



The emergence of fatty acids—Aquatic insects as vectors along a productivity gradient

Kristin Scharnweber | Fernando Chaguaceda | Erik Dalman | Lars Tranvik | Peter Eklöv

Department of Ecology and Genetics;
Limnology, Uppsala University, Uppsala,
Sweden

Correspondence

Kristin Scharnweber, Department of
Ecology and Genetics; Limnology, Uppsala
University, Uppsala, Sweden.
Email: kristin.scharnweber@ebc.uu.se

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Abstract

1. Aquatic and terrestrial ecosystems differ fundamentally in the abundance of long-chained polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA; 20:5n-3), which are produced by aquatic algae, but only in low quantities by terrestrial plants. Aquatic insects, such as Chironomidae (non-biting midges) feed on algae during their larval stage, making them rich in EPA and therefore high-quality prey for insectivores after emergence. However, the magnitude of EPA subsidies from aquatic insects may be different among water bodies in response to abiotic (e.g. nutrient load) as well as biotic factors (e.g. food web structure).
2. To test the predation effects of crucian carp (*Carassius carassius*), nutrient concentrations, and Chironomidae community composition on the fatty acid export from aquatic ecosystems, we conducted a 25-day experiment across 20 1,500-L mesocosms covering a total phosphorus (TP) gradient of 20–1,000 $\mu\text{g/L}$.
3. Twice a week, we collected adult emerging Chironomidae and found differences in fatty acid composition in the two most abundant chironomid species emerging from the mesocosms. Two PUFAs, α -linolenic acid and EPA, contributed to most of the variation in Chironomidae fatty acid content across the nutrient gradient. Whereas the proportions of α -linolenic acid were positively correlated to the mesocosm TP concentration, we found a negative correlation for the proportions of Chironomidae EPA and mesocosm TP concentration. However, despite lower biomass-specific EPA content at higher TP, higher biomass of emerging Chironomidae at intermediate TP concentrations resulted in higher total export of PUFAs from water to land.
4. Predation pressure from carp decreased the biomass of emerging Chironomidae on average 8-fold. Chironomidae biomass showed a hump-shaped relationship along the TP-gradient and was strongly influenced by periphyton biomass.
5. Export rates of EPA and fatty acids in general responded in a quadratic manner along the nutrient gradient, reaching a maximum value at a TP of 400 $\mu\text{g/L}$ and decreasing thereafter.

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6. These findings highlight that the export of fatty acids from aquatic systems via adult Chironomidae is highly dependent on fish predation pressure, but also the nutrient concentrations of the system.

KEYWORDS

aquatic-terrestrial linkage, Chironomidae, mesocosms, nutrient gradient, polyunsaturated fatty acids (PUFA)

1 | INTRODUCTION

Aquatic and terrestrial ecosystems are tightly linked via multiple connections and fluxes that can occur in both directions, from land to water, but also from water to land (Polis, Anderson, & Holt, 1997; Vander Zanden & Gratton, 2011). A meta-analysis by Bartels et al. (2012) suggests that the flow of energy and nutrients from water to land are of higher quality than vice versa. One important food quality aspect is the abundance of polyunsaturated fatty acids (PUFAs), which are generally defined as fatty acids with two or more double bonds (e.g. Parrish, 1999). These components are important for several physiological processes in the metabolism of organisms, for instance the regulation of immune functions, but also the makeup of cell membranes (Parrish, 2009), and they are considered essential for the growth and reproduction of consumers (Brett & Müller-Navarra, 1997).

Consumers receive most of the PUFAs they need, directly or indirectly, from photosynthetic primary producers (Bell & Tocher, 2009) and the fatty acid composition of primary consumers usually reflects that of primary producers (Guo, Kainz, Sheldon, & Bunn, 2016; Torres-Ruiz, Wehr, & Perrone, 2007). Higher-level consumers in the food web may then obtain PUFAs via feeding on lower trophic levels that serve as integrators of the high-quality food (Strandberg et al., 2015; Twining, Brenna, Hairston, & Flecker, 2016). Compared to terrestrial plants, aquatic algae produce much higher amounts of long-chained PUFAs, such as eicosapentaenoic acid (EPA; 20:5n-3), which is especially valuable for the metabolism of consumers and this results in a fundamental imbalance between the food qualities of those two ecosystems (Ahlgren, Gustafsson, & Boberg, 1992; Hixson, Sharma, Kainz, Wacker, & Arts, 2015).

Aquatic insects act as one of the major pathways to export EPA from water to land (Borisova, Makhutova, Gladyshev, & Sushchik, 2016; Gladyshev, Sushchik, & Makhutova, 2013; Popova et al., 2017). Many aquatic insects have a complex life cycle with an aquatic larval stage that feeds on algae, but, after reaching maturity, emerges and flies out to the terrestrial ecosystem where they may serve as prey for many terrestrial predators, such as bats, birds, and spiders (Schreiber & Rudolf, 2008). In a global estimate of the export of long-chained PUFAs from aquatic to the terrestrial ecosystems, emerging aquatic insects contributed on average to >27% of the long-chained PUFAs transported from waters to land (Gladyshev et al., 2013). Several studies including lake and stream surveys have found that among emerging insects, the group Chironomidae (non-biting midges) form

the highest proportion and biomass of emerging individuals (Baxter, Fausch, & Saunders, 2005; Hoekman, Dreyer, Jackson, Townsend, & Gratton, 2011; Martin-Creuzburg, Kowarik, & Straile, 2017).

While recent studies highlight the importance of PUFA-rich aquatic insects for the fitness of terrestrial predators (Fritz et al., 2017; Twining, Shipley, & Winkler, 2018), so far, our knowledge is limited about the variability in the export of fatty acids from aquatic ecosystems. First, predation can strongly regulate the abundance and biomass of emerging insects (Knight, McCoy, Chase, McCoy, & Holt, 2005; Wesner, 2016), including Chironomidae (Pitcher & Soluk, 2018). It can therefore be hypothesised that in systems where fish predators are absent, the export rate of fatty acids via Chironomidae is higher than in systems where fish are present. Second, it can be assumed that the fatty acid composition of aquatic insects is highly influenced by the trophic state of the ecosystem. Therefore, the quantity of algae available as food for Chironomidae may be a consequence of nutrient availability in the system that might be translated to Chironomidae growth and abundance (de Haas, van Haaren, Koelmans, Kraak, & Admiraal, 2005; Vos, Peeters, Gylstra, Kraak, & Admiraal, 2004). Third, interspecific variation in Chironomidae may contribute to variable export rates of fatty acids. Chironomidae have diverse modes of feeding (Monakov, 1972), and ecologically similar species are commonly regarded as functional feeding groups (Cummins, 1973). Collector-gatherers are deposit feeding larvae and this feeding mode is also the most common one in Chironomidae. Collector-filterers are suspension feeders that filter food particles from the water column, scrapers acquire food by shearing material from surfaces, whereas shredders use chewing, mining, gouging, or rasping when feeding, and predators ingest parts or whole organism as prey (Armitage, Cranston, & Pinder, 1997). PUFA composition in Chironomidae may therefore depend on the species-specific feeding specialisation, and the aquatic-terrestrial export of PUFA might strongly depend on the community composition. For example, predatory species are generally found to be rich in PUFAs (Guo et al., 2018; Lau, Vrede, Pickova, & Goedkoop, 2012; Strandberg et al., 2015), but overall, our knowledge is limited on species-specific fatty acid composition of Chironomidae (but see Makhutova, Borisova, Shulepina, Kolmakova, & Sushchik, 2017).

In this study, we tested the export of EPA and other fatty acids via emergence of Chironomidae, as a response to fish predation, trophic state, and Chironomidae species composition. We conducted an experiment consisting of floating mesocosms in which we established a nutrient gradient covering concentrations of total

phosphorus (TP) from 20 to 1,000 $\mu\text{g/L}$ and analysed the fatty acid composition of the emerging Chironomidae. We hypothesised that: (1) export rates are related to predation pressure, i.e. Chironomidae abundance decreases in the presence of fish predators and therefore higher export rates occur in mesocosms without fish; (2) export of EPA and other fatty acids in general via emerging Chironomidae varies with nutrient levels and is related to the production of periphyton that chironomid larvae feed on; and (3) fatty acid composition in Chironomidae depends on species composition.

2 | METHODS

2.1 | Experimental set-up

To test the effects of increasing nutrient concentrations on the fatty acid composition of emerging Chironomidae we conducted a mesocosm experiment that allows the realism of natural ecosystems, and at the same time the possibilities of controlled and replicated experimental design needed for hypothesis testing. However, this particular design does not allow quantifying natural biomasses and emergence rates. Our mesocosm facility was set-up in Lake Erken (59°51'N, 18°36'E) in Central Sweden, which is a meso-eutrophic lake with ambient concentrations of TP at 31 $\mu\text{g/L}$ and total nitrogen (TN) at 0.7 mg/L (unpublished average of 25 years of monitoring). The mesocosms consisted of 20 white opaque, open-top cylinders with a flat bottom, made of high-density polyethylene that were 2 m deep and had a diameter between 92 and 101 cm. The floating mesocosms were attached to a dock close to the lakeshore.

