Signs of the times: Isotopic signature changes in several fish species following invasion of Lake Constance by quagga mussels

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A R T I C L E   I N F O

Article history:
Received 26 October 2021
Accepted 14 March 2022
Available online 24 March 2022
Communicated by Orlane Anneville

Keywords:
Coregonus wartmanni
Energy pathways
Food web
Invasive species
Isotopic niche

A B S T R A C T

Since the arrival of the invasive quagga mussels Dreissena rostriformis bugensis in Lake Constance, significant changes in the zooplankton and benthic invertebrate community were observed. Five years later the quagga mussel has become the dominating species of the benthic community. Its effects on other components of the food web, especially those at higher trophic levels such as fish, remain unclear around the world. To evaluate the actual impact of quaggas on the local food web of Lake Constance, the stable isotope compositions of pelagic whitefish and different benthic fish species from before and after the quagga invasion were compared. A significant increase in δ13C was detected in pelagic whitefish one year after the establishment of the quagga mussel in the lake. This change was most likely the consequence of an increase in benthic-derived nearshore primary production and a shift towards more littoral feeding, than a change in dietary composition. Stomach content analysis of contemporary samples revealed that pelagic whitefish (Coregonus wartmanni) still feed exclusively on pelagic zooplankton. In contrast, benthic whitefish (Coregonus macrophthalmus), roach (Rutilus rutilus) and tench (Tinca tinca) show today high levels of quagga consumption. However, this behaviour alone could not explain the observed differences in δ15N from periods before and after the quagga invasion. The results suggest that energy sources and pathways have changed considerably for both pelagic and benthic dwelling fish species in Lake Constance following the establishment of quaggas.

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Dreissena spp. (Molloy et al., 1997). Furthermore, the increase in benthic invertebrate biomass typically observed after a quagga invasion is often followed by an increase in biomass of benthivorous fish in the littoral zone (Higgins and Vander Zanden, 2010; Karatayev et al., 2015).

The extent to which quagga mussels impact higher trophic levels remains debatable. This is especially true in areas where quaggas potentially affect the production of economically important fish stocks (Bunnell et al., 2014; He et al., 2015; Riley and Dunlop, 2016). For example in North America, a number of studies alluded to potential shifts in energy pathways between invasive quaggas and fish populations (Rennie et al., 2009; Turschak et al., 2014), but direct comparisons between post- and pre-invasion scenarios are scarce. However a large study explored links between quaggas and resource use by the benthivorous lake whitefish Coregonus clupeaformis using stable isotopes ratios (δ13C and δ15N) determined from archived scale samples collected from many different lakes before and after the mussels arrived (Fera et al., 2017). The authors showed that δ13C of this keystone native fish species increased following the mussel invasion. Trends in δ15N were less clear but generally showed a decline in most lakes after the establishment of the mussel (Fera et al., 2017). This result provided important support for the theory that fundamental energy pathways are changed following quagga invasion.

The present study was conducted in Lake Constance, the second largest lake in Central Europe. Here, the presence of quagga mussels was first reported in 2016 (Hydra, 2021). Since then, the species has spread rapidly. Quaggas are now hyperabundant and a dominant feature of the benthic community lake-wide, occurring in densities of more than 2,000 individuals per square meter (personal communications T. Basen) on sandy bottoms down to 40 m depth and on stones and rocks as deep as 100 m, with further records even in the deepest parts of the lake at 250 m (Hydra, 2021). Quagga mussels are today the dominant members of the bivalve community, having almost completely displaced the previously abundant non-native zebra mussel Dreissena polymorpha (Pallas 1771) (Hydra, 2021), introduced in the mid-1960 s (Cleven and Frenzel, 1993; Werner et al., 2005). Currently, zebra mussels are hard to detect and occur isolated only in some shallower parts of the lake (T. Basen, per. comm.). The extent to which quagga mussels impact the fundamental energy pathways within the lake remains unknown, but results from elsewhere suggest that zooplankton density in the pelagic zone will have decreased due to the filtration by the quaggas while production in the littoral zone will have increased (Evans et al., 2011; Fahrenstiel et al., 2010; Vanderploeg et al., 2010). It is hypothesised that recent declines in both growth rates and catches (Rösch et al., 2018) of the most commercially important fish species in Lake Constance, the pelagic feeding whitefish Coregonus warthmani (Bloch 1784) (Baer et al., 2022, 2017) may be a result of the species being forced to feed more in littoral waters. A similar shift was previously documented for the sister species C. clupeaformis in the Great Lakes (Rennie et al., 2009). If this is the case, pelagic whitefish might be expected to show an increase in δ13C after quagga invasion, because primary producers of shallow waters are more enriched in δ13C than those living in pelagic zone (Fera et al., 2017; Rennie et al., 2009; Vander Zanden and Rasmussen, 1999). Furthermore, it is hypothesised that a fundamental shift in energy pathways similar to that outlined above has taken place post-invasion for many other fish taxa. To test both hypotheses, the present study compares the δ13N and δ15C values from pelagic feeding whitefish and benthic species of different feeding guilds before and after the invasion of the quagga mussel in Lake Constance. Further analyses of stomach contents, isotopic niche overlap of the different feeding guilds, and isotopic composition of energetic baseline organisms (mussels and snails) were used to interpret the results. Those have implications beyond Lake Constance and may inform responses in other large lakes in Central Europe facing invasion by quagga mussels.

