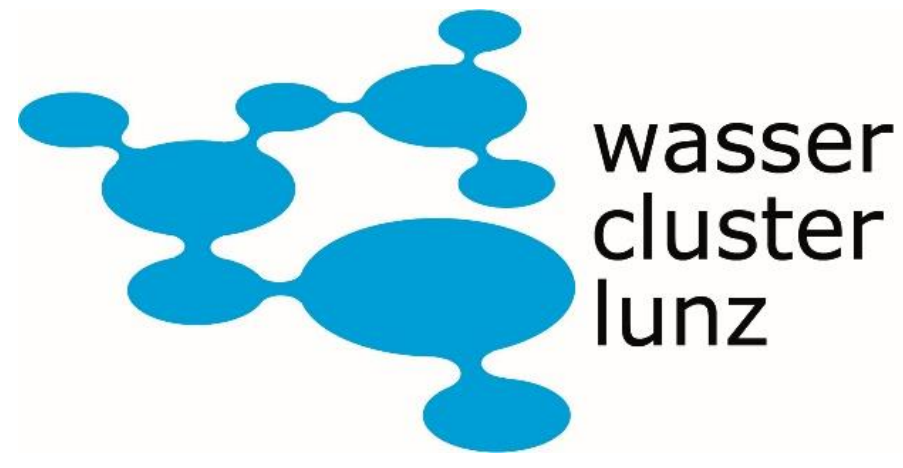


Assessing zooplankton foraging depths using a Bayesian fatty acid-specific stable isotope mixing model



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Abstract

Aquatic consumers require dietary energy and essential dietary micronutrients, which they cannot synthesize de novo, to survive, grow and reproduce. Fatty acids (FA) supply both, dietary energy along food chains and essential omega-3 and omega-6 polyunsaturated FA (PUFA) for various physiological processes of consumers. Tracing PUFA sources is thus important for understanding which diet conveys required nutrients to consumers. We used compound-specific stable hydrogen and carbon isotope analysis (CSIA) to investigate the trophic transfer of linoleic acid (LIN, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3) in a controlled laboratory study. This analytical approach was applied to assess specific feeding depths of *Bosmina*, *Daphnia*, cyclopoid and calanoid copepods in Lake Lunz during a 6-weeks summer field study. The isotope fractionation coefficients were larger for $\delta^2\text{H}$ (ALA: $-12.4 \pm 3.8\%$ and LIN: $-22.2 \pm 5.8\%$) than for $\delta^{13}\text{C}$ (ALA: $-0.05\% \pm 1.32$ and LIN: $-1.55\% \pm 1.2$). The CSIA values of epi-, meta- and hypolimnetic seston were clearly different at any sampling time, in contrast to bulk stable isotope values, which could not be used to distinguish between diet sources. Based on our models, *Daphnia* were mainly retaining epilimnetic diet sources, while calanoid copepods were mainly feeding on hypolimnetic resources. Dual-isotope CSIA of FA are a promising tool to provide long-term information on specific feeding habitats of aquatic ecosystems and appear to be robust to time-dependent environmental fluctuations.