On 4 July 2017, the mesocosms were filled with lake water and a sediment layer of about 10 cm sediment was collected from the profundal area of the lake, and added to each mesocosm. We anticipated nutrient resuspension from the added sediment and therefore, we exchanged the water 1 week after the sediment addition. Recruitment of Chironomidae to the mesocosms was possible via the addition of the sediments or through oviposition from the populations occurring naturally in Lake Erken. We established a 10-step-nutrient gradient from ambient, mesotrophic lake conditions to hyper-eutrophic conditions (TP: 20–1,000 $\mu\text{g/L}$, TN: 0.45–11.3 mg/L) by adding KH_2PO_4 and NH_4NO_3 (see Figure 1a,b for scheme of the facility), using an N:P ratio of 11.3:1, which is similar to the one used in previous studies (e.g. Cole, Pace, Carpenter, & Kitchell, 2000; Cottingham, Carpenter, & St Amand, 1998; Davidson et al., 2015). Each nutrient-treatment level was maintained in replicate mesocosms, with nutrient concentrations measured weekly and adjusted to maintain target levels. Besides the nutrient gradient, we further established a top-down treatment by adding two juvenile fish (crucian carp, *Carassius carassius*) with an average total length of 6.8 ± 1.0 cm to 10 mesocosms across the entire nutrient gradient to test the effects of increased predation pressure on Chironomid emergence. Fish individuals were matched according to their weight to obtain a similar fish biomass

in all mesocosms. Crucian carp is an omnivorous fish species with a mixed diet of macroinvertebrates (especially Chironomidae) and zooplankton (Penttinen & Holopainen, 1992) and we aimed for a biomass that is within the range of naturally occurring carp biomass (Holopainen & Pitkänen, 1985).

Emerging adult Chironomidae were sampled using floating cone-shaped traps similar to the ones described by LeSage and Harrison (1979). Emergence traps were placed on a floating ring of Styrofoam (diameter: 61 cm), with walls of mosquito net (mesh size: 2 mm) and a funnel with a dry collection chamber on top (Figure 1c). To cover the typical early-autumn peak of emerging Chironomidae of Lake Erken (Sandberg, 1969), emergence traps were mounted on the mesocosms on 15 August and emptied with the help of a small exhauster twice a week until 19 September. The fish addition occurred on 25 August and for the purpose of this study, we only considered samples taken after the fish addition, i.e. the experiment lasted 25 days with seven sampling campaigns.

Sampled adult Chironomidae were transported to the laboratory at Uppsala University where they were killed by freezing and sorted into species and sexes according to their morphological appearance using a stereo microscope. Example specimens of each species were stored in ethanol for species identification. Functional feeding groups of the species were assigned according to Armitage et al. (1997) and Mandaville (2002). Samples were freeze-dried and stored at -80° for fatty acids analyses.

To obtain an estimate of biomass development during the course of the experiment (25 days) of the most abundant species across the gradient, Chironomidae dry weight (DW) was measured using a microbalance for up to five individuals of the same species from each mesocosm and time point. For less abundant species, averages of individual biomass were obtained by weighing several individuals from the same species, mesocosm and time point.

Polypropylene strips were placed along the inside of each mesocosm (7 cm wide, extending from top to bottom) in order to monitor periphyton growth on the container wall. Every 2 weeks, we removed and scraped the biofilm from the entire strip, and replaced the strip in the mesocosm. Collected biofilm was dried in an oven at 60°C for 24 h in pre-weighed vials, which were reweighed to obtain DW. A subsample of the dried material was taken and acidified using 5% HCl to remove inorganic carbon, dried again, and then combusted for carbon analyses in an elemental analyser (Costech Analytical Technologies Inc., Valencia, CA, USA). To obtain an estimate of the carbon content in the periphyton biomass (g C/m^2), we multiplied the obtained values of biofilm DW (g/m^2) growing during the experiment with the carbon content and we summed the values of the three biofilm sampling campaigns conducted during the emergence experiment. Following each sampling, periphyton growing on the walls of the mesocosms was scraped off and allowed to sediment. We assumed that periphyton removal lead to a translocation of Chironomidae inhabiting the walls to the bottom of the mesocosms. These individuals were subsequently caught in the emergence traps that mostly covered the centre of the mesocosms (Figure 1c).

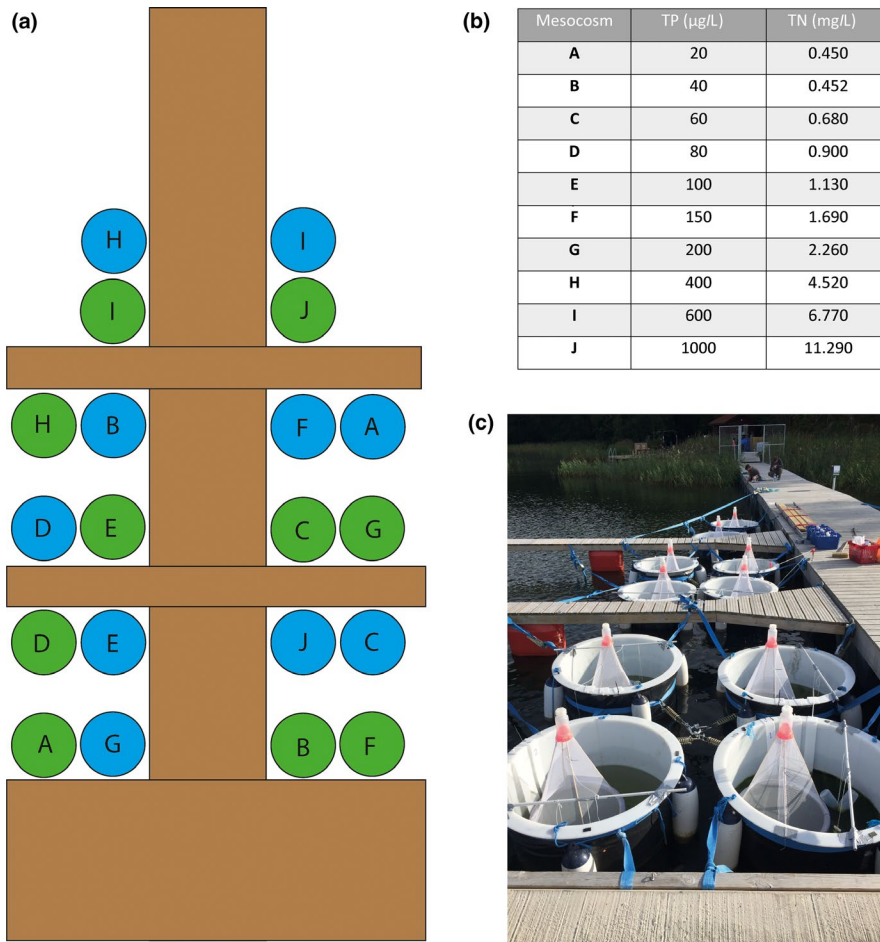


FIGURE 1 Representation of the mesocosm facility. (a) Scheme of the experimental set-up. Capital letters refer to nutrient concentrations, green colour depicts mesocosms without fish addition and blue mesocosms where two juvenile crucian carp were added; (b) table with concentration of total phosphorus (TP) and total nitrogen (TN) used for each step of the nutrient gradient; (c) photo of floating mesocosms with mounted emergence traps

The study was approved by the Uppsala Animal Ethic Committee with permit number 5.8.18-03672/2017.

2.2 | Fatty acid analysis

Fatty acid analysis was conducted according to previously published protocols (Scharnweber, Strandberg, Karlsson, & Eklöv, 2016; Scharnweber, Strandberg, Marklund, & Eklöv, 2016). Adult Chironomid species differed greatly in biomass. Thus, 2–11 individuals needed to be pooled to obtain 0.6–5.6 mg DW of each species. For lipid extraction, a mixture of chloroform/methanol (2:1, by volume) was used and 0.88% KCl was added to remove non-lipids. This procedure was repeated a second time. Samples were sonicated for 10 min to enhance extraction. After evaporation under a nitrogen stream, lipids were dissolved in hexane and transmethylated at 90°C for 90 min, using acidic catalyst (1% H_2SO_4 in methanol). Analyses of fatty acid methyl esters were conducted at Uppsala University using an Agilent 7890B Gas Chromatographer (Agilent Technologies) equipped with a DB-23 column (length 30 m, ID 0.25 mm, film thickness 0.25 μm , Agilent). A splitless injection was applied, using an initial oven temperature of 50°C for 1 min, which then was increased by 15°C/min to 150°C, 1°C/min to 180°C, 10°C/min to 210°C to a final temperature of 210°C that was maintained for 10 min. Helium

gas was used as a carrier with an average velocity of 36 cm/s. Fatty acid methyl ester peaks were identified using retention times and mass spectra using the software MSD ChemStation (F01.01.2317; Agilent Technologies). Heneicosanoic acid (Nu-Chek Prep, Inc.) was used as an internal standard. Using calibration curves of standard solutions of known lipid mixtures (Nu-Chek Prep, Inc.), we calculated fatty acid content, and data were expressed as percentages relative to total fatty acid (fatty acid %) and as content related to insect DW (mg g/DW).