Material and methods

Approval by a review board institution or ethics committee for procedures undertaken in the present study was not necessary because all fish were caught under permission granted by the local fisheries administration (Regierungspräsidium Tübingen) by licenced personnel. All captured species were handled according to the German Animal Protection Law (§ 4) and the ordinance on slaughter and killing of animals (Tierschutzschlachtverordnung § 13).

Sampling

Lake Constance is situated between Austria, Germany and Switzerland (Fig. 1) and is part of the Rhine drainage basin. It has a total surface area of 536 km², including a 472 km² large, deep, oligotrophic Upper Lake (ULC) and a 63 km² large, shallower, mesotrophic Lower Lake (LLC). The lake has undergone intensive re-oligotrophication between 1990 and 2010 (Baer et al., 2017; Stich and Brinker, 2010), and its fish community comprises a minimum of 30 species (Eckmann and Rösch, 1998) of which about 10 are targeted by the fishery (Rösch, 2014). Of these, whitefish (Coregonus spp.) make up the economically most important catch (Baer et al., 2017).

Before the quagga invasion took place, no specific monitoring of quaggas and their influence on the fish community was conducted. However, in two different scientific studies tissue samples from a variety of fish species were taken and stored before the quagga invasion took place. Due to this fortunate circumstance it was possible to get some tissue samples from eight different fish species of the pre-quagga period. Therefore, the present study focused on the following fish species from five different feeding guilds: 1) zooplanktivorous pelagic whitefish from ULC; 2) benthivorous whitefish (Coregonus macrophthalmus Nüsslin 1882) from ULC, plus bleak (Alburnus alburnus L.), roach (Rutilus rutilus L.) and tench (Tinca tinca L.) from both lakes; 3) benth-zooplanktivorous benthic whitefish (Coregonus spp.) from LLC; 4) herbivorous redd (Scardinius erythrophthalmus L.) from ULC and 5) piscivorous pike (Esox lucius L.) from both lakes.

In a study analysing the energy content of whitefish, pelagic whitefish of ULC were sampled from July to September every year from 2014 to 2020, using drifting gill nets. For each year, tissue samples were prepared for analysis by oven-drying at around 60 °C for 48 h and grinding to fine powder. These powders were stored in vacuum-insulated plastic bags for later analysis. From ten powders per year; we used sub-samples for our analysis (n = 70). The seven other fish species were sampled during two surveys looking for the overall composition and changes in the local fish community: 1) an intensive four week fishing campaign in both parts of the lake in September 2014 (Alexander et al., 2015) and 2) an additional survey in August and September 2020, again covering both parts of the lake. All fish were caught using benthic gill nets in the littoral zone and euthanized with an overdose of clove oil (1 mL L⁻¹) and a gill cut. In 2014, (hereafter called “pre-quagga” period), samples of the white muscle of the seven additional species were stored in a freezer at −20 °C for further analysis. Those samples were used to compare their stable isotope contents with samples taken in 2020 (hereafter called “post-quagga” period). The samples of 2020 were stored until stable isotope analysis in the same way as the samples from 2014. Preliminary studies (Baer et al., 2022) identified benthic whitefish, roach, and tench as “quagga consumers” and pelagic
whitefish, rudd, pike, and bleak as “non-quagga consumers” (Table 1).

