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Escaping malnutrition by shifting habitats: A driver of three-spined stickleback invasion in Lake Constance

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Abstract

Fatty acids, and especially long-chain polyunsaturated fatty acids, are biologically important components in the metabolism of vertebrates, including fish. Essential fatty acids (EFA) are those that in a given animal cannot be synthesized or modified from precursors and must therefore be acquired via the diet. Because EFAs are often unevenly distributed in nature, this requirement may drive species to make behavioral or ecological adaptations to avoid malnutrition. This is especially true for fish like the three-spined stickleback (*Gasterosteus aculeatus* L.) of Upper Lake Constance (ULC), whose recent marine ancestors evolved with access to EFA-rich prey, but which found themselves in an EFA-deficient habitat. An unexpected and unprecedented ecological shift in the ULC stickleback population from the littoral to pelagic zones in 2012 might be linked to EFA availability, triggering ecological release and enabling them to build a hyperabundant population while displacing the former keystone species, the pelagic whitefish *Coregonus wartmanni*. To test this hypothesis, sticklebacks from the littoral and pelagic zones of ULC were sampled seasonally in two consecutive years, and their stomach contents and fatty acid profiles were analysed. Pelagic sticklebacks were found to possess significantly higher values of an important EFA, docosahexaenoic acid (DHA), especially during autumn. Evaluation of the DHA supply suggests that sticklebacks feeding in the littoral zone during autumn could not meet their DHA requirement, whereas DHA availability in the pelagic zone was surplus to demand. During autumn, pelagic sticklebacks consumed large amounts of DHA-rich prey, that is, copepods, whereas littoral sticklebacks relied mainly mostly on cladocerans, which provide much lower quantities of DHA. Access to pelagic zooplankton in 2012 was possibly facilitated by low densities of previously dominant zooplanktivorous whitefish. The present study offers a convincing physiological explanation for the observed expansion of invasive sticklebacks from the littoral to the pelagic zones of Lake Constance, contributing to a phase shift with severe consequences for fisheries.

1 | INTRODUCTION

Fatty acids known as long-chain polyunsaturated fatty acids (LC-PUFA, carbon chain ≥20) are of great biological importance for vertebrates, especially fish, because they are essential for metabolic processes, including endocrine regulation, immune function, cognition, and reproduction (Das, 2006). Deficiency of these compounds can lead to reduced fitness through physiological impairments like reduced growth, reduced immune competence, and increased mortality (Sargent et al., 1995).

The omega-6 (n-6) and omega-3 (n-3) fatty acids such as α-linolenic acid (ALA; C18:3n-3), eicosapentaenoic acid (EPA; C20:5n-3), or docosahexaenoic acid (DHA; C22:6n-3) are particularly important compounds for fish (Castro et al., 2012; Glencross, 2009), but they have an uneven distribution in nature (Hudson et al., 2022). This places selective pressure on individual consumers to optimize dietary intake. Essential fatty acids (EFA) are relatively abundant in marine organisms, so for marine fish in their ancestral setting, a balanced diet is easily achievable (Tocher, 2010) compensating their limited ability to desaturate and elongate fatty acids themselves (Parrish, 2009). Conversely, if species of marine origin colonize EFA-poor habitats, they can suffer from nutrient deficiencies (Parzanini et al., 2020). Most freshwater systems have limited availability of LC-PUFAs, so over time freshwater fish have evolved an ability to synthesize them efficiently through desaturation of fatty acids by the enzyme "fatty acid desaturase 2" (FADS2) (Bláhová et al., 2020; Castro et al., 2012). Fish species of marine origin to colonize freshwater habitats require adaptation to EFA-poor environments, and ongoing evolution in fresh water often involves an increase in the number of copies of FADS2 genes compared to their marine conspecifics (Ishikawa et al., 2019).

A prominent example of this nutritional adaptation is the three-spined stickleback (Gasterosteus aculeatus L.). Genetic isolation resulting from differential ice coverage and glacial retreat during the last Ice Age resulted in several distinct lineages of this species in freshwater habitats across Europe (Fang et al., 2020; Sanz et al., 2015). The species colonized Lake Geneva, one of the largest lakes north-west of the Alps, before the last Ice Age, whereas the drainage system of Lake Constance, another large lake north of the Alps, was most likely colonized much later, after the ice retreated (Hudson et al., 2022). More recently still, sticklebacks from a marine lineage (Hudson et al., 2021) were transferred directly into Lake Constance. This probably happened during the 1940s, with the first confirmed record dating to 1951 (Roch et al., 2018). Sticklebacks from Lake Geneva, with 10,000 years of isolated evolution to EFA-poor freshwater habitat, were found to have significantly more copies of the FADS2 genes than those of Lake Constance (Hudson et al., 2022; Ishikawa et al., 2019).

Until relatively recently the sticklebacks of Lake Constance occupied the littoral zone year-round (Roch et al., 2018). However, in autumn 2012, their population increased abruptly and unexpectedly. The increase coincided with a marked shift from an exclusively shore-line lifestyle into a pelagic one (Eckmann and Engesser 2019). A lake-wide survey in September 2014 revealed that the new pelagic ecotype of stickleback now represented 96% of all individuals in the pelagic fish community, accounting for 28% of total fish biomass (Alexander et al., 2016). Twice yearly hydroacoustic scans of the pelagic zone conducted from 2009 to 2018 revealed that the density of stickleback population increased exponentially in 2012 up to a plateau, with seasonal variations after 2014 peaking at 7990 individuals/ha (Eckmann and Engesser 2019). Surveys with pelagic trawls between 2017 and 2019 identified densities exceeding 10,000 individuals/ha (Gugele et al., 2020). To the best of our knowledge, such a niche shift has not yet been described in any other large freshwater lake (and must be considered unique for Lake Constance).

