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Taxonomic composition and lake bathymetry influence fatty acid export via emergent insects

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Abstract

1. The ecological role of emergent aquatic insects from lakes in exporting dietary polyunsaturated fatty acids (PUFA) across the freshwater-land interface is still poorly understood.

2. In this field study, we explored the seasonal biomass export of emergent insects from three subalpine lakes and investigated how lipids of emergent insects were related to lake bathymetry, lipids of organic matter in lake sediments (i.e., basal resources), and the taxonomic composition of insects.

3. The total lipid and PUFA fluxes of emergent insects were strongly related to taxonomy and lake bathymetry, but weakly associated with the PUFA content of the uppermost lake sediment layers. PUFA flux estimates of the dominant taxon, Chironomidae, from the shallowest lake (3 m depth; 125 g PUFA m^{-2} season^{-1}) were considerably higher than those from the deepest lake (33 m depth; 56 g PUFA m^{-2} season^{-1}), due to the higher per area biomass of emergent insects from this shallow lake. Insect taxonomy also affected the composition of PUFA transfer to land: Chironomidae were richer in ω-6 PUFA, such as linoleic acid (18:2n-6) and arachidonic acid (20:4n-6), whereas Ephemeroptera and Trichoptera contained more ω-3 PUFA, especially α-linolenic acid (18:3n-3) and eicosapentaenoic acid (20:5n-3).

4. Our findings suggest that taxon-specific differences in PUFA content and lake bathymetry jointly shape PUFA fluxes and thus the provisioning of emergent insects as dietary sources of physiologically important PUFA for riparian consumers.

KEYWORDS
aquatic–terrestrial linkage, dietary energy fluxes, lipids, semi-aquatic insects, ω-3 polyunsaturated fatty acids

1 | INTRODUCTION

Nutrient fluxes across ecosystem boundaries via animals connect aquatic and terrestrial food webs. For instance, fish, amphibians, and emergent aquatic insects, provide nutrient subsidies for a diversity of terrestrial consumers in the riparian zone (Bartels et al., 2012; Nakano & Murakami, 2001; Twining et al., 2019). Based on differences in the magnitude of organic carbon fluxes, Bartels et al. (2012) suggested that subsidies from terrestrial to freshwater ecosystems are typically larger than those from freshwater to terrestrial systems. However, subsidies differ not only in quantity, but also in biochemical composition (Marcarelli et al., 2011). Compared to terrestrial resources, aquatic subsidies are rich in several physiologically important fatty acids (FA) that make them particularly valuable resources for both aquatic and terrestrial consumers (Twining et al., 2019). Assessing the magnitude and nutritional/biochemical composition of cross-habitat nutrient movement is thus crucial for understanding the spatiotemporal dynamics of subsidies and their consequences for recipient communities (Bartels et al., 2012; Gratton & Vander Zanden, 2009; Polis et al., 2004).

Emergent insects provide the main flow of energy from freshwater to recipient terrestrial ecosystems (Nakano & Murakami, 2001; Polis et al., 2004) and are rich in long-chain polyunsaturated FA (LC-PUFA), especially eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6) (Martin-Creuzburg et al., 2017; Scharnweber et al., 2019; Twining et al., 2019). These LC-PUFA are mainly produced by aquatic primary producers, whereas their precursors, the shorter chain PUFA α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LIN, 18:2n-6) are synthesised by both aquatic and terrestrial primary producers (Guo et al., 2016; Hixson et al., 2015). The LC-PUFA (≥C20) help maintain vital physiological processes of animals (Fritz et al., 2017; Twining, Brenna, Hairston et al., 2016), but many animals cannot biosynthesise these fats *de novo* (Cook and McMaster 2004), or from precursors in high enough quantity to meet physiological demands (e.g., Twining et al., 2018). Thus, many consumers, including those in riparian areas (Twining et al., 2018), must obtain LC-PUFA from their diet. At the lake sediment–water interface, aquatic insect larvae feed on a diversity of aquatic basal resources (e.g., biofilm; Kautza & Sullivan, 2016) that are rich in LC-PUFA (Guo et al., 2016). In contrast, terrestrial insects generally rely upon terrestrial primary producers, which contain mostly shorter chain PUFA, including ALA and LIN (Hixson et al., 2015; Twining et al., 2016). As a consequence of these differences at the base of food webs, aquatic and terrestrial insects differ in their biochemical composition and thus in the composition of fatty acids that can be transferred to consumers in surrounding ecosystems (Twining et al., 2019).

It is difficult to estimate aquatic insect fluxes from lakes because they may vary based on lake bathymetry, physicochemical parameters (e.g., temperature, trophic status), productivity, community composition, and predator–prey interactions (Gratton & Vander Zanden, 2009; Martin-Creuzburg et al., 2017). The productivity and thus organic matter transfer between aquatic and terrestrial ecosystems also changes seasonally (Burpee & Saros, 2020). Therefore, the export of aquatic subsidies by emergent insects probably varies from lake to lake and needs to be investigated to better understand the transfer of dietary energy via emergent lake insects.

The aims of this field study were: (1) to quantify the export of total lipids and FA via emergent insects from three subalpine lakes; and (2) to explore how the magnitude and composition of insect export is affected by the taxonomic composition of insects, the FA content of basal resources, lake bathymetry, and season. First, we investigated dietary lipid composition (i.e., total lipid and FA) of organic matter at the sediment–water interface of lakes as a potential diet source for insect larvae. Subsequently, we estimated the export of total lipids and FA from emergent insects throughout the study period (June–September) for the three study lakes and for different depths within each lake. This allowed us to assess the FA exported by different taxa during the season of greatest insect emergence, and to examine the importance of different taxa as potential food sources for riparian consumers.