All fish were measured and weighed wet shortly after capture to the nearest millimetre total length (TL) and gram. In the above-mentioned survey of benthivorous species in 2014, no stomach content analysis was conducted. During the sampling of pelagic whitefish between 2014 and 2019, stomachs were opened in irregular intervals and the food components were macroscopically identified (R. Rösch, personal communication). To get a deeper insight into the actual, post-quagga diet composition, in total 345 fish were caught in 2020 and used for stomach content analysis (Table 1). In each, an abdominal incision was made with small scissors and the digestive tract (stomach and intestine) was removed, separated from other visceral organs and remains and stored in methylated ethanol (70 %). Digestive tracts were dried with tissue paper and weighed to the nearest 0.01 g on a micro-balance, opened by a longitudinal slit so that food items could be carefully pushed out into a glass frame. The empty digestive tract was reweighed. Food items were identified and counted under a binocular (Zeiss Stemi 508, Carl Zeiss Microscopy GmbH, Jena, Germany), using ten-fold magnification, following the procedure described in (Brandner et al., 2013). Due to the fact that shell fragments from zebra mussels could be much easier identified correctly than those from quaggas (Beggel et al., 2015), it was especially examined for fragments with the typical features of D. polymorpha, i.e. the arched and flattened ventral surface of the shell and the sharp angled transition of the ventral and dorsal surface. If none of those were found, in the next step fragments with the typical features of quaggas were sought. If no discrimination was possible (<5% of all stomachs with mussels), the fragments were defined as pieces from Dreissena spp. Because more than 90% of all consumed dreissenids were categorized as quagga mussels (although the protocol favoured the classification as zebra mussel) and that zebra mussels were rare in the lake in 2020, all found dreissenids were assigned to quagga mussels. The food items were categorized as follows: 1) quagga mussels, 2) Asian clam (Corbicula fluminea), 3) snails (Lymnaea stagnalis, Bithynia tentaculata, Viviparus viviparus), 4) benthic macroinvertebrates (Gammarus spp. and larvae, pupae and adults of different insect species), 5) pelagic zooplankton (Bosmina, Copepoda, Daphnia/Diaphanosoma), 6) aquatic plants (remains of submerged macrophytes), 7) fish (Perca fluviatilis, Cyprinidae). For each analysed digestive tract, the observed food categories were weighed as a group to the nearest 0.01 g and the proportion of food in each category was calculated by wet weight (%W).

Preliminary studies showed that benthic whitefish, roach, and tench consume large quantities of quagga mussels and several species of snail (Baer et al., 2022). To compare the isotopic value in the source and the consumer and to discuss the possibility that a switch from snail to mussel feeding could be responsible for

**Table 1**

Relative proportions of different food categories by wet weight (%W ± standard deviation SD) in the diets of seven different fish species from Upper (ULC) and Lower (LLC) Lake Constance.

<table>
<thead>
<tr>
<th>Species</th>
<th>Lake (sample size)</th>
<th>%W (±SD)</th>
<th>Quagga mussel</th>
<th>Asian clam</th>
<th>Snails</th>
<th>Benthic macroinvertebrates</th>
<th>Pelagic zooplankton</th>
<th>Aquatic plants</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benthic whitefish</td>
<td>ULC (76)</td>
<td>14 (±31)</td>
<td>1 (±6)</td>
<td>1 (±7)</td>
<td>43 (±40)</td>
<td>100</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td></td>
<td>LLC (55)</td>
<td>7 (±24)</td>
<td>6 (±17)</td>
<td>0 (±6)</td>
<td>29 (±41)</td>
<td>0 (±0)</td>
<td>100</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td>Pelagic whitefish</td>
<td>ULC (10)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>100</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td></td>
<td>LLC</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Roach</td>
<td>ULC (66)</td>
<td>81 (±35)</td>
<td>0 (±0)</td>
<td>5 (±19)</td>
<td>6 (±18)</td>
<td>0 (±0)</td>
<td>7 (±23)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td></td>
<td>LLC (55)</td>
<td>61 (±47)</td>
<td>15 (±34)</td>
<td>23 (±44)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>2 (±6)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td>Tench</td>
<td>ULC (30)</td>
<td>23 (±41)</td>
<td>0 (±2)</td>
<td>60 (±46)</td>
<td>15 (±13)</td>
<td>0 (±0)</td>
<td>25 (±6)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td></td>
<td>LLC (10)</td>
<td>43 (±46)</td>
<td>0 (±0)</td>
<td>25 (±42)</td>
<td>32 (±45)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td>Bleak</td>
<td>ULC (19)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>66 (±5)</td>
<td>0 (±0)</td>
<td>34 (±11)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td></td>
<td>LLC (20)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>59 (±10)</td>
<td>0 (±0)</td>
<td>41 (±13)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td>Rudd</td>
<td>ULC (11)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>100</td>
<td>0 (±0)</td>
</tr>
<tr>
<td></td>
<td>LLC (15)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pike</td>
<td>ULC (18)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>0 (±0)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>LLC (15)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Fig. 1. Location of Lake Constance in Europe (left side, black square) and the position of Upper and Lower Lake Constance (right side).
changes in the isotopic composition, the common pond snail *Lymnea stagnalis* (L.) (n = 20) and the faucet snail *Bithynia tentaculata* (L.) (n = 10) were additionally collected from the littoral of ULC in August 2020. The widespread freshwater snail *Viviparous viviparus* (L.) was collected by snorkelling in LLC in September 2020 (n = 20), and quagga mussels were collected from piles at depth of 0.5 – 2 m in both parts of the lake (n = 100 each lake). Soft tissue excised from snails and mussels was frozen at −20 °C until further stable isotope analysis.