Although the two populations of stickleback remain morphometrically and genetically very similar, and the pelagic type remains associated with the littoral zone for purposes of breeding, they already show early signs of sympatric speciation (Dahms et al., 2022). In May and June they build nests in shallow areas, spawn, and guard their young alongside the littoral type (Gugele et al., 2020), but from July to April they expand into pelagic habitats. The full extent of the ecological impact of this pelagic invasion is yet to be evaluated, but direct effects are apparent on the pelagic whitefish Coregonus wartmanni (Bloch 1784), once the dominant keystone pelagic fish species and the main target of local fisheries (Baer et al., 2017). These impacts include interspecific competition for food leading to reduced whitefish growth and survival, and predation by sticklebacks on whitefish larvae and probably eggs, hampering recruitment (Baer et al., 2021; DeWeber et al., 2022; Gugele et al., 2023; Roch et al., 2018; Ros et al., 2019; Rösch et al., 2018). These observations coincide with a sharp decline in whitefish fishery yield, from around 300–600 mt (metric tons) before stickleback invasion to less than 22 mt in 2022 (www.ibkf.org).

The rapid invasion of the pelagic zone by sticklebacks appears to be unique for a large deep oligotrophic lake and is yet to be fully explained (Baer et al., 2022). Based on the outcome of a recent study using common garden trials, sticklebacks originating from Lake Constance exhibited low numbers of FADS2 copies, low growth, and low DHA levels (Hudson et al., 2022), leading to the hypothesis that EFA-rich prey in the pelagic zone could be an important driver of habitat expansion. The relatively recent marine ancestry of sticklebacks from Lake Constance means they possess only few copies of the FADS2 genes and thus possess limited ability to synthetize LC-PUFAs like EPA or DHA (Hudson et al., 2022). They rely mainly on direct dietary sources of EFAs, especially EPA and DHA. Sticklebacks are omnivorous and forage opportunistically according to optimal forage theory (Nomakuchi et al., 2009; Ogorelec, Brinker, & Straile, 2022; Roch et al., 2018; Walker, 1997). Their requirement for dietary EFAs must be met by zooplankton such as calanoid or cyclopid copepods, which

are the only relevant source of significant levels of DHA in pelagic fresh water (Persson & Vrede, 2006). Studies in other freshwater systems identified gammarids as important prey in benthic habitats, providing significant amounts of EPA (Kolanowski et al., 2007). An extensive literature survey concluded that although terrestrial and benthic prey items contain higher levels of EPA than pelagic zooplankton, the latter are a better source of DHA (Hudson et al., 2022). In view of this divergent habitat-specific availability, it seems likely that littoral and pelagic sticklebacks in Upper Lake Constance (ULC) may exhibit different fatty acid profiles and that littoral populations may be limited by deficiencies in DHA. Therefore, we hypothesized that a lower EFA content in littoral v. pelagic sticklebacks might be explained by dietary differences and provide a possible explanation for the sudden and highly successful expansion of the species into the pelagic zone. To test this hypothesis, sticklebacks from both habitats were caught seasonally during two consecutive years, and their fatty acid profiles and stomach contents were characterized. These data were analysed for evidence of DHA deficit, which might have been counterbalanced by a habitat shift to the pelagic zone.

2 | MATERIALS AND METHODS

All fish were caught and processed by licensed personnel, by permission of the local fisheries administration (Regierungspräsidium Tübingen) and according to the German Animal Protection Law (§ 4) and the ordinance on slaughter and killing of animals (Tierschutzschlachtverordnung § 13).

2.1 | Sampling of sticklebacks

Lake Constance is bordered by Austria, Germany, and Switzerland (47°38′ N; 9°22′ E). The total surface area of 536 km^2 is divided between the large (472 km^2), deep (>250 m) Upper Lake (ULC) and the smaller (63 km^2), shallower Lower Lake (LLC). This paper deals solely with the warm, monomictic, oligotrophic, and better-documented ULC. Lack of data and knowledge about the stickleback situation and different conditions in the mesotrophic basin of LLC precluded it from consideration in the present study.

Seasonal stickleback sampling of ULC was conducted four times a year over two consecutive years, from spring 2017 through summer 2019, using littoral (bottom) and pelagic (floating) gillnets with mesh-sizes of 10–12 mm. All nets had a deployed height of 3 m, whereas length varied with mesh-size: 30 m for nets with 10 mm mesh and 15 m for the 12 mm mesh net. Pelagic nets were deployed to drift freely behind those used in the monthly monitoring of whitefish (mesh-sizes 36–44 mm), at depths of 3–15 m in the areas where greatest stickleback abundances were recorded during hydroacoustic surveys (Gugele et al., 2020), that is, near the center of the lake where water depths range between 180 and 250 m. Littoral nets were set near the shore in the littoral zone at depths from 6 to 20 m. All nets were set overnight, with a soak time of about 15 h. A total of 16 sampling events covered each habitat (pelagic and littoral zones) and each season (spring from March to May, summer from June to August, autumn from September to November, and winter from December to February) twice. Further details of sampling protocols are given in Baer et al. (2022). Sampled sticklebacks were killed with an overdose of clove oil (1 mL L^{-1}) and a gill cut. They were measured *post mortem* (total length [TL] to the nearest millimeter), wet weighed to the nearest 0.01 mg, and their sex was recorded. Sticklebacks infested with the pseudophyllidean cestode *Schistocephalus solidus* were excluded from the dataset (*n* = 8). Furthermore, to avoid sex-biased outcomes (males have fewer copies of FADS2, Ishikawa et al., 2019), only females were selected for further analysis. In total, 141 sticklebacks were subject to lipid analyses, 72 from the littoral zone, 69 from the pelagic zone (for each quarterly sampling 6 to 11 sticklebacks per habitat, see Table S1 in supplements). Hereafter sticklebacks caught in the pelagic zone are called "pelagic sticklebacks," and individuals caught in the littoral zone "littoral sticklebacks." Prior to fatty acid profiling the whole sticklebacks were freeze-dried, stored at −80°C, and analysed after the end of the sampling period.

