RESEARCH ARTICLE

Stable isotope values and trophic analysis of invasive three-spined stickleback in Upper Lake Constance points to significant piscivory

Sarah Maria Gugele[1,2,3], Jan Baer[1], Christina Spießl[1,4],
Elizabeth Yohannes[2,5], Steve Blumenshine[6], Barnaby J. Roberts[1,2],
Mario R. Mota-Ferreira[1], Alexander Brinker[1,2]

1 *Fisheries Research Station Baden-Württemberg, Argenweg 50/1, 88085, Langenargen, Germany* **2** *University of Constance, Institute for Limnology, Mainaustraße 252, 78464, Konstanz, Germany* **3** *State Government of Vorarlberg, Division Agriculture and Rural Area, Department Fisheries and Water Ecology, Auhafendamm 1, 6972, Hard, Austria* **4** *Aquatic Systems Biology Unit, Technical University of Munich, TUM School of Life Science, Department of Life Science Systems, Mühlenweg 22, 85354, Freising, Germany* **5** *Max-Planck Institute of Animal Behavior, Am Obstberg 1, 78315, Radolfzell, Germany* **6** *CSU-WATER, 2703 E. Barstow, MS JC133, Fresno, CA 93740, USA*

Corresponding author: Jan Baer (jan.baer@lazbw.bwl.de)

Citation: Gugele SM, Baer J, Spießl C, Yohannes E, Blumenshine S, Roberts BJ, Mota-Ferreira MR, Brinker A (2023) Stable isotope values and trophic analysis of invasive three-spined stickleback in Upper Lake Constance points to significant piscivory. NeoBiota 87: 73–102. https://doi.org/10.3897/neobiota.87.100355

Abstract

The three-spined stickleback *Gasterosteus aculeatus* was introduced into Lake Constance in the 1940s and occupied a limited range until late 2012. Since then the species has expanded from a solely littoral habitat in Upper Lake Constance, but now makes seasonal migrations into the pelagic zone. This behavioral change has been accompanied by a drastic increase in stickleback abundance. In order to integrate information about feeding of sticklebacks in Upper Lake Constance over two consecutive years, stomach content analysis was combined with seasonal stable isotope analysis on two types of tissue (muscle and liver). Isotope values were also obtained for zooplankton, whitefish larvae and eggs. We calculated the contribution of potential food sources for sticklebacks' diet using a Bayesian mixing model (SIMMR). Furthermore, we determined stickleback trophic position, and $\delta^{15}N$ and $\delta^{13}C$ values were compared with those of other fish species of Lake Constance. The results of the Bayesian model as well as the stomach content analysis showed clear evidence of stickleback predation on fish eggs and larvae. Stickleback $\delta^{15}N$ values were elevated during winter and comparable to those of piscivorous pike, while $\delta^{15}N$ values of zooplankton were reduced, and those of whitefish larvae were similar to those of sticklebacks after accounting trophic fractionation of N isotopes. Trophic position calculations further identified sticklebacks as piscivorous,

while the δ¹³C values of the liver and stomach content analysis suggests that a benthic-pelagic species pair may exist in Lake Constance. These findings support the hypotheses that sticklebacks in Lake Constance can display piscivorous feeding behaviour on sympatric fish species, most likely whitefish larvae and eggs.

Keywords

Carbon littoral source, *Gasterosteus aculeatus*, larvae predation, niche overlap, trophic position, whitefish

Introduction

Aquatic invasive species (AIS) are of concern worldwide due to their devastating impacts on ecosystems and economies (Havel et al. 2015; Treanor et al. 2017). Furthermore, the number of AIS in freshwater ecosystems will increase in the future because AIS are still translocating outside of their native ranges and in many cases AIS have not fully occupied their potential ranges (Strayer 2010a). Additionally, the introduction of AIS drives changes in the structure and functioning of existing ecosystems and complicates their management (Strayer 2010b). Therefore, knowing the trophic niches and consumption impacts of AIS is crucial to addressing the implications for food webs and ecosystems (Bodey et al. 2011). This is especially true in Lake Constance, one of the largest lakes in Central Europe, where the three-spined stickleback (*Gasterosteus aculeatus* L., 1758), hereafter referred to as stickleback, was introduced accidentally in the mid-1940s (Roch et al. 2018), because it is known that sticklebacks could have detrimental effects on native fish community if hyperabundant stickleback population established (Bergström et al. 2015; Byström et al. 2015). For over half a century the species existed almost exclusively in small swarms near the shore but, since the end of 2012, has expanded from littoral habitats into the pelagic zone of Upper Lake Constance (ULC) and undergone a drastic increase in abundance (Eckmann and Engesser 2019; Gugele et al. 2020). Only two years later, in September 2014, stickleback shoals represented 96% of all pelagic fish and 28% of the total pelagic fish biomass (Eckmann and Engesser 2019), a globally unique situation for a large and deep oligotrophic lake. After 2014, average stickleback abundances plateaued to between 1280 and 7990 individuals/ha in the pelagic zone of Lake Constance (Eckmann and Engesser 2019), with a peak abundance of 19100 individuals/ha recorded in September 2017 (Gugele et al. 2020). Recent stomach content analysis of sticklebacks has identified zooplankton (*Daphnia* spp.) as their main prey (Ogorelec et al. 2022). Since the native pelagic whitefish (*Coregonus wartmanni* Bloch, 1784) also mainly feed on zooplankton (Eckmann and Rösch 1998; Ogorelec et al. 2022), high stickleback abundances may have deleterious impacts on native whitefish via interspecific competition. By 2015 the yield of native whitefish, the main target species of the local fishery (Baer et al. 2017), had declined drastically from around 300–600 mt (metric tons) before stickleback invasion to less than 150 mt (Roch et al. 2018). At the same time, the average weight of whitefish (age classes 3–5) captured in the pelagic zone has undergone a 17%–51% decline (arithmetic mean: 33%) (Rösch et al. 2018). Besides, integrated bioenergetics

and growth modelling revealed increasing competition with sticklebacks as a chief factor in reduced growth of whitefish in the lake (DeWeber et al. 2022).

There is also evidence that sticklebacks in Lake Constance may impact whitefish as predators of whitefish larvae and eggs (Roch et al. 2018; Rösch et al. 2018; Ros et al. 2019; Gugele et al. 2020; Baer et al. 2021). Whitefish spawn in November and December (Eckmann 1991) and whitefish larvae with a total length below 4 cm, which resembles the gape limitation of sticklebacks (cf. Roch et al. 2018) can be found in Lake Constance from February until the end of May (Eckmann 1989). Additionally, stickleback aggregations were documented in habitats where the newly hatched whitefish larvae were found from March to April, both in the pelagic zone and near the shoreline (Gugele et al. 2020). However, it should be kept in mind that pelagic whitefish spawn near the water surface in the open waters of the lake (Eckmann 1991) and their eggs sink relatively quickly to depths as great as 250 m (Eckmann and Rösch 1998), leaving a short time window to predate on them. Nevertheless, sticklebacks are able to locate whitefish eggs even during complete darkness (Baer et al. 2021) but only forage in depths down to around 30 m (Eckmann and Engesser 2019; Gugele et al. 2020), so any consumption of whitefish eggs must take place before they sink and become unattainable.

Furthermore, the distribution of the whitefish larvae is normally patchy (Ransom et al. 2022) and evidence of predation of whitefish larvae by sticklebacks may be only coincidentally available for single sticklebacks. These factors mean the role of sticklebacks in Lake Constance food web, including their impact on native lake whitefish, is not entirely simple, and predation of whitefish eggs and larvae may be a rare behaviour, posing only a minor threat to naturally hatched or stocked whitefish larvae. However, each year 100–200 million larvae between 18–25 mm in size are stocked in the littoral and pelagic zone across the whole lake (Baer et al. 2023). Consequently, it is hypothesized that the stocked larvae may act as an easily available, nutrient and energy rich food source in the oligotrophic water that might facilitate the stickleback invasion's development, permitting the species to fully exploit the pelagic habitat while simultaneously reducing recruitment of whitefish (Roch et al. 2018).

Therefore, to get more insight into the feeding ecology of sticklebacks in ULC, we performed monthly stable isotope analysis of stickleback muscle and liver tissue over a two-year period. In addition to providing information on the diet of an organism over time, stable isotope analysis can illuminate feeding habitats, quantify complex interactions, and be used to track elements, energy, or mass through food webs and ecosystems (Post 2002; Janjua and Gerdeaux 2011). Stable isotope ratios of carbon and nitrogen have been applied extensively in food web studies over the past 30 years (Janjua and Gerdeaux 2011) and have proved useful in assessing the impacts of invasive species on trophic structures (Vander Zanden and Rasmussen 1999; Bodey et al. 2011). In the present study, stable isotope analysis was carried out on stickleback and their potential prey in the pelagic zone (zooplankton, whitefish eggs and larvae) alongside classic stomach content analysis and stable isotope mixing models to address the following four research questions: 1) Do the $\delta^{15}N$ values and trophic position of sticklebacks reflect the seasonal feeding of whitefish larvae and eggs or that of other

food sources, such as zooplankton? 2) Where do invasive sticklebacks sit relative to other fish species in the trophic structure of Lake Constance? 3) How does the trophic position of sticklebacks in Lake Constance compare to that of conspecifics in similar ecosystems? 4) Are there any differences in isotopic signature between sticklebacks caught in littoral and pelagic habitats? The answers to these questions have implications beyond Lake Constance because similar tendencies, i.e. increased predation by introduced sticklebacks on native fish species, are also being observed elsewhere (Ljunggren et al. 2010; Bergström et al. 2015; Byström et al. 2015).

Materials and methods

Ethics statement

Approval of the present study by a review board institution or ethics committee was not necessary because all fish were caught under permits issued by the local fisheries administration (Regierungspräsidium Tübingen), by qualified (license-holding) personnel subject to regular checks by the local fisheries administration (Regierungspräsidium Tübingen). All fish were caught according to the German Animal Protection Law (Tierschutzgesetz § 4) and the ordinance on slaughter and killing of animals (Tierschutzschlachtverordnung § 13).

Sampling of sticklebacks

Lake Constance is part of the Rhine drainage basin and is bordered by Austria, Germany and Switzerland (47°38'N, 9°22'E). The total surface area of 536 km² is divided between the large (472 km²), deep (>250 m) Upper Lake (ULC) and the smaller (63 km²), shallower Lower Lake (LLC). Due to missing data and lack of knowledge about the stickleback situation in LLC and different type of lake (warm, mesotrophic), this basin was excluded in the present study. Thus, the current study only focusses on warm monomictic, large oligotrophic pre-alpine basin of ULC. The fish community of ULC comprises a minimum of 30 species (Eckmann and Rösch 1998) of which about 10 are targeted by professional fishermen (Rösch 2014). Of these, whitefish (*Coregonus* spp.) are the most economically important, and fisheries management is based on routine monitoring of this important group (www.ibkf.org). An overview of the fisheries situation is given by Baer et al. (2017).

Stickleback sampling of ULC was conducted monthly, from March 2017 until November 2018, using littoral and pelagic gillnets with mesh sizes of 10–12 mm. All nets had a height of 3 m, while length varied with mesh size: 30 m for nets with 10 mm mesh and 15 m for the 12-mm mesh net. All pelagic nets were deployed to drift freely behind the nets used in the monthly monitoring of whitefish (mesh sizes 36–44 mm), at depths of 3–15 m according to the areas of greatest stickleback abundance recorded during hydroacoustic surveys (Gugele et al. 2020). Benthic nets were set at depths from 6 to 20 m. All nets were set overnight, with a soak time of about 15 h. The overall

catch in the pelagic gillnets (number per unit effort, NPUE, as n/m² per net) was low from January to September (NPUE 0.03–0.25) and peaked between November and December (NPUE 1.1–7.7), the spawning season of whitefish (Baer et al. 2022b). Catches in the benthic gillnets were highest during the stickleback spawning season between May and July (NPUE 11.8–59.0) and a second peak was again observed during November and December (NPUE 6.0–79.0; Baer et al. 2022b).