A total of 401 samples of white muscle tissues were taken from fish captured in ULC and LLC before (2014) and after (2020) the quagga invasion (Table 2). In 2020, samples were taken from fish used for stomach content analysis.

For benthic whitefish (here: *Coregonus macrophthalmus* from ULC and *Coregonus* spp. from LLC) and roach, both known to be potential quagga consumers (Baer et al., 2022), post-invasion samples for stable isotope analysis were taken from fish with and without mussels in the digestive tract. This was done to test whether changes in isotopic values could be associated with quagga consumption or to indicate specialist quagga feeding by single individuals. Therefore, tissues samples from ULC included 11 benthic whitefish and 25 roach with mussels in the stomachs and 7 whitefish and 9 roach without mussels in the stomachs. For LLC the corresponding numbers were 5 whitefish and 4 roach with mussels and 15 whitefish and 16 roach without mussels.

### Stable isotope analysis

Samples of mussels, snails and fish were prepared for analysis via freeze drying at −50 °C and pressurization at <1 mbar, then ground to homogenous powder using a mixer mill. Sample powder (0.3 – 0.4 mg) was weighed into tin capsules and combusted in an isotope ratio mass spectrometer (Delta plus, Finnigan MAT, MassCom GmbH, Bremen, Germany), interfaced (viaConFlo II, Finnigan MAT, MassCom 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 (δ13C, δ15N) in parts per thousand deviations (%), where δ = 1000 × (Rsample/Rstandard − 1) relative to the Pee Dee Belemnite (PDB) standard for carbon and atmospheric N2 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 δ13C and δ15N respectively.

When comparing organic materials from different sampling years, the Suess effect has to be considered (Verburg, 2007), taking into account changes in δ13C in any carbon reservoir resulting from anthropogenic release of CO2 to the atmosphere (Keeling, 1979; Verburg, 2007). To this end, atmospheric CO2 data measured at Mauna Loa by the Scripps CO2 program (Keeling and Keeling, 2017) was used, in which mean annual δ13C of atmospheric CO2, linearly increased by 0.0281‰ per year between 1994 and 2020 (r² = 0.9877; n = 27). Thus to account for the Suess effect and make the data comparable, 0.0281‰ was added to the δ13C values for each year of pelagic whitefish data and 0.169‰ (+6 × 0.0281) was added to the 2020 data of all other analyzed species.

### Statistical analysis

Temporal trends in the stable isotope ratios of pelagic whitefish (sampled yearly from 2014 to 2020) were investigated using a segmented least squares linear regression (i.e., “breakpoint analysis”) to identify potential breakpoints in the slopes of δ15N and δ13C through time, and test their temporal relationship with quagga establishment in ULC during 2016. The statistical significance of the breakpoint was set at P < 0.05 and tested by respective confidence values.

To test the effects on δ15N and δ13C values of quagga consumers (benthic whitefish, roach, and tench) and non-quagga consumers (bleak, Rudd, and pike) sampled before (2014) and after (2020) quagga invasion in both parts of the lake (ULC and LLC) the following general linear model (GLM) (Sachs, 1997) was used:

\[
Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \delta_{ijklm}
\]

where \(Y_{ijklm}\) is the δ15N (or δ13C) value of muscle tissue; \(\mu\) is the overall mean, \(\alpha_i\) denotes feeding behaviour (quagga consumer, non-quagga consumer), \(\beta_j\) is period (2014 and 2020), \((\alpha\beta)_{ij}\) is the interaction between feeding behaviour and period, \(\gamma_k\) represents lake (ULC and LLC), and \(\delta_{ijklm}\) is the random residual error. Single outliers were excluded from the dataset (selection criterion: more than eight times standard deviation). Student-t tests were used for post hoc comparisons between period, feeding behaviour, and lake.

To test whether quaggas found in the digestive tracts of roach and benthic whitefish could be related to highly specialized quagga feeding of single individuals, the following GLM was used:

\[
Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\gamma\beta)_{jk} + \delta_{ijklm}
\]

where \(Y_{ijklm}\) is the δ15N (or δ13C) value of muscle tissue in roach and benthic whitefish; \(\mu\) is the overall mean, \(\alpha_i\) denotes quagga presence in the digestive tract (yes/no), \(\beta_j\) is length (TL in cm), \(\gamma_k\) represents lake (LLC and ULC), \((\alpha\beta)_{ij}\) is the interaction between quaggas in the digestive tracts and TL, \((\gamma\beta)_{jk}\) is the interaction between TL and lake, and \(\delta_{ijklm}\) is the random residual error. Student-t tests were used for post hoc comparison between lake and quagga presence or absence the digestive tracts (yes vs. no).