2.3 | Data analyses

Happel, Czesny, Rinchar, and Hanson (2017) compared several methods of data transformations for proportional data of fatty acids. They found no clear advantage of any transformation compared to no transformation. For our dataset of adult Chironomidae collected from the mesocosms without fish, the major outcome of the analyses remained the same when comparing the untransformed data with an arcsin square-root transformed data set. Thus, we decided to use raw values to make the interpretation of results easy and straightforward.

To understand the general variation in fatty acid composition, we first used multivariate analyses including all fatty acids. Non-metric

multidimensional scaling was used to display variation of fatty acid composition in emerging Chironomidae. Non-parametric PERMANOVA with species and TP was used to test the overall effects of taxonomy and nutrient concentrations on the variation of Chironomidae fatty acid proportions within the whole fatty acid dataset. We used permutation of residuals under a reduced model (9,999 permutations) with type III sums of squares (Anderson, Gorley, & Clarke, 2008). Proportion of variance explained was calculated from sums of squares. A similarity percentages routine (SIMPER) helped us to identify target fatty acids, which were fatty acids that contributed most to the observed differences in Chironomidae between mesocosms at the low- and high-end of the concentration gradient.

To compare the variation in the target fatty acids, univariate analyses were conducted. We calculated a general linear model with proportion target fatty acids as dependent variable and TP, species, and sex as fixed factors. We used independent *t*-tests to compare differences in target fatty acid proportions between males and females of the different species. Linear regressions

were used to compute species-specific significance of relationship between target fatty acids and TP. We used quadratic regressions to assess significance of the relationship between periphyton biomass and TP.

To estimate the export of fatty acids via emerging Chironomidae (estimated as mg per 25 days per trap) and to compare these values between the mesocosms, average content of α -linolenic acid (ALA), EPA, total sum of fatty acids and PUFAs in mg g/DW were calculated for each of the two major functional feeding groups (i.e. predators and collector-gatherers), for each mesocosm, and for both sexes. To do so, Chironomidae species were assigned to functional feeding groups and average content was multiplied with the average biomass of emerging Chironomidae in g DW per trap obtained over the 25-day experiment for each species, sex and mesocosm. Unidentified species were assigned to averages of all species and samples. We used quadratic regressions to assess significance of the relationship between the export of fatty acids and TP.

TABLE 1 Species list of the Chironomidae caught and assignment to functional feeding groups

Species	Functional feeding group	Number of males caught	Number of females caught
Subfamily Chironomini			
<i>Chironomus annularius</i>	Collector-gatherer	1	11
<i>Chironomus (Chironomus) luridus</i>	Collector-gatherer	134	146
<i>Glyptotendipes palles</i>	Collector-gatherer	6	0
<i>Microtendipes pedellus</i>	Collector-filterer	36	2
<i>Parachironomus gracilior</i>	Collector-gatherer	48	55
<i>Polypedilum (Polypedilum) nubeculosum</i>	Shredder	91	183
Subfamily Orthocladiinae			
<i>Cricotopus (Isocladius) intersectus</i>	Shredder	25	0
<i>Cricotopus (Isocladius) pilitarsis</i>	Shredder	3	0
<i>Cricotopus (Isocladius) sylvestris</i>	Shredder	26	22
<i>Corynoneura</i> sp.	Collector-filterer	1	0
<i>Parakiefferiella smolandica</i>	Collector-gatherer	2	0
Subfamily Tanytopodinae			
<i>Ablabesmyia monilis</i>	Predator	292	434
<i>Procladius (Holotanypus) choreus</i>	Predator	1	2
Subfamily Tanytarsini			
<i>Paratanytarsus dissimilis</i>	Collector-filterer	2	0
<i>Paralauterborniella nigrohalteralis</i>	Collector-gatherer	1	0
<i>Tanytarsus ejuncidus</i>	Collector-gatherer	1	0
<i>Tanytarsus gibbosicceps</i>	Collector-gatherer	10	0
<i>Tanytarsus brundini</i>	Collector-gatherer	25	15
Not identified		64	
	Total	1,654	

We used multiple regression models to understand the main drivers of the export rate of ALA, EPA, total PUFA content (sum of all PUFAs, defined as fatty acids with multiple double bonds) and total fatty acid content (sum of all 22 fatty acids analysed) from mesocosms with and without fish (i.e. eight models in total), similar to the approach of Martin-Creuzburg et al. (2017). In the models, we included the emerging biomass, the ratio of collector-gatherer/predators as a measure for community composition, the mean fatty content (ALA, EPA, total PUFAs, or total fatty acids, respectively), periphyton biomass, TP, and an interaction term of all variables as predictors. Prior to analyses, we mean-centred the predictors to improve the quality of the statistical tests by reducing multicollinearity. Due to the low number of emerging Chironomidae from the mesocosms of the fish treatment, we were not able to include the ratio of collector-gatherer/predators for the analyses of this treatment. We used stepwise backward model selection with adjusted R^2 to identify the model that explained best the relationships between the predictor variables and the export rate of ALA, EPA, total PUFA, and total fatty acid content for the different treatments (fish and no fish).

We used Primer 7.0.6 with the PERMANOVA add-on (Primer E Ltd.) for the multivariate analyses and IBM SPSS statistics V24 for all univariate analyses.

3 | RESULTS

In total, we caught 1,625 individual adult Chironomidae and determined 21 species (Table 1). Rare species accounted for 3.9% of individuals and were not identified. Five species, accounting for 85.9% of all individuals, were used in the fatty acid analyses: the predator

Ablabesmyia monilis, the shredder *Polypedilum (Polypedilum) nubeculosum*, the collector-filterer *Microtendipes pedellus*, and the two collector-gatherers *Chironomus luridus* and *Parachironomus gracilior*. Overall, the number of the emerging Chironomidae was six times lower in the mesocosms with fish compared with those without fish. We therefore focused the fatty acid analyses on samples from the treatment without fish only, assuming no difference in fatty acid composition between mesocosms with and without fish.

We identified 33 fatty acids, but did not consider 11, as they had proportions below 1% (Appendix S1). As depicted in the non-metric multidimensional scaling, the two species of collector-gatherers were characterised by high proportions of ALA, whereas the shredder *P. nubeculosum* showed high proportions of 16:0, and *A. monilis* as a predator had high proportions of EPA (Figure 2). Fatty acid composition differed significantly between the species (pseudo-F: 14.233; $p = .0001$, 42.8% of the variation explained) and mesocosms of different TP concentrations (pseudo-F: 3.8149; $p = .0001$, 16.1% of the variation explained).

Two chironomid species (*C. luridus* and *A. monilis*) made up 60.8% of all individuals sampled and were collected in sufficient numbers across the whole nutrient gradient to allow comparisons. SIMPER analyses performed for each of the species separately identified that ALA and EPA were the strongest contributors to differences in fatty acid composition across the gradient (see Appendix S2 for pairwise comparisons between mesocosms of the low- and high-end of the gradient). We therefore focused on these two target fatty acids to enhance our understanding on the species-specific variation of fatty acids across the nutrient gradient.

Total phosphorous significantly explained the variation in ALA (GLM: $F_{7,46} = 7.092$; $p < .0001$) and EPA (GLM: $F_{7,46} = 5.126$; $p < .0001$). In both species, increasing nutrient concentrations were related to

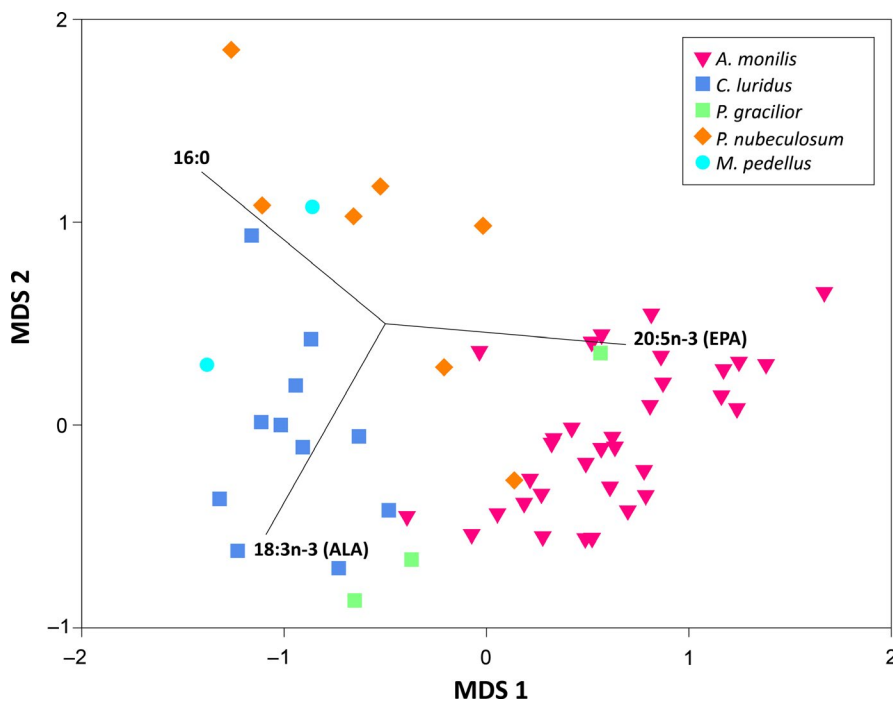


FIGURE 2 Two-dimensional representation of non-metric multidimensional scaling (MDS) of fatty acid composition (%) from the emerging Chironomidae of the mesocosms with stress level 0.05. Vectors from fatty acids were identified to have a correlation coefficient of $R^2 > 0.9$ to the observed difference in the composition between the Chironomidae species and the vectors depict the respective dimension. Symbols depict different functional feeding groups: predator (triangles), collector-gatherers (squares), shredder (diamond), and filter-feeder (circle). ALA, α -linolenic acid; EPA, eicosapentaenoic acid