10 to 34 samples of stickleback white muscle and liver tissue were taken from each monthly catch. Catches of fewer than 10 individuals (recorded in August and September each year, plus April 2017, July 2017 and October 2018) were excluded from analysis. C and N stable isotope analysis was run on 275 sticklebacks. Of these, 193 were caught in the littoral zone and 82 in the pelagic zone. All fish were euthanised 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 mm), weighed to the nearest 0.01 mg and sex was recorded. Some sticklebacks were infested with the pseudophyllidean cestode *Schistocephalus solidus*, and because it is known that the health status of a fish can have direct effects on the stable isotope values (Karlson et al. 2018), a combined parasite-to-host biomass ratio (parasite index, *PI*) was calculated as an indirect measure of the severity of infestation (Baer et al. 2022b). All parasites were counted per host, blotted, and weighed to the nearest 0.01 mg and *PI* was determined using the formula

$$PI = P/H \qquad\qquad (1)$$

where P is the total weight of the parasites and H is the mass of the host without the parasite.

Due to internal procedures, 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 (TL 68 mm ± 6 mm standard deviation SD). Samples were taken during all four seasons (autumn 2017, winter 2017, spring 2018, and summer 2018), and for each season and each habitat, the gut contents of at least 10 individuals were analysed with the exception of some sampling dates (20 during winter and summer in both pelagic and littoral zone, and 19 during spring in the pelagic zone). Food items were identified and counted in a zooplankton counting chamber and categorised into five groups, namely copepods (nauplii, copepodites and copepods of Cyclopoida, Calanoida and Harpacticoida); *Bosmina* (all members of the genus *Bosmina*); other herbivorous/detritivorous cladocerans (*Daphnia* spp., *Diaphanosoma brachyurum* and Chydoridae); predatory cladocerans (*Bythotrephes longimanus* and *Leptodora kindtii*); fish (eggs and larvae) and other (Chironomidae, Annelida, Bivalvia, Collembola, Ceratopogonidae, Ephemeroptera, adult Heteroptera, Hydrachnidia, adult Mysidae, Nematoda, Ostracoda, Plecoptera, Simuliidae and Trichoptera). Diet quantification of sticklebacks followed the use of the numerical method and diet was calculated as a percentage of the total number of prey items eaten per stickleback (Amundsen and Sánchez-Hernández 2019). Furthermore, we calculated for each season and habitat the mean number (± standard deviation SD) of consumed food items per category.

Stable isotope analysis of sticklebacks, the diet of sticklebacks and other fish species

Although stable isotope analyses on fish C and N are generally performed using muscle tissue alone, the more rapid turnover of liver tissue means isotope signatures there reflect more recent feeding (Boecklen et al. 2011). Repeated at suitable intervals, in this case monthly, these tissue-based differences in signal lag can help resolve the timing of hard-to-predict peaks in seasonal prey availability, such as that of whitefish eggs and larvae.

Tissue samples from sticklebacks caught in 2017–18 were prepared for analysis by drying them in an oven at around 60 °C for 48 hr and grinding them into a fine powder. Lipid extraction was performed on the samples because some studies have shown that in tissues with C:N ratios greater than 3.5, such treatment reduces bias in $\delta^{13}C$ values (Skinner et al. 2016). Therefore, lipid extraction of samples was conducted by adding 200 μL of 2:1 Chloroform:Methanol mixture to the powdered tissue. Afterwards, samples were vortexed and centrifuged for two minutes at 4000 rpm. The excess sample was discarded, and centrifugation was repeated 2–4 times until the sample colour changed from yellow to colourless. Samples were then washed in 200 μL Milli-Q water, followed by further vortexing and centrifugation for 2 min (4000 rpm). Again, the excess sample was discarded, and washing was repeated multiple times during the lipid extraction. Next, samples were dried in a fume hood for 48 hr, then ground again to a fine powder and weighed (ca. 0.7 mg) to the nearest 0.001 mg in tin capsules, using a microbalance (Chyo Balance Corporation, Kyoto, Japan).

To measure isotopic ratios samples were combusted in a vario MICRO cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The emerging gases were separated via gas chromatography and passed into a Micromass Isoprime isotope mass spectrometer (Isoprime Ltd., Cheadle Hulme, UK) for determination of the 13C/12C and 15N/14N ratios (R). Measurements are reported in δ-notation ($\delta^{13}C$, $\delta^{15}N$) in parts per thousand deviations (‰), where $\delta = 1000 \times (R_{sample}/R_{standard} - 1)$ relative to the Pee Dee Belemnite (PDB) for carbon and atmospheric N_2 for nitrogen. Two sulphanilamide (Isoprime internal standards) and two casein samples were used as laboratory standards for every 10 unknowns in the sequence. Replicate assays of internal laboratory standards indicated measurement errors (SD) of ± 0.05% and 0.15% for $\delta^{13}C$ and $\delta^{15}N$, respectively.

To compare the isotopic values of sticklebacks with those of other species of fish in ULC, 128 additional sampled of muscle tissue were analysed from bleak (*Alburnus alburnus* L., 1758; n = 18; mean TL 60 mm ± 6 mm SD) roach (*Rutilus rutilus* L., 1758; n = 34; mean TL 263 mm ± 71 mm SD); rudd (*Scardinius erythrophthalmus* L., 1758; n = 12; mean TL 217 mm ± 40 mm SD); tench (*Tinca tinca* L., 1758; n = 19; mean TL 226 mm ± 147 mm SD); pelagic whitefish (n = 21; mean TL 308 mm ± 55 mm SD); burbot (*Lota lota* L., 1758); n = 19; mean TL 374 mm ± 38 mm SD); and pike (*Esox lucius* L., 1758; n = 5; mean TL 289 mm ± 44 mm SD). All were sampled with gill nets during August and September 2020. All fish were euthanised with an overdose of clove oil (1 mL L^{-1}) and a gill cut. Using data from stomach content analyses carried

out in prior studies, all fish species were divided into different feeding guilds: white-fish were categorised as zooplanktivorous; burbot as partly piscivorous (Hansen et al. 2022); pike as piscivorous; roach, bleak, and tench as benthivorous/insectivorous; and rudd as herbivorous (Baer et al. 2022a). Due to the stomach content analysis from this study, sticklebacks were categorised as omnivorous. Samples of white muscle were excised and frozen (-20 °C) until further processing.

For calculating the trophic position of sticklebacks, pike and burbot, faucet snails (*Bithynia tentaculata*, n = 10) were collected in August 2020 from the littoral habi-tat and used for the estimation of the littoral baseline (=$\delta^{15}N_{lit.\ base}$). Quagga mussels (*Dreissena rostriformis bugensis*, n = 200) were collected from free-standing piles in the pelagic zone 0.5–2 m depth in the upper mixed layer of Lake Constance and used for the estimation of the pelagic baseline (=$\delta^{15}N_{pel.\ base}$).

To gain more insight into the isotopic signatures of potential stickleback prey dur-ing winter and spring, five samples of zooplankton (wet weight (g): mean = 2.34, SD = 2.53) were netted with 300 μm mesh in the epilimnion of ULC, first in October and December 2021, then in February, March, and early May 2022. An abundance of pollen in the lake epilimnion during April 2022 prevented an uncontaminated sam-ple being taken during that month. In addition, in December 2021, 36 females of *C. wartmanni* (pelagic whitefish) and 42 *C. macrophthalmus* (benthic whitefish) were caught during spawning at their spawning grounds in ULC as part of routine sam-pling conducted by the Fisheries Research Station of Baden-Württemberg. To get the isotopic signature of whitefish eggs and larvae, a small sample of eggs was taken from each individual and larvae hatched from the eggs of pelagic whitefish (kept at a hatch-ery facility in Langenargen, Baden-Württemberg) were also sampled. After hatching, larvae were held in rearing vats until the yolk sac was partly absorbed and larvae had begun to exhibit normal swimming behaviour. From these non-fed, free-swimming larvae, four subsamples of multiple individuals (*n*: mean = 172, SD = 100) were taken and euthanised with an overdose of carbonated water. Clove oil was avoided in this in-stance as it may have biased isotopic readings, and unlike larger fish, the delicate larvae cannot be easily washed without damage. To remove potential biases due to the length of time between the main stickleback sampling (2017–2018) and the sampling of the zooplankton, whitefish eggs and larvae (2021–2022) (Fig. 1), a further 50 sticklebacks were caught for additional stable isotope analysis in January, February and April 2022 (25 each in the pelagic and littoral zone, in total 150) using gill nets.

Samples of sticklebacks caught in 2022 and other fish were prepared for stable isotope analysis via freeze drying at -50 °C under pressurisation (<1 mbar), and ground to homogenous powder using a mixer mill. Whitefish egg samples were dried at 60 °C in a drying oven, before being stored in a glass desiccator filled with silica desiccator beads; the desiccator was stored in a cool, dark environment. Samples of plankton and pelagic whitefish larvae were dried overnight in a drying oven (60 °C), then stored in freezers at -20 °C. Each individual dried sample of plankton, whitefish larvae and whitefish eggs were then separately homogenised using a BeadRupture Homogenizer (Omni International, Kennesaw, Georgia, United States) by dispensing the sample
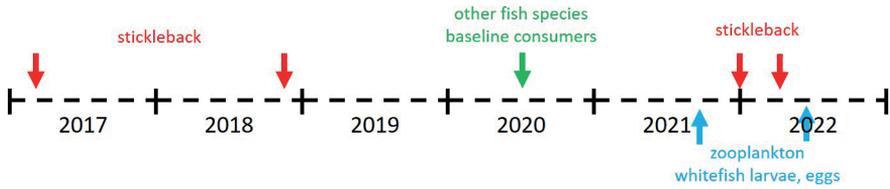
Figure 1. Timeline of sampling in the present study.

into a plastic microtube, with a number of sterilised metal beads (<0.5 mL), and processing the sample into a fine, homogenous powder. The times and speeds used in the homogenisation process were adapted according to the individual condition of the samples. After homogenisation, samples were stored in freezers at -20 °C. Sample powder (0.3–0.4 mg) was weighed into tin capsules and combusted in an isotope ratio mass spectrometer (Delta plus, Finnigan MAT, Mas-Com GmbH, Bremen, Germany), interfaced (viaConFlo II, Finnigan MAT, MasCom GmbH, Bremen, Germany) with an elemental analyser (EA 1108, CarloErba, Thermo Fisher SCIENTIFIC, Milan, Italy). Because the mean C:N values (±SD) of all fish samples were below 3.5, lipid extraction of fish muscle tissue was not conducted (Matthews et al. 2010; Skinner et al. 2016). Measurements are reported in δ -notation ($\delta^{13}C$, $\delta^{15}N$) in parts per thousand deviations (‰), where δ = 1000 × (Rsample/Rstandard – 1) relative to the Pee Dee Belemnite (PDB) standard for carbon and atmospheric N_2 for nitrogen. Finely ground animal horn (keratin) was used as a laboratory standard for every 10 unknowns in sequence. Replicate assays of internal laboratory standards indicated measurement errors (SD) of ± 0.05% and 0.15% for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Trophic position of sticklebacks

The trophic position of sticklebacks was calculated according to the protocol established by Post (2002) for a two-source food web:

$$\text{Tophic position} = \lambda_{Base} + (\delta^{15}N_{stickleback} - [\delta^{15}N_{lit. base} * \alpha + \delta^{15}N_{pel. base} * (1 - \alpha)]) / \Delta_n \quad (2)$$

$$\alpha = \frac{\delta^{13}C_{stickleback} - \delta^{13}C_{pel. base}}{\delta^{13}C_{lit. base} - \delta^{13}C_{pel. base}} \quad (3)$$

where λ_{Base} denotes the trophic position of the consumer (λ_{Base} = 2) used for the estimation of the littoral (=$\delta^{15}N_{lit. base}$) and pelagic (=$\delta^{15}N_{pel. base}$) baseline. The isotope values of faucet snails and quagga mussels were used for $\delta^{15}N_{pel. base}$ and $\delta^{13}C_{pel. base}$. As filter feeders, quagga mussels are an ideal integrator species for representing the consumer base of the pelagic food web, and are favoured over bulk seston or plankton samples, which may include non-consumer material and undifferentiated detritus and thus bias stable isotope ratio signatures. The isotopic ratios of quagga mussels and faucet snails were assessed using the same method applied for plankton, whitefish eggs and larvae (see

above). $\delta^{13}C_{stickleback}$ is the measured $\delta^{13}C$ value of sticklebacks muscle. $\delta^{15}N_{stickleback}$ is the measured $\delta^{15}N$ value of sticklebacks muscle, Δ_n is the enrichment in $\delta^{15}N$ per trophic level (Δ_n = 3.4 (Minagawa and Wada 1984; Vander Zanden and Rasmussen 1999; Post 2002; Reimchen et al. 2008)) and α denotes the proportion of sticklebacks carbon derived ultimately from littoral sources. A Δ_n value of 3.4 is commonly used for sticklebacks (Post 2002; Matthews et al. 2010), but in the light of recent reviews suggesting a lower level of $\delta^{15}N$ enrichment for carnivorous fish (Vanderklift and Ponsard 2003; Boecklen et al. 2011; Blanke et al. 2017; Kambikambi et al. 2019), a further analysis as included using Δ_n = 2.0 as a value for the trophic enrichment of $\delta^{15}N$ in sticklebacks.