Data from all examined fish species from both lakes and for both periods (before and after quagga invasion, and for ULC pelagic whitefish sampled in 2014 and 2020) were pooled according to feeding guild (benthivorous, zooplanktivorous, benthizooplanktivorous, herbivorous, and piscivorous) in order to identify potential breakpoints in the slopes of δ15N and δ13C through time, and test their temporal relationship with quagga establishment in ULC during 2016. The statistical significance of the breakpoint was set at P < 0.05 and tested by respective confidence values.

### Table 2

Species, total length (TL) range, and number of fish used for stable isotope analysis (before and after the quagga invasion) from Upper (ULC) and Lower (LLC) Lake Constance.

<table>
<thead>
<tr>
<th>Species</th>
<th>Quagga consumer?</th>
<th>Min.–max. TL in cm</th>
<th>Samples (n) before quagga invasion</th>
<th>Samples (n) after quagga invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULC</td>
<td>LLC</td>
</tr>
<tr>
<td>Benthic whitefish</td>
<td>Yes</td>
<td>26 – 61</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Pelagic whitefish</td>
<td>No</td>
<td>17 – 40</td>
<td>30</td>
<td>–</td>
</tr>
<tr>
<td>Roach</td>
<td>Yes</td>
<td>9 – 37</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>TENCH</td>
<td>Yes</td>
<td>14 – 53</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>BLEAK</td>
<td>No</td>
<td>5 – 16</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Rudder</td>
<td>No</td>
<td>5 – 30</td>
<td>16</td>
<td>–</td>
</tr>
<tr>
<td>Pike</td>
<td>No</td>
<td>23 – 60</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td><strong>103</strong></td>
<td><strong>40</strong></td>
</tr>
</tbody>
</table>
to calculate the standard ellipse area corrected for small samples (SEA_c). The SEA_c represents the core isotopic niche of each guild after factoring in maximum likelihood, similar to a two-dimensional measurement of standard deviation, and is drawn from around 40% of data (Jackson et al., 2011). The isotopic niche overlap of the different feeding guilds was subsequently calculated as a proportion of the sum of the non-overlapping area of SEA_c. All analyses were performed with help of 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).

All other statistics were run on JMP Pro 16.1 (64 bit, SAS Institute).

Results

Stomach content

In both lakes, the highest proportions of quaggas by weight were found in the digestive tract of roach (Table 1), in which species all other food categories appeared of minor importance (Table 1). Quaggas were also consumed by tench and benthic whitefish, but in smaller amounts (Table 1). For tench, snails were of much greater importance than mussels, making up nearly 60% of all tench prey in ULC. The tench in LLC also ate far more snails and other benthic macroinvertebrates than mussels (including both quagga and Asian clam). For benthic whitefish in both lakes, the food category with the highest %W-values in the digestive tracts was benthic macroinvertebrates, especially gammarids (Table 1), while the diet of bleak was dominated by insect pupae (Table 1). Pelagic whitefish fed exclusively on zooplankton, rudd on aquatic plants and pike solely on fish (Table 1).

Breakpoint analysis of pelagic whitefish

In the segmented regression analysis for pelagic whitefish sampled yearly between 2014 and 2020, a significant breakpoint was detected in the slope of δ13C values in 2017, one year after the establishment of the quagga mussel in the lake (Fig. 2). Values of δ15N on the other hand remained relatively consistent between pre- and post-quagga periods (Fig. 2).

δ15N and δ13C values before and after quagga establishment for other fish species

For all other analyzed fish species, clear differences were observed in δ15N between the periods with and without quaggas. In benthic whitefish in both lakes δ15N values increased between 2014 and 2020 (Fig. 3), while for roach the δ15N values decreased from 2014 to 2020 (Fig. 3). In tench differences in δ15N were only observed for fish from LLC (Fig. 3). Bleak exhibited lower δ15N values in 2020 than in 2014 in both lakes, similar to rudd, which was only available for ULC (Fig. 3). For pike in both lakes, δ15N increased between 2014 and 2020 (Fig. 3). These observations were corroborated by the GLM for δ15N (r2 adjusted = 0.31, n = 346, P < 0.0001), indicating that period and lake both had a highly significant impact on the outcome of the model (P < 0.0001). Overall δ15N-values recorded from LLC fish were significantly larger than those from ULC (t-test, α < 0.05) while in both lakes values were significantly lower in the post-quagga invasion period (2020) than they had been pre-invasion (2014) (t-test, α < 0.05). Neither feeding behaviour (quagga consumption or non-quagga consumption) nor the interaction of feeding behaviour and period had an impact on the outcome of the model.

Compared to δ15N, the changes observed in δ13C between the pre- and post-quagga periods among the sampled fish species were small (Fig. 3), a result underlined by the outcome of the GLM for δ13C (r2 adjusted = 0.50, n = 346, P < 0.0001) in which period had no impact on the output of the model (P < 0.05) and according to the profiler only a small effect strength in the interaction leading to a marginal overall impact on the model output. In contrast, feeding behaviour was highly influential on the outcome of the model (P < 0.0001). Lake had also a significant influence (P = 0.002). Post hoc comparisons revealed that non-quagga consumers showed significantly higher δ13C values than quagga consumers (t-test, α < 0.05) and δ13C values in LLC were significantly higher than in ULC (t-test, α < 0.05).