Introduction

Zooplankton as part of the trophic cascade transfer dietary energy from primary producers to consumers at higher trophic levels. In lakes, dietary access to the edible particle size (seston) for zooplankton varies with depth and season. Lake zooplankton typically perform diel vertical migration (DVM) by moving to deeper, dark water layers during the daytime to minimize predation risk and moving to the algae-rich upper water layers at night to optimize feeding success (Ringelberg, 2009). Such diel vertical migration should thus result in potentially low feeding activities below the chlorophyll-a maxima at low and dark lake depths. Despite relying on n-3 LC-PUFA for improved performance of neuronal structures, most animals cannot synthesize n-3 or n-6 PUFA de novo and hence depend on the dietary acquisition of precursor fatty acids from each PUFA class. These fatty acid precursors include linoleic acid (18:2n-6, LIN) and α -linolenic acid (18:3n-3, ALA), which can be converted to the n-6 PUFA arachidonic acid (20:4n-6, ARA), or the n-3 PUFA eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). While n-3 LC-PUFA are generally scarce in diets and might require endogenous metabolic conversion to meet physiological requirements (Pilecky et al., 2021), they support neuronal signal transmission of animals more efficiently compared to their precursors. Consequently the most efficient feeding strategies correspond with physiological PUFA requirements of zooplankton to be able to invest all the obtained dietary energy into growth and reproduction, rather than metabolising precursors. Understanding spatial and temporal zooplankton feeding dynamics throughout the lake water column requires high temporal sampling resolution because the presence of zooplankton at a specific depth does not necessarily correlate with its feeding location. In a 6-weeks long lake study, we examined bulk stable isotopes and the compound-specific stable-isotopes of fatty acids of seston in the epi-, meta-, and hypolimnion, and of the zooplankton taxa *Daphnia longispina*, *Bosmina longirostris*, *Eudiaptomus gracilis*, and *Cyclops abyssorum* permanently resident in Lake Lunz, Austria. We tested the hypothesis that CSIA of essential fatty acids can discern the foraging depth of different zooplankton species. In addition to providing more detailed feeding information for zooplankton it was expected that using stable isotopes of fatty acids will result in a source-specific metric of diet quality across the lake water column for zooplankton because the CSIA also yield data on fatty acid contents.

Methods

Study site and sampling

Lake Lunz (68 ha; 47°51'10"N, 15°3'10"E, 608 m a.s.l., 34 m maximum depth) is a pristine subalpine, oligotrophic lake. Lake seston and zooplankton samples were collected three times a week from a stationary platform above deepest point of the lake from July 7 to August 11, 2021. Lake water was collected from the epilimnion (2-3 m below the surface), metalimnion (8-10 m) and hypolimnion (25 m) using a Schindler trap. To retrieve Seston samples 3L lake water were filtered through a 30 μm mesh, retained on pre-combusted WhatmanTM GF/C filters (0.7 μm) per triplicate, freeze dried and stored at -80°C . Zooplankton was collected in triplicates by vertically hauling a plankton net (100 μm mesh size, 36 cm diameter) from 25 m depth to the surface. Bulk zooplankton were retained on 500 μm and 250 μm filter cups, transferred into FalconTM tubes (50 mL), freeze-dried, separated to different species, and stored at -80°C .

Extraction of lipids and Gas chromatography – flame ionization detector (GC-FID) and isotope-ratio mass spectrometry (GC-IRMS)

Lipids were extracted according to standard procedures (Heissenberger et al., 2010). The freeze-dried samples were set up in 2 ml of Chloroform for the first step of extraction. With adding Methanol, chloroform:methanol (2:1 vol/vol) and 0.8ml of 0.9% NaCl with following sonication, vortexing and centrifuging two layers formed. The yields obtained from the bottom layer in three consecutive “washing steps” were concentrated under N_2 . For FAME formation this extracts were evaporated to change the carrier solute to Toluene and subsequently incubating with sulfuric acid:methanol (1:100 vol/vol) for 16 h at 50°C , following addition of KHCO_3 and hexane. Samples were shaken, vortexed and centrifuged and the upper organic layers collected, pooled and concentrated under N_2 . Fatty acid methyl esters (FAME) were analyzed using a gas chromatograph (TRACE GC, ThermoFisher Scientific) equipped with an FID and a SUPELCOTM SP-2560 column (100 m, 25 mm i.d., 0.2 μm film thickness). Chromeleon 7TM was used for peak quantification. FAME were identified by comparison of their retention times with known standards (37-component FAME mix, 47885-U, Supelco; Sigma-Aldrich, Bellefonte, Pennsylvania). Fatty acid concentrations were determined using external calibration curves based on known standard concentrations.