To compare the trophic position of sticklebacks with piscivorous fish species, the trophic position for pike and burbot was then calculated for each pike and burbot using the formula: Trophic position = [(piscivorous fish $\delta^{15}N$ − $\delta^{15}N_{lit.\ base}$)/ Δ_n] + λ_{Base} (Nyqvist et al. 2018).

Statistical analysis

To test the effects of covariates on the $\delta^{15}N$ or $\delta^{13}C$ of muscle or liver tissue and the trophic position of sticklebacks, the following general linear model (GLM) (Sachs 1997) was used:

$$Y_{ijklmno} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \delta_l + \varepsilon_m + \zeta_n + \eta_o + \theta_{ijklmno} \qquad (4)$$

where $Y_{ijklmno}$ is $\delta^{15}N$ or $\delta^{13}C$ in muscle or liver tissue or the trophic position of sticklebacks; μ is the overall mean, α_i denotes month, β_j is total length, $(\alpha\beta)_{ij}$ is the interaction between month and total length, γ_k represents year and was added to the model as a random factor, δ_l is habitat (pelagic or littoral zone), ε_m is sex (male or female), ζ_n denotes the infection state (yes/no), η_o is parasite index and $\theta_{ijklmno}$ is the random residual error. Model requirements, *i.e.* 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 were excluded from the dataset (selection criteria: more than eight times standard deviation). Student's t-test was used for *post hoc* comparisons between habitat and sex after testing for homoscedasticity (Levene test) and by building contrasts (Sokal and Rohlf 2003). The GLM for trophic position of sticklebacks was also run with Δ_n set at 2.0.

Differences in mean $\delta^{15}N$ and $\delta^{13}C$ values among Lake Constance fish species were examined using Tukey-Kramer HSD-tests.

The contribution rate of potential food sources for sticklebacks' diet was estimated using the Bayesian mixing model in the SIMMR package (Parnell and Inger 2019), which was based on the SIAR package (Parnell et al. 2010), and implemented in the R 4.04 software (R Core Team 2020). We run separated mixing models for isotopic signatures of the liver samples of sticklebacks collected in winter (December to March) and in the summer (June and July). We choose to use only liver values because isotope values of the liver react faster than those of the muscles (time lag of only one month, Perga and

Gerdeaux 2006). We include as potential food sources the isotopic values for whitefish larvae and eggs of both whitefish species, plankton, burbot, pike, and chironomids from both pelagic and littoral habitats. Whitefish eggs and larvae are only available for stickleback during winter (Roch et al. 2018; Baer et al. 2021); as such, they were not included in the model for summer samples. Furthermore, we used the isotopic values from burbot and pike as a proxy for the food source "fish", because eggs and larvae of pike are known to be a food source for sticklebacks (Bergström et al. 2015) and the larvae of burbot are small and are available for sticklebacks the whole year round (Probst and Eckmann 2009). The δ^{15}N and δ^{13}C values of chironomids from both habitats were taken from previous samplings in Upper Lake Constance, made in 2015–2017, representing mean yearly values (kindly provided by M. Sabel and D. Straile). As correction factors, we used the widely accepted trophic enrichment factors (TEFs) (Cui et al. 2021) to estimate the direct contribution of food sources to detritivores (chironomids) and planktivores (whitefish) and omnivores (stickleback), and the indirect contribution of food sources to sticklebacks caught in the pelagic and littoral zone during winter and summer. In order to simplify the model and to enhance interpretability, we performed *a-posteriori* combination of some of the food sources that fell on similar regions of the isospace plot (Suppl. material 1: fig. S1). We combined the isotopic values of both species of whitefish eggs and larvae to category "Whitefish eggs" and "Whitefish larvae", the chironomids from both habitats to "Chironomids", and the burbot and pike to new category "Fish". Furthermore, to get more insight into the importance of the prey category "Fish", we also ran the model without this category. SIMMR relies on a Markov Chain Monte Carlo to find possible solutions and disregards those not probabilistically consistent with the data. The iterations run were 10^4, the burn-in was 10^3, the posterior was thinned by 10, and the number of chains fit was 4.

Furthermore, data from all examined fish species were pooled according to feeding guild in order to calculate a standard ellipse area corrected for small samples (SEA$_c$). The SEA$_c$ represents the core isotopic niche of each guild after factoring in maximum likelihoods. It comprises around 40% of data and resembles a two-dimensional measurement of standard deviation (Jackson et al. 2011). The small sample size-corrected standard ellipse area (SEAc) represents the core isotopic niche area of the individuals sampled. The isotopic niche overlap of omnivorous sticklebacks with the remaining feeding guilds was subsequently calculated as a proportion of the sum of non-overlapping areas of the SEA$_c$s. All analyses were performed using the SIBER package (Stable Isotope Bayesian Ellipses in R, v. 2.15; (Jackson et al. 2011)) in R (v. 4.04, (R Core Team 2020)).

To compare the trophic position of sticklebacks in Lake Constance to that of conspecifics in similar ecosystems (to see if the position in ULC is common), the trophic positions of sticklebacks from Lake Constance (here: mean value of all sticklebacks, independent of habitat) were compared to lake populations from North America (Matthews et al. 2010) and Norway (Østbye et al. 2016) using a z-test on arithmetic means and standard deviation with *post hoc* Bonferroni correction. We used for this comparison all lakes cited by Matthews et al. (2010) and only lake populations from Norway with a clear assignment to the isotopic values of sticklebacks (Einletvatn and Farstadtvatn lake).

The same trophic position calculation (according to Post 2002) and the same trophic enrichment factor (Δ_n = 3.4) used in this study also applied to analyses of these lake systems.

Unless further specified, all statistics were performed in JMP Pro 15.1 (64 bit, SAS Institute).

Results

Stable isotope values of sticklebacks (main sampling)

For sticklebacks sampled between 2017 and 2018 (mean TL 66 mm $\pm$ 5 mm ($\pm$ SD)), the mean δ^{15}N value was 14.9 $\pm$ 1.2‰ ($\pm$ SD) for muscle and 14.6 $\pm$ 2.2‰ ($\pm$ SD) for liver tissue. The lowest δ^{15}N values for stickleback muscle tissue were recorded in summer (June-July), with mean values between 14.1‰–14.3‰ (Fig. 2). During autumn and winter (October-February) intermediate values of 14.5‰–15.2‰ were measured, and the highest values of between 15.3‰–15.7‰ occurred during spring in March and April (Fig. 2). Similar trends were observed for δ^{15}N in stickleback liver tissue, with lowest mean values occurring during the summer months (12.3‰–12.4‰), intermediate values from October to December (14.6‰–15.7‰) and highest values in March and April (16.1‰–16.6‰) (Fig. 2). δ^{15}N in the liver showed more monthly variation than muscle tissues in both littoral and pelagic habitats (F-tests on within-group variations; $3.88_{185,180}$ and $3.25_{82,70}$ respectively, both $P \leq 0.001$). Month had a significant influence, with the effect strength greatest in muscle tissue δ^{15}N (GLM, r^2 = 0.23, n = 252, $P < 0.0001$, Table 1). No other parameters had a significant effect (Table 1). The GLM for δ^{15}N values of liver tissue (r^2 = 0.60, n = 266) showed a similar result for month and year effects ($P < 0.0001$ and $P < 0.01$, respectively). The interaction between TL and month also significantly affected liver δ^{15}N, while no other parameters showed any significant effect (Table 1).

Mean δ^{13}C values averaged -30.5 $\pm$ 0.8‰ ($\pm$ SD) for stickleback muscle tissue and -31.2 $\pm$ 1.5‰ ($\pm$ SD) for liver tissue. Muscle δ^{13}C was lowest during July (-29.5‰) and fluctuated slightly between -30.1‰–-31.0‰ (Fig. 2) during all other sampling months. Stickleback liver δ^{13}C values were highest during July (-28.5‰) and lowest during March (-32.2‰) and varied across other sampling months between -31.0‰–-32.0‰ (Fig. 2). The model testing effects on δ^{13}C muscle values (GLM, r^2 = 0.27, n = 251, $P < 0.0001$) revealed that only month and sex had a significant influence ($P < 0.0001$). *Post hoc* comparison found that female fish had significantly lower muscle δ^{13}C values than males (Student-t, $P < 0.05$), but the effect strength of sex was 5× smaller than for month (Table 1). All other parameters had either no impact or exhibited only weak effects (Table 1). Month and sex had a significant influence ($P < 0.0001$) on liver δ^{13}C (GLM, r^2 = 0.46, n = 255, $P < 0.0001$), and as with muscle δ^{13}C, females exhibited lower values than males (Student-t, $P < 0.05$). In contrast to the δ^{13}C level in muscle tissue, however, habitat had a significant effect ($P < 0.05$) on liver δ^{13}C, with significantly lower values in sticklebacks from the pelagic zone compared to littoral specimens (Student-t,
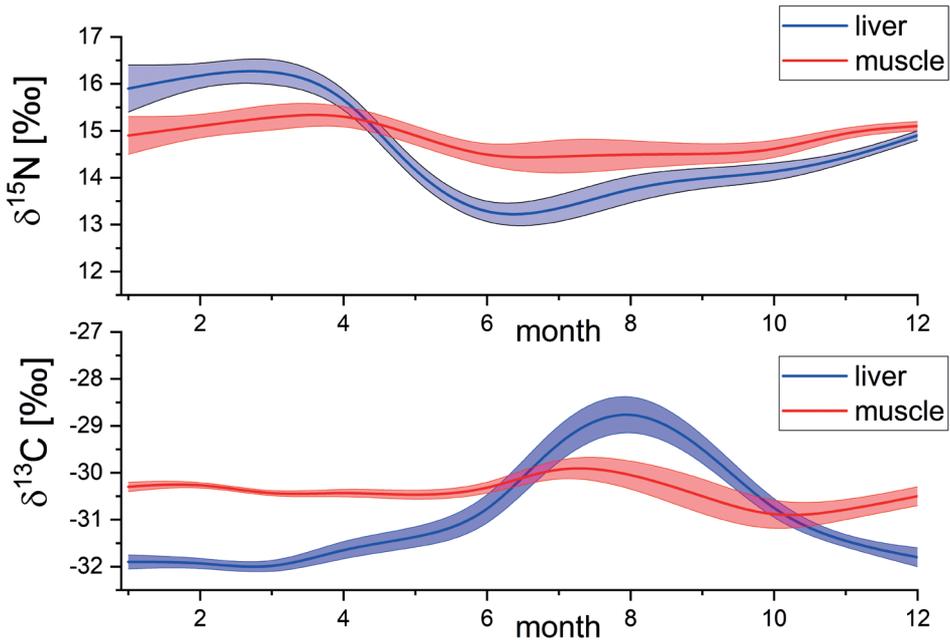
Figure 2. Fitted spline intervals of $\delta^{15}N$ and $\delta^{13}C$ in the muscle and liver of sticklebacks sampled in 2017–2018. Solid lines are the mean values, and shaded areas represent upper and lower 95% confidence intervals during the course of the year in Lake Constance.