increasing ALA proportions, but decreasing EPA proportions, but regression was significant for *A. monilis* only (Figure 3a,b). The GLM also highlighted the significantly different proportions of ALA and EPA between the species with *C. luridus* being higher in ALA (GLM: $F_{1,46} = 31.819$; $p < .0001$) and *A. monilis* being higher in EPA (GLM: $F_{1,46} = 203.707$; $p < .0001$). Furthermore, EPA proportions (GLM: $F_{1,46} = 6.355$; $p = .016$) differed significantly between males and females, but this was not significant in ALA proportions. However, when analysed separately, only the differences between the proportions of EPA in males and females in *C. luridus* were significant (t test: $t_{11} = -4.088$, $p = .002$) with males having higher proportions of EPA (Figure 3b).

We found high variation in the biomass of adult Chironomidae emerging from the mesocosms (Figure 4a). Overall, the biomass was 8-fold higher in the mesocosms without fish compared to the mesocosms where crucian carp was added. Furthermore, the biomass of

Chironomidae emerging from the mesocosms responded to the nutrient addition with higher biomasses in higher concentrations. In mesocosms with fish, the highest biomass emerged in the mesocosm at a TP of 600 $\mu\text{g/L}$, whereas quadratic regression indicated a distinct peak in biomass emergence in the fishless mesocosms at a TP of 400 $\mu\text{g/L}$. For both treatments, a decrease in the emerging biomass was found at the highest nutrient concentration (TP of 1000 $\mu\text{g/L}$; Figure 4a).

Periphyton biomass showed a similar pattern to the Chironomidae biomass and was highest in the mesocosm with a TP of 400 $\mu\text{g/L}$ in the fishless treatment. In the fishless treatments, we found a significant quadratic relationship between periphyton carbon and TP, but no significant relationships were found in the mesocosms with fish (Figure 4b).

The export of fatty acids along the nutrient gradient in mesocosms with and without fish generally followed the pattern of the

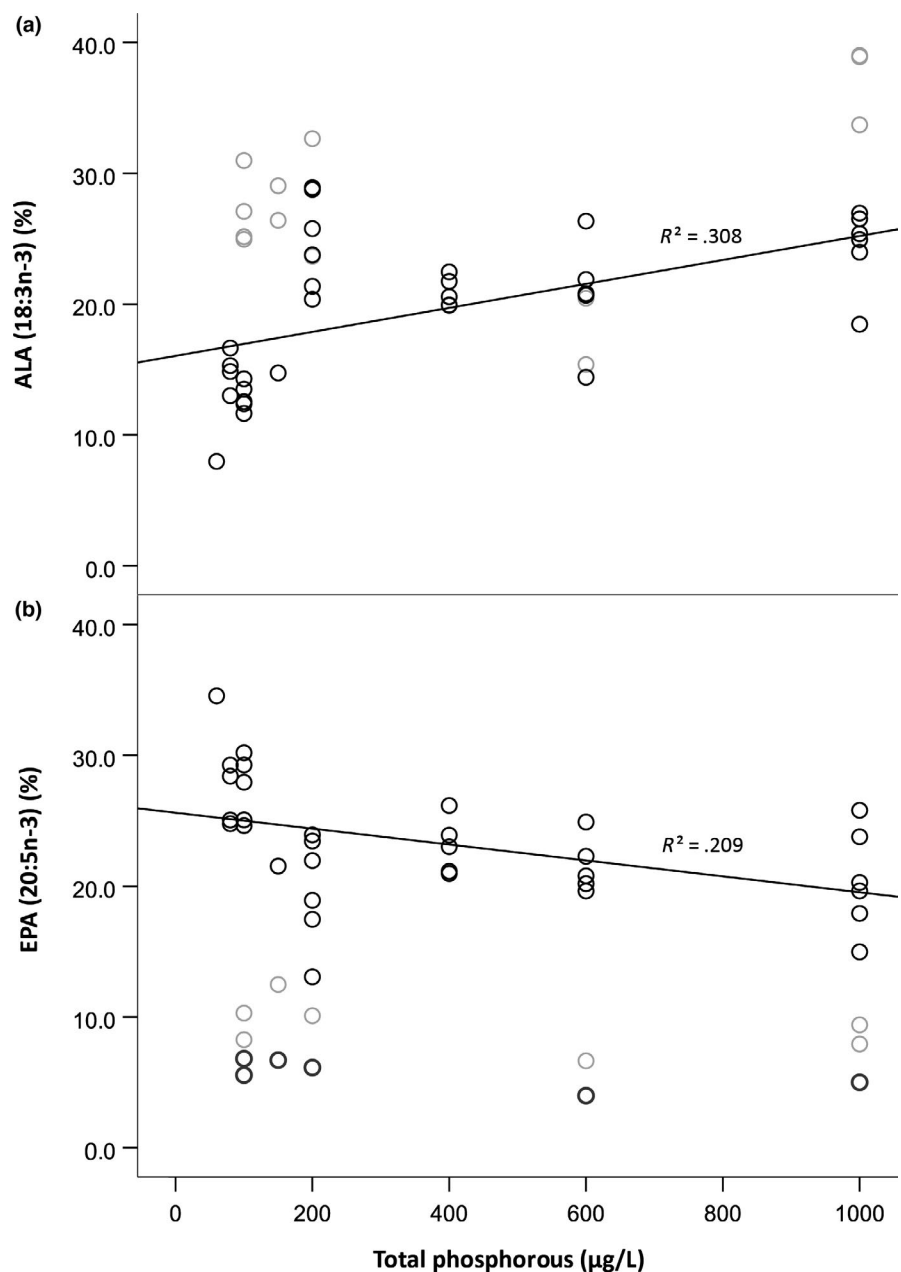


FIGURE 3 Proportions of (a) α -linolenic acid (ALA; 18:3n-3) and (b) eicosapentaenoic acid (EPA; 20:5n-3) in total fatty acids of emerging *Chironomus luridus* (grey symbols) and *Ablabesmyia monilis* (black symbols) across the nutrient gradient in the fishless treatments. Regression line (including goodness-of-fit; R^2) depict significant relations of fatty acid proportions in *A. monilis* along the gradient. As EPA proportions differed significantly between *C. luridus* sexes, males (light grey symbols) and females (dark grey symbols) were analysed separately, but regressions were not significant