2 | METHODS

2.1 | Study sites and sampling

Emergent insects were collected over a period of 117 days, from June to September 2019, in three oligotrophic subalpine lakes close to Lunz am See, Austria. Lake Lunz (47°51′13″N, 15°03′11″E), Lake Mittersee (47°49′38″N, 15°04′32″E), and Lake Obersee (47°48′17″N, 15°04′29″E) are located only a few km from one another, but vary considerably in altitude and depth (601, 767, and 1,113 m above sea level and 33, 3, and 14 m depth, respectively).

Floating emergence traps were placed at the water surface of each of the lakes above multiple depths per lake: one trap was placed at each depth of 1, 3, 5, 10, 20, and 33 m in Lake Lunz, at 1 m (2 times) and 3 m in Lake Mittersee, and at 1, 3, 5, and 14 m in Lake Obersee. Each transect covers the main depth zones which can be find in each lake. Each trap covered a surface area of 0.36 m² (see Martin-Creuzburg et al., 2017). Emergent insects were collected twice a week from Lake Lunz and once a week from Lake Mittersee and Lake Obersee. Insects were transported to the laboratory immediately after collection, frozen at −80°C, freeze-dried (Genesis Freeze dryer, Virtis Inc.) and assigned to different taxonomic groups at the family level. Insects were counted and put in pre-weighed tin cups, weighed, and stored at −20°C until further analyses.

2.2 | Sediment organic matter analysis

Sediment samples were collected below each of the emergent traps in all three study lakes using a sediment corer (UWITEC, Mondsee, Austria). First, the macrophytes present in the corer have been removed. Then, the top 0.5-cm layer below the sediment–water

interface was sampled, including detritus and microalgae, as insect larvae were mostly present in this top sediment layer (visual inspection), stored at −80°C and freeze-dried for further biochemical analyses. At Lake Mittersee, no sediments could be obtained at the deepest spot (3 m) because of the rocky lake bottom. Lipids and FA were analysed in organic matter of the sediment samples.

2.3 | Insect emergence

The main taxonomic groups investigated were Chironomidae, Ephemeroptera, Trichoptera, Microsema, and Odonata. Insect emergence was reported as the sum of insect biomass (dry mass) per m² for the entire sampling period. This comprised most of the annual insect emergence from these subalpine lakes, except for Lake Lunz, which also had considerable emergent insect biomass during the spring (personal observation). Because our estimates are not representative of annual emergence, but only of the emergences from June to September, we chose to represent data as seasonal emergence for the study period (expressed in m^{-2} $season^{-1}$). Total sampled emergence was then extrapolated to the surface areas of the different depth zones of each lake and to total lake surface area. The taxonomy and biomass of all emergent insects collected from the three lakes is provided in Table S1. To explore spatial differences in insect emergence among lakes, we compared seasonal emergence per m² at 1 m water depth because this depth zone was available for all three lakes and because the highest insect biomass was exported from 1 m water depth (see below). PUFA fluxes per m² and sampling season (mg m^{-2} $season^{-1}$) were calculated as:

$$PUFA\,flux = \sum insect\,emergence \times \sum PUFA \qquad (1)$$

where insect emergence is expressed per square meter and sampling period (g m^{-2} $season^{-1}$), and Σ PUFA is the mean $\pm SE$ per unit biomass (in mg PUFA g^{-1}) of samples.

2.4 | Lipids and fatty acid analyses

Freeze-dried insect taxa (>2 mg from each week) and organic matter of sediment samples (>20 mg from September) were selected for further analyses. All samples were homogenised and lipids were extracted as described by Guo et al. (2016). Total lipids were transmethylated to FA methyl esters (FAME) and analysed using a TRACE GC Ultra gas chromatograph (Thermo Fisher Scientific) using a Supelco^{TM} SP-2560 column (100 m, 0.25 mm i.d., 0.2 μm film thickness), identified by comparison of their retention times with standards (37-component FAME Mix, Supelco 47885-U; Bacterial Acid Methyl Ester Mix, Supelco 47080-U), then quantified with reference to seven-point calibration curves based on known standard dilution raw concentrations. Chromeleon 7 (Software, Thermo Fisher Scientific) was used for calculation and, if necessary, manual resetting of the chromatograms. All FA were measured and reported as FAMEs, expressed as mg FAME g^{-1} dry weight and/or as mass percentage (%) of total reported FA.

2.5 | Data analyses

Non-parametric tests were performed because conditions of normally-distributed data (Shapiro–Wilks test) and homoscedasticity (Bartlett test) were not satisfied. Differences in emergent biomass among taxa in each lake and among lakes were independently tested with Kruskal–Wallis (KW tests) tests and with Conover–Iman's adjustment method (Table 1).

Dissimilarities of global FA composition (>1% of total FA), using Euclidean distances as dissimilarity values among taxa, were analysed by permutational analysis of variance (PERMANOVA, Anderson 2014) considering lake, depth and month variables. When interactions were not significant, the difference in the content of each independent FA among taxa was tested independently by KW tests as described above (Table 2). The difference of global FA compositions across lakes and depths of Chironomidae and of the organic matter in the upper lake sediment layer was tested as described above by PERMANOVA, while differences of individual FA were tested using KW analysis (Table 2).

Linear models were used to assess how taxa, lake, depth and/or month of sampling explained variations in PUFA export. The different models were ranked and compared based on Akaïke information criterion (AIC) adjusted for small sample sizes (Meredith, 2019). Adjusted r^2 was obtained to compare the predictive power of these models (Jaeger, 2016).

Regression tree analyses were applied to structure clusters between individual FA and the six main insect taxa. As homogeneous covariance matrices are not required in regression tree analysis and variables do not need to be normally distributed (Budge et al., 2002), relative FA data (mass percentage, %) were used. Explanatory variables were FA composition of insect taxa. Each of the splits (non-terminal nodes) were labelled with the variable and its values that determined the split, while the leaves (terminal nodes) were labelled with insect taxa (De'ath & Fabricius, 2000).