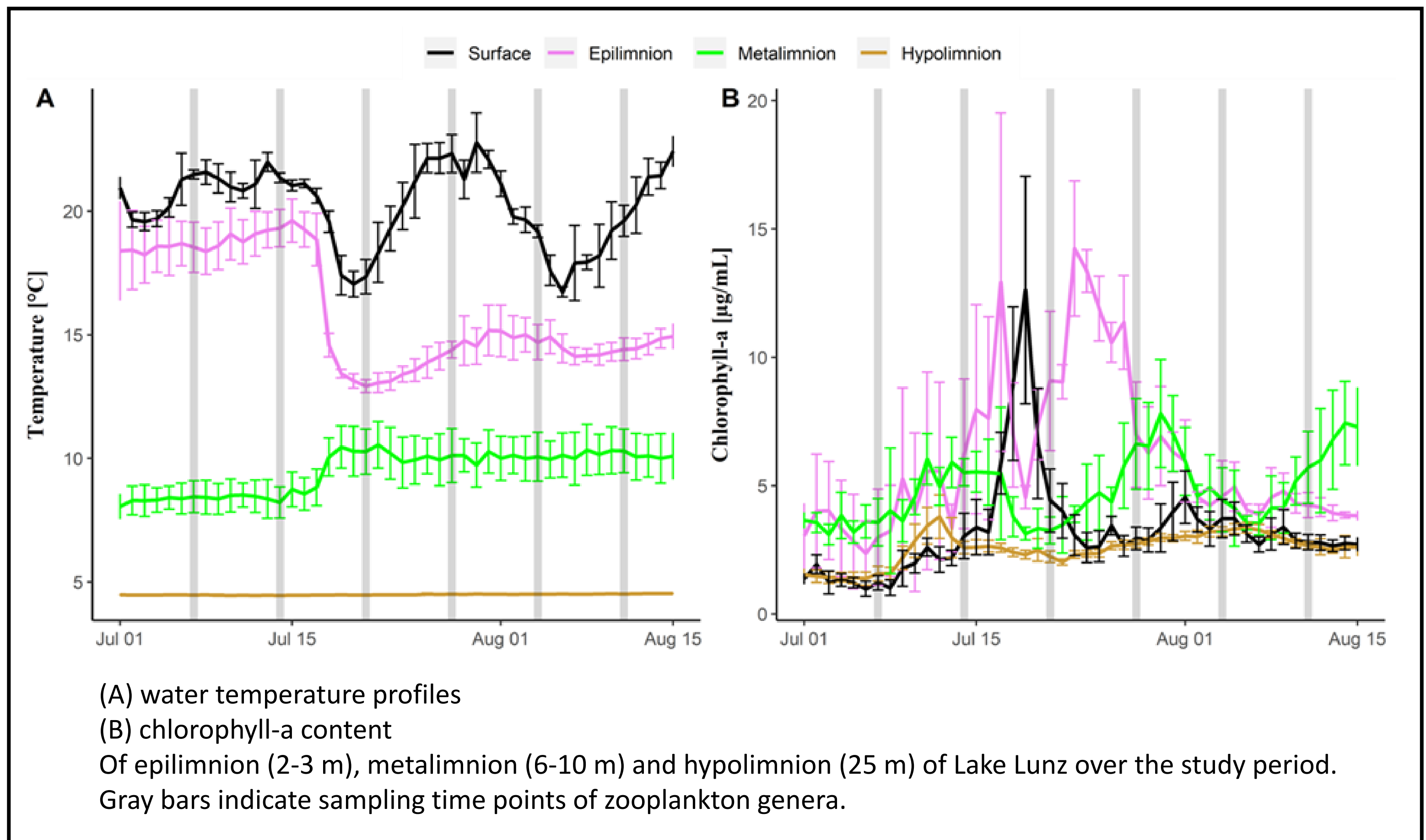


Fig. 2. Isotopic biplot of $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values of polyunsaturated fatty acids.