2.2 | Lipid analysis

Total lipids from defrosted, freeze-dried, and homogenized samples (whole sticklebacks, powdered with "Tube-mill 100 control," IKA-Werke GmbH & CO. KG, Staufen, Germany) were dissolved according to Folch et al. (1957) in chloroform (3–5 mL) and stored at −20°C for 24 h to improve lipid extraction efficiency. Samples were extracted in chloroform–methanol (2:1), vortexed, and centrifuged. Afterwards, mixtures were gassed for 2 h with nitrogen (evaporation system "XcelVap," Horizon Technology, Inc., New Hampshire, USA) to remove chloroform. Lipids were subsequently analysed gravimetrically and quantified using gas chromatography–flame ionization detection ("Nexis GC 2030," Shimadzu, Kyoto, Japan). LC-PUFAs were identified based on retention times relative to a laboratory standard (Supelco 37-component FAME-mix, Sigma-Aldrich, St. Louis, MO, USA). Concentrations of ALA, EPA, DHA, and LC-PUFA were calculated in micrograms per gram (µg g^{-1}) and for total lipid content per gram (g g^{-1}) for each sample in relation to wet weight. The ratio of n-3 to n-6 acids of each sample was determined, and other important fatty acids were analysed so that their concentration in relation to wet weight (µg g^{-1}) would also be calculated (see Table S2 in supplements). The contribution of all analysed lipids was measured in relation to total lipid weight (%).

2.3 | Statistics

We performed a principal component analysis (PCA) on the correlation matrix of three time variables (summer, autumn, and winter; excluding spring as the season when pelagic and littoral sticklebacks share the same habitat while spawning) and two habitats (littoral and pelagic), using the quantity of measured fatty acids (µg g^{-1}) in relation to the lipid dry mass of each stickleback. A linear discriminant analysis (LDA) was then used to predict stickleback residence

(littoral or pelagic) from individual lipid acid profiles (each fatty acid in µg g^{-1}).

To investigate drivers of ALA, EPA, and DHA occurrence in sticklebacks, the following general linear model (GLM) (Sachs, 1997) was used:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\beta\gamma)_{jk} + \zeta_l + \varepsilon_{ijkl} \qquad (1)$$

where Y_{ijkl} is the content of ALA, EPA, and DHA in sticklebacks in µg g^{-1}; µ is the overall mean, α_i denotes season, β_j is habitat (pelagic or littoral zone), $(\alpha\beta)_{ij}$ is the interaction between season and habitat, γ_k is total length, $(\beta\gamma)_{jk}$ is the interaction of total length and habitat, γ_l represents year, added to the model as a random factor, and ε_{ijkl} is the random residual error. Requirements of the model, that is, residuals not violating linearity, normality, or non-independence, were checked by inspecting residuals (predicted *vs.* expected plots) and multicollinearity by inspection correlation of independent variables. Single outliers with extreme values ($n = 2$) were excluded from the dataset (selection criteria: more than eight times S.D.). Student's *t*-test was used for post hoc comparisons between habitats, and Tukey's honestly significant difference (HSD)-test was applied to compare seasons (Sokal & Rohlf, 2003).

All statistics were performed in JMP Pro 17.1.0 (64 bit, SAS Institute).

2.4 | Stomach content analysis

Gastrointestinal tracts (stomach and intestine) were analysed from a subsample of 109 sticklebacks: 69 caught in the pelagic zone and 40 caught in the littoral zone. Samples were taken during all four seasons (autumn 2017, winter 2017, spring 2018, and summer 2018), and for each season and each habitat, and the gut contents of at least 10 individuals were analysed. Food items were identified and counted in a zooplankton counting chamber (also known as Bogorov counting chambers) and assigned into five food categories: (a) copepods (nauplii, copepodites, and copepods of *Cyclopoida*, *Calanoida*, *Harpacticoida*); (b) cladocerans (*Bosmina* spp., *Daphnia* spp., *Diaphanosoma brachyurum*, *Chydoridae*, *Bythotrephes longimanus*, *Leptodora kinditii*); (c) benthic organisms (*Chironomidae*, *Annelida*, *Bivalvia*, *Collembola*, *Ceratopogonidae*, *Hydrachnidia*, *Mysidae*, *Nematoda*, *Ostracoda*, *Plecoptera*, *Simuliidae*); (d) terrestrial organisms (adult *Trichoptera*, *Ephemeroptera*, *Heteroptera*); and (e) fish (eggs and larvae). The mean number and S.D. of consumed prey per stickleback was calculated as the number of individuals per prey type, averaged, and dispersed across all individuals.

2.5 | DHA estimated consumption

To calculate DHA availability for sticklebacks from both habitats during autumn, the season with the highest density of sticklebacks in the pelagic habitat during the study period (Gugele et al., 2020), we used the dry weight of consumed food items derived from the mean

number of consumed individuals and multiplied them by the average dry weight of cladocerans (3.8 µg) and copepods (2.7 µg). To address and depict the variation in cladocerans in size and dry weight, we used the mean dry weight of juvenile *Bosmina coregoni* and *Bosmina longicornis* (0.57 µg) to calculate a minimum value and the mean dry weight of adult *B. longimanus* and *L. kindtii* (145.9 µg) to calculate a possible maximum value (unpublished data of autumn zooplankton samples of Lake Constance, Institute of Lake Research). We used chironomids as a proxy for consumed benthic organisms with a dry weight of 1 mg per individual (Borisova et al., 2016; Makhutova et al., 2014). The consumed dry mass was multiplied by the mean dry matter lipid content for cladocerans (31%, Mariash et al., 2017), copepods (30%, Vlymen, 1970), and chironomids (8%, Goedkoop et al., 1998) and finally multiplied by the DHA content of total extracted lipid for copepods (17.2%) and cladocerans (0.8%) (Persson & Vrede, 2006) and for chironomids (0.03%, Makhutova et al., 2014). To assess the DHA content of consumed organisms by wet weight, we multiplied the amount of DHA in dry matter by 8.33 (Dumont et al., 1975). This outcome was then set into context with the DHA requirements of sticklebacks, which we assumed in the absence of specific data, to be similar to that of other fish species of marine origin at 5 g kg^{-1} diet (0.5% per diet, see Glencross, 2009).

3 | RESULTS

The arithmetic mean TL of all sampled sticklebacks was 60 ± 7 mm (±S.D.), and their mean wet weight 2.0 ± 0.8 g (±S.D.). TL and weight data are provided in the supplementary information sorted by season, year, and habitat (Table S1). Sticklebacks caught in pelagic waters exhibited greater total lipid contents than those from the littoral zone, by wet weight (11.0% *v.* 7.5%) and in relation to dry weight (29.9% *v.* 22.2%). Furthermore, sticklebacks caught in pelagic waters showed from spring to winter an increasing trend in the total lipid content in relation to wet weight (from 8.6% in spring to 14.3% in winter) and in relation to dry weight (from 22.1% in spring to 39.2% in winter). In contrast, the total lipid content in sticklebacks from the littoral zone showed from spring to autumn a decreasing trend (in relation to wet weight from 7.2% to 5.9% and in relation to dry weight from 23.1% to 16.5%), but highest levels in winter, too (10.0% in relation to wet weight and 28.4% in relation to dry weight).