Stable isotope values of mussels and snails

Quagga mussels and snails exhibited highly divergent stable isotope values in both lakes (Table 3). δ15N values of quaggas were
lower than those of snails and only the great pond snail *Lymnea stagnalis* exhibited a δ<sup>13</sup>C value as low as that of quaggas (Table 3).

### Specialized quagga feeding

The GLM showed that presence or absence of quaggas in the digestive tracts of benthic whitefish had no impact on the outcomes for either the δ<sup>13</sup>C model ($r^2_{\text{adjusted}} = 0.22$, $n = 38$, $P = 0.02$) or the δ<sup>15</sup>N model ($r^2_{\text{adjusted}} = 0.14$, $n = 38$, $P = 0.02$). Furthermore, there was no significant effect of the other tested parameters (length, lake or the interaction of length with lake or quagga consumption) on stable isotopes in benthic whitefish. The δ<sup>13</sup>C model for roach ($r^2_{\text{adjusted}} = 0.39$, $n = 54$, $P < 0.0001$) showed comparable results, with no influence of quagga presence in the digestive tract on outcomes and no impact of lake (ULC or LLC), interaction of length with lake or quagga-consuming tendency. However, there was a significant positive influence of total length of this species on δ<sup>13</sup>C ($P < 0.05$). The GLM for δ<sup>15</sup>N ($r^2_{\text{adjusted}} = 0.15$, $n = 54$, $P = 0.02$) showed a positive correlation between total length of roach in LLC and δ<sup>15</sup>N and a significant influence of the interaction between total length and lake ($P = 0.04$). As for all other models the remaining parameters showed no impact on roach model ($P$ greater than 0.05).

### Isotopic niche overlap

**Fig. 4** displays core isotopic niches of each feeding guild before and after the quagga invasion of Lake Constance. In ULC during the pre-quagga period, the standard ellipse area corrected for small samples (SEAc) ranged from 1.6 for zooplanktivorous fish to 6.6 for benthivorous species while in LLC the range was 2.7 for piscivorous to 3.3 for benthiv-zooplanktivorous whitefish (Fig. 4). Post-invasion, in 2020, the margin of SEAc values was higher, ranging in ULC from 2.0 for piscivorous species to 7.8 for benthivorous and in LLC from 2.0 for piscivorous to 5.1 in benthivorous fish. Pre-invasion niche overlaps observed between benthivorous and piscivorous fish amounted to 19.1% in ULC and 51.2% in LLC (Fig. 4), but by 2020 no such overlaps were apparent in either ULC or in LLC despite the larger standard ellipse areas (Fig. 4).

### Discussion

The data presented here corroborates the first hypothesis, that following quagga mussel invasion the pelagic living whitefish *Coregonus wartmanni* in ULC has been forced into littoral waters, comparable to its sister species *C. clupeaformis* in the Great Lakes (Rennie et al., 2009). This change is evidenced by the break point analysis and an enrichment of δ<sup>13</sup>C in pelagic whitefish shortly (one year) after the mussel invasion. It is well established that primary producers in shallower waters are enriched in δ<sup>13</sup>C relative to those in deeper waters (Fahnenstiel et al., 2010; Vander Zanden and Rasmussen, 1999; Vanderploeg et al., 2010) and this general trend seems also to hold for higher trophic levels (Fera et al., 2017). Therefore, the δ<sup>13</sup>C value of a consumer can serve as an indicator of feeding location, whereby higher δ<sup>13</sup>C values point to a greater importance of nearshore sources (Rennie et al., 2009).
Interestingly in ULC, there is no evidence that pelagic whitefish have changed their foraging habits to favor benthic organisms. The stomach analyses reported here reveal no macrozoobenthic content and suggest that pelagic zooplankton continue to dominate the diet, as it has for decades (Eckmann and Rösch, 1998). One possible explanation for the observed increase in $\delta^{13}C$ in pelagic whitefish is an overall shift in energy pathways in ULC whereby the entire lake system, including pelagic zooplankton, is now more driven by benthic primary production. This shift in energy production from the open pelagic to the benthic region is called benthification (Mayer et al., 2014) and was also documented after the invasion of the quagga mussel in Lake Simcoe, Canada, (Rennie et al., 2013). Thus, changes in the stable isotope pattern of analyzed fish may be part of an increasing $\delta^{13}C$ across both primary and secondary nearshore consumers. If this is the case, a profound shift in the source of carbon at the base of the food web could be responsible for the observed changes (Rennie et al., 2013) rather than a change in diet composition. Future studies analyzing benthic and pelagic samples from different biota and from different depths in Lake Constance will be required to further develop this hypothesis, and sediment samples from periods before and after quagga invasion may help to resolve historic and existing energy pathways.