Results

The $\delta^2\text{H}_{\text{ALA}}$ values showed the largest differences among the lake water layers of all FA (epilimnion: $-207\% \pm 22$; metalimnion: $-232\% \pm 15$; hypolimnion: $-309\% \pm 7$, ANOVA, $F_{2,40} = 118.5$, $p < 0.001$, Tukey all-comparisons $p < 0.001$). $\delta^2\text{H}_{\text{LIN}}$ values were also significantly different (epilimnion: $-300\% \pm 19$; metalimnion: $-286\% \pm 37$; hypolimnion $261\% \pm 22$, ANOVA, Tukey all-comparisons $p < 0.001$) between the lake layers. Dual-carbon and hydrogen analysis revealed differences in ALA and LIN (MANOVA, Pillai, $p < 0.05$) between the four zooplankton genera. The pooled $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values of LIN and ALA matched between seston and zooplankton, whereas $\delta^2\text{H}_{\text{EPA}}$ values were lower in zooplankton than in seston ($-394.7\% \pm 18.9$ vs. $-337.1\% \pm 41.9$, Tukey, $p < 0.001$), but no difference was found in $\delta^{13}\text{C}_{\text{EPA}}$ values between seston and zooplankton (Fig. 2). A Bayesian stable-isotope mixing model was performed using the stable-isotope values of LIN and ALA. *Daphnia* showed the highest probability of feeding on epilimnetic seston (0.59 ± 0.13) followed by *Cyclops* (0.37 ± 0.05), *Bosmina* (0.33 ± 0.05) and calanoids (0.16 ± 0.05). On the other hand, calanoids were the only zooplankton group with significant diet contributions attributed to hypolimnetic seston (0.32 ± 0.06). While the model suggested a higher preference of larger-sized *Daphnia* and *Eudiaptomus* for feeding on metalimnetic seston, *Cyclops* feeding was more pronounced in the epilimnion. No difference in feeding preferences was observed between the two *Bosmina* size classes (Fig. 3).

Conclusion

The presented dual-isotope ($\delta^{13}\text{C}$ and $\delta^2\text{H}$) CSIA of FA approach for discerning feeding grounds of primary consumers provides an alternative to bulk stable isotope analyses, particularly in cases in which the potential dietary resources exhibit low isotopic differentiation of bulk C or H. The identification of zooplankton feeding grounds within lake depths using CSIA also comes along with the provision of dietary quality data because on the one hand dietary FA mass fractions at specific lake layers are concurrently identified and can thus be used as a measure for dietary energy acquisition and on the other hand a decrease in $\delta^2\text{H}_{\text{FA}}$ values indicates FA conversion and thus a potential mismatch between diet and physiological requirements. The isotopic values for LIN and ALA of consumers, corrected for trophic fractionation, perfectly reflected the range of values found in the diet, thereby revealing the feeding ground within the water column of different zooplankton species.

(A) water temperature profiles

(B) chlorophyll-a content

Of epilimnion (2-3 m), metalimnion (6-10 m) and hypolimnion (25 m) of Lake Lunz over the study period. Gray bars indicate sampling time points of zooplankton genera.

Data Analysis

Data analysis and graphics were performed in R (Version 4.0.2) using the packages *rstatix*, *ggplot2*, *ggpubr*, *lme4*, *vegan*, and *corrplot*. Bayesian isotopic mixing models were generated in R software, using the *simmr* package. Consumer-diet isotope discrimination factors used in the models were for $\delta^2\text{H}_{\text{ALA}}$ $-12.4 \pm 3.8\%$, for $\delta^2\text{H}_{\text{LIN}}$ $-22.2 \pm 5.8\%$, for $\delta^{13}\text{C}_{\text{ALA}}$ $-0.05\% \pm 1.32$, and for $\delta^{13}\text{C}_{\text{LIN}}$ $-1.55\% \pm 1.2$ based on the experimental and field stable-isotope data from Pilecky et al. (2022).