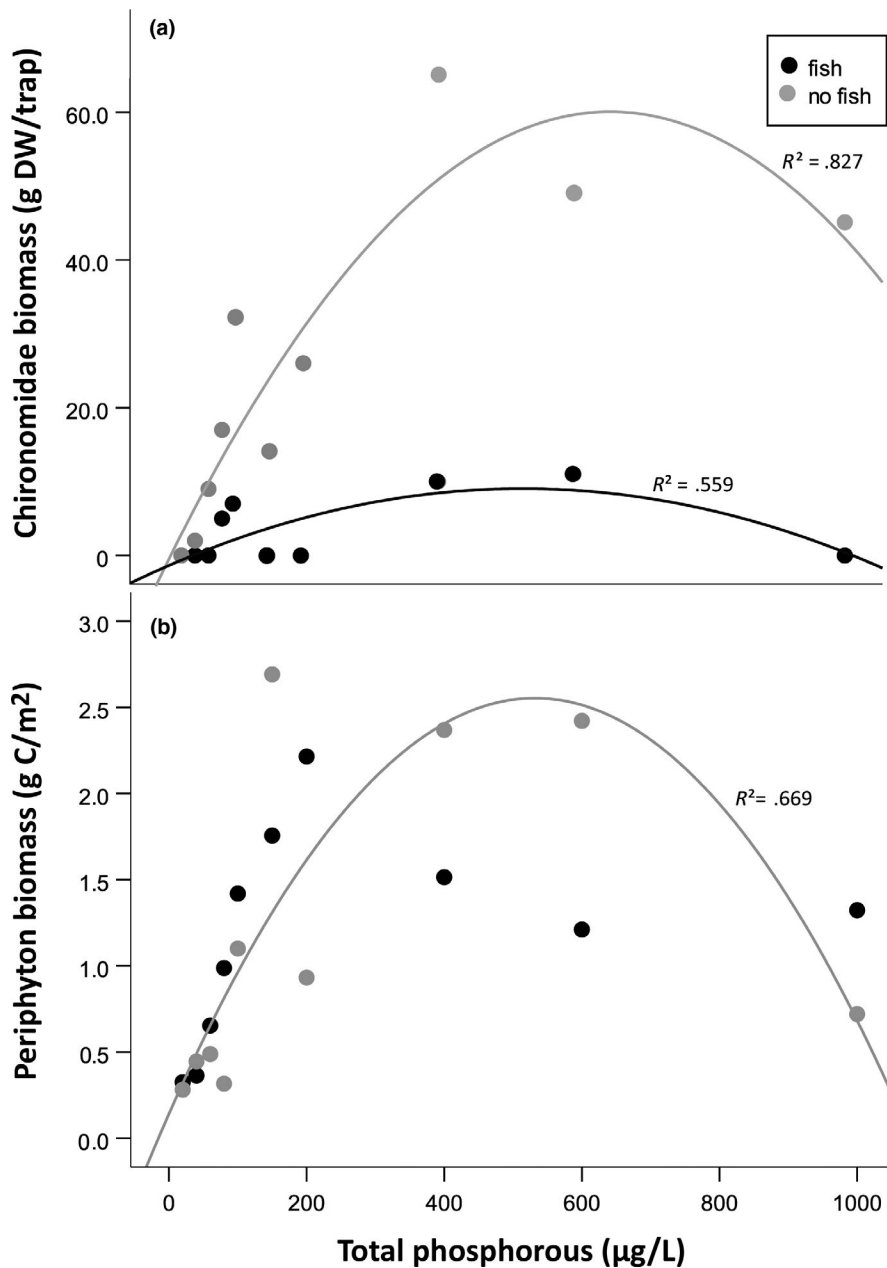


FIGURE 4 Relationships of (a) total biomass of emerging Chironomidae and (b) biomass of periphyton growing on the wall of the mesocosms to mesocosm phosphorus concentration, with fish (black symbols) and without fish (grey symbols). Significant quadratic relationships are depicted by curves (including goodness-of-fit; R^2). DW, dry mass

biomass emergence and quadratic regression lines appeared similar. The amount of ALA, EPA, total PUFAs, and total fatty acids were 7.4–8.1-fold higher in the fishless mesocosms compared to the ones with fish (Figure 5). A distinct peak in maximum export was found for EPA, total fatty acids, and total PUFAs at the TP concentration of 400 µg/L in the mesocosms without fish. This pattern was less distinct at a TP level between 400 and 600 µg in the fish treatments. At the highest nutrient concentrations, the export decreased in both treatments (Figure 5b,c,d). For ALA, the highest export rates were found at a TP level of 400 µg/L in both treatments (Figure 5a). Furthermore, the amount of exported ALA did not decrease in the highest nutrient concentration in the absence of fish. Instead, the two mesocosms of highest nutrient concentrations had a similar export rate (Figure 5a).

The variables included in the multiple regression models were able to explain 88.3–99.4% of the variation in the export of ALA, EPA, total PUFAs and total fatty acids (Table 2). Predictors included in the model differed between the eight models, but overall, the biomass of emerging Chironomidae was the strongest predictor (explaining 87.8–99.4% of all variance, Table 3). In the fishless mesocosms, mean ALA concentration further had a significant effect on the export of ALA ($t = 3.433$, $p = .019$). For the export of the total PUFAs from mesocosms where fish were added, two additional predictors were significant in the model: the mean PUFA concentration ($t = 3.159$, $p = .034$) and the biomass of periphyton ($t = 2.780$, $p = .050$). For the total fatty acid export from the mesocosms with fish, the mean fatty acid concentration ($t = 2.818$, $p = .037$) was a second significant predictor in the model, besides the highly significant effect of the Chironomidae biomass (Table 3).

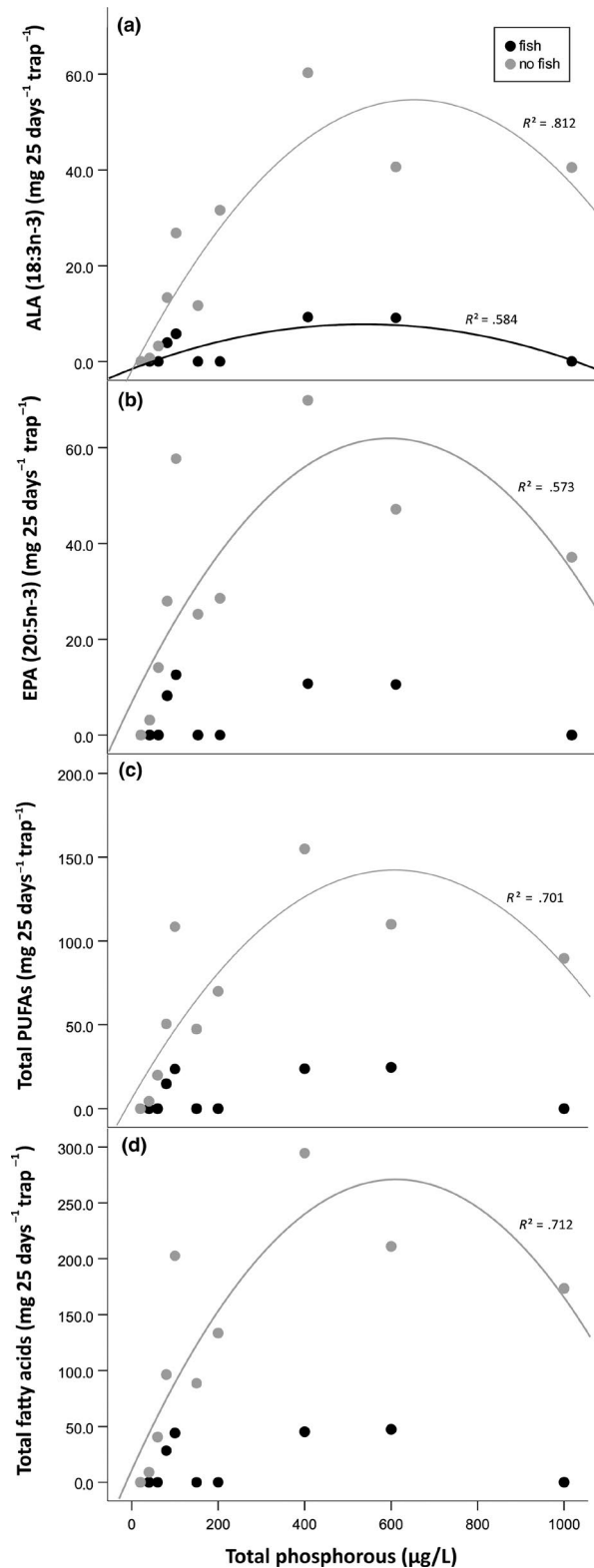


FIGURE 5 Export via emerging Chironomidae of (a) α -linolenic acid (ALA; 18:3n-3); (b) eicosapentaenoic acid (EPA; 20:5n-3); (c) total polyunsaturated fatty acids (PUFAs); and (d) total fatty acids per mesocosm along the nutrient gradient. Black symbols represent mesocosms with added crucian carp, grey symbols represent mesocosms without fish. Significant quadratic relationships are depicted by curves (including goodness-of-fit; R^2)

4 | DISCUSSION

Our study suggests that the export of fatty acids from aquatic systems via adult Chironomidae is highly dependent on fish predation pressure, but also on the nutrient concentrations of the system. In the mesocosms without crucian carp, the amount of ALA, EPA, total fatty acids, and total PUFAs were 7.4–8.1 times higher than in the mesocosms where fish were present. Predation is known to create strong direct, but also indirect effects on food webs (e.g. Sinclair, Mduma, & Brashares, 2003; Terborgh et al., 2001). Fish predation can have direct effects on the abundance of emerging insects, including Chironomidae (Knight et al., 2005; Pitcher & Soluk, 2018; Wesner, 2010). In our mesocosms, the presence of two juvenile crucian carp reduced the number of emerging insects, which directly translated into the quantity of fatty acids exported from the aquatic system to the terrestrial environment. Thus, when predation pressure is high, fewer fatty acids will be exported from aquatic habitats. As a consequence, less EPA will also be transferred to the terrestrial environment, which is severely limited in these habitats as terrestrial vascular plants produce it only in low quantities (Twining et al., 2016). Recent studies highlight the importance of aquatic-derived EPA on the fitness of terrestrial predators. For example, Fritz et al. (2017) found that wolf spiders (*Tigrosa georgicola*) from wetland areas had a higher level of long-chained aquatic PUFAs and further a better immune response than spiders in upland habitats. Using a 24-year dataset of tree swallows (*Tachycineta bicolor*), Twining et al. (2018) were able to connect the breeding success in this bird species with availability of EPA-rich aquatic insects.

However, PUFAs tend to accumulate in planktivorous fish (Strandberg et al., 2015), which are especially rich in docosahexaenoic acid (22:6n-3), and therefore provide the potential to maintain and store PUFAs that could be available for harvesting by humans or other piscivores.

We found that periphyton biomass, until a certain threshold value, is positively related to the nutrient concentration, which is connected to Chironomidae biomass. Those findings are in line with previous studies showing that population sizes of Chironomidae larvae are positively correlated with the amount of algae produced (de Haas et al., 2005; Vos et al., 2004). We therefore conclude that fatty acid export rates are determined by the biomass of aquatic insects, and that the export is influenced by the top-down effect from fish predators. However, the hump-shaped relationship with increasing total phosphorus suggests that the export is also driven by bottom-up effects. Increasing nutrients supported higher periphyton biomass but this decreased at high nutrient levels, probably as a result of shading of high nutrient conditions favouring phytoplankton growth (Liboriussen & Jeppesen, 2006; Vadeboncoeur, Lodge, & Carpenter, 2001).