Principal component analyses were performed to investigate the variation in FA composition (%) of Chironomidae among lakes. Euclidean distance on FA content were performed to prevent excessive weight to rare species (Legendre & Gallagher, 2001), and a test of similarity percentages analyses (SIMPER) was carried out to assess the most discriminant FA responsible of the difference among lakes (>80%). The FA accumulation factors (FAAF (Hessen & Leu, 2006); using FA mass fractions, i.e., mg FA g^{-1}) were calculated for consumers (insects) and food sources, i.e., organic matter at the lake sediment–water interface as;

$$FAAF = \frac{FA_{insects}}{FA_{sediments}} \qquad (2)$$

FAAF > 1 indicate the extent of FA increase from organic matter of sediments to insects, and FAAF < 1 are a measure of FA decrease in insects relative to FA in organic matter of the sediment. FAAF were associated with Spearman's rank correlation (where r_s is the correlation coefficient) to assess relationships among the FA contents of organic matter in sediments and insects.

All statistical analyses and graphics were performed with R (R. Core Team, 2020). PERMANOVA and principal component analyses were carried out using *vegan*, KW tests were calculated using *stats*, while post-hoc tests were explored using *PMCMR*. Linear models (LM) and their AIC scores were calculated using *stats* and *wiqid*, while the regression tree analysis was performed using both *rpart* and *rpart.plot*. Spearman's correlations were calculated using *ggpubr*.

3 | RESULTS

3.1 | Insect emergences from lakes

The main taxa in Lake Lunz were Chironomidae, Ephemeroptera, Microsema and Trichoptera, and Chironomidae, Ephemeroptera and Odonata in Lake Obersee. For Lake Mittersee, only Chironomidae, found at all three lakes, had sufficient biomass for biochemical analyses (for the list of all others taxon found in Lake Mittersee, see Table S1). During the sampling period, the overall estimate of insect emergence from Lake Lunz was 31 times higher (68 ha; 462 kg insects season[-1]) than from Lake Mittersee (2.8 ha; 15 kg insects season[-1]), but only 1.4 times higher than from Lake Obersee (14.5 ha; 334 kg insects season[-1]; Table 1).

Chironomidae contributed most to the total insect biomass export from all three lakes, but their overall seasonal emergence from Lake Lunz was 22.8 and 3.6 times higher than those from Lake Mittersee and Lake Obersee, respectively (Table 1). The highest insect biomasses exported from Lake Lunz and Lake Obersee occurred in June and July, while the highest biomass export from the shallow Lake Mittersee occurred in September (Figure 1). The highest exported insect biomass for all lakes was recorded above 1 m water depth and the total insect biomass export decreased with lake depth for all three lakes (Figure 1). The overall insect emergence per m² at 1 m water depth did not differ significantly among lakes (KW test, $H25 = 1.40$, $p = 0.50$; Table 1). However, the seasonal Chironomidae biomass exported from Lake Mittersee was 4.3 and 1.5 times higher than those from Lake Obersee and Lake Lunz, respectively (Table 1).

3.2 | Export of lipids by insect taxa

The taxonomic composition of emergent insects had the greatest predictive power for total PUFA export from the lakes (LM: PUFA~taxa, AIC = 2,270.90, $r^2 = 0.42$, $p < 0.001$). Emergent insect taxa from Lake Mittersee had significantly higher total lipid contents (193.10 ± 8.70 mg g^{-1}; Table 2) than those from Lake Lunz (174.10 ± 5.30 mg g^{-1}, Table 2), while total lipid content in insects

from Lake Obersee were not significantly different than those from Lake Mittersee (201.30 ± 12.90 mg g^{-1}, Table 2).

PUFA composition separated insect taxa independently of lake identity (regression tree analysis; Figure 2). Chironomidae and Odonata contained lower amounts of ALA (<5.53%) but higher amounts of LIN (>7.79%). The left subcluster (Figure 2), mainly composed of Chironomidae, was characterised by high contents of ω-6 PUFA (LIN and ARA). Ephemeroptera and Trichoptera formed a series of subclusters characterised by a lower content of LIN (<7.70%). The right subclusters were mainly separated based on a high content of ω-3 PUFA (ALA, 18:4n-3, and EPA).

Within each lake, insects varied in lipid and fatty acid contents. Total lipids exported by insects from Lake Lunz did not significantly differ among taxa during the sampling period (KW test, $H25 = 0.50$, $p = 0.92$), ranging from 163 ± 12 to 184.30 ± 13.80 mg g^{-1} (Table 2). Insects from Lake Obersee varied significantly in their total lipid content (KW test, $H25 = 33.90$, $p < 0.001$; Table 2) with Ephemeroptera containing twice as much lipids (336 ± 28 mg g^{-1}, Table 2) as Chironomidae and Odonata (157 ± 10 and 174 ± 28 mg g^{-1}, respectively). The overall FA content of insect was similar among taxa from Lake Lunz (PERMANOVA: $df = 3$, $r^2 = 0.03$, $p = 0.16$), but differed significantly among taxa from the other two lakes (PERMANOVA, Lake Mittersee: $df = 8$, $r^2 = 0.81$, $p < 0.001$; Lake Obersee: $df = 10$, $r^2 = 0.74$, $p < 0.001$). Fluxes of ω-3 and ω-6 PUFA from 1 m water depth via Ephemeroptera were 184 and 402 times higher at Lake Obersee than at Lake Lunz, respectively (Table 1).

PUFA fluxes were influenced by lake depth, but due to the low number of trap replicates per depth and replicate of depth among lakes, it was only possible to compare fluxes from 1 and 3 m depths across all lakes. Generally, fluxes were higher at shallow depths, with the highest fluxes observed at 1 m depth and then decreasing with lake depth. PUFA fluxes were 1.3 times higher from 1 m depth than those from 3 m depth in Lake Lunz and Lake Mittersee, while in Lake Obersee PUFA fluxes from 1 m depth were 2.5 times higher compared to those from 3 m depth (Table 1). With the exception of Ephemeroptera from Lake Lunz and Chirnomidae from Lake Obersee, PUFA fluxes from 1m depths were always higher compared to those 3 m depths (Table 1).