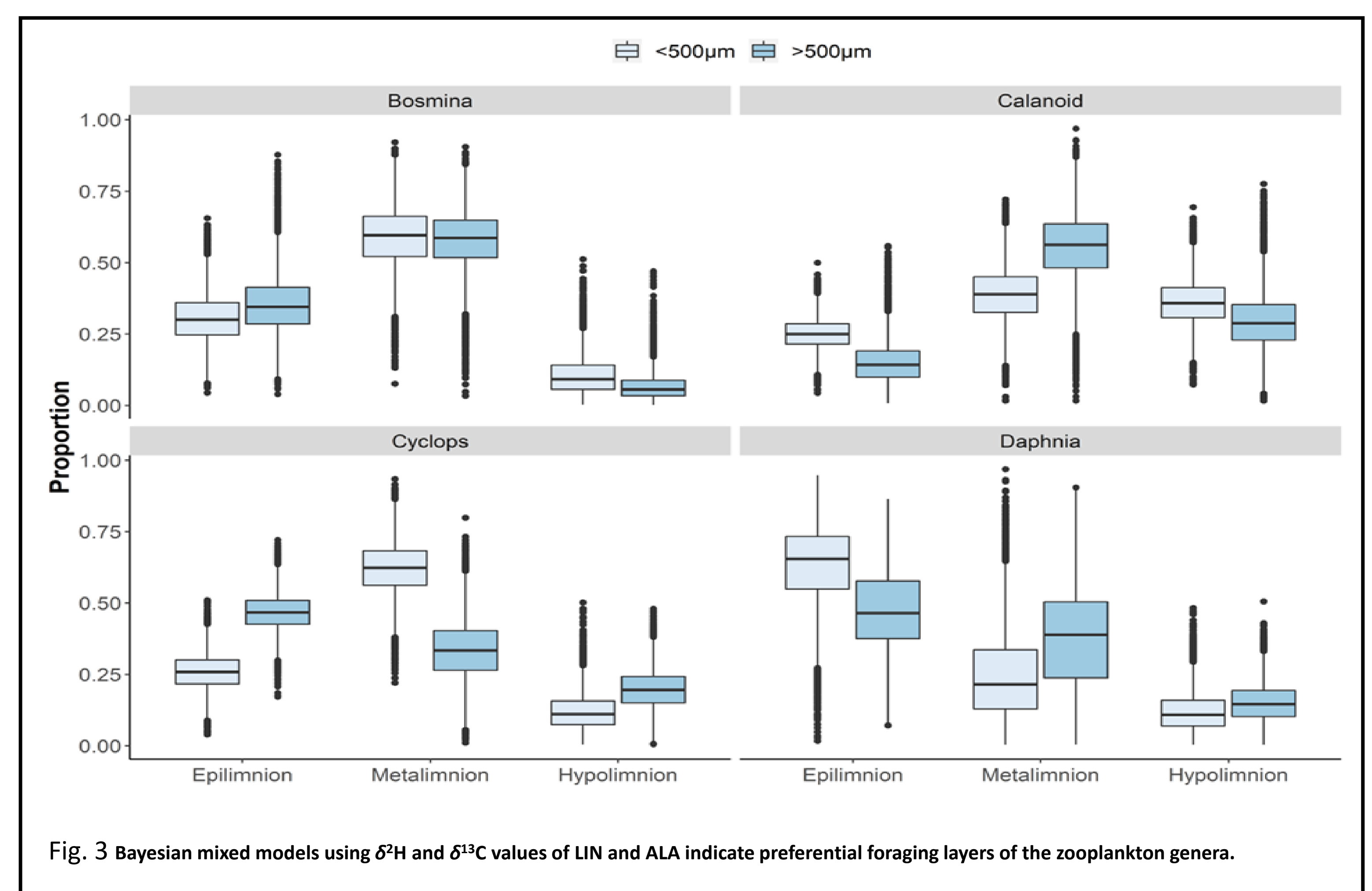


Fig. 3. Bayesian mixed models using $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values of LIN and ALA indicate preferential foraging layers of the zooplankton genera.

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Wiener Wasser