3.1 | Lipid profile

The PCA of fatty acid profiles effectively grouped the data according to habitat and season based on a scree plot identifying three relevant principal components and accounting for 65.6% of total variance (see supplements, Figures S1 and S2). The first two principal components (PC1 and PC2) already accounted for 54% of total variance. PC1 explained 32% of variance, with differences especially apparent during autumn, when sticklebacks from the littoral zone were most strongly
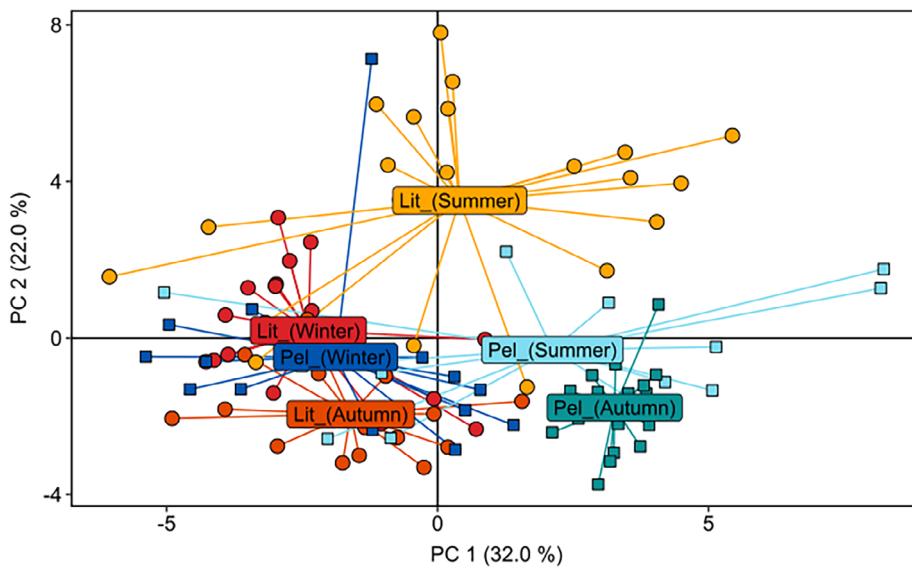
FIGURE 1 Results of the principal component analysis, showing first two principal components (PC), which explain most of the variance in the data; "Lit" and circles stand for littoral sticklebacks, "Pel" and boxes represent pelagic sticklebacks *Gasterosteus aculeatus* L.

separated from pelagic sticklebacks (Figure 1). Differences were smaller during winter, whereas during summer differences between pelagic and littoral sticklebacks were apparent in PC2 (explaining 22% of variance) (Figure 1). The LDA assigned 92.6% of sticklebacks correctly to littoral or pelagic habitat based on the lipid acid profile. By habitat 94.7% of sticklebacks assigned to the littoral group were correctly placed, and 90.2% of the pelagic group.

Sticklebacks caught in the pelagic zone during summer, autumn, and winter exhibited higher lipid contents, higher values of LC-PUFA, and higher n-3/n-6 ratios (Figure 2 and Table S3 in supplements).

The overall ALA content of sticklebacks in the littoral zone was 4.1 ± 3.5 µg g^{-1} (±S.D.), distinctly lower than the 6.8 ± 4.7 µg g^{-1} (±S.D.) recorded in pelagic specimens. However, the GLM ($n = 141$, $r^2_{adjusted} = 0.20$, $p < 0.0001$) revealed that this difference was not significantly linked to habitat ($p > 0.05$). Season had a significant influence on ALA levels ($p < 0.05$), and *post hoc* comparison showed significantly higher values in spring than in autumn and winter (*t*-test, $p < 0.05$). ALA levels were not correlated to TL (Table 1). The interaction between length and habitat as well as between season and habitat had no significant influence on ALA (Table 1).

The EPA levels of sticklebacks caught in the littoral zone averaged 4.5 ± 3.0 µg g^{-1} (±S.D.), compared to 6.6 ± 4.0 µg g^{-1} (±S.D.) in pelagic specimens. However as for ALA, the model (GLM, $n = 141$, $r^2_{adjusted} = 0.22$, $p < 0.0001$) revealed that higher levels of EPA in pelagic sticklebacks were not significantly linked to habitat ($p > 0.05$). Neither season nor the interaction between habitat and total length had any influence on the EPA level (Table 1), but the effect of TL was highly significant ($p < 0.0001$) with a positive correlation to EPA level (Table 1). The interaction of season and habitat was also significant ($p < 0.001$), and respective *post hoc* comparisons revealed significantly higher levels of EPA during summer and autumn in pelagic sticklebacks compared to sticklebacks caught during autumn and winter in the littoral zone (Tukey–Kramer HSD, $p < 0.05$).

The overall DHA content of pelagic sticklebacks was 5.0 ± 2.6 µg g^{-1} (±S.D.), distinctly higher than that for littoral specimens, which averaged 3.1 ± 2.6 µg g^{-1} (±S.D.) DHA. In contrast to ALA and EPA, the GLM for DHA ($n = 141$, $r^2_{adjusted} = 0.40$, $p < 0.0001$) revealed that this difference was highly significantly linked to habitat ($p < 0.001$). Season also exerted a highly significant influence ($p < 0.0001$), and post hoc comparisons revealed that sticklebacks caught in autumn in the pelagic zone had significantly higher levels of DHA than those from the littoral zone (Tukey–Kramer HSD, $p < 0.05$). As for EPA, TL also had a significant positive influence on the outcome of the DHA-model (Table 1), but to a lower degree than habitat and season (Table 1). The interactions between habitat and TL and between season and habitat had no significant influence on the DHA value (Table 1).