3.3 | Lipids of emerged Chironomidae

The total lipid content of Chironomidae was significantly higher at Lake Mittersee (189 ± 5 mg g^{-1}) than at Lake Lunz (169 ± 6 mg g^{-1}) and Lake Obersee (157 ± 10 mg g^{-1}, Table 2). Total PUFA fluxes from Chironomidae at Lake Mittersee from 1 m depth were 2 and 7 times higher, fluxes of ω-3 PUFA were 1.5 and 4.3 higher, and fluxes of ω-6 PUFA were 2 and 7 times higher than fluxes from Chironomidae from 1 m depths at Lake Lunz and Lake Obersee, respectively (Table 1).

Lake identity explained the greatest amount of variation in exported PUFA by Chironomidae (LM, AIC = 1,163.60, $r^2 = 0.33$, $p < 0.001$). Chironomidae from Lake Mittersee contained 1.5 times more PUFA (41.60 ± 1.80 mg FAME g[-1], Table 2) than other lakes,

TABLE 1 Emerged insect biomass per lake (kg per sampling period, season^{-1}), by m^2 from 1 m depth (g m^{-2} season^{-1}), and PUFA, ω-3 and ω-6 fluxes from 1 m depth (in mg m^{-2} season^{-1}) of main insect taxa from the three lakes

	Lake Lunz					Lake Mittersee		Lake Obersee			
	All taxa	Chironomidae	Ephemeroptera	Micrasema	Trichoptera	All taxa	Chironomidae	All taxa	Chironomidae	Ephemeroptera	Odonata
Emergence during sampling period (days)	117	117	111	32	111	104	104	104	90	104	82
Emergent biomass per lake (kg season^{-1})	461.7^B	313.2^{A,c}	8^a	30.7^{ab}	86.8b	15.1^A	13.7^B	333.5^B	86.8^C	87.5	107.4
Emergent biomass from 1 m depth (g m^{-2} season^{-1})	2.8	2^b	0.01^a	0.2^a	0.5^a	3.5	3	3.2	0.7^a	0.9^{ab}	1.1^b
PUFA flux from entire lake (kg season^{-1})	13.2	8.7	0.2	0.7	2	0.6	0.6	11.6	2.2	6.1	3.4
PUFA flux from 1 m depth (mg m^{-2} season^{-1})	79.8	55.6	0.3	4.7	16.5	133	124.8	111.4	17.9	63.3	35.3
PUFA flux from 3 m depth (mg m^{-2} season^{-1})	59.9	44.5	0.6	1.9	9.6	102.6	112.3	45.2	19.1	7	3.2
ω-3 flux from 1 m depth (mg m^{-2} season^{-1})	45.4	25.4	0.3	4.2	11.2	94.9	87.3	72.6	9.7	46	22.2
ω-6 flux from 1 m depth (mg m^{-2} season^{-1})	27.2	12	0.04	0.5	3.7	34.7	34.2	36.2	7.8	16.1	12.2

Note: Lower case letters indicate the significant differences between taxa of each lake, capital letters indicate significant differences among the three lakes (Kruskal–Wallis, at the level $p < 0.05$).

TABLE 2 Total lipids (mg g^{-1}) and fatty acid (FA) content (mg FA methyl esters g^{-1}; mean ± *SE*) of organic matter of sediment, and of main insect taxa emerged from the three lakes during the sampling season (season^{-1})

	Lake Lunz					
	Organic matter of sediment	All taxa	Chironomidae	Ephemeroptera	Micrasema	Trichoptera
Total Lipids	24.5 ± 16	174.1 ± 5.3 [A]	169.3 ± 5.5[A]	163 ± 18.9	180.8 ± 18.6	184.3 ± 13.8
Σ BFA	0.2 ± 0[A]	5.3 ± 0.3[A]	6.8 ± 0.2[a,A]	8.8 ± 2[a]	1.3 ± 0.1[b]	2.5 ± 0.3[b]
Σ SFA	2.5 ± 0.1[A]	21.4 ± 0.9	21.5 ± 0.6[A]	19.7 ± 4.2	16.8 ± 1.6	28 ± 2.9
16:1n-7	0.2 ± 0[A]	7.5 ± 0.4	8.2 ± 0.4[b]	6.8 ± 3[ab]	5.9 ± 1[ab]	6.6 ± 1[a]
18:1n-9	0.1 ± 0[A]	8.7 ± 0.2[A]	5.7 ± 0.1[a,A]	5.4 ± 0.6[a]	13.5 ± 1[b]	14.3 ± 1.1[b]
Σ MUFA	0.6 ± 0.1[A]	20.2 ± 0.7 [A]	19 ± 0.6	20.4 ± 5.1	20.8 ± 1.8	22.8 ± 2.1
18:2n-6 (LIN)	0 ± 0[A]	7.6 ± 0.3	9.8 ± 0.3[c]	2.6 ± 0.3 [ab]	1.7 ± 0.4 [a]	5 ± 0.6 [b]
18:3n-3 (ALA)	0 ± 0[A]	5.6 ± 0.4 [A]	3.4 ± 0.3[b,A]	9.6 ± 0.9 [a]	4.9 ± 0.6 [a]	10.1 ± 1 [a]
18:4n-3 (SDA)	0 ± 0[A]	0.8 ± 0.1 [A]	0.6 ± 0[ab,A]	1.8 ± 0.6[a]	0.5 ± 0.1[b]	1 ± 0.3[b]
20:4n-6 (ARA)	0 ± 0[B]	1.5 ± 0.1 [A]	1.6 ± 0.1[a,A]	0.6 ± 0.2[b]	0.4 ± 0[b]	1.8 ± 0.2[a]
20:5n-3 (EPA)	0 ± 0[A]	12.2 ± 0.4 [A]	11.6 ± 0.4[A]	13.3 ± 1.3	13.6 ± 1	13.3 ± 1.3
Σ PUFA	0.3 ± 0[A]	28.5 ± 0.8 [A]	27.8 ± 0.8[ab,A]	28.9 ± 3.1 [ab]	21.5 ± 1.1[a]	32.1 ± 2.1[b]
Σ Fatty acids	3.5 ± 0.3[A]	71.1 ± 2.1 [A]	69.5 ± 1.8[A]	69.4 ± 12	59.4 ± 4.2	78.8 ± 5.9
Σ ω-3	0.2 ± 0[A]	16.2 ± 0.8[A]	12.7 ± 0.8[b,A]	25 ± 2.6[a]	19.1 ± 1[a]	22.4 ± 1.9[a]
Σ ω-6	0.1 ± 0 [A]	9.7 ± 0.4	12 ± 0.4[c]	3.9 ± 0.5 [ab]	2.4 ± 0.4 [a]	7.3 ± 0.7 [b]
ω-3/ω-6	1.8 ± 0.1	2.7 ± 0.4[A]	1.1[c,A]	6.5 [ab]	9.2 [a]	3.9[b]