The profound changes in $\delta^{13}C$ of pelagic whitefish observed here also corroborate the second hypothesis of the present study, that sources of energy, and thus energy pathways, have changed considerably in Lake Constance since the establishment of quaggas. This change is supported by the elimination of pre-invasion isotopic niche overlaps between benthivorous and piscivorous feeding guilds previously (before quagga invasion) amounting to 19% and 51% niche overlaps in ULC and LLC respectively.

On first view it is surprising that no changes were observed in $\delta^{15}N$ of pelagic whitefish between pre- and post-quagga periods. However, this is consistent with results from other large lakes where hyperabundant quaggas are associated with greatly altered energy pathways. For example in Lake Huron whitefish exhibited large changes in $\delta^{13}C$ after quagga invasion, but no or only slightly (dependent on sampling site) changes in $\delta^{15}N$ (Fera et al., 2017). Meanwhile the model for $\delta^{15}N$ revealed that in Upper and Lower Lake Constance the occurrence of quaggas significantly changed the isotopic values of other analyzed fish species. Highly variable shifts in $\delta^{15}N$ across taxa like in Lake Constance have also been observed in other lakes (Rennie et al., 2013; Turschak et al., 2014). For herbivorous rudd in Upper Lake Constance, the observed shift may be associated with changes in the submerged macrophytes community following quagga invasion (Geisler et al., 2016; Zhu et al., 2006). For roach, tench, bleak, and benthic whitefish the variations in the isotopic signature may be associated with changes in the benthic macrozoobenthos community, because aggregations of quaggas (druses) rapidly develop into three-dimensional reef-like structures that provide shelter and food for invertebrates, thereby boosting diversity and species interactions (Karatayev et al., 2015; Umek et al., 2010). Such changes at the base of the food web could easily influence consumers at higher trophic levels, such as piscivorous fish (Turschak and Bootsma, 2015) and thus account for large difference in the $\delta^{15}N$ value of piscivorous pike in Lake Constance before and after quagga invasion. While it is possible that overarching impacts, such as watershed influences from sewage or agricultural inputs or an increase in N-fixers (i.e., cyanobacteria) among the algal community (Rennie et al., 2013) might cause to decline the $\delta^{15}N$ value of fishes, there is no evidence that such impacts exist on a large scale and hence cannot explain different directional shifts observed here. Indeed,
there appears to be no single explanation for the variable changes in the $\delta^{15}N$ values of different endemic fish species. Additional work is required to determine the causes for varying $\delta^{15}N$ values in particular benthic fish species in Lake Constance.

Meanwhile the changes in the isotopic signature of endemic benthivorous fish species in Lake Constance point to a general change in feeding behavior. Before quagga mussel invasion, whitefish were only rarely found in benthivorous whitefish, and only a few individual mussels when mussels were found (R. Rösch, unpublished data). In recent years, more than 1000 quaggas can be found in large benthic whitefish (data not shown). Furthermore, the $\delta^{13}C$ model reveals that quagga consumers exhibit significantly lower $\delta^{13}C$ values than non-quagga consumers and that the interaction of feeding behaviour and period had a significant impact. The analyses of stomach contents revealed that roach in both lakes and tench in LLC consume large quantities of quagga mussels. Roach are opportunistic feeders (Nahon et al., 2020), and appear to have switched from their previously preferred diet of zooplankton and insect larvae (Hölker and Breckling, 2001; Persson, 1987) in response to this new, superabundant food source. A similar opportunistic switch could be assumed for tench in LLC (Boedeltje et al., 2019). Nevertheless, other interpretations are possible, given the isotopic values of several other potential diet components. For example, zooplankton and insect larvae also differ significantly from dreissenids (Verstijnen et al., 2019), and highly divergent values are to be expected between quagga consumers and non-quagga-consumers. Furthermore, snail species commonly found in the digestive tracts of roach and tench in this study, such as the very abundant Bithynia tentaculata in ULC or Viviparus viviparus in LLC, exhibit considerably higher $\delta^{13}C$ values than quaggas. The conclusion that observed changes in $\delta^{13}C$ in roach and tench reflect a shift away from previously high levels of snail consumption is supported by stomach content analysis from tench: in tench from ULC, the diet comprised 60 % snails and 23 % quaggas, while those from LLC consumed fewer snails (25 % of diet) and more quaggas (43 % of the diet). This difference alone could be the explanation for the differences in stable isotope signatures between tench from the two lakes.