Table 1. The significance and effect strength of study parameters on the $\delta^{15}N$ and $\delta^{13}C$ values of muscle and liver tissue of sticklebacks from Lake Constance.

$\delta^{15}N$		
	muscle	**liver**
parameter	effect strength ($\pm$ standard error)	effect strength ($\pm$ standard error)
month	0.96^{xxx} ($\pm$ 0.005)	0.986^{xxx} ($\pm$ 0.01)
habitat	0.001 ($\pm$ 0.001)	0.007 ($\pm$ 0.001)
total length	0.247 ($\pm$ 0.01)	0.059 ($\pm$ 0.005)
sex [m/f]	0.004 ($\pm$ 0.001)	0.006 ($\pm$ 0.001)
infested [yes/no]	0.023 ($\pm$ 0.001)	0.001 ($\pm$ 0.002)
parasite index	0.001 ($\pm$ 0.001)	0.004 ($\pm$ 0.001)
TL*month	n.a.	n.a.
$\delta^{13}C$		
	muscle	**liver**
parameter	effect strength ($\pm$ standard error)	effect strength ($\pm$ standard error)
month	0.513^{xxx} ($\pm$ 0.01)	0.776^{xxx} ($\pm$ 0.009)
habitat	0.011 ($\pm$ 0.001)	0.062^{x} ($\pm$ 0.005)
total length	0.187 ($\pm$ 0.01)	0.099 ($\pm$ 0.006)
sex [m/f]	0.127^{xxx} ($\pm$ 0.004)	0.146^{xxx} ($\pm$ 0.007)
infested [yes/no]	0.0069 ($\pm$ 0.003)	0.006 ($\pm$ 0.005)
parasite index	0.26 ($\pm$ 0.007)	0.022 ($\pm$ 0.003)
TL*month	n.a.	n.a.

Model terms: x = $P < 0.05$; xx = $P < 0.01$; xxx = $P < 0.0001$; effect strength is a dimensionless factor assessing the impact of a variable in the model formula.

$P < 0.05$). Overall, the effects of sex and habitat on the whole model were low (Table 1). There were no significant differences in the isotopic signature of sticklebacks caught in the pelagic and littoral habitat, except for the liver $\delta^{13}C$ values.

Stable isotope values of zooplankton, whitefish and sticklebacks (additional sampling)

The $\delta^{15}N$ value of zooplankton increased from October (10.6‰) until December (13.4‰), showed the highest peak in February (14.4‰) and decreased until April to a value of 7.9‰ (Fig. 3). Eggs of pelagic whitefish showed a mean $\delta^{15}N$ value of 13.9 $\pm$ 0.5‰ ($\pm$ SD) and did not differ significantly from those of benthic whitefish, which had a mean $\delta^{15}N$ value of 14.1 $\pm$ 1.1‰ ($\pm$ SD) (t-test, $P > 0.05$). The combined mean $\delta^{15}N$ value for eggs of both species was 14.0 $\pm$ 0.8‰ ($\pm$ SD), significantly higher than that of zooplankton sampled in the same months (Fig. 3). The mean $\delta^{15}N$ value of sampled whitefish larvae, 15.3 $\pm$ 0.2‰ ($\pm$ SD), was also higher than that for zooplankton sampled in the same months (Fig. 3). Sticklebacks sampled in 2021–2022 exhibited a mean $\delta^{15}N$ value of 15.4 $\pm$ 0.5‰ ($\pm$ SD) and a mean $\delta^{13}C$ value of -31.0 $\pm$ 0.6‰ ($\pm$ SD) and were close to results from winter 2017–2018. No differences were observed in mean $\delta^{15}N$ and $\delta^{13}C$ values between month and habitats (Tukey-Kramer-HSD, $P > 0.0.5$). The $\delta^{15}N$ values of sticklebacks showed no difference from those of whitefish larvae but were notably higher than those for zooplankton (Fig. 3). In March and April, when the zooplankton showed decreasing $\delta^{15}N$ values, the $\delta^{15}N$ values of sticklebacks continued on the same level as the month before (Fig. 3).

The $\delta^{13}C$ values of sampled zooplankton showed no clear temporal trend (-32.9‰ in October, -34.2‰ in December, -34.0‰ in February, -35.9‰ in March, and -34.8‰ in April). The mean $\delta^{13}C$ value of pelagic whitefish eggs was -33.7 $\pm$ 0.4‰ ($\pm$ SD), significantly different to that of benthic whitefish eggs at -33.1 $\pm$ 0.6‰ ($\pm$ SD) (t-test, $P < 0.05$). The mean $\delta^{13}C$ value of whitefish larvae was -33.2 $\pm$ 0.5‰ ($\pm$ SD).

Stomach content analysis

Nearly all analysed sticklebacks had food in their digestive tracts. Only one individual sampled in the pelagic zone during spring had an empty stomach. The numerically dominant food source for sticklebacks during spring and winter, independent of sampled habitat, were copepods (Table 2). Interestingly, in 4 out of 10 sticklebacks (40%) sampled during spring in the littoral zone, fish eggs of unknown taxa were recorded, and in winter, the stomachs of two out of 20 (10%) pelagic sticklebacks contained fragments of fish eggs and partially digested fish larvae. Differences between the stomach contents of sticklebacks sampled in the pelagic or littoral zone became more obvious during other seasons. In summer, pelagic sticklebacks consumed mostly *Daphnia* (Table 2) while littoral sticklebacks fed mostly (73%) on benthic macroinvertebrates, mainly chironomids (here: other, Table 2). One stickleback caught in the littoral zone during summer had consumed 14 fish eggs of unknown taxa, amounting to 38% of all food items present in
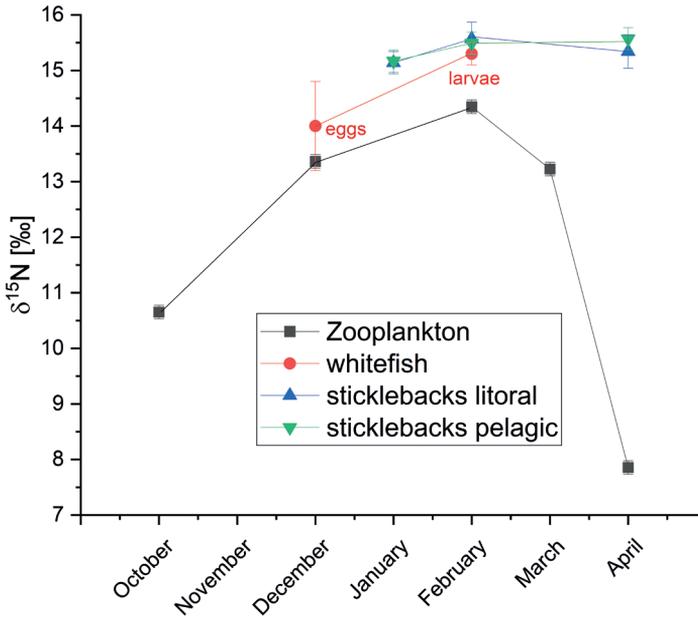
Figure 3. Arithmetic mean $\delta^{15}N$ values with standard deviation of zooplankton, whitefish eggs and larvae and sticklebacks from the pelagic and littoral zone of Upper Lake Constance sampled in winter 2021–2022.

that individual's digestive tract. In autumn, copepods remained the most common prey category (81%) consumed by pelagic sticklebacks, whilst *Bosmina* (74%) were the most frequent prey category in littoral. Autumn was the only season when no fish (larvae or eggs) were found in either pelagic or littoral stickleback digestive tracts (Table 2).

Bayesian mixing model

The results of the SIMMR mixing model suggested a clear seasonal distinction in the contribution of food sources to sticklebacks (Fig. 4). During winter, whitefish larvae and whitefish eggs contributed a mean proportion of 30–40% (the highest contribution) to the diet, while zooplankton and chironomids are of lesser importance (mean values between 10–15%), independent of sampling habitat (littoral or pelagic zone) (Fig. 4). During summer, the importance of chironomids and zooplankton increased to proportions of 25–30% (chironomids) and 35–40% (zooplankton). During summer, neither whitefish eggs nor whitefish larvae are available. However, other protein-rich sources, *i.e.* fish larvae from later spawning species, increased in importance during that time to provide a mean value of 30% (pelagic zone) and 40% (littoral zone), compared to winter, when fish (non-whitefish) contribute a proportion of around 15% to the diet of sticklebacks (Fig. 4). When we excluded the category "Fish" from the model, the importance of chironomids and plankton increased during summer to proportions of 30–35% for chironomids and 65–70% for zooplankton (Suppl. mate-

Table 2. Diet composition of sticklebacks (prey types expressed as a percentage of the total number of prey items eaten: during spring, summer, autumn and winter, and as the mean number of consumed individuals per stickleback ± SD) sampled in pelagic and littoral zone of Upper Lake Constance. h/d cladocera = other herbivorous/detritivorous cladocera.

diet	habitat	spring	summer	autumn	winter
copepods	pelagic	85.95%	12.12%	81.44%	56.06%
		371 ± 254	124 ± 215	596 ± 304	172 ± 76
	littoral	58.18%	12.49%	15.50%	68.98%
		124 ± 215	7 ± 7	28 ± 36	122 ± 158
Bosmina	pelagic	2.41%	5.63%	6.82%	0.13%
		10 ± 7	7 ± 8	50 ± 36	1 ± 1
	littoral	0.67%	6.54%	73.83%	1.13%
		1 ± 2	2 ± 3	111 ± 74	1 ± 1
h/d cladocera	pelagic	10.66%	78.83%	11.47%	43.69%
		46 ± 29	96 ± 108	84 ± 71	134 ± 61
	littoral	0.96%	4.16%	2.41%	13.55%
		1 ± 3	2 ± 1	2 ± 2	17 ± 23
predatory cladocera	pelagic	0.05%	3.00%	0.27%	0.06%
		1 ± 1	4 ± 5	4 ± 4	1 ± 0.6
	littoral	0.00%	0.36%	0.04%	0.11%
		0	1 ± 0	1 ± 0	1 ± 0
fish (eggs, larvae)	pelagic	0.00%	0.00%	0.00%	0.03%
		0	0	0	1 ± 0
	littoral	2.34%	3.78%	0.00%	0.00%
		1 ± 1	1 ± 4	0	0
other	pelagic	0.93%	0.41%	0.00%	0.02%
		5 ± 5	1 ± 1	0	1 ± 0
	littoral	37.84%	72.67%	8.22%	16.22%
		13 ± 8	35 ± 29	4 ± 11	7 ± 5

rial 1: fig. S2), however, during winter, the importance of whitefish eggs and larvae was (with a mean contribution of 30–45% to the diet) similar to the outcome of the model which included the category "Fish" (Suppl. material 1: fig. S2). The detailed summary of both Bayesian mixing models (SIMMR) outputs and matrix plots of source contribution proportions are given in the supplements (Suppl. material 1: tables S1–S4).