Abbreviations: BFA, bacterial FA (sum of iso- and anteiso-15:0, 15:0, iso-16:0, iso- and anteiso-17:0, 17:0, 18:1n-7); MUFA, monounsaturated FA; ω-3, sum of all omega-3 FA; ω-6, sum of all omega-6 FA; PUFA, polyunsaturated FA; SFA, saturated FA.

Note: Lower case letters indicate the significant differences between taxa inside each lake, capital letters indicate significant differences among the three lakes (Kruskal–Wallis, at the level $p < 0.05$). Only the fatty acids presenting mass fractions >to 0.5 μg FAME g^{-1} dry weight are shown.

while there was no significant difference in the PUFA content of Chironomidae from Lakes Lunz and Obersee (28 ± 1 and 26 ± 2 mg FAME g^{-1}, respectively; Table 2).

The FA composition of Chironomidae varied among the lakes (PERMANOVA, $df = 2$, $r^2 = 0.09$, $p < 0.001$; Figure 3), with Chironomidae origin (lake) being mainly discriminated by the first principle component (Figure 3). Chironomidae from Lake Mittersee had significantly higher contents of ALA and EPA than Chironomidae from other lakes (Figure 3; Table 2). Across lakes, Chironomidae had significantly lower ω-3/ω-6 PUFA ratios and generally contributed more ω-6 PUFA to fluxes than other insects (Table 2), as shown in the regression tree analysis (Figure 2), in which Chironomidae clusters were separated from other taxa by LIN and ARA.

3.4 | Nutritional value of the organic matter in the upper lake sediment layer

Total lipids and total FA contents of the organic matter in the upper sediment layer (0.5 cm below the sediment–water interface) did not differ significantly among lakes or with lake depth (lipids: KW tests, $H25 = 0.96$ and 7.89; $p = 0.62$ and 0.44, respectively; FA: PERMANOVA, $df = 2$, $r^2 = 0.18$, $p = 0.09$, and $df = 10$, $r^2 = 0.68$, $p = 0.13$, respectively). However, the PUFA contents of organic

matter in sediments of Lake Mittersee were 32.6 and 5.4 times higher than at Lake Lunz and Lake Obersee, respectively (Table 2). This was due to the high ω-6 PUFA content of the uppermost sediment layer from Lake Mittersee, which was 69 and 11 times higher than those from Lake Lunz and Lake Obersee, respectively (Table 2).

In general, the FA contents of emergent insects were higher than those in the uppermost sediment layer. The FAAF revealed that the EPA, LIN, and ALA contents of emergent insects were 6.2, 5.8, and 5.4 times higher than in organic matter of sediments, respectively. However, 16:1n-7 and ARA contents were lower in insects compared to organic matter of sediments (FAAF: 0.93 and 1.8, respectively). Short chain FA were significantly correlated between organic matter of sediments and insects (Spearman's correlations: 16:1n-7, $r_s = 0.56$; 18:1n-9, $r_s = 0.44$; ALA, $r_s = 0.81$; $p < 0.05$), as ARA ($r_s = 0.82$, $p < 0.05$). No significant interactions were found for total lipid contents and EPA ($r_s = 0.23$ and -0.79, respectively, $p > 0.05$).

4 | DISCUSSION

The magnitude of the insect-mediated FA export from the three sub-alpine lakes studied here was largely determined by insect taxonomy