While stomach content analysis reveals that some roach and whitefish in Lake Constance do consume mussels, the instantaneous presence of few individual mussels when mussels were found (R. Rösch, unpublished data). In recent years, more than 1000 quaggas can be found in large benthic whitefish (data not shown). Furthermore, the $\delta^{13}C$ model reveals that quagga consumers exhibit significantly lower $\delta^{13}C$ values than non-quagga consumers and that the interaction of feeding behaviour and period had a significant impact. The analyses of stomach contents revealed that roach in both lakes and tench in LLC consume large quantities of quagga mussels. Roach are opportunistic feeders (Nahon et al., 2020), and appear to have switched from their previously preferred diet of zooplankton and insect larvae (Hölker and Breckling, 2001; Persson, 1987) in response to this new, superabundant food source. A similar opportunistic switch could be assumed for tench in LLC (Boedeltje et al., 2019). Nevertheless, other interpretations are possible, given the isotopic values of several other potential diet components. For example, zooplankton and insect larvae also differ significantly from dreissenids (Verstijnen et al., 2019), and highly divergent values are to be expected between quagga consumers and non-quagga-consumers. Furthermore, snail species commonly found in the digestive tracts of roach and tench in this study, such as the very abundant Bithynia tentaculata in ULC or Viviparus viviparus in LLC, exhibit considerably higher $\delta^{13}C$ values than quaggas. The conclusion that observed changes in $\delta^{13}C$ in roach and tench reflect a shift away from previously high levels of snail consumption is supported by stomach content analysis from tench: in tench from ULC, the diet comprised 60 % snails and 23 % quaggas, while those from LLC consumed fewer snails (25 % of diet) and more quaggas (43 % of the diet). This difference alone could be the explanation for the differences in stable isotope signatures between tench from the two lakes.

While stomach content analysis reveals that some roach and whitefish in Lake Constance do consume mussels, the instantaneous presence or absence of quaggas in the digestive tract was not reflected in the $\delta^{15}N$ or $\delta^{13}C$ archived in the tissue of analysed individuals. Furthermore, there was no evidence that some individuals became specialists on mussel feeding while others preferred a different diet. However, in the case of roach, there is a clear positive correlation between body size (length) and both $\delta^{13}C$ and $\delta^{15}N$, suggesting that mussel predation may be length-dependent in this species. This result confirms the findings of previous authors suggesting that gape size, the dimensions of pharyngeal teeth and chewing ability limit the range of quagga sizes available to roach (Nagelkerke and Sibbing, 1996; Sibbing, 1988) but contradicts another study in Lake Constance (Baer et al., 2022) where no correlation was observed between total length and mussel consumption by roach. Additional studies are needed to evaluate the importance of roach length for mussel consumption.

Despite the distinct changes in the energy pathways indicated by stable isotope composition, the overall impact of quagga invasion on the commercially important whitefish seems to be of minor significance. The high proportions of either zooplankton or benthic macroinvertebrates in the digestive tracts of Lake Constance whitefish suggest that adequate food remained available at the time of sampling and that whitefish have not thus far been forced to supplement their usual diet with quaggas (Pothoven et al., 2001; Pothoven and Madenjian, 2008). However, if the filtration of quaggas does decrease zooplankton density in Lake Constance in the future (Karatayev et al., 2015), forcing whitefish to begin targeting quaggas as prey, the impact on growth and recruitment could be severe (Hoyle et al., 2008; Rennie et al., 2009) because of the relatively low caloric value and high handling cost of consuming mussels (Baer et al., 2022; Nagelkerke and Sibbing, 1996).

Conclusion

The present study highlights pronounced changes in energetic pathways in a large aquatic ecosystem, Lake Constance, following invasion of quagga mussel. For pelagic whitefish, observed increases in stable isotope values are most likely the result of increasing benthic-derived nearshore primary production and feeding closer to shore, rather than a change in dietary composition. In contrast, other fish species including benthic whitefish, roach and tench, showed marked qualitative changes in feeding behaviour and now rely on quagga mussels for at least part of their diet. This novel food source in combination with likely changes in the benthic macrozoobenthos and submerged macrophytes community appears to be driving changes in the isotopic composition of the fish community in Lake Constance. It is likely that similar changes will occur in other large lakes in Central Europe facing invasion by quagga mussels.

CRediT authorship contribution statement

Jan Baer: Formal analysis, Data curation, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. Christina Spiessl: Formal analysis, Data curation, Software, Investigation. Karl Auerswald: Validation, Visualization, Writing – review & editing. Juergen Geist: Validation, Visualization, Writing – review & editing. Alexander Brinker: Formal analysis, Project administration, Resources, Software, Supervision, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This project was funded by the Fischereiabgabe Baden-Württemberg. The help by Rudi Schäufele (Technical University of Munich) and Helga Bentele (Fisheries Research Station Lange-nargen) during isotope measurement is gratefully acknowledged. The authors would like to thank Amy-Jane Beer for the language correction and improvement of the manuscript.

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