3.2 | Stomach contents

Nearly all analysed sticklebacks had food in their digestive tracts. Only one individual sampled from the pelagic zone during spring was empty. During spring the numerically dominant food source for sticklebacks, independent of habitat, was copepods (Table 2). In summer, pelagic sticklebacks consumed mostly cladocerans, whereas littoral specimens consumed mostly benthic organisms, mainly chironomids (Table 2). In autumn, copepods were again the most common prey category for pelagic sticklebacks (81%), whereas cladocerans (78%) were the most frequent food for littoral specimens. In winter, the proportion of copepods in the stomachs of stickleback from the pelagic zone decreased (56%), and cladocerans became more common (44%), whereas copepods were the dominant food source for sticklebacks caught in the littoral zone, as in spring (84%). Remains of fish eggs and larvae were found in all seasons except autumn, but were most abundant in spring. Terrestrial organisms were found only occasionally in single sticklebacks (Table 2).
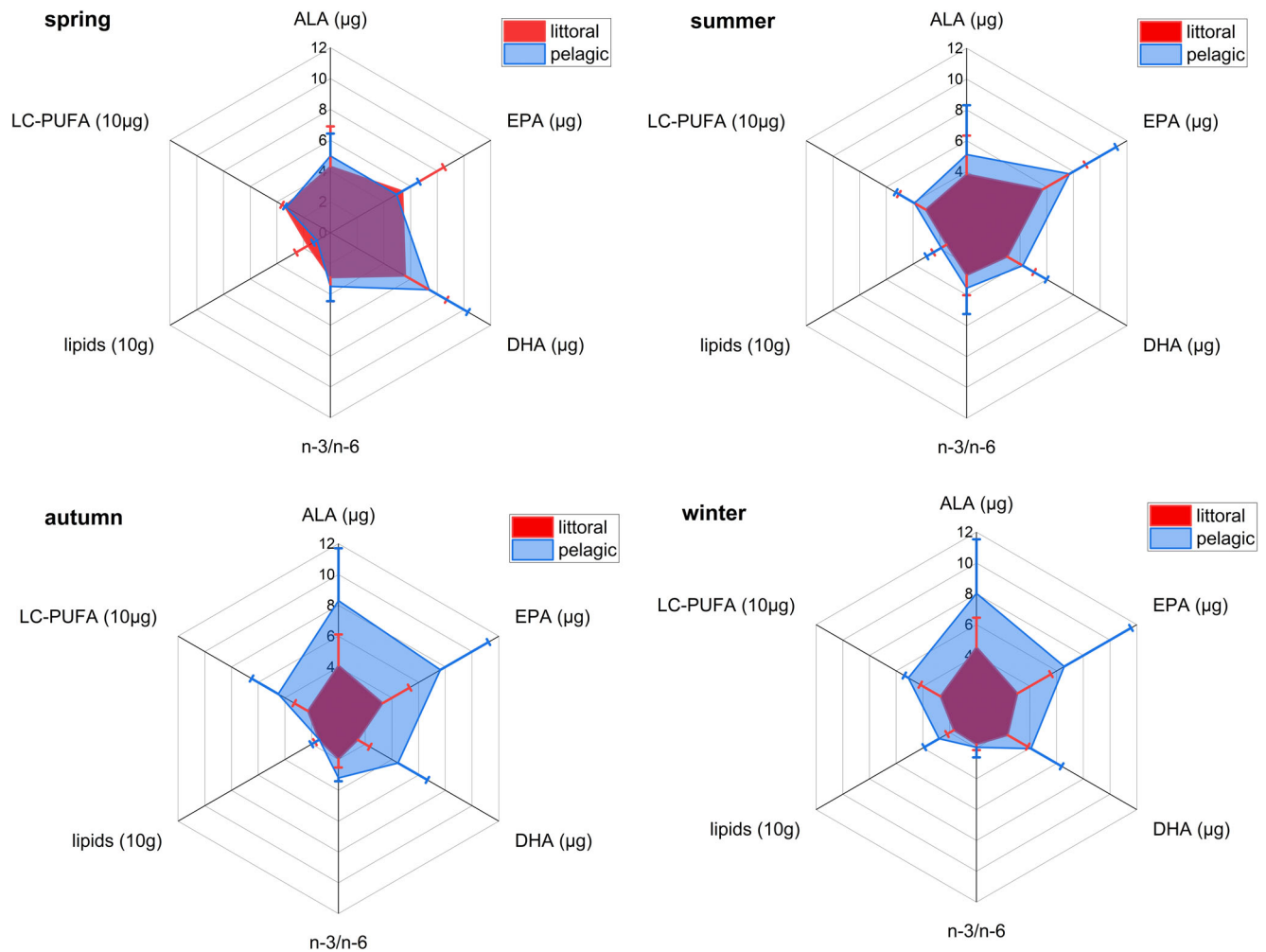
FIGURE 2 Radar chart with arithmetic means and positive standard deviations of α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), total lipid content, and long-chain polyunsaturated fatty acids (LC-PUFA) values as well as the n-3/n-6 ratio of sticklebacks *Gasterosteus aculeatus* L from the littoral and pelagic zones of Lake Constance caught during spring, summer, autumn, and winter.

TABLE 1 The significance and effect strength of different parameters on concentrations of ALA, EPA, and DHA in sticklebacks in Upper Lake Constance.

	ALA		EPA		DHA	
Model terms	**Significance/ correlation***	**Effect strength**	**Significance/ correlation***	**Effect strength**	**Significance/ correlation***	**Effect strength**
Habitat	n.s.	0.668	n.s.	0.585	xx/pelagic = +, littoral = −	0.399
Season	x/ spring = +, summer = +, autumn = −, winter = −	0.379	n.s.	0.218	xxx/spring = −, summer = +, autumn = +, winter = −	0.519
TL	n.s.	0.139	xxx/+	0.353	xx/+	0.177
Habitat* TL	n.s.	n.a.	n.s.	n.a.	n.s.	n.a.
Season* habitat	n.s.	n.a.	xx/n.a.	n.a.	n.s.	n.a.

*xxx = $p < 0.0001$; xx = $p < 0.01$; x = $p < 0.05$; +, positive correlation; −, negative correlation; effect strength is a dimensionless factor assessing the impact of a variable in the model formula. Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n.a., not applicable; n.s., not significant; TL, total length.