Stable isotope values of other fish species from Lake Constance

For the other fishes species examined from Lake Constance, mean $\delta^{15}N$ values for muscle tissue varied by up to 4.0‰, with a minimum of 9.6 ± 1.1‰ for herbivorous rudd and a maximum of 13.6 ± 0.1‰ for piscivorous pike with whitefish, bleak, roach, burbot, and tench exhibiting intermediate values (Fig. 5). At the time of sampling (summer), mean stickleback muscle $\delta^{15}N$ (14.3 ± 1.2‰) did not differ from that of exclusively piscivorous pike (Tukey-Kramer-HSD, $P > 0.05$) and was significantly higher than that of all other fish species (Tukey-Kramer-HSD, $P < 0.0.5$). The $\delta^{15}N$ values of quagga mussels and faucet snails were 5.6 ± 0.1‰ and 6.9 ± 0.2‰ respectively (Fig. 5).
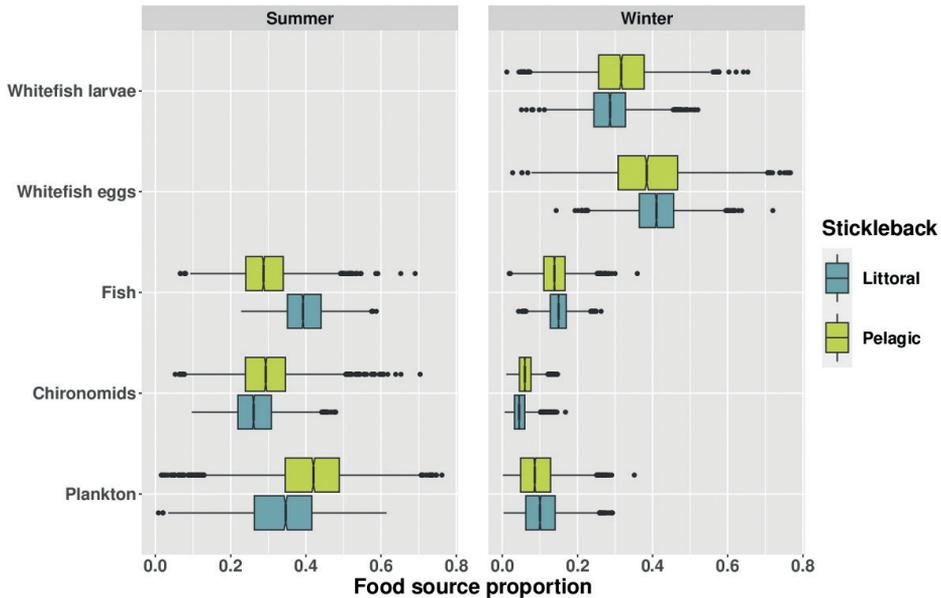
Figure 4. Posterior distribution of dietary proportion estimates of different food sources from sticklebacks from the pelagic and littoral zone of ULC during summer and winter, according to Bayesian modelling, expressed as Box-and-Whisker plots with median values and interquartile range (IQR), and minimum and maximum if it doesn't extend the IQR value beyond 1.5. Data outside this range are plotted individually.

The mean muscle $\delta^{13}C$ of all analysed fish species, except sticklebacks, ranged from -29.6 ± 1.4‰ for whitefish to -21.4 ± 1.9‰ for rudd, while mean values for bleak, roach, tench, pike and burbot ranged between -28.4‰ and -25.1‰ (Fig. 5). At the time of sampling, in summer, the mean muscle $\delta^{13}C$ of sticklebacks (-30.5 ± 1.0‰) was significantly lower than that of all other fish species (Tukey-Kramer-HSD, $P < 0.0.5$). The $\delta^{13}C$ values of quagga mussels and faucet snails were -30.1 ± 0.3‰ and -24.3 ± 0.9‰ respectively (Fig. 5).

Fig. 5 shows the core isotopic niche of each feeding guild. SEAc values range from 2.43 (zooplanktivorous) to 6.56 (benthivorous). The niche overlap between omnivorous sticklebacks and other feeding guilds was limited to a 9% overlap with zooplanktivorous whitefish.

Trophic position of sticklebacks

The statistical model testing effects on the stickleback trophic position (GLM, $r^2 = 0.29$, n = 249, $P < 0.0001$) identified a significant influence of month ($P < 0.0001$), which comprised > 64% of total effect strength revealed the model (Table 3). When using a Δ_n value of 3.4, the mean trophic position of sticklebacks in Lake Constance was 4.7 ± 0.6 (± SD). The reduced Δ_n value of 2.0 yielded stickleback trophic positions of 6.7 ± 1.0 (± SD). This is unrealistic given the food web and feeding guilds of Lake Constance. Consequently, further analyses of trophic position are based solely
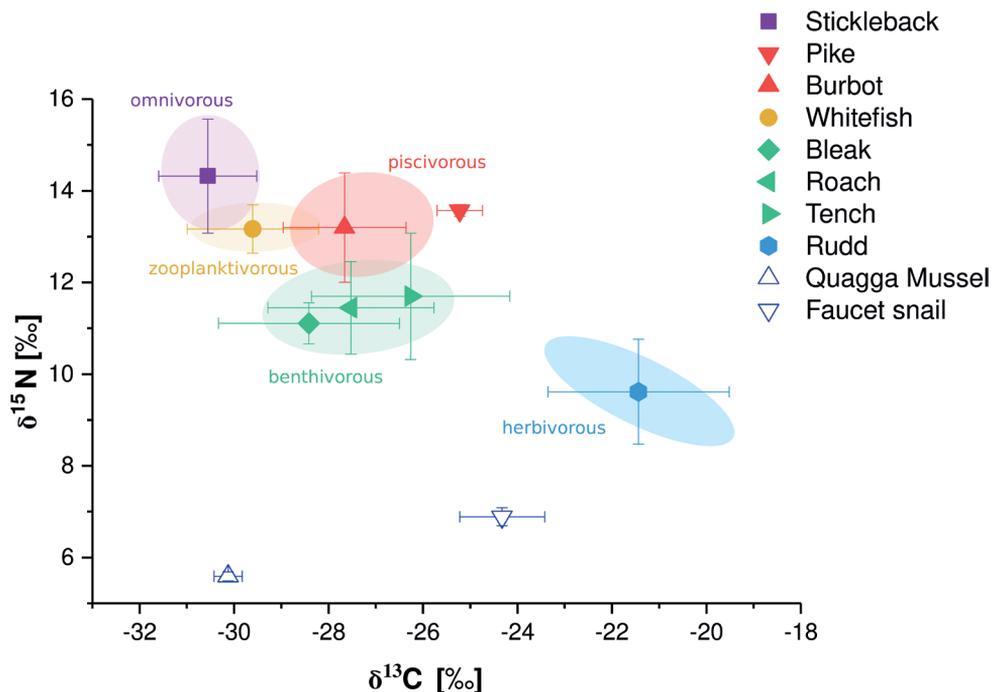
Figure 5. $\delta^{15}N$ versus $\delta^{13}C$ bi-plot showing the mean isotope values of aquatic consumers in Lake Constance during summer (August and September). Horizontal and vertical bars represent ± SD of total pooled data. The standard ellipse areas (SEAc) represent the core isotopic niche for each trophic guild (comprising ~ 40% of the data; (Jackson et al. 2011)).

on results from the Δ_n = 3.4 iterations of the model. The lowest trophic positions for sticklebacks were calculated for the summer months of June and July (4.4–4.6) and the highest for the spring months of March and April (4.9–5.0). No other tested parameters had a significant effect on trophic position (Table 3).

The trophic position for piscivorous pike in ULC was 4.2 ± 0.2 (± SD) and for partly piscivorous burbot 4.2 ± 0.4 (± SD).

Table 3. The significance and effect strength of different parameters on the trophic position of sticklebacks in Lake Constance.

parameter	effect strength
month	0.969[xxx] (± 0.005)
habitat	0.001 (± 0.001)
total length	0.235 (± 0.01)
sex [m/f]	0.001 (± 0.001)
infested [yes/no]	0.012 (± 0.001)
parasite index	0.011 (± 0.0011)
TL*month	n.a.

Model terms: x = $P < 0.05$; xx = $P < 0.01$; xxx = $P < 0.0001$; effect strength is a dimensionless factor assessing impact of variable in model formula.
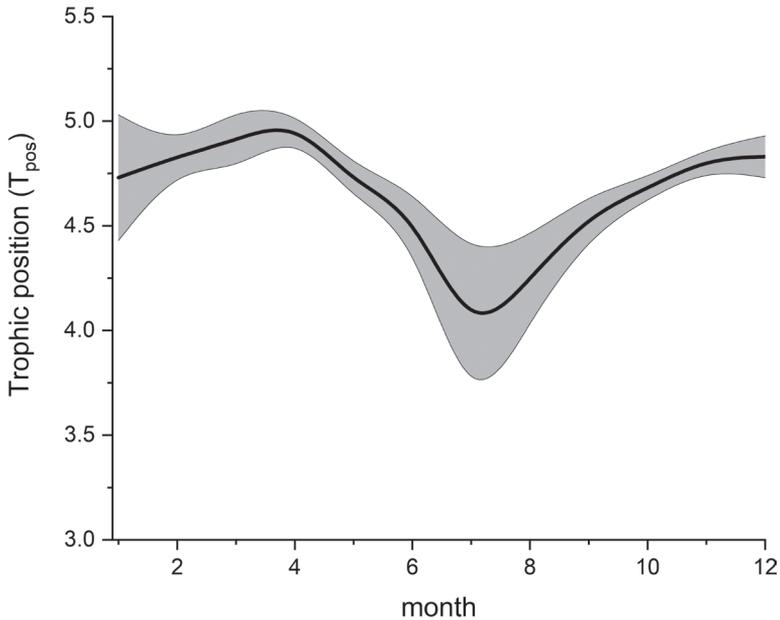
Figure 6. Fitted spline intervals of the trophic position of sticklebacks in Upper Lake Constance. Solid lines are the mean values, and shaded areas represent upper and lower 95% confidence intervals during the course of the year.

A seasonal trend in stickleback trophic position was apparent, with lowest values during the stickleback spawning season (summer months), and increasing in the spawning season of whitefish during autumn and winter (Fig. 6).

Trophic position of sticklebacks in other lakes and in comparison to Lake Constance

Sticklebacks from Lake Constance have a significantly higher trophic position than investigated populations in North America and Norway (z-test, $P < 0.001$) (Fig. 7). The proportion of littoral carbon in the diet of sticklebacks (α) from Lake Constance (mean = 0.06, standard error = 0.01) is comparable with that observed in populations with a "limnetic-like" phenotype from Norway and North America ($\alpha \leq 0.4$; Fig. 7) but far from that documented for benthic populations ($\alpha \geq 0.6$; Fig. 7).

Discussion

A key insight from the present study is the seasonal trend in $\delta^{15}N$ from stickleback muscle and liver, with the highest values occurring during winter and spring. These values assist us to answer research question 1 (Do the $\delta^{15}N$ values and trophic position of sticklebacks reflect the seasonal feeding of whitefish larvae and eggs or that of
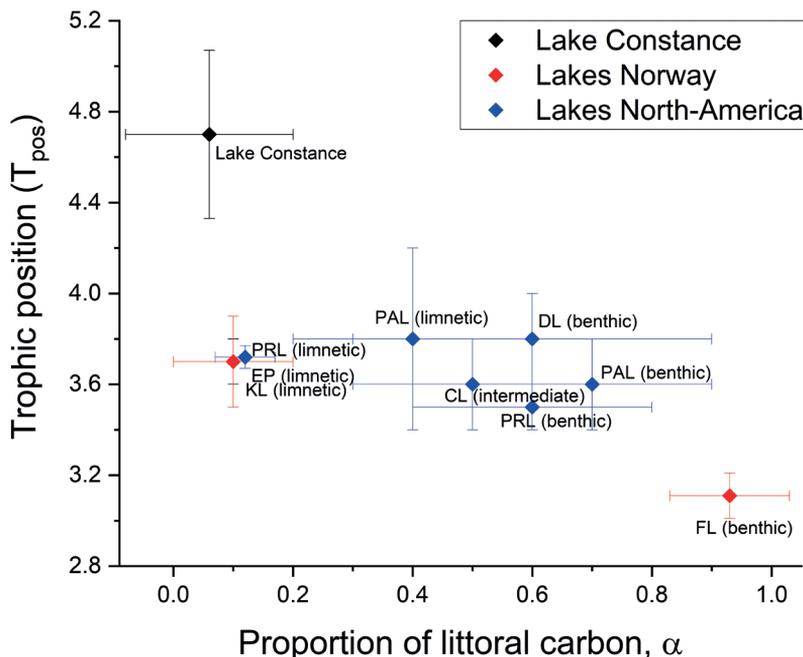
Figure 7. Comparison of trophic position and littoral carbon in the diet of three-spined sticklebacks. Values for sticklebacks from Lake Constance in black (mean value of all analysed sticklebacks, independent of habitat), from North America in blue and Norway in red; error bars indicating standard deviation. The eight North American stickleback populations (Matthews et al. 2010) (PRL = Priest Lake, KL = Kennedy Lake, PAL = Paxton Lake, CL = Cranby Lake, DL = Dugout Lake) and the two from Norway (Østbye et al. 2016) (EP = Einletvatn pond, FL = Farstadtvatn lake) are subdivided into limnetic (n = 4), benthic (n = 4) and intermediate (n = 1) ecophenotypes.