In our study, the regression between periphyton biomass and phosphorus concentration was not significant in the mesocosms where fish were added. Furthermore, the trends of the quadratic relationships between export rates of EPA, total PUFAs, and total fatty acids with TP were not significant in mesocosms with fish. In

TABLE 2 Analysis of variance for the overall significance of the multiple regression models predicting export of (a) α -linolenic acid (18:3n-3), (b) eicosapentaenoic acid (20:5n-3), (c) total polyunsaturated fatty acids (PUFAs) and (d) total fatty acids (FAs) from the mesocosms

	Treatment	Source	df	SS	MS	F	p	Adj. R ²
(a) 18:3n-3	Fish	Regression	1	137.793	137.793	1,470.651	<.001	0.994
		Residual	8	0.750	0.094			
		Total	9	138.543				
	No fish	Regression	5	3,703.258	740.652	110.213	<.001	0.984
		Residual	4	26.881	6.720			
		Total	9	3,730.138				
(b) 20:5n-3	Fish	Regression	2	251.036	125.518	34.916	<.001	0.883
		Residual	7	25.164	3.595			
		Total	9	276.201				
	No fish	Regression	3	4,320.221	1,435.753	29.919	.001	0.906
		Residual	6	274.966	47.988			
		Total	9	4,595.187				
(c) Total PUFAs	Fish	Regression	5	1,186.233	237.247	83.469	<.001	0.979
		Residual	4	11.369	2.842			
		Total	9	1,197.602				
	No fish	Regression	3	22,586.696	7,528.899	92.075	<.001	0.968
		Residual	6	490.615	81.769			
		Total	9	23,077.311				
(d) Total FAs	Fish	Regression	4	4,245.699	1,061.425	88.014	<.001	0.975
		Residual	5	60.299	12.060			
		Total	9	4,305.998				
	No fish	Regression	3	81,429.945	27,143.315	107.269	<.001	0.973
		Residual	6	1,518.236	253.039			
		Total	9	82,948.182				

Abbreviations: F: F-statistic; df, degrees of freedom; MS, mean square; p: probability value; SS, sum of squares.

the mesocosms where crucian carp were added, the highest periphyton biomass was measured at a TP concentration of 200 $\mu\text{g TP/L}$, and was not synchronised to the peak of Chironomidae emergence. We assume that the added fish exerted a high predation pressure, controlling the Chironomid population. In support of this theory, emergence rates decreased almost to zero at the end of the experiment in the fish treatment, whereas in the fishless treatment, they remained at higher rates (Appendix S3). Furthermore, the fish could have caused indirect effects on periphyton biomass via the fish-invertebrate-periphyton cascade (Brönmark & Weisner, 1992; Jones & Sayer, 2003). Predation from crucian carp might have decreased the abundance of Chironomidae, which then released periphyton from grazing pressure, allowing it to increase in biomass. This could potentially explain higher periphyton biomass at the lower end of the nutrient gradient, at a TP of 150 $\mu\text{g/L}$. However, crucian carp are opportunistic feeders (Penttinen & Holopainen, 1992) that can adapt their feeding strategy when specific prey items are scarce (Chaguaceda, Eklöv, & Scharnweber, in preparation). Therefore, the indirect effects from crucian carp on periphyton abundance via feeding on Chironomidae grazers might be related to several additional parameters, e.g. zooplankton abundance.

Our study highlights the differences in fatty acid composition between Chironomidae of different species. Generally, the predatory species *A. monilis* was characterised by higher proportions of EPA and lower proportions of ALA, whereas the opposite was the case in the collector-gatherer *C. luridus*. Similar to our study, Makhutova et al. (2017) found that ALA and EPA made a major contribution to the differences in fatty acid variation between five different Chironomidae species of a saline lake in Siberia. They further indicated a significant difference in fatty acid composition of Chironomidae inhabiting different ecological niches of the lake, while the fatty acid composition did not differ significantly between larvae and adults. Larvae of the genus *Chironomus*, which live at greater depths with black silt deposits had higher contents of bacterial fatty acid markers (i17:0, ai17:0, 17:0, 17:1, 18:1n7) compared to *Glyptotendipes* larvae that were characterised with a higher content of ALA, living in sediments of the littoral zone with higher content of clay and sand. We show that differences in specific fatty acid composition among species may also occur among species inhabiting the same spatial area within a lake (i.e. the littoral zone) but belonging to different functional feeding groups. Due to the interspecific differences in fatty acid composition, it is reasonable to assume that the export rate of fatty acids

TABLE 3 Results of regression models including predictor variables predicting export of (a) α -linolenic acid (18:3n-3), (b) eicosapentaenoic acid (20:5n-3), (c) total polyunsaturated fatty acids (PUFAs) and (d) total fatty acids (FAs) from the mesocosms

	Treatment	Variable	Coefficient	SE	t	p
(a) 18:3n-3	Fish	Chironomidae biomass	0.861	0.022	38.349	<.001
		TP	-0.014	0.006	-2.447	.071
	No fish	Chironomidae biomass	0.981	1.107	12.805	<.001
		Collector/predator ratio	-2.073	1.142	-1.815	.144
		Mean ALA content	16.538	4.789	3.453	.026
	Periphyton biomass	-1.130	1.208	-0.935	.403	
(b) 20:5n-3	Fish	Chironomidae biomass	1.188	0.143	8.320	<.001
		TP	-0.002	0.002	-1.148	.289
	No fish	TP	-0.021	0.012	-1.784	.125
		Chironomidae biomass	1.170	0.168	6.970	<.001
		Collector/predator ratio	3.549	2.711	1.309	.238
(c) Total PUFAs	Fish	TP	-0.003	0.002	-1.413	.230
		Chironomidae biomass	2.088	0.196	10.655	<.001
		Mean PUFA content	21.228	6.719	3.159	.034
		Periphyton biomass	0.905	0.325	2.780	.050
		Interaction term	0.337	0.262	1.285	.268
	No fish	TP	-0.030	0.016	-1.884	.109
Chironomidae Biomass		2.553	0.219	11.647	<.001	
	Collector/predator ratio	5.995	3.538	1.694	.141	
(d) Total FAs	Fish	Chironomidae biomass	4.317	0.294	15.089	<.001
		Mean FA content	18.659	6.622	2.818	.037
		Periphyton biomass	1.621	0.723	2.242	.075
		Interaction term	0.415	0.360	1.151	
	No fish	TP	-0.052	0.028	-1.894	.107
		Chironomidae biomass	4.822	0.386	12.507	<.001
		Collector/predator ratio	10.579	6.224	1.700	.140

Note: Bold font depicts significant results. Values are based on centred values.

from water to land depends on the Chironomidae community composition. However, in our mesocosm Chironomidae total biomass was the strongest predictor for the export rates, suppressing the more subtle effects resulting from changes in the community composition, measured as the ratio of collector-gatherers to predators.

By analysing specific Chironomidae species separately, we could further identify variation in the fatty acid composition in adult Chironomidae across the nutrient gradient. The proportion of EPA in *A. monilis*, which was the most abundant Chironomidae species (accounting for 43.9% of all individuals caught) was negatively correlated with the increase in nutrients along the mesocosm gradient. A similar trend, albeit not significant, was visible in the second most abundant Chironomidae species *C. luridus*. Lower proportions of EPA in a higher nutrient concentration suggest that hypertrophic aquatic ecosystems export lower quality prey for terrestrial predators. However, the increase in the number of emerging individuals overrode the relative decrease of PUFAs when calculating the total export of PUFAs from water to land and EPA content was a non-significant predictor in the multiple regression determining EPA export rates.

In contrast to the low EPA proportion, the ALA proportion in *A. monilis* significantly increased with increasing nutrient concentration analysed from the fishless mesocosms. A similar, but not significant trend could be seen in *C. luridus*. Mean ALA content was a significant predictor in the multiple regression of ALA export rates in the fishless treatments and the high ALA proportion in Chironomidae individuals at the end of the nutrient concentration spectrum slightly dampened the decreasing slope of export rates at high nutrient concentrations.

We found elevated proportions of ALA in Chironomidae collected from mesocosms of higher nutrient concentrations. These findings might be related to physiological responses of periphyton to differences in nutrient concentrations. Under low nutrient conditions, excess carbon can accumulate and this might dilute PUFA content similar to the pattern observed in stoichiometry (Hessen, Elser, Sterner, & Urabe, 2013; Hill, Rinchar, & Czesny, 2011). In phytoplankton, low nutrient concentrations lead to the production of PUFA-poor triacylglycerols, whereas high nutrient concentrations lead to an increase of PUFA-rich galactolipids in the algae (Guschina & Harwood, 2009). Although there has been intense research on

stoichiometry of algae and their potential to respond to different nutrient levels, little is known about the cellular responses in periphyton. Some studies have reported that added nutrients lead to an increase in the proportions of ALA in periphyton (Cashman, Wehr, & Truhn, 2013; Guo et al., 2016; Hill et al., 2011), but unfortunately we did not analyse periphyton fatty acids in this study.