Lake Mittersee			Lake Obersee				
Organic matter of sediment	All taxa	Chironomidae	Organic matter of sediment	All taxa	Chironomidae	Ephemeroptera	Odonata
59 ± 27.4	193.1 ± 8.7 [B]	189.3 ± 4.9[B]	71.2 ± 27.4	201.3 ± 12.9[AB]	157.3 ± 10.1[a,A]	336.4 ± 14.2[b]	173.7 ± 27.9[a]
0.4 ± 0.1[B]	4.6 ± 0.4[A]	5 ± 0.4[B]	0.5 ± 0.1[B]	17.7 ± 4.3[B]	8.7 ± 0.5[a,C]	40 ± 2.2[b]	11.4 ± 1.7[a]
4.4 ± 3[B]	27.1 ± 3.1	25.1 ± 0.8[B]	4 ± 0.6[C]	34.1 ± 3.1	23 ± 1.8[a,AB]	78.2 ± 4.1[b]	24.8 ± 3.2[a]
0.7 ± 0.3[AB]	11 ± 2.7	8 ± 0.6	1 ± 0.3[B]	12.9 ± 1.5	9.8 ± 1.2[a]	34.4 ± 2.5[b]	5 ± 1.1[c]
1.2 ± 1.1[AB]	11.8 ± .2[AB]	8.2 ± 0.6[B]	0.3 ± 0.1[B]	12.8 ± 1.2[B]	5.9 ± 0.6[a,A]	22.4 ± 0.8[b]	11.8 ± 2.4[c]
2.1 ± 1.5 [AB]	26.3 ± 5[B]	20 ± 1.1	1.6 ± 0.4[B]	41.5 ± 5.5[AB]	21.5 ± 1.9[a]	93.5 ± 4.4[b]	27.1 ± 3.9[a]
4.3 ± 4.2 [B]	8 ± 0.6	9.3 ± 0.5	0.4 ± 0.2[B]	8.4 ± 0.6	8.9 ± 0.7[a]	12.8 ± 0.5[b]	7.8 ± 1.5[a]
1.8 ± 1.6 [B]	14.5 ± 1 [B]	15 ± 1.1[B]	0.8 ± 0.7[B]	11.3 ± 1.9[C]	6.2 ± 0.6[a,C]	24.7 ± 2.4[b]	7.3 ± 1.1[a]
0.1 ± 0.1[B]	1 ± 0.1[AB]	1.2 ± 0.1[B]	0.1 ± 0[B]	1.5 ± 0.3[B]	0.6 ± 0.1[a,A]	4.9 ± 0.5[b]	1.5 ± 0.6[a]
0.3 ± 0[AB]	1 ± 0.1[B]	1.1 ± 0.1[B]	0.1 ± 0[A]	2.1 ± 0.1[C]	1.7 ± 0.1[a,A]	3.1 ± 0.2[b]	2.5 ± 0.3[b]
0.4 ± 0.3[AB]	12.2 ± 0.7[A]	13.5 ± 0.6 [B]	0.2 ± 0.1[B]	10.2 ± 0.8[B]	7.5 ± 0.7[a,C]	22 ± 1.3[b]	11.4 ± 1.4[c]
9.8 ± 9.3[B]	38 ± 1.9[B]	41.6 ± 1.8[B]	1.8 ± 0.9[B]	34.8 ± 3[A]	25.5 ± 1.7[a,A]	70.3 ± 3.9[b]	32.1 ± 4.1[a]
16.4 ± 13.8[A]	92.2 ± 7.9[B]	87.5 ± 3.2[B]	7.7 ± 1.5[B]	111.5 ± 10.2[B]	71.6 ± 5[a,A]	243.9 ± 11.2[b]	84.6 ± 10.2[a]
2.8 ± 2.5[AB]	27.1 ± 1.6[B]	29.1 ± 1.6 [B]	1.2 ± 0.7[B]	22.7 ± 2.6[A]	13.9 ± 1.3[a,A]	51.1 ± 4.2[b]	20.2 ± 3.4[a]
6.9 ± 6.8 [AB]	9.9 ± 0.7	11.4 ± 0.6	0.6 ± 0.2[B]	11.3 ± 0.7	11.1 ± 0.8 [a]	17.9 ± 0.6[b]	11.1 ± 1.6 [a]
1.4	3.6 ± 0.4[B]	2.7[B]	1.5	1.9 ± 0.1[A]	1.4[a,A]	2.8[b]	1.9[a]

and lake bathymetry. Our study highlights that the littoral zones of lakes supply higher insect biomass and LC-PUFA per surface area to riparian ecosystems than deeper zones across lakes. From a diet composition perspective, we show that LC-PUFA retention in emergent insects is independent of dietary LC-PUFA supply from organic matter of sediments. These results indicate that insect diversity and lake bathymetry (i.e., area covered by shallow littoral zones) are key for understanding the trophic transfer of PUFA from lakes to riparian ecosystems.