other food sources, such as zooplankton?). It could be hypothesised that the seasonal trend is linked to elevated $\delta^{15}N$ values of the main food resource during winter (here copepods and cladocera), as observed in Lake Geneva where whitefish consume mainly zooplankton during the winter months (Perga and Gerdeaux 2005, 2006). There, the seasonal nature of zooplankton $\delta^{15}N$ was echoed in the tissues of whitefish after a lag of one month in the case of liver tissue, and four to five months later in the case of muscle tissue. Sticklebacks from Lake Constance also consume large quantities of zooplankton (Ogorelec et al. 2022). However, it appears that stickleback $\delta^{15}N$ values are only partly dictated by the isotopic signature of zooplankton. On the one hand, we can see a winter increase and decrease in the $\delta^{15}N$ values of stickleback liver (as in Lake Geneva) and observe a switch from the summer diet of *Daphnia* and macrozoobenthos to copepods during winter. Much the same diet shift and increases in $\delta^{15}N$ were observed in a study from North America (McIntyre et al. 2006). On the other hand, $\delta^{15}N$ values in stickleback tissue from ULC increase up to 16.6‰ and are distinctly higher than those observed in local zooplankton (7.9‰–14.4‰). Moreover, values in stickleback muscle (which typically respond more slowly to dietary change) decreased only slightly after

winter, and never fell below 14.1‰ throughout the year. In contrast, the δ^{15}N value of zooplankton decreased to below 8‰ in April. Therefore, seasonal changes in zooplankton feeding from *Daphnia* to copepods alone cannot be responsible for the distinctly elevated δ^{15}N values observed in ULC sticklebacks during winter or the moderately high values (well above those recorded in other fish species) observed during the rest of the year. Were that the case, we would expect to observe much lower δ^{15}N values year-round and a much stronger decrease after winter. Additionally, during summer, the mean δ^{15}N value of stickleback muscle (14.3 ± 1.2‰) was significantly higher than that of adult whitefish (13.2 ± 0.5‰), indicating differences in feeding ecology. Our stomach content analyses partially support the stable isotope results, as in winter the stomachs of 20% of pelagic sticklebacks contained fragments of fish eggs and partially digested fish larvae, while in spring 40% of littoral sticklebacks sampled contained fish eggs of unknown taxa. Judging by the season and pigmentation, the eggs and the larvae most likely belong to whitefish. Furthermore, stable isotope analysis of whitefish eggs and larvae revealed much higher δ^{15}N levels than were identified in zooplankton. Thus, it is highly likely that the δ^{15}N increase in the liver of sticklebacks during winter and spring and the high δ^{15}N values in the muscle during spring are at least partly due to piscivory. Moreover, the outcome of our stable isotope mixing models estimates that whitefish larvae and whitefish eggs were the dominant prey items of sticklebacks during winter. This outcome suggests that we severely underestimate the predation rate during winter using the stomach content analysis alone. We guess that two factors are responsible for this outcome: first, the stormy winds during and after the spawning seasons, which hampered a normal sampling and second, the extremely patchy distribution of whitefish larvae (Ransom et al. 2022) and therefore clutched appearance of larvae in the diet of sticklebacks, which is further supported by mass feeding (Roch et al. 2018). It could be hypothesised that during food scarcity in winter, when the density of zooplankton and benthic macroinvertebrates is much lower compared to summer (Ogorelec et al. 2022), whitefish eggs and larvae are an exceptionally valuable additional diet component for sticklebacks due to their easy availability and undisputed caloric value (Ros et al. 2019; Baer et al. 2021). Furthermore, the Bayesian model calculated for summer a proportion of 30% (pelagic zone) and 40% (littoral zone) of fish in the diet of sticklebacks. As for winter, this outcome starkly contrasts with our stomach content analysis. We found only one female stickleback, caught in August, which had eaten 14 fish eggs. However, even if cannibalistic behaviour is well known for sticklebacks (FitzGerald and van Havre 1987; Mehlis et al. 2010), those eggs were highly likely not from sticklebacks, because sticklebacks in ULC spawn much earlier, between April and June (Gugele et al. 2020). Different cyprinids, like bleak *Alburnus alburnus* and the white bream *Blicca bjoerkna*, are multiple spawners and some spawn until the end of July (Rinchard and Kestement 2003). Thus, it seems likely that sticklebacks from ULC consume, if the opportunity arises, eggs from other fish species, *i.e.* cyprinids, as has been observed in other water systems before (Dukowska and Grzybkowska 2014). Furthermore, nearly all fish from ULC spawn in the littoral zone, not in pelagic waters. Therefore, the difference in fish consumption from sticklebacks caught during sum-

mer in both habitats most probably reflects the availability of fish eggs and larvae in both habitats. All in all, the results from the stable isotope mixing models pointed to a significant piscivorous behaviour of sticklebacks in ULC. It is well known from other parts of the world that sticklebacks can consume high amounts of fish seasonally: in the Baltic Sea sticklebacks were observed to migrate purposely to the spring spawning grounds of perch and pike, where they fed on eggs and larvae of those species. This leads to significant stock reductions of the latter two species (Ljunggren et al. 2010; Bergström et al. 2015; Byström et al. 2015). In ULC, similar patterns were observed (sticklebacks migrated to the spawning grounds of whitefish, Gugele et al. 2020).

The answers to research questions 2 (Where do invasive sticklebacks sit relative to other fish species in the trophic structure of Lake Constance?) and 3 (How does the trophic position of sticklebacks in Lake Constance compare to that of conspecifics in similar ecosystems?) are somewhat contradictory. Generally, Lake Constance stickle-backs occupied an extraordinarily high trophic position (mean = 4.7), even using a conservative estimate of trophic enrichment (Δ_n = 3.4). Other studies, using the same calculation and the same values for trophic enrichment and trophic position of the consumer (λ_{Base} = 2), yielded markedly lower scores: The mean trophic positions calculated for sticklebacks in lakes of North America and Norway ranged from 2.9 to 3.7 (Matthews et al. 2010; Østbye et al. 2016), and in Japan, the species occupies a mean trophic position of 2.8 (Ravinet et al. 2014), however, in those lakes no piscivorous behaviour of sticklebacks was observed. In large oligotrophic lakes, trophic positions higher than 4 are normally occupied by at least partly piscivorous fish like arctic charr (*Salvelinus alpinus* L., 1758) or perch (*Perca fluviatilis* L., 1758) (Eloranta et al. 2015). In ULC, pike and burbot showed trophic positions of around 4.2, and their mean δ^{15}N values are highly comparable to sticklebacks. However, this does not mean that they were all at the same trophic level because it is proven that the intra- and interindividual variation in the isotopic composition depends not only on the diet, but also on the amount of isotopic variations among the food sources (Matthews and Mazumder 2005). We found large seasonal variations in the isotopic signatures of zooplankton in ULC as other authors did (Perga and Gerdeaux 2006; Janjua and Gerdeaux 2011). In those cases, modelling the trophic position of fish species is hardly reliable because many sources of δ^{15}N variation are, therefore, unrelated to trophic variation (Matthews and Mazumder 2005; Janjua and Gerdeaux 2011). Another explanation for the high trophic position of sticklebacks in ULC sticklebacks could be the δ^{15}N or δ^{13}C values (here: baseline values) of other consumers represented here by faucet snail and quagga mussel. It is known that interannual variation in the δ^{15}N or δ^{13}C values of snails and mussels can occur (Westrelin et al. 2023). We sampled snails and mussels two years after we sampled the sticklebacks which may have biased our outcomes. Furthermore, it is known that the δ^{15}N or δ^{13}C values for dreissenids in Lake Constance (zebra mussel *Dreissena polymorpha* Pallas, 1771), are depth, water temperature and season dependent (Yohannes et al. 2014). However, even if we use this seasonal variation to consider potential innerannual variation and to adjust the δ^{15}N or δ^{13}C values for the season (late summer) to a range of -30.5‰ to -34.5‰ for δ^{13}C and 6‰ to 9‰ for δ^{15}N (cf. Yohannes et al. 2014) for the baseline values,

stickleback trophic position always exceeds 4. In other lakes, for example, the western basin of Lake Erie in the US, $\delta^{13}C$ values of around -22‰ were reported for quagga mussels (Guzzo et al. 2011). Those values would lead, even in Lake Constance, to a different α-value, and a trophic position of around 3.5 and are, thus, very comparable to other lakes in North America (Matthews et al. 2010). However, the western basin of Lake Erie is much warmer than Lake Constance and mesotrophic (Guzzo et al. 2011), and it is generally known that $\delta^{15}N$ values in warmer ecosystems are generally lower while $\delta^{13}C$ values tend to be elevated in cooler systems (Yohannes et al. 2014). These factors must be considered when looking to trophic position results for sticklebacks from other parts of the world, even if they are acquired using the same equation (Post 2002) and the same method. Ecosystems operating under different temperature regimes may not be strictly comparable. However, comparisons between this study and those from Canadian and Norwegian lakes with similar cooler temperature regimes are valid, and by these benchmarks, the trophic position of stickleback in Lake Constance is remarkably high. Further investigations are needed to disentangle the effects of temperature on stable isotope turnover rates and diet-tissue discrimination patterns.

The diet and stable isotope mixing model evidence of regular or at least occasional piscivory by Lake Constance sticklebacks are corroborated by comparison of the mean $\delta^{15}N$ muscle values of sticklebacks with those of fish species from a range of foraging guilds. Sticklebacks possessed significantly higher muscle $\delta^{15}N$ values (up to 5‰) than the zooplanktivorous and/or benthivorous and herbivorous fish in our analysis, including whitefish, bleak, roach, tench, and rudd. Other studies have shown $\delta^{15}N$ differences between years for various fish species, but the values of the fish species from one foraging guild tend to be more or less stable from year to year (Janjua and Gerdeaux 2011). Furthermore, mean stickleback $\delta^{15}N$ muscle values in Lake Constance are much the same as those of pike here and in other lakes (Juanes et al. 2002; Craig 2008). This indicates that a considerable portion of stickleback diet is derived from protein-rich prey (Gu et al. 1996; Frisch et al. 2014), such as fish eggs or larvae. Recent studies of sticklebacks in Lake Constance in which stomach content analysis identified zooplankton as the only prey (Bretzel et al. 2021; Ogorelec et al. 2022), should be scrutinised in the light of these findings, as indigestible arthropod body parts will remain longer in alimentary tract than protein-rich prey items (Preston et al. 2017). And the extremely patchy distribution of whitefish larvae (Ransom et al. 2022) makes regular feeding highly doubtful. Not only the present study but also other studies dealing with invasive fish species have shown that stable isotope analysis, together with the modelling of contribution rate of potential food sources to consumers, can provide a more effective assessment of an invader's feeding behaviour and potential environmental impacts than classical stomach content analysis alone (Brush et al. 2012).