3.3 | DHA estimated consumption

Sticklebacks feeding in the littoral zone of ULC during autumn consumed food items containing roughly 0.1% DHA by wet weight

(Table 3). Most of this was derived from copepods, the smaller parts originated from medium-sized cladocerans (Table 3). The calculated content of 0.093% g DHA kg^{-1} diet (Table 3) equates to 18.7% of the 0.5% requirement considered necessary for marine fish species

TABLE 2 Diet composition of sticklebacks (food source expressed as a percentage of total prey items eaten during spring, summer, autumn, and winter, and as the mean number of consumed individuals per stickleback ±S.D.) sampled in the pelagic and littoral zones of Upper Lake Constance.

	Spring		Summer		Autumn		Winter	
Diet	Littoral	Pelagic	Littoral	Pelagic	Littoral	Pelagic	Littoral	Pelagic
Copepods	89.02% 125 ± 204	85.97% 371 ± 254	14.64% 7 ± 7	12.12% 15 ± 12	19.03% 28 ± 39	81.44% 596 ± 304	83.56% 122 ± 150	56.06% 172 ± 76
Cladocerans	1.28% 2 ± 3	13.13% 57 ± 29	8.37% 4 ± 3	87.46% 107 ± 116	78.41% 114 ± 71	18.56% 135 ± 79	11.92% 17 ± 21	43.89% 135 ± 61
Benthic organisms	9.34% 13 ± 8	0.61% 6 ± 6	73.43% 35 ± 27	0.33% 2 ± 1	2.56% 4 ± 11	0% 0	4.45% 7 ± 5	0.02% 0 ± 1
Fish (eggs, larvae)	0.36% 1 ± 1	0% 0	2.93% 1 ± 4	0% 0	0% 0	0% 0	0% 0	0.03% 1 ± 0
Terrestrial organisms	0% 0	0.29% 2 ± 1	0.63% 1 ± 1	0.08% 1 ± 0	0% 0	0% 0	0.07% 0 ± 1	0% 0

(Glencross, 2009). Assuming that sticklebacks feed only on small cladocerans (*B. coregoni* and *B. longicornis*), the calculated content of DHA kg^{-1} diet decreases to 0.083%, which equates to 16.6% of the requirement while assuming that sticklebacks feed only on large cladocerans (*B. longimanus* and *L. kindtii*), the calculated content of DHA kg^{-1} diet increased to 0.182%, which equates to 36.4% of the requirement.

In the pelagic zone, the total proportion of 3.292% g DHA kg^{-1} diet (Table 3) equates to 658.3% of the requirement. DHA derived from the consumption of copepods was responsible for 98.5% of this intake, with cladocerans (of a mean weight of 3.7 μg) contributing 1.5% (Table 3). If it is assumed that sticklebacks fed only on small cladocerans, this proportions dropped to 0.2%, and if it is assumed that only large cladocerans were eaten by sticklebacks, this proportion increased to 37.5%. In both cases the daily demands were over-fulfilled (102% and 817% of the daily requirement).

4 | DISCUSSION

A key insight from the present study is the significantly higher concentration of DHA, one of the most important fatty acids (Sargent et al., 1995), in pelagic sticklebacks compared to littoral sticklebacks from ULC. This difference was greatest during autumn and is most likely related to foraging on different prey. Stomach content data suggest that littoral sticklebacks feed mainly on cladocerans during autumn, a food source almost completely deficient in DHA (Burns et al., 2011). In contrast, sticklebacks in the pelagic zone tend to feed almost exclusively on copepods, which, in freshwater lakes (Persson & Vrede, 2006) including ULC (Hartwich et al., 2013), tend to be rich in DHA. Studies of feeding behavior and food preference in pelagic sticklebacks from Lake Constance underline the importance of copepods as an autumn food resource (Bretzel et al., 2021; Ogorelec et al., 2022b); however, no data from the literature are available for littoral feeding behavior of sticklebacks in ULC. Data from other lakes show that benthic-orientated sticklebacks feed mainly on DHA-deficient prey such as chironomids, whereas pelagic sticklebacks forage more regularly on DHA-rich prey, that is, copepods (Ishikawa et al., 2021). How such differences might impact the fitness of littoral sticklebacks is uncertain, but it might be expected that given their recent marine ancestry, Lake Constance sticklebacks will struggle to meet their nutritional demand for DHA from their common prey. A diet that delivers around 0.1% g DHA kg^{-1} during autumn accounts for only one fifth of the estimated 0.5% g DHA kg^{-1} generally required by marine fish (Glencross, 2009). This outcome is robust to the selected size of the consumed cladocerans, because even if sticklebacks were exclusively feeding on large cladocerans in addition to their copepod diet, they still could not fulfill their daily demand. In contrast, sticklebacks feeding in the pelagic waters of ULC consume approximately seven times more DHA (3.3% g DHA kg^{-1} diet) than required. And even if assuming they only feed exclusively on small cladocerans, the proportion of consumed copepods is sufficient to distinctly exceed their daily requirement of DHA. To clarify the situation, it would be valuable to ascertain the exact species-specific demands

TABLE 3 Mean number of consumed prey items of littoral and pelagic sticklebacks in Upper Lake Constance during autumn, their dry weight, and the DHA content by dry and wet weight.

Habitat	Food source	Consumed individuals (n)	Dry weight per individual (mg)	Total dry weight (mg)	Consumed dry mass (%)	% DHA in dry diet	% DHA in wet diet
Littoral	Copepods	28	0.0027	0.076	1.677	0.860	0.072
Littoral	Cladocerans	114	0.0038	0.433	9.608	0.238	0.020
Littoral	Chironomids	4	1.000	4.000	88.715	0.021	0.002
Littoral	All	146					0.093
Pelagic	Copepods	596	0.0027	1.609	75.827	38.899	3.242
Pelagic	Cladocerans	135	0.0038	0.513	24.173	0.599	0.050
Pelagic	Chironomids	0					0
Pelagic	All	731					3.292

Abbreviation: DHA, docosahexaenoic acid.