It is still largely unknown to what extent the fatty acid composition found in the diet is reflected in the consumers. Consumers have recently been found to be capable of the *de novo* biosynthesis of long-chained PUFAs (Kabeya et al., 2018), but little is known about the efficiency of this route and the costs involved. For example, Guo et al. (2016) indicated different capacities in this respect between a mayfly and a caddisfly species. Also, Guo et al. (2018) conducted a field study of stream macroinvertebrates in order to investigate the divergence between the fatty acid compositions of the consumers in comparison with their basal resource, periphyton. They found a strong variance in periphyton quality, but the integration of fatty acids, especially EPA into macroinvertebrate tissue was dependent on the functional feeding group. This finding suggests that herbivores are able to accumulate EPA, either by selective feeding on EPA-rich sources, preferential assimilation, or through active control of the internal EPA content via physiological pathways (Guo et al., 2018). We are not aware of any investigation of how the fatty acid composition of Chironomidae depends on periphyton food sources.

To our knowledge, nothing is known about the differences in fatty acid composition between female and males in Chironomidae. We found a significant effect of sex on the proportion of EPA in one of the species (*C. luridus*) only. However, a trend towards higher proportions of ALA in females of *A. monilis* was visible. In mayflies (*Ephemeroptera sylvicola*), a lower content of fatty acids was found in females and this was related to the production of eggs (Meier, Meyer, & Meyns, 2000). We did not monitor the reproductive status of the emerging Chironomidae, and cannot be certain if oviposition had occurred prior to sampling. In Chironomidae, the behavior after emergence is species-specific with some species showing instant mating and oviposition after emergence and other species displaying swarming and delayed oviposition (Armitage et al., 1997).

To summarise, our study demonstrates the high variability in export rates of fatty acids via emerging adult Chironomidae from aquatic systems, and shows that this depends on predation pressure, trophic status, species composition, and sex differences. This variability also includes the export rates of EPA, which has due to its low abundance in terrestrial habitats an important role for the fitness of terrestrial consumers (Twining et al., 2016), and our study highlights the strong dynamics involved in the provision of this highly unsaturated fatty acid. This study is one of the first attempts to evaluate the export of fatty acids from aquatic ecosystems in responses to several abiotic and biotic factors.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the DiVA (Digitala Vetenskapliga Arkivet) repository of Uppsala University at <https://uu.diva-portal.org>, reference number: urn:nbn:se:uu:diva-389470 (Scharnweber, 2019).

ORCID

Kristin Scharnweber  <https://orcid.org/0000-0003-2858-5947>

Fernando Chaguaceda  <https://orcid.org/0000-0002-0827-2110>

Lars Tranvik  <https://orcid.org/0000-0003-3509-8266>

Peter Eklöv  <https://orcid.org/0000-0002-8981-1453>

REFERENCES

- Ahlgren, G., Gustafsson, I. B., & Boberg, M. (1992). Fatty acid content and chemical composition of freshwater microalgae. *Journal of Phycology*, 28(1), 37–50. <https://doi.org/10.1111/j.0022-3646.1992.00037.x>.
- Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to software and statistical methods*. Devon, UK: Primer-E Ltd.
- Armitage, P., Cranston, P. S., & Pinder, L. C. V. (1997). *The Chironomidae. The biology and ecology of non-biting midges*. London, UK: Chapman & Hall.
- Bartels, P., Cucherousset, J., Steger, K., Eklöv, P., Tranvik, L. J., & Hillebrand, H. (2012). Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics. *Ecology*, 93(5), 1173–1182.
- Baxter, C. V., Fausch, K. D., & Saunders, W. C. (2005). Tangled webs: Reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biology*, 50(2), 201–220.
- Bell, M. V., & Tocher, D. (2009). Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In M. T. Art, M. T. Brett, & M. Kainz (Eds.), *Lipids in aquatic ecosystems* (pp. 211–236). Dordrecht, The Netherlands/Heidelberg, Germany/London, UK/New York, NY: Springer.
- Borisova, E. V., Makhutova, O. N., Gladyshev, M. I., & Sushchik, N. N. (2016). Fluxes of biomass and essential polyunsaturated fatty acids from water to land via chironomid emergence from a mountain lake. *Contemporary Problems of Ecology*, 9(4), 446–457. <https://doi.org/10.1134/s199542551604003x>.
- Brett, M. T., & Müller-Navarra, D. C. (1997). The role of highly unsaturated fatty acids in aquatic food web processes. *Freshwater Biology*, 38(3), 483–499. <https://doi.org/10.1046/j.1365-2427.1997.00220.x>.
- Brönmark, C., & Weisner, S. E. B. (1992). Indirect effects of fish community structure on submerged vegetation in shallow, eutrophic lakes—An alternative mechanism. *Hydrobiologia*, 243, 293–301. <https://doi.org/10.1007/bf00007045>.