4.1 | Emergence and export of dietary nutrients among lakes

Our two larger study lakes, Lake Lunz and Lake Obersee, exported a higher total insect biomass than the smaller and shallower Lake Mittersee. However, across all lakes, per area insect emergence was much higher from shallow depths. This is consistent with previous findings, suggesting that littoral zones in lakes have higher insect emergence compared to the deeper pelagic areas (Martin-Creuzburg et al., 2017). Results from our study lakes further suggest that the deeper the lake is, the lower the emergence of aquatic insect, and thus PUFA fluxes, are. Thus, deeper lakes may provide less dietary energy to riparian consumers on a per area basis. Moreover, when comparing the emergence of Chironomidae from 1 m² surface area above 1 m water depth among lakes, the shallow Lake Mittersee was the most productive. The higher invertebrate export from Lake Mittersee may be attributed to the relatively higher abundance of benthic algae and macrophytes (Beckett et al., 1992; Waters & Giovanni, 2002) compared to the other two deeper lakes (visual observations). The presence of macrophytes in Lake Mittersee, and probably in shallow lakes in general, may protect insect larvae better against fish predation (Cardinale et al., 1998; Tolonen et al., 2003). In addition, the higher availability of PUFA in the upper sediment layers at Lake Mittersee, probably induced due to the presence of macroalgae at the sediment–water interface, promoted insect emergence and the considerably higher PUFA flux. The correlation of FAAF between organic matter of sediments and insects showed that insects probably obtained C16 and C18 FA from the upper lake sediment layers, but may have acquired EPA, which is required for their adult life stage (Borisova et al., 2016), either from other diet sources (e.g., seston), and/or may have biosynthesised EPA from dietary precursors (Kabeya et al., 2018; Strandberg et al., 2020). This finding points to the importance of lake bathymetry and basal resource nutritional compositions as potential predictors of insect emergence for assessing the export of dietary nutrients from lakes, but that LC-PUFA retention in emergent insects was independent of dietary LC-PUFA supply from organic matter of sediments.
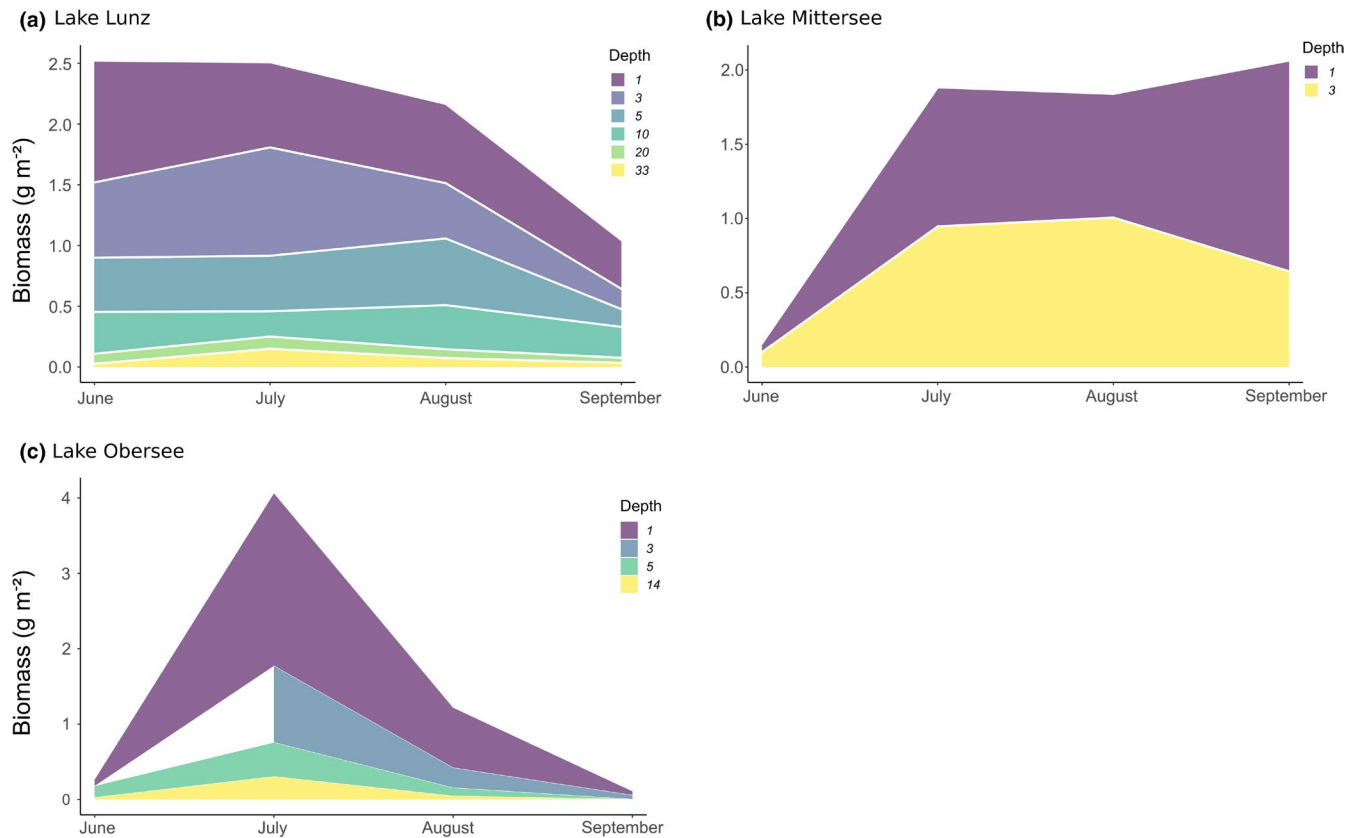
FIGURE 1 Emerged biomass of insect taxa per month (g m⁻²) from different lake depths from; (a) Lake Lunz, (b) Lake Mittersee, and, (c) Lake Obersee at each month of the sampling period (June–September)
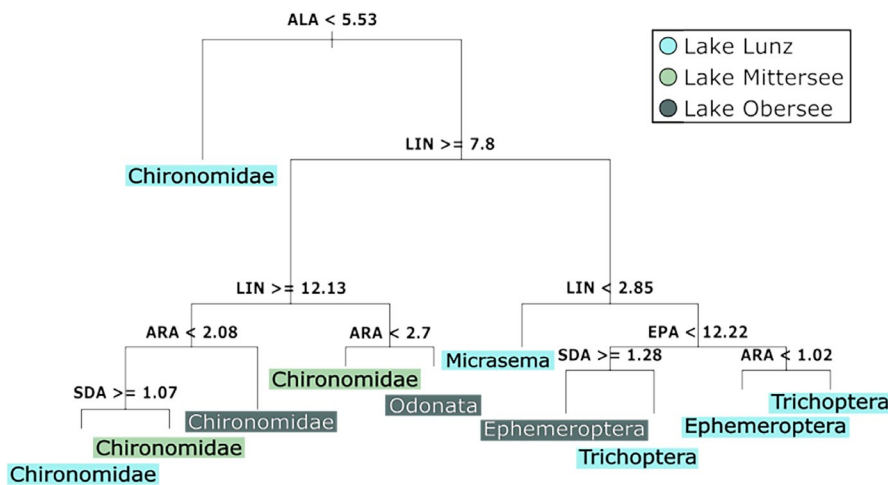

FIGURE 2 Regression tree analysis examining configurations between individual fatty acids (%; only fatty acids that represented >3% of in total fatty acids at least one sample are shown) and each taxon from the three lakes. ALA = 18:3n-3, LIN = 18:2n-6, ARA = 20:4n-6, EPA = 20:5n-3, SDA = 18:4n-3