The answer to research question 4 (Are there any differences in isotopic signature between sticklebacks caught in littoral and pelagic habitats?), if only the $\delta^{13}C$ values recorded in stickleback muscle tissue are considered, is relatively clear: the answer is no. The low values (-30.5 ± 0.8‰) point to pelagic feeding of the species (France 1995). The apparently low proportion (<10%) of littoral carbon (α) assimilated from the diet

of ULC sticklebacks strongly suggests that the population comprises predominantly pelagic feeders. In benthic-feeding individuals of this species, the proportion of littoral carbon is normally around 60% or higher (Matthews et al. 2010; Østbye et al. 2016). However, it is somewhat surprising that littoral habitat has so little influence on stickleback muscle δ^{13}C values, as stomach content analysis suggests that during summer and autumn, littoral and pelagic sticklebacks consume distinctly different diets: significantly, dietary overlap was only observed in winter and spring (Table 3). Muscle tissue is typically favoured in stable isotope analysis to characterise longer-term feeding patterns and to differentiate consumers into ecotypes or guilds (Matthews et al. 2010; Østbye et al. 2016), while the rapid turnover of liver tissue means it incorporates and processes diet components faster than muscle tissue and is thus more sensitive to short-term feeding changes (Perga and Gerdeaux 2006). These trends are borne out in the current study, where a seasonal habitat shift appears to significantly affect δ^{13}C liver levels but not muscle tissue δ^{13}C levels. It may be that the duration of littoral feeding is too short to register in muscle or that a potentially reduced feeding rate during the littoral phase (during/after spawning in summer and autumn) is compensated by an increased feeding rate during the pelagic phase (winter and spring), or that an interplay of these effects occurs. Thus, further studies of stable isotope signatures of the liver will be needed to ascertain which of these three possibilities is the case and also to further test the recent theory that a benthic-pelagic species pair (one littoral, one pelagic, cf. Dahms et al. 2022) exist in Lake Constance. The latter question may require the deployment of specific markers, such as fatty acids (Hou et al. 2020; Rubenson et al. 2020) or sophisticated genetic analysis (Marques et al. 2016). Further laboratory studies are also required to attain more detailed and species-specific information regarding the effects of different diets (zooplankton, fish eggs, fish larvae, etc.), the impact of starvation, spawning, and parental care on the isotopic expression in stickleback muscle and liver tissue. The hypothesis that stocking with recently hatched whitefish larvae (as has been the practice in ULC for over a century) facilitates stickleback population growth and spread (Roch et al. 2018) also requires further investigation in order to ascertain whether stocking is a factor prompting the stickleback invasion of the pelagic zone in ULC.

Results of this study pertinent to the original research questions include confirmation from stomach content analysis that sticklebacks feed on whitefish larvae and eggs as well as fish of unknown taxa: corroborated by δ^{15}N values, the outcome of stable isotopes mixing models, and trophic profiling. Stable isotope analysis revealed significantly elevated δ^{15}N values comparable to those of pike and consistent with piscivory, and δ^{13}C profiles identified stickleback as mostly pelagic feeders. Furthermore, it appears that while the trophic position of sticklebacks is independent of their littoral and pelagic foraging habitats, differences in the isotopic signature of littoral and pelagic captures were visible in the liver, offering support for the idea that stickleback has a key role coupling the littoral and pelagic food webs of Lake Constance. However, actual data (not shown) of stomach contents of piscivorous fish, such as pike, catfish, char or trout (*Salmo trutta*), revealed that sticklebacks were only eaten occasionally by other fish and stickleback predation seems instead to be mostly from fish-eating birds, which would render this

avenue a dead end for the aquatic ecosystem. However, a similar assessment was hypothesized for the dreissenid mussels in the Great Lakes, where the lakewide degree of mussel predation by fish was believed to be limited, but when round goby (*Neogobius melanostomus*) and lake whitefish (*Coregonus clupeaformis*) began to feed intensively on quagga mussels they contributed significantly to Great Lakes' food webs (Madenjian et al. 2010). Whether the predatory fish of Lake Constance will adapt to the stickleback resource, especially in the pelagic zone, is an open question for the future.

Conclusion

The study results support the hypothesis that Lake Constance sticklebacks feed occasionally but rather intensively on the eggs and larvae of whitefish and some other fish species. This finding is in line with findings from the Baltic Sea, which highlight the negative impacts of stickleback predation on other fish species (Ljunggren et al. 2010; Bergström et al. 2015; Byström et al. 2015). The data in this study sheds important light on the puzzle of the Lake Constance stickleback invasion, in particular the question of how they have become such an important component of the food web of this large ecosystem. Our results suggest that sticklebacks in ULC are predators of whitefish larvae and eggs with significant negative consequences for their recruitment, and that this behaviour should be considered in the future management of the lake.

Acknowledgements

References

Amundsen P-A, Sánchez-Hernández J (2019) Feeding studies take guts – critical review and recommendations of methods for stomach contents analysis in fish. Journal of Fish Biology 95(6): 1364–1373. https://doi.org/10.1111/jfb.14151

Baer J, Eckmann R, Rösch R, Arlinghaus R, Brinker A (2017) Managing Upper Lake Constance fishery in a multi-sector policy landscape: beneficiary and victim of a century of anthropogenic trophic change. In: Song AM, Bower SD, Onyango P, Cooke SJ, Chuenpagdee R (Eds) Inter-Sectoral Governance of Inland Fisheries. TBTI Publication Series, E-01/2017. Too Big To Ignore-WorldFish, St. John's, Canada, 32–47.

Baer J, Gugele SM, Bretzel J, DeWeber JT, Brinker A (2021) All day-long: Sticklebacks effectively forage on whitefish eggs during all light conditions. PLoS ONE 16(8): e0255497. https://doi.org/10.1371/journal.pone.0255497

Baer J, Spiessl C, Brinker A (2022a) Size matters? Species- and size-specific fish predation on recently established invasive quagga mussels *Dreissena rostriformis bugensis* Andrusov 1897 in a large, deep oligotrophic lake. Journal of Fish Biology 100(5): 1272–1282. https://doi.org/10.1111/jfb.15043

Baer J, Gugele SM, Roch S, Brinker A (2022b) Stickleback mass occurrence driven by spatially uneven parasite pressure? Insights into infection dynamics, host mortality, and epizootic variability. Parasitology Research 121(6): 1607–1619. https://doi.org/10.1007/s00436-022-07517-4

Baer J, Kugler M, Schubert M, Schotzko N, Rösch R, Vonlanthen P, DeWeber JT (2023) A matter of time – efficacy of whitefish stocking in a large pre-alpine lake. Fisheries Management and Ecology fme.12624. https://doi.org/10.1111/fme.12624

Bergström U, Olsson J, Casini M, Eriksson BK, Fredriksson R, Wennhage H, Appelberg M (2015) Stickleback increase in the Baltic Sea – A thorny issue for coastal predatory fish. Estuarine, Coastal and Shelf Science 163: 134–142. https://doi.org/10.1016/j.ecss.2015.06.017

Blanke CM, Chikaraishi Y, Takizawa Y, Steffan SA, Dharampal PS, Zanden MJV (2017) Comparing compound-specific and bulk stable nitrogen isotope trophic discrimination factors across multiple freshwater fish species and diets. Canadian Journal of Fisheries and Aquatic Sciences 74(8): 1291–1297. https://doi.org/10.1139/cjfas-2016-0420

Bodey TW, Bearhop S, McDonald RA (2011) Invasions and stable isotope analysis – informing ecology and management. In: Veitch CR, Clout MN, Towns DR (Eds) Island Invasives: Eradication and Management. IUCN, Gland, 148–151.

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics 42(1): 411–440. https://doi.org/10.1146/annurev-ecolsys-102209-144726

Bretzel JB, Geist J, Gugele SM, Baer J, Brinker A (2021) Feeding Ecology of Invasive Three-Spined Stickleback (*Gasterosteus aculeatus*) in Relation to Native Juvenile Eurasian Perch (*Perca fluviatilis*) in the Pelagic Zone of Upper Lake Constance. Frontiers in Environmental Science 9: e670125. https://doi.org/10.3389/fenvs.2021.670125

Brush JM, Fisk AT, Hussey NE, Johnson TB (2012) Spatial and seasonal variability in the diet of round goby (*Neogobius melanostomus*): Stable isotopes indicate that stomach contents

overestimate the importance of dreissenids. Canadian Journal of Fisheries and Aquatic Sciences 69(3): 573–586. https://doi.org/10.1139/f2012-001

Byström P, Bergström U, Hjälten A, Ståhl S, Jonsson D, Olsson J (2015) Declining coastal piscivore populations in the Baltic Sea: Where and when do sticklebacks matter? Ambio 44(S3): 462–471. https://doi.org/10.1007/s13280-015-0665-5

Craig JF (2008) A short review of pike ecology. Hydrobiologia 601(1): 5–16. https://doi.org/10.1007/s10750-007-9262-3

Cui L, Jiang Z, Huang X, Wu Y, Liu S, Chen Q, Li J, He J (2021) Carbon Transfer Processes of Food Web and Trophic Pathways in a Tropical Eutrophic Seagrass Meadow. Frontiers in Marine Science 8: 725282. https://doi.org/10.3389/fmars.2021.725282

Dahms C, Roch S, Elmer KR, Ros A, Brinker A, Jacobs A (2022) Rapid intralacustrine evolution of an invasive pelagic three-spined stickleback (*Gasterosteus aculeatus*) ecotype in Lake Constance. bioRxiv preprint. https://doi.org/10.1101/2022.09.01.506194

DeWeber JT, Baer J, Rösch R, Brinker A (2022) Turning summer into winter: nutrient dynamics, temperature, density dependence and invasive species drive bioenergetic processes and growth of a keystone coldwater fish. Oikos: e09316. https://doi.org/10.1111/oik.09316

Dukowska M, Grzybkowska M (2014) Coexistence of Fish Species in a Large Lowland River: Food Niche Partitioning between Small-Sized Percids, Cyprinids and Sticklebacks in Submersed Macrophytes. PLoS ONE 9(11): e109927. https://doi.org/10.1371/journal.pone.0109927

Eckmann R (1989) The distribution of coregonid larvae (*Coregonus lavaretus* and *C. fera*) from Lake Constance in a vertical temperature gradient. Polskie Archiwum Hydrobiologii 36: 485–494.

Eckmann R (1991) A hydroacoustic study of the pelagic spawning behavior of whitefish (*Coregonus lavaretus*) in lake Constance. Canadian Journal of Fisheries and Aquatic Sciences 48(6): 995–1002. https://doi.org/10.1139/f91-116

Eckmann R, Engesser B (2019) Reconstructing the build-up of a pelagic stickleback (*Gasterosteus aculeatus*) population using hydroacoustics. Fisheries Research 210: 189–192. https://doi.org/10.1016/j.fishres.2018.08.002

Eckmann R, Rösch R (1998) Lake constance fisheries and fish ecology. Advances in Limnology 53: 285–301.

Eloranta AP, Kahilainen KK, Amundsen P-A, Knudsen R, Harrod C, Jones RI (2015) Lake size and fish diversity determine resource use and trophic position of a top predator in high-latitude lakes. Ecology and Evolution 5(8): 1664–1675. https://doi.org/10.1002/ece3.1464

FitzGerald GJ, van Havre N (1987) The adaptive significance of cannibalism in sticklebacks (Gasterosteidae: Pisces). Behavioral Ecology and Sociobiology 20(2): 125–128. https://doi.org/10.1007/BF00572634

France RL (1995) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnology and Oceanography 40(7): 1310–1313. https://doi.org/10.4319/lo.1995.40.7.1310

Frisch AJ, Ireland M, Baker R (2014) Trophic ecology of large predatory reef fishes: Energy pathways, trophic level, and implications for fisheries in a changing climate. Marine Biology 161(1): 61–73. https://doi.org/10.1007/s00227-013-2315-4

Gu B, Schelske CL, Hoyer MV (1996) Stable isotopes of carbon and nitrogen as indicators of diet and trophic structure of the fish community in a shallow hypereutrophic lake. Journal of Fish Biology 49(6): 1233–1243. https://doi.org/10.1111/j.1095-8649.1996.tb01792.x

Gugele SM, Baer J, Brinker A (2020) The spatiotemporal dynamics of invasive three-spined sticklebacks in a large, deep lake and possible options for stock reduction. Fisheries Research 232: e105746. https://doi.org/10.1016/j.fishres.2020.105746

Guzzo MM, Haffner GD, Sorge S, Rush SA, Fisk AT (2011) Spatial and temporal variabilities of δ¹³C and δ¹⁵N within lower trophic levels of a large lake: Implications for estimating trophic relationships of consumers. Hydrobiologia 1(1): 41–53. https://doi.org/10.1007/s10750-011-0794-1

