thus potentially alter the EPA and ARA content in chironomids (Strandberg et al. 2020a, Pietz et al. 2023). The lower LC-PUFA content in chironomids sampled in July than in May might have been a temperature-induced effect or a result of the phenology of various species (Los and Murata 1998). The increased desaturase activity and subsequent increase in LC-PUFA levels at lower temperatures may be linked with mechanical properties of lipid membranes, or motion of transmembrane proteins (Harayama and Antonny 2023, Renne and Ernst 2023). Additionally, low ambient temperatures slow the growth rate (Eggermont and Heiri 2012) and thus may lead to a higher relative accumulation of LC-PUFA in chironomids. In contrast, under global climate change scenarios, increasing temperatures in the shallow littoral zone may lead to decreased LC-PUFA levels in chironomids. Consequently, this would lead to a general decrease of the PUFA levels in the aquatic and riparian food web, e.g. fishes that feed on chironomids, and lastly affect the PUFA availability in human fish-based diets. Additionally, decreased LC-PUFA levels in chironomid larvae corresponded with decreased levels in imagines, adversely impacting the cross-ecosystem transfer of LC-PUFA. Lower chironomid LC-PUFA content in summer may be compensated by an increase in total chironomid biomass at the ecosystem level (Einarsson et al. 2002, Dessborn et al. 2009, Fehlinger et al. 2022). Further controlled experiments are required to better understand linkages between diet quality/quantity, ambient temperature, and the growth rates of chironomids, and how these factors affect the accumulation and metabolism of PUFA in chironomids and thus their role as aquatic-terrestrial vectors supplying riparian consumers with essential PUFA.

Entrekin et al. (2007) suggested that some chironomid taxa rely more on terrestrial inputs than others. Thus, varying levels of retention of terrestrial resources at group level might reflect differences in the taxonomic composition of this very species-rich group (more than 650 species in Finland alone). We acknowledge that LC-PUFA mass fractions and bioconversion rates, as well as the identified diet source retention may have been affected by the community composition of our samples, specifically the relative abundance of predatory taxa. Particularly, predatory chironomids, such as Tanypodinae, may have lower LC-PUFA bioconversion rates because of a higher dietary intake of EPA and ARA than omnivorous/detrivorous chironomids. However, only few Tanypodinae are strictly carnivorous, and most species also feed on detritus and diatoms (Pinder 1986). We cannot evaluate the relative importance of interspecific variation versus ecophysiological adjustment on the Chironomidae EPA and ARA levels in different lakes because the low samples sizes did not allow consistent analysis at the species or genus level. The effects of lake size and season, explaining 11% of the variation in the entire fatty acid profile in Chironomidae, may represent differences in feeding modes (i.e. trait/species distribution within the community) and/or diet. However, regardless of the potential mode of action, no significant associations between the EPA and ARA levels in the Chironomidae community and the selected environmental parameters were observed. Additionally, the analyzed environmental parameters did not correlate with the δ^2H_{ARA} and δ^2H_{EPA} values, indicating that any taxonomic differences were not related with the prevalence of the PUFA bioconversion pathway in chironomids. This suggests that efficient PUFA bioconversion may be a general feature for the entire Chironomidae family.

In conclusion, we provide field and experimental evidence that chironomids are PUFA regulators that maintain LC-PUFA levels under varying dietary and physio-chemical conditions. This also includes upgrading of allochthonous organic matter sources. The uniformly standard nutritional quality of chironomids is supported by constantly producing LC-PUFA even from nutritionally poor terrestrial diet. These results together with the ubiquitous presence and high abundance of chironomids indicate that these emergent insects are a key taxon in maintaining and increasing the LC-PUFA levels in aquatic and terrestrial food webs and thus supporting consumers at higher trophic levels.

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Author contributions

Matthias Pilecky: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (supporting); Investigation (lead); Methodology (lead); Project administration (equal); Validation (lead); Visualization (lead); Writing – original draft (lead). **Aatu Turunen**: Conceptualization (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Resources (equal). **Mohammad S. Sohrabi**: Investigation (supporting); Methodology (supporting). **Sadikshya Ghimire**: Investigation (supporting); Methodology (supporting). **Timo Ilo**: Investigation (supporting); Methodology (supporting). **Petri Kesti**: Investigation (supporting). **Simon Vitecek**: Investigation (supporting); Methodology (supporting); Validation (supporting); Writing – review and editing (equal). **Lena Fehlinger**: Writing – review and editing (equal). **Jarkko Akkanen**: Resources (supporting); Supervision (supporting). **Sami J. Taipale**: Investigation (supporting); Methodology (supporting); Resources (supporting); Supervision (supporting); Validation (supporting); Writing – review and editing (equal). **Anssi Vainikka**: Conceptualization (supporting); Methodology (supporting); Resources (equal); Supervision (equal); Writing – review and editing (equal). **Kimmo K. Kahilainen**: Conceptualization (supporting); Investigation (supporting); Methodology (supporting); Resources (equal); Supervision (equal); Validation (equal); Writing – review and editing (equal). **Martin J. Kainz**: Conceptualization (supporting); Funding acquisition (equal); Supervision (equal); Writing – review and editing (equal). **Ursula Strandberg**: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (lead); Validation (equal); Visualization (equal); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

The collective data can be accessed via DOOR: https://door.donau-uni.ac.at/o:3726; Meta-information: https://doi.org/10.48341/ma49-pa49; Laboratory study data: https://doi.org/10.48341/ezwd-t608; Field study data: https://doi.org/10.48341/nrwy-pd16.

Supporting information

The Supporting information associated with this article is available with the online version.

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