- Cashman, M. J., Wehr, J. D., & Truhn, K. (2013). Elevated light and nutrients alter the nutritional quality of stream periphyton. *Freshwater Biology*, 58(7), 1447–1457. <https://doi.org/10.1111/fwb.12142>.
- Chaguaceda, F., Eklöv, P., & Scharnweber, K. under review. Regulation of fatty acid composition related to ontogenetic changes and niche differentiation of a common aquatic consumer. *Oecologica*.
- Cole, J. J., Pace, M. L., Carpenter, S. R., & Kitchell, J. F. (2000). Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. *Limnology and Oceanography*, 45(8), 1718–1730.
- Cottingham, K. L., Carpenter, S. R., & St Amand, A. L. (1998). Responses of epilimnetic phytoplankton to experimental nutrient enrichment in three small seepage lakes. *Journal of Plankton Research*, 20(10), 1889–1914. <https://doi.org/10.1093/plankt/20.10.1889>.
- Cummins, K. W. (1973). Trophic relations of aquatic insects. *Annual Review of Entomology*, 18, 183–206. <https://doi.org/10.1146/annurev.en.18.010173.001151>.
- Davidson, T. A., Audet, J., Svenning, J. C., Lauridsen, T. L., Søndergaard, M., Landkildehus, F., ... Jeppesen, E. (2015). Eutrophication effects on greenhouse gas fluxes from shallow-lake mesocosms override those of climate warming. *Global Change Biology*, 21(12), 4449–4463. <https://doi.org/10.1111/gcb.13062>.
- de Haas, E. M., van Haaren, R., Koelmans, A. A., Kraak, M. H. S., & Admiraal, W. (2005). Analyzing the causes for the persistence of chironomids in floodplain lake sediments. *Archiv für Hydrobiologie*, 162(2), 211–228. <https://doi.org/10.1127/0003-9136/2005/0162-0211>.
- Fritz, K. A., Kirschman, L. J., McCay, S. D., Trushenski, J. T., Warne, R. W., & Whiles, M. R. (2017). Subsidies of essential nutrients from aquatic environments correlate with immune function in terrestrial consumers. *Freshwater Science*, 36(4), 893–900. <https://doi.org/10.1086/694451>.
- Gladyshev, M. I., Sushchik, N. N., & Makhutova, O. N. (2013). Production of EPA and DHA in aquatic ecosystems and their transfer to the land. *Prostaglandins & Other Lipid Mediators*, 107, 117–126. <https://doi.org/10.1016/j.prostaglandins.2013.03.002>.
- Guo, F., Bunn, S. E., Brett, M. T., Fry, B., Hager, H., Ouyang, X. G., & Kainz, M. J. (2018). Feeding strategies for the acquisition of high-quality food sources in stream macroinvertebrates: Collecting, integrating, and mixed feeding. *Limnology and Oceanography*, 63(5), 1964–1978. <https://doi.org/10.1002/lno.10818>.
- Guo, F., Kainz, M. J., Sheldon, F., & Bunn, S. E. (2016). Effects of light and nutrients on periphyton and the fatty acid composition and somatic growth of invertebrate grazers in subtropical streams. *Oecologia*, 181(2), 449–462. <https://doi.org/10.1007/s00442-016-3573-x>.
- Guschina, I. A., & Harwood, J. L. (2009). Algal lipids and effect of the environment on their biochemistry. In M. T. Arts, M. T. Brett, & M. J. Kainz (Eds.), *Lipids in aquatic ecosystems*. Dordrecht, The Netherlands/Heidelberg, Germany/London, UK/New York, NY: Springer.
- Happel, A., Czesny, S., Rinchar, J., & Hanson, S. D. (2017). Data pre-treatment and choice of resemblance metric affect how fatty acid profiles depict known dietary origins. *Ecological Research*, 32(5), 757–767. <https://doi.org/10.1007/s11284-017-1485-9>.
- Hessen, D. O., Elser, J. J., Sterner, R. W., & Urabe, J. (2013). Ecological stoichiometry: An elementary approach using basic principles. *Limnology and Oceanography*, 58(6), 2219–2236. <https://doi.org/10.4319/lno.2013.58.6.2219>.
- Hill, W. R., Rinchar, J., & Czesny, S. (2011). Light, nutrients and the fatty acid composition of stream periphyton. *Freshwater Biology*, 56(9), 1825–1836. <https://doi.org/10.1111/j.1365-2427.2011.02622.x>.
- Hixson, S. M., Sharma, B., Kainz, M. J., Wacker, A., & Arts, M. T. (2015). Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: A fundamental dichotomy between freshwater and terrestrial ecosystems. *Environmental Reviews*, 23(4), 414–424. <https://doi.org/10.1139/er-2015-0029>.
- Hoekman, D., Dreyer, J., Jackson, R. D., Townsend, P. A., & Gratton, C. (2011). Lake to land subsidies: Experimental addition of aquatic insects increases terrestrial arthropod densities. *Ecology*, 92(11), 2063–2072.
- Holopainen, I. J., & Pitkänen, A. K. (1985). Population size and structure of crucian carp (*Carassius carassius* (L.)) in two small, natural ponds in Eastern Finland. *Annales Zoologici Fennici*, 22(4), 397–406.
- Jones, J. I., & Sayer, C. D. (2003). Does the fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes? *Ecology*, 84(8), 2155–2167.
- Kabeya, N., Fonseca, M. M., Ferrier, D. E. K., Navarro, J. C., Bay, L. K., Francis, D. S., ... Monroig, O. (2018). Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. *Science Advances*, 4(5), 8. <https://doi.org/10.1126/sciadv.aar6849>.
- Knight, T. M., McCoy, M. W., Chase, J. M., McCoy, K. A., & Holt, R. D. (2005). Trophic cascades across ecosystems. *Nature*, 437(7060), 880–883. <https://doi.org/10.1038/nature03962>.
- Lau, D. C. P., Vrede, T., Pickova, J., & Goedkoop, W. (2012). Fatty acid composition of consumers in boreal lakes—Variation across species, space and time. *Freshwater Biology*, 57(1), 24–38. <https://doi.org/10.1111/j.1365-2427.2011.02690.x>.
- LeSage, L., & Harrison, A. D. (1979). Improved traps and techniques for the study of emerging aquatic insects. *Entomological News*, 90(2), 65–78.
- Liborius, L., & Jeppesen, E. (2006). Structure, biomass, production and depth distribution of periphyton on artificial substratum in shallow lakes with contrasting nutrient concentrations. *Freshwater Biology*, 51(1), 95–109. <https://doi.org/10.1111/j.1365-2427.2005.01481.x>.
- Makhutova, O. N., Borisova, E. V., Shulepina, S. P., Kolmakova, A. A., & Sushchik, N. N. (2017). Fatty acid composition and content in chironomid species at various life stages dominating in a saline Siberian lake. *Contemporary Problems of Ecology*, 10(3), 230–239. <https://doi.org/10.1134/s1995425517030064>.
- Mandaville, S. M. (2002). *Benthic macroinvertebrates in freshwaters: Taxa tolerance values, metrics, and protocols*. Dartmouth, Canada: Soil & Water Conservation Society of Metro Halifax.
- Martin-Creuzburg, D., Kowarik, C., & Straile, D. (2017). Cross-ecosystem fluxes: Export of polyunsaturated fatty acids from aquatic to terrestrial ecosystems via emerging insects. *Science of the Total Environment*, 577, 174–182. <https://doi.org/10.1016/j.scitotenv.2016.10.156>.
- Meier, G. M., Meyer, E. I., & Meyns, S. (2000). Lipid content of stream macroinvertebrates. *Archiv für Hydrobiologie*, 147(4), 447–463.
- Monakov, A. V. (1972). Review of studies on feeding of aquatic invertebrates conducted at Institute of Biology of Inland Waters, Academy of Science, USSR. *Journal of the Fisheries Research Board of Canada*, 29(4), 363. <https://doi.org/10.1139/f72-064>.
- Parrish, C. C. (1999). Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In M. T. Arts & B. B. Wainman (Eds.), *Lipids in freshwater ecosystems* (pp. 4–20). New York, NY: Springer.
- Parrish, C. C. (2009). Essential fatty acids in aquatic food webs. In M. T. Arts, M. T. Brett, & M. Kainz (Eds.), *Lipids in aquatic ecosystems* (pp. 309–326). Dordrecht, The Netherlands/Heidelberg, Germany/London, UK/New York, NY: Springer.
- Penttinen, O. P., & Holopainen, I. J. (1992). Seasonal feeding activity and ontogenetic dietary shifts in Crucian carp, *Carassius carassius*. *Environmental Biology of Fishes*, 33(1–2), 215–221. <https://doi.org/10.1007/bf00002566>.
- Pitcher, K. A., & Soluk, D. A. (2018). Fish presence and inter-patch connectivity interactively alter the size of emergent insects in experimental enclosures. *Ecosphere*, 9(3), 17. <https://doi.org/10.1002/ecs2.2118>.
- Polis, G. A., Anderson, W. B., & Holt, R. D. (1997). Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics*, 28, 289–316.

- Popova, O. N., Haritonov, A. Y., Sushchik, N. N., Makhutova, O. N., Kalachova, G. S., Kolmakova, A. A., & Gladyshev, M. I. (2017). Export of aquatic productivity, including highly unsaturated fatty acids, to terrestrial ecosystems via Odonata. *Science of the Total Environment*, 581, 40–48. <https://doi.org/10.1016/j.scitotenv.2017.01.017>.
- Sandberg, G. (1969). A quantitative study of chironomid distribution and emergence in Lake Erken. *Archiv fur Hydrobiologie*, 2, 119–201.
- Scharnweber, K., Strandberg, U., Karlsson, K., & Eklöv, P. (2016). Decrease of population divergence in Eurasian perch (*Perca fluviatilis*) in browning waters: Role of fatty acids and foraging efficiency. *Plos One*, 11(9), 20. <https://doi.org/10.1371/journal.pone.0162470>.
- Scharnweber, K., Strandberg, U., Marklund, M. H. K., & Eklöv, P. (2016). Combining resource use assessment techniques reveal trade-offs in trophic specialization of polymorphic perch. *Ecosphere*, 7(8), e01387, 01310.01002/ecs01382.01387.
- Scharnweber, K. (2019). Chironomidae fatty acid proportions. DiVA, urn:nbn:se:uu:diva-389470.
- Schreiber, S., & Rudolf, V. H. W. (2008). Crossing habitat boundaries: Coupling dynamics of ecosystems through complex life cycles. *Ecology Letters*, 11(6), 576–587. <https://doi.org/10.1111/j.1461-0248.2008.01171.x>.
- Sinclair, A. R. E., Mduma, S., & Brashares, J. S. (2003). Patterns of predation in a diverse predator-prey system. *Nature*, 425(6955), 288–290. <https://doi.org/10.1038/nature01934>.
- Strandberg, U., Hiltunen, M., Jelkänen, E., Taipale, S., Kainz, M., Brett, M. T., & Kankaala, P. (2015). Selective transfer of polyunsaturated fatty acids from phytoplankton to planktivorous fish in large boreal lakes. *Science of the Total Environment*, 536, 858–865.
- Terborgh, J., Lopez, L., Nunez, P., Rao, M., Shahabuddin, G., Orihuela, G., ... Balbas, L. (2001). Ecological meltdown in predator-free forest fragments. *Science*, 294(5548), 1923–1926. <https://doi.org/10.1126/science.1064397>.
- Torres-Ruiz, M., Wehr, J. D., & Perrone, A. A. (2007). Trophic relations in a stream food web: Importance of fatty acids for macroinvertebrate consumers. *Journal of the North American Benthological Society*, 26(3), 509–522. <https://doi.org/10.1899/06-070.1>
- Twining, C. W., Brenna, J. T., Hairston, N. G., & Flecker, A. S. (2016). Highly unsaturated fatty acids in nature: What we know and what we need to learn. *Oikos*, 125(6), 749–760. <https://doi.org/10.1111/oik.02910>.
- Twining, C. W., Shipley, J. R., & Winkler, D. W. (2018). Aquatic insects rich in omega-3 fatty acids drive breeding success in a widespread bird. *Ecology Letters*, 21(12), 1812–1820. <https://doi.org/10.1111/ele.13156>.
- Vadeboncoeur, Y., Lodge, D. M., & Carpenter, S. R. (2001). Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. *Ecology*, 82(4), 1065–1077. <https://doi.org/10.2307/2679903>.
- Vander Zanden, M. J., & Gratton, C. (2011). Blowin' in the wind: Reciprocal airborne carbon fluxes between lakes and land. *Canadian Journal of Fisheries and Aquatic Sciences*, 68(1), 170–182. <https://doi.org/10.1139/f10-157>.
- Vos, J. H., Peeters, E., Gylstra, R., Kraak, M. H. S., & Admiraal, W. (2004). Nutritional value of sediments for macroinvertebrate communities in shallow eutrophic waters. *Archiv fur Hydrobiologie*, 161(4), 469–487. <https://doi.org/10.1127/0003-9136/2004/0161-0469>.
- Wesner, J. S. (2010). Aquatic predation alters a terrestrial prey subsidy. *Ecology*, 91(5), 1435–1444. <https://doi.org/10.1890/09-1532.1>.
- Wesner, J. S. (2016). Contrasting effects of fish predation on benthic versus emerging prey: a meta-analysis. *Oecologia*, 180(4), 1205–1211. <https://doi.org/10.1007/s00442-015-3539-4>.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Appendix

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