of DHA for the sticklebacks of ULC through controlled follow-up studies in which sticklebacks are fed with dose–response test diets to identify the precise DHA levels required for parameters like growth, immune response, and survival. Nonetheless, the present data strongly point to enhanced availability of DHA as a distinct nutritional benefit of feeding in pelagic waters. Further advantages may derive from increased absolute lipid levels, enhanced supply of ALA and EPA, and improved n-3/n-6 ratios, especially during summer, autumn, and winter. Sticklebacks from the pelagic zone showed significantly higher EPA levels during summer and autumn than individuals caught in autumn and winter in the littoral zone, and the total number of copepods and cladocerans consumed by sticklebacks was distinctly higher in the pelagic zone than the littoral zone, independent of season. Overall, both diet and lipid value data indicate that it could be highly beneficial to feed in pelagic waters: with more and higher-quality food available year-round, and especially with respect to DHA, which is highly deficient in the littoral zone. This is especially true in autumn, the season that coincidentally shows the greatest numbers of sticklebacks in the pelagic zone (Gugele et al., 2020). For sticklebacks feeding in the littoral zone in autumn, they might be expected to suffer nutritional deficits. These results thereby support the hypothesis that the invasion of the pelagic waters of Lake Constance was triggered or promoted by nutritional benefits; the shift making it is much easier for individuals to balance their diet, find the optimal n-3/n-6 ratio, and most importantly to overcome DHA deficits. Nevertheless, the question why not all sticklebacks move during times of DHA deficits into the pelagic zone remains open. The abundance of sticklebacks in the pelagic zone is very high, probably causing density-dependent effects like increased intraspecific competition, which might hinder parts of the stickleback population to enter the pelagic zone, or guide them back to the littoral zone. Further studies, for example, including tagged specimens, may help clarify this question.

It has previously been shown that DHA deficiency can result in reduced growth and increased mortality (Sargent et al., 1995). Neither of these impacts could be adequately addressed with the field data at hand. In autumn, the mean length of sticklebacks from the littoral zone was distinctly greater than that of pelagic sticklebacks

(supplements, Table S1). However, this result is biased by the fact that young-of-the-year sticklebacks migrate in high numbers into the pelagic zone during autumn, resulting in a reduction in the mean length of sticklebacks in this habitat (Gugele et al., 2020). In contrast, during other seasons, pelagic sticklebacks were on average longer and heavier by wet weight than littoral specimens (see supplements, Table S1). However, when the influence of length was controlled in the models, the results show that the amount of ALA, EPA, or DHA was less influenced by length than by habitat or season. Generally, the amount of ALA, EPA, and DHA was positively correlated with total length, as reported by other authors (Iverson et al., 2002; Mohanty et al., 2012). However, in the absence of age data and with large variation in the timing of egg deposition among sticklebacks in ULC (from April to end of July), it was not possible to calculate separate growth rates for pelagic and littoral sticklebacks.

Genetic studies by Dahms et al. (2022) identified multiple outlier loci between littoral and pelagic ecotypes across the genome, which may be early signs of sympatric speciation. Therefore, the hypothesis arose that a benthic-pelagic species pair in ULC with reduced genetic exchange in this system exists (cf. Dahms et al., 2022). Our data support this hypothesis, highlighting differences in the diets and fatty acid profiles of littoral and pelagic groups. Our PCA based on the fatty acid profiles demonstrates a clear separation between pelagic and littoral sticklebacks (especially during autumn), and our LDA does so with a certainty of more than 90%. The differences in EFA profiles, especially DHA, become obvious in autumn. It appears likely that after spawning together in the littoral zone, the stickleback populations separate into littoral and pelagic groups, with different diets and consequently different EFA profiles. Mixing of both populations after spawning and parental care seems unlikely, as this would result in very similar DHA values during summer. However, given that all sticklebacks spawn in the same shallow waters of ULC (Gugele et al., 2020), the mechanism of separation (e.g., mate choice) is not known at this stage. Nevertheless, our results together with the genetic evidence by Dahms et al. (2022) point to a rare case of littoral-pelagic ecotype divergence, with food selectivity as an important factor.
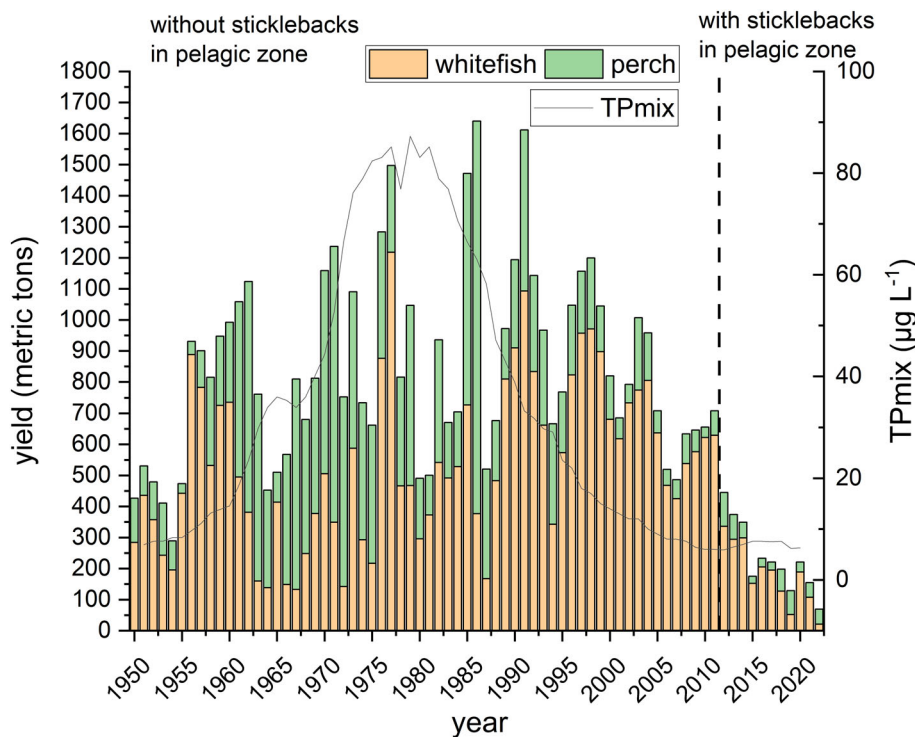
FIGURE 3 Annual commercial yield of perch (green bars, *Perca fluviatilis* L.) and whitefish (orange bars, *Coregonus* spec.) from Upper Lake Constance, and volume-weighted average PO_4-P content during spring mixing (TPmix, line) during the time without (1950–2011) and with (2012–2022) sticklebacks in the pelagic zone.