Hansen AG, Gardner JR, Connelly KA, Polacek M, Beauchamp DA (2022) Resource use among top-level piscivores in a temperate reservoir: Implications for a threatened cold-water specialist. Ecology Freshwater Fish 31(3): 469–491. https://doi.org/10.1111/eff.12644

Havel JE, Kovalenko KE, Thomaz SM, Amalfitano S, Kats LB (2015) Aquatic invasive species: Challenges for the future. Hydrobiologia 750(1): 147–170. https://doi.org/10.1007/s10750-014-2166-0

Hou Z, Faulk CK, Fuiman LA (2020) Dynamics of diet-egg transfer of fatty acids in the teleost fish, red drum (*Sciaenops ocellatus*). Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences 375(1804): e20190646. https://doi.org/10.1098/rstb.2019.0646

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80(3): 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Janjua MY, Gerdeaux D (2011) Evaluation of food web and fish dietary niches in oligotrophic Lake Annecy by gut content and stable isotope analysis. Lake and Reservoir Management 27(2): 115–127. https://doi.org/10.1080/07438141.2011.566413

Juanes F, Buckel JA, Scharf FS (2002) Feeding Ecology of Piscivorous Fishes. In: Hart PJB, Reynolds JD (Eds) Handbook of Fish Biology and Fisheries (Vol. 1). Blackwell Publishing Ltd, Oxford, 267–283. https://doi.org/10.1002/9780470693803.ch12

Kambikambi MJ, Chakona A, Kadye WT (2019) The influence of diet composition and tissue type on the stable isotope incorporation patterns of a small-bodied southern African minnow *Enteromius anoplus* (Cypriniformes, Cyprinidae). Rapid Communications in Mass Spectrometry 33(6): 613–623. https://doi.org/10.1002/rcm.8393

Karlson AML, Reutgard M, Garbaras A, Gorokhova E (2018) Isotopic niche reflects stress-induced variability in physiological status. Royal Society Open Science 5(2): e171398. https://doi.org/10.1098/rsos.171398

Ljunggren L, Sandström A, Bergström U, Mattila J, Lappalainen A, Johansson G, Sundblad G, Casini M, Kaljuste O, Eriksson BK (2010) Recruitment failure of coastal predatory fish in the Baltic Sea coincident with an offshore ecosystem regime shift. ICES Journal of Marine Science 67(8): 1587–1595. https://doi.org/10.1093/icesjms/fsq109

Madenjian CP, Pothoven SA, Schneeberger PJ, Ebener MP, Mohr LC, Nalepa TF, Bence JR (2010) Dreissenid mussels are not a "dead end" in Great Lakes food webs. Journal of Great Lakes Research 36: 73–77. https://doi.org/10.1016/j.jglr.2009.09.001

Marques DA, Lucek K, Meier JI, Mwaiko S, Wagner CE, Excoffier L, Seehausen O (2016) Genomics of rapid incipient speciation in sympatric threespine stickleback. PLoS Genetics 12(2): e1005887. https://doi.org/10.1371/journal.pgen.1005887

Matthews B, Mazumder A (2005) Temporal variation in body composition (C : N) helps explain seasonal patterns of zooplankton $\delta^{13}C$. Freshwater Biology 50(3): 502–515. https://doi.org/10.1111/j.1365-2427.2005.01336.x

Matthews B, Marchinko KB, Bolnick DI, Mazumder A (2010) Specialization of trophic position and habitat use by sticklebacks in an adaptive radiation. Ecology 91(4): 1025–1034. https://doi.org/10.1890/09-0235.1

McIntyre JK, Beauchamp DA, Mazur MM, Overman NC (2006) Ontogenetic trophic interactions and benthopelagic coupling in Lake Washington: Evidence from stable isotopes and diet analysis. Transactions of the American Fisheries Society 135(5): 1312–1328. https://doi.org/10.1577/T05-099.1

Mehlis M, Bakker TCM, Engqvist L, Frommen JG (2010) To eat or not to eat: Egg-based assessment of paternity triggers fine-tuned decisions about filial cannibalism. Proceedings of the Royal Society B, Biological Sciences 277(1694): 2627–2635. https://doi.org/10.1098/rspb.2010.0234

Minagawa M, Wada E (1984) Stepwise enrichment of 15N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48(5): 1135–1140. https://doi.org/10.1016/0016-7037(84)90204-7

Nyqvist MJ, Cucherousset J, Gozlan RE, Britton JR (2018) Relationships between individual movement, trophic position and growth of juvenile pike (*Esox lucius*). Ecology Freshwater Fish 27(1): 398–407. https://doi.org/10.1111/eff.12355

Ogorelec Ž, Brinker A, Straile D (2022) Small but voracious: Invasive generalist consumes more zooplankton in winter than native planktivore. NeoBiota 78: 71–97. https://doi.org/10.3897/neobiota.78.86788

Østbye K, Harrod C, Gregersen F, Klepaker T, Schulz M, Schluter D, Vøllestad LA (2016) The temporal window of ecological adaptation in postglacial lakes: A comparison of head morphology, trophic position and habitat use in Norwegian threespine stickleback populations. BMC Evolutionary Biology 16(1): e102. https://doi.org/10.1186/s12862-016-0676-2

Parnell AC, Inger R (2019) Stable Isotope Mixing Models in R With Simmr. https://cran.r-project.org/web/packages/simmr/vignettes/simmr.html

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: Coping with too much variation. PLoS ONE 5(3): e9672. https://doi.org/10.1371/journal.pone.0009672

Perga ME, Gerdeaux D (2005) 'Are fish what they eat' all year round? Oecologia 144(4): 598–606. https://doi.org/10.1007/s00442-005-0069-5

Perga M-E, Gerdeaux D (2006) Seasonal variability in the $\delta^{13}C$ and $\delta^{15}N$ values of the zooplankton taxa in two alpine lakes. Acta Oecologica 30(1): 69–77. https://doi.org/10.1016/j.actao.2006.01.007

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83(3): 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Preston DL, Henderson JS, Falke LP, Novak M (2017) Using survival models to estimate invertebrate prey identification times in a generalist stream fish. Transactions of the American Fisheries Society 146: 1303–1314. https://doi.org/10.1080/00028487.2017.1370018

Probst WN, Eckmann R (2009) The influence of light on the diel vertical migration of young-of-the-year burbot Lota lota in Lake Constance. Journal of Fish Biology 74(1): 150–166. https://doi.org/10.1111/j.1095-8649.2008.02120.x

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/

Ransom AL, Houghton CJ, Hanson SD, Hansen SP, Shaffer M, Forsythe PS (2022) Larval lake whitefish distribution in the open waters of Green Bay, Lake Michigan. Journal of Great Lakes Research 48(1): 229–237. https://doi.org/10.1016/j.jglr.2021.11.003

Ravinet M, Takeuchi N, Kume M, Mori S, Kitano J (2014) Comparative analysis of japanese three-spined stickleback clades reveals the Pacific Ocean lineage has adapted to freshwater environments while the Japan Sea has not. PLoS ONE 9(12): e112404. https://doi.org/10.1371/journal.pone.0112404

Reimchen TE, Ingram T, Hansen SC (2008) Assessing niche differences of sex, armour and asymmetry phenotypes using stable isotope analyses in Haida Gwaii Sticklebacks. Behaviour 145(4–5): 561–577. https://doi.org/10.1163/156853908792451449

Rinchard J, Kestemont P (2003) Liver changes related to oocyte growth in roach, a single spawner fish, and in bleak and white bream, two multiple spawner fish. International Review of Hydrobiology 88: 68–76. https://doi.org/10.1002/iroh.200390006

Roch S, von Ammon L, Geist J, Brinker A (2018) Foraging habits of invasive three-spined sticklebacks (*Gasterosteus aculeatus*) – impacts on fisheries yield in Upper Lake Constance. Fisheries Research 204: 172–180. https://doi.org/10.1016/j.fishres.2018.02.014

Ros A, Dunst J, Gugele S, Brinker A (2019) Anti-predator mechanisms in evolutionarily predator-naïve vs. adapted fish larvae. Ecosphere 10(4): e02699. https://doi.org/10.1002/ecs2.2699

Rösch R (2014) Lake Constance fish and fisheries. In: Welcomme RL, Valbo-Jorgensen J, Walls AS (Eds) Inland Fisheries Evolution and Management – Case Studies from four Continents. FAO Fisheries and Aquaculture Technical Paper. FAO, Rome, 21–32.

Rösch R, Baer J, Brinker A (2018) Impact of the invasive three-spined stickleback (*Gasterosteus aculeatus*) on relative abundance and growth of native pelagic whitefish (*Coregonus wartmanni*) in Upper Lake Constance. Hydrobiologia 824(1): 243–254. https://doi.org/10.1007/s10750-017-3479-6

Rubenson ES, Lawrence DJ, Olden JD (2020) Threats to rearing juvenile chinook salmon from nonnative smallmouth bass inferred from stable isotope and fatty acid biomarkers. Transactions of the American Fisheries Society 149(3): 350–363. https://doi.org/10.1002/tafs.10237

Sachs L (1997) Angewandte Statistik. Springer, Berlin, 884 pp. https://doi.org/10.1007/978-3-662-05746-9

Skinner MM, Martin AA, Moore BC (2016) Is lipid correction necessary in the stable isotope analysis of fish tissues? Rapid Communications in Mass Spectrometry 30(7): 881–889. https://doi.org/10.1002/rcm.7480

Sokal RR, Rohlf JF (2003) Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freemann and Company, New York.

Strayer DL (2010a) Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology 55: 152–174. https://doi.org/10.1111/j.1365-2427.2009.02380.x

Strayer DL (2010b) Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology 55: 152–174. https://doi.org/10.1111/j.1365-2427.2009.02380.x

Treanor HB, Ray AM, Layhee M, Watten BJ, Gross JA, Gresswell RE, Webb MAH (2017) Using carbon dioxide in fisheries and aquatic invasive species management. Fisheries (Bethesda, Md.) 42(12): 621–628. https://doi.org/10.1080/03632415.2017.1383903

Vander Zanden MJ, Rasmussen JB (1999) Primary consumer δ 13C and δ 15N and the trophic position of aquatic consumers. Ecology 80(4): 1395–1404. https://doi.org/10.1890/0012-9658(1999)080[1395:PCCANA]2.0.CO;2

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δ15N enrichment: A meta-analysis. Oecologia 136(2): 169–182. https://doi.org/10.1007/s00442-003-1270-z

Westrelin S, Balzani P, Haubrock PJ, Santoul F (2023) Interannual variability in the trophic niche of young-of-year fish belonging to four piscivorous species coexisting in a natural lake. Freshwater Biology 68(3): 487–501. https://doi.org/10.1111/fwb.14041

Yohannes E, Franke L, Rothhaupt KO (2014) Zebra mussel δ13C and δ15N as a proxy for depth-specific pelagic isotope profiles and lake temperature. Hydrobiologia 731(1): 191–198. https://doi.org/10.1007/s10750-013-1786-0

Supplementary material 1

Supplementary information
Authors: Sarah Maria Gugele, Jan Baer, Christina Spießl, Elizabeth Yohannes, Steve Blumenshine, Barnaby J. Roberts, Mario R. Mota-Ferreira, Alexander Brinker
Data type: docx
Explanation note: **table S1.** Quantiles of the posterior distribution of the Bayesian mixing model parameter estimation. **table S2.** Correlation of the posterior distribution of the Bayesian mixing model parameter estimation. **figure S1.** Isospace plot representing the isotopic signatures of the stickleback liver samples caught in summer (June and July) and in winter (December to March). **table S3.** Quantiles of the posterior distribution of the Bayesian mixing model parameter estimation for models without the fish sources. **table S4.** Correlation of the posterior distribution of the Bayesian mixing model parameter estimation for models without the fish sources. **figure S2.** Posterior distribution of dietary proportion estimates of different food sources from sticklebacks from the pelagic and littoral zone of ULC during summer and winter, according to Bayesian modelling without fish sources, expressed as Box-and-Whisker plots with median values and interquartile range (IQR).

Link: https://doi.org/10.3897/neobiota.87.100355.suppl1