4.2 | Difference in food quality exported by insect taxa

The differences in exported FA quantity and composition that we observed were largely due to differences in insect taxonomy. Therefore, the changes in insect taxa among these lakes resulted in variable PUFA supply to riparian consumers, which probably affect their access to high nutritional quality food resources. For example, Chironomidae exported high amounts of ω-6 PUFA, whereas other taxa (e.g. Ephemeroptera and Trichoptera) exported predominantly ω-3 PUFA. In addition to taxonomic differences in FA composition, the FA composition of emergent insects is also associated with their larval feeding modes (Guo et al., 2018; Makhutova et al., 2017; Scharnweber et al., 2019; Torres-Ruiz et al., 2007). For example, predatory larvae, such as Chaoboridae, Odonata, and some Chironomidae, are richer in LC-PUFA than non-predatory larvae (Martin-Creuzburg et al., 2017; Popova et al., 2017; Scharnweber et al., 2019). As a result, it is important to consider the taxonomic
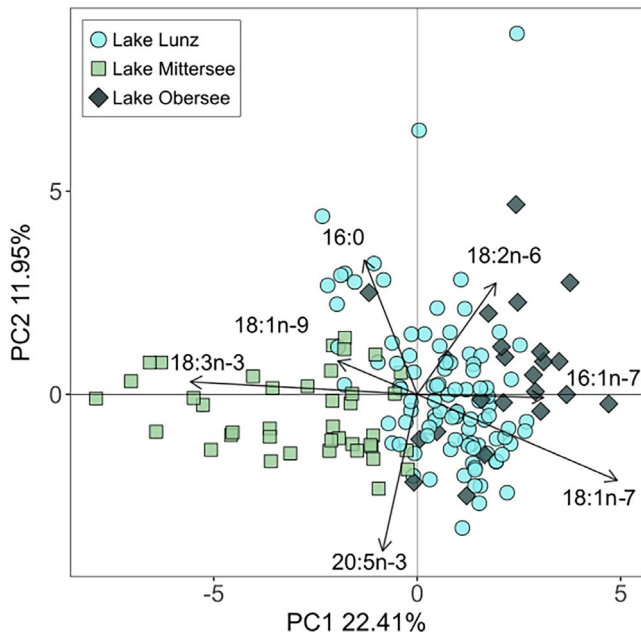
FIGURE 3 Principal component analysis using fatty acid profiles (%) of Chironomidae among the three study lakes. Only fatty acids that account for >80% of the contribution of dissimilarity between lakes are shown (SIMPER test)

composition and the feeding modes of emergent insects when quantifying the export of FA from lakes to riparian food webs. Because the quantity, but moreover the quality, of dietary nutrients exported via insects can impact the physiology and breeding success of riparian consumers (Fritz et al., 2017; Génier et al., 2021; Twining et al., 2016; Twining et al., 2018, 2019), understanding emergent insect diversity is key for assessing their nutritional value for riparian consumers.

Although the food resources availability and the various dietary traits among emergent insect taxa appear to drive differences in their FA composition, we have shown that the same insect taxon can synthesise and/or retain PUFA differently. Indeed, the FA content of the same insect taxon (i.e., Ephemeroptera or Chironomidae) differed among lakes, highlighting the importance of understanding intra-taxon variability in the export of PUFA. It was previously reported that differences in PUFA content between larvae and adults are negligible and that PUFA fluxes can be estimated from PUFA contents of insect larvae and emergence data (Gladyshev et al., 2009). In addition, Martin-Creuzburg et al., (2017) suggested that taxon-specific emergence data and seasonal PUFA contents of emergent adult insects could predict PUFA fluxes. However, we show here that depending on the lake, emergent insects from the same order (e.g., Ephemeroptera) or family (e.g., Chironomidae) can provide subsidies that differ in both quantity and quality. Therefore, characterising species assemblages of different insect taxon seems paramount of importance in order to understand the different effects on consumer diet of riparian ecosystems.

4.3 | Differences in lipids exported by Chironomidae among lakes

Chironomidae from Lake Mittersee contained higher FA contents, especially PUFA, than Chironomidae from the other lakes, suggesting that provision of PUFA for riparian consumers is lake-dependent and can potentially result in different food quality for consumers. The higher PUFA contents in both the upper sediments layers and Chironomidae from Lake Mittersee suggest that Chironomidae in this lake accumulated FA from organic matter of sediments. Nevertheless, differences in the FA content of Chironomidae between lakes were mainly explained by differences in the accumulation of ALA and EPA. While ALA levels in Chironomidae were positively related to ALA levels in the upper sediment layers, the EPA content of Chironomidae was much greater than the EPA contents of organic matter in sediments. Previous studies have demonstrated that macroinvertebrates predators (Mayntz et al., 2005) and insect herbivores (Behmer, 2009) can employ selective retention to redress specific nutritional imbalances, here, we suggest that this is also likely to be the case for emergent insects. Overall, we found that the PUFA content of the upper lake sediment layer alone cannot predict the content of LC-PUFA in emergent insects.

Differences in the FA content of Chironomidae across lakes may be related to PUFA assimilation from other food sources that we did not sample (e.g., seston, macrophyte detritus or epibionts) and/or to variations in Chironomidae taxonomy among lakes (Borisova et al., 2016; Makhutova et al., 2017; Scharnweber et al., 2019). In addition, differences in the FA content of Chironomidae may be related to the acquisition of LC-PUFA via conversion from precursors, despite previous reports that other emergent insects have low PUFA conversion abilities (Strandberg et al., 2020). As insects were assigned to orders or families in our study, it is important to acknowledge that the FA content was not evaluated at the species-level.

5 | CONCLUSION

In conclusion, we show here that lake depth, a geomorphological factor, and insect taxonomy, a biological factor, can predict/affect the quantity and quality of subsidies from lakes to riparian ecosystems. Although further studies are required to fully characterise the insect biomass and PUFA fluxes exported from lakes depending on their bathymetry, trophic status, and temperature. As freshwater-derived PUFA can influence the growth, immune status, and survival of consumers from riparian habitats (Fritz et al., 2017; Twining et al., 2019; Twining, et al., 2018), it is crucial to protect the diversity of aquatic insect species and their habitats. Finally, because the PUFA content of the upper lake sediment layers predicted C16 and C18 FA of emergent insects, but not their LC-PUFA (>20C) content, this suggests that emergent insects may obtain and preferentially retain LC-PUFA from other diet sources and/or biosynthesised LC-PUFA from dietary precursors. Future studies are thus warranted

to investigate the processes of PUFA conversion and retention in emergent insects that supply dietary energy to riparian consumers.

CONFLICT OF INTERESTS

We declare there is no conflict of interest associated with any of the decisions made or components of this study.

DATA AVAILABILITY STATEMENT

Emerged biomass data of all taxa underlying the analyses in this study are given in Table S1.

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