Given that the apparently superior dietary opportunities of the ULC pelagic zone have existed since sticklebacks arrived >70 years ago (Roch et al., 2018), a question remains as to why the invasion did not happen earlier. However, the niche width of a species is balanced between the diversifying forces of intra- and interspecific competition (Taper & Chase, 1985; Van Valen, 1965). When those forces change, the niche width may also change, and the form of niche expansion known as "ecological release" becomes possible (Bolnick et al., 2010; Feinsinger & Swarm, 1982; Persson, 1985). This phenomenon has been observed for several different fish species, most usually when species living in highly competitive environments have an opportunity to migrate into habitats with fewer competitors (Persson & Hansson, 1999; Robinson et al., 2000). It might be supposed that the sudden, massive invasion of the pelagic zone of ULC by sticklebacks in 2012 may have been triggered by a reduced density of competitors, that is, pelagic whitefish. Interestingly in ULC, the yield of commercial whitefish, previously a highly competitive zooplanktivorous keystone species (Ogorelec et al., 2022a), fell by more than 50% in 2012 with further declines thereafter, for unknown reasons (Figure 3). From 2012 onwards there has been a massive reduction in the year-class strength of young whitefish (Rösch et al., 2018). Furthermore, yields of perch (*Perca fluviatilis* L.), another zooplanktivorous fish, were also very low in 2012 (Eckmann et al., 2006) (Figure 3), and autumn pelagic densities of this species stayed low in the ensuing years, especially in the upper 25 m of the water column, while remaining high in the littoral zone (Gugele et al., 2020). Thus, from 2012 on, with whitefish and perch biomasses unusually low (as reflected in fishery yields, Figure 3), sticklebacks may have been presented with an "underpopulated" habitat with minimal interspecific competition for food.

There are other rare but well-documented examples of sticklebacks making lasting takeovers of large aquatic habitats. In the Baltic Sea the species was observed to migrate purposefully to the spring spawning grounds of perch and pike, where they fed on eggs and larvae. This led to significant stock reductions of the latter two species and a population increase of sticklebacks (Bergström et al., 2015; Byström et al., 2015; Ljunggren et al., 2010). In ULC in the years after the invasion in 2012, similar patterns were observed when sticklebacks began to migrate to the spawning grounds of whitefish (Gugele et al., 2020). Lake Constance sticklebacks are routinely piscivorous (Gugele et al., 2023) and target whitefish larvae and eggs during and shortly after that species' spawning period (Baer et al. 2021). In the present study, remains of fish eggs and larvae in the guts of stickleback were found in all seasons except autumn, but they were most abundant in spring. Due to the fact that freshwater fish accumulate significant quantities of DHA (Scharnweber et al., 2021), this behavior may help optimize EFA uptake for sticklebacks. It is possible that such predation pressure may directly impact whitefish recruitment (Baer et al., 2021) and thereby also reduce future interspecific competition in the pelagic zone, as the declining density of whitefish from 2012 suggests.

In contrast to other parts of the world, sticklebacks have never been actively targeted by fisheries in Lake Constance (Ojaveer, 1999). However, preliminary attempts have been made to test the potential for population control by trawling (Gugele et al., 2020). The results of that trial suggested early autumn would be the best time for stock reduction, with highest abundance of sticklebacks and low potential for by-catch (0%–0.04%) of other species. If such trawling were to happen, it would impact mainly the pelagic sticklebacks that feed on copepods, probably likely resulting in an overall reduction in feeding

pressure on the pelagic zooplankton community. This "niche compression" for sticklebacks might be highly beneficial for whitefish, the natural keystone species (Gaedke, 1998), with reduced interspecific competition, leading to greater accessibility of DHA-rich prey and, most likely, improved growth rates (DeWeber et al., 2022) and fitness (Muir et al., 2014).

Being of recent marine ancestry (Hudson et al., 2021), sticklebacks from Lake Constance possess low numbers of the FADS2 genes and a limited ability to synthetize DHA (Hudson et al., 2022). They would therefore benefit significantly from feeding on the DHA-rich prey found in the pelagic zone, and this is a likely factor in their niche expansion in ULC. It seems likely that similar outmigrations elsewhere will also be limited to sticklebacks of recent marine origin, whether they arrive by stocking or natural immigration. The risk of comparable invasions in EFA-poor freshwater ecosystems where sticklebacks have lived for many thousands of years is therefore small, as such populations have had time to accumulate significantly more copies of the FADS2 genes. On the contrary, managers of stickleback-free ecosystems should be particularly alert to the risk of the invasion by marine sticklebacks, to avoid similar situations with dramatic consequences for the natural food web and long-established fisheries.

5 | CONCLUSIONS

To conclude, a combination of two factors provides an explanation for the recent invasion, hyperabundance, and persistence of sticklebacks in the pelagic of ULC: (a) low density of other zooplanktivorous fish in pelagic waters leading to decreased interspecific competition, and therefore (b) access to DHA-rich prey, that is, copepods, providing a dietary advantage. However, factors leading to the low density of other zooplanktivorous fish, especially whitefish, in the pelagic zone in 2012, and thus to the "wide open door" for stickleback niche expansion, need further investigation. Follow-up studies are also needed to elucidate mechanisms of habitat segregation further, and to identify functional connections. The findings of this study illustrate that consideration of food web factors generally, and analyses of fatty acid composition in particular, can be important in investigating habitat shifts and invasion success in species such as sticklebacks. These principles hold for aquatic ecosystems beyond Lake Constance, given the need to understand biological community shifts in a time of multiple anthropogenic impacts and climate change, particularly if they result in ecological phase shifts with strong consequences for fisheries and other ecosystem services.

AUTHOR CONTRIBUTIONS

Alexander Brinker developed the initial ideas. Sabrina Ziegaus and Mark Schumann provided the analysis of the lipid acid values. Jan Baer, Juergen Geist, and Alexander Brinker undertook the data analysis and the writing and preparation of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.