



# Trophic ecology of invasive marbled and spiny-cheek crayfish populations

Stefan M. Linzmaier · Camille Musseau · Sven Matern · Jonathan M. Jeschke

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**Abstract** North American cambarid crayfish have been highly successful in establishing and spreading across Europe and are now over-invading earlier arrivals in many water bodies. Parthenogenetic marbled crayfish (*Procambarus virginalis*), which originated from aquarium stocks, are relatively recent invaders and have established in lakes previously invaded by spiny-cheek crayfish (*Faxonius limosus*). However, the feeding ecology of marbled crayfish and consequential impacts on the non-native species' coexistence are largely unexplored. By combining

laboratory experiments with stable isotope analyses of field samples, we were able to (1) determine food preferences of both species under controlled conditions and (2) explore their trophic niches in three lakes where both species co-occur. In the food-choice laboratory experiments, the two species showed similar food preferences and consumption rates. Consistently, the stable isotope analyses ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) highlighted the intermediate trophic position of both species. Marbled crayfish and spiny-cheek crayfish occupied a wide range of trophic positions corresponding to a very generalist diet. However, marbled crayfish were more relying on arthropod prey than spiny-cheek crayfish which fed more on mollusks. This is the first work providing evidence for trophic plasticity of marbled crayfish in lake food webs. Our results suggest that the addition of marbled crayfish increases grazing pressure on macrophytes and macrophyte-dependent organisms and the allochthonous detritus decomposition in ecosystems already invaded by spiny-cheek crayfish. Since both species are listed as invasive alien species of EU concern, further assessments of potentially endangered food organisms are needed.

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## Introduction

As the number of invasions increases worldwide, many formerly successful invasive species compete with new non-native species in so-called over-invasion scenarios (Russell et al. 2014; Seebens et al. 2017). These new waves of species invasions can have unpredictable, yet wide-ranging impacts on food webs. Invasive crayfish species in Europe are an illustrative example for aquatic over-invasions, as they can induce profound changes in aquatic systems and compete for food and shelter with other crayfish species (Gherardi 2007). Nowadays, invasive crayfish rarely compete with native ones in Europe, as the latter have disappeared from many water bodies due to pollution, habitat destruction or crayfish plague (*Aphanomyces astaci*; Holdich 2002).

Instead, formerly successful invaders are now facing competition with new non-native species which are introduced via different pathways (Chucholl 2013b; Chucholl and Wendler 2017). For example, spiny-cheek crayfish (*Faxonius limosus*) were introduced for aquaculture and are now co-occurring with marbled crayfish (*Procambarus virginalis*) coming from the aquarium pet trade (Chucholl et al. 2012). Co-occurrences of two or more non-native crayfish species are becoming increasingly common in Europe, but the interactions between these species and those they consume have rarely been studied (Hudina et al. 2011; James et al. 2016; Linzmaier et al. 2018). Every established population will have to adapt its trophic niche yet again if the invader has a similar functional role (Tilman 2004). Established crayfish species have been shown to shift or constrict trophic niches if new non-native crayfish exhibit similar niches (Jackson et al. 2016; Larson et al. 2017). Although omnivorous crayfish are usually very flexible in their diet, some species can be more specialized than others, and their invasion impact and response to competition may differ (Johnston et al. 2011; Stites et al. 2017). Potential invaders would thus benefit from a larger trophic niche and could cause more substantial changes in the food web.

Marbled crayfish are one of several new non-native crayfish invading fresh waters (Kouba et al. 2014). This species is unique among decapods, as it is able to reproduce through parthenogenesis and was probably created in aquaria (Gutkunst et al. 2018; Scholtz et al. 2003). Marbled crayfish were described in a lake for

the first time in Germany in 2003 (Marten et al. 2004). Its taxonomic status, morphology and development have been extensively studied, but the ecology of the species has rarely been looked at (Gutkunst et al. 2018; Vogt et al. 2018). To our knowledge, there is only one study on the trophic ecology of marbled crayfish (Lipták et al. 2019) and some studies on its wild progenitor, the slough crayfish (*Procambarus fallax*) which is native to the south-eastern USA (Sargeant et al. 2010; VanArman 2011), both species have been associated with low trophic levels. Whether invasive marbled crayfish utilizes similar resources as slough crayfish is currently unclear.

Most known water bodies invaded by marbled crayfish are lentic habitats, such as gravel pit lakes, close to larger cities (Chucholl et al. 2012). In Central Europe, these habitats have mostly been invaded by spiny-cheek crayfish (Kouba et al. 2014). The number of naturalized marbled crayfish populations increases in Europe (especially Germany; Chucholl 2015), and the European Union included it in the “List of Invasive Alien Species of Union Concern” (EU Regulation 1143/2014). Thus, the question of its ecological impact becomes ever more pressing if management plans should be implemented.

Generally, crayfish are omnivorous generalists and often situated at the center of aquatic food webs occupying intermediate trophic positions (TPs; secondary consumers; Roth et al. 2006; Usio and Townsend 2002). The individual TP of crayfish is, however, related to body size, and usually increases as they grow (Roth et al. 2006). Yet, some studied species show a reverse pattern of decreasing TPs with body size (Taylor and Soucek 2010). Thus, invaded food webs are affected by crayfish on many levels, as crayfish can utilize a wide range of food items and food sizes (Souty-Grosset et al. 2006). As prey, they also support native predators’ diets for several species of birds (Poulin et al. 2007), reptiles (Ottonello et al. 2005), mammals (Fischer et al. 2009) and fish (Haertel Borer et al. 2005).

However, non-native crayfish can destructively affect food webs. Their grazing activity can reduce aquatic vegetation, leading to strong habitat loss for many species like birds, amphibians and freshwater macroinvertebrates (Gherardi and Acquistapace 2007; van der Wal et al. 2013). Through predation, they can threaten amphibians (Kats and Ferrer 2003) and dragonfly larvae (Siesa et al. 2014), mollusks

(Chucholl 2013a) and other prey organisms (Bubb et al. 2009). Finally, some species can crucially affect ecosystem functions due to their high rate of litter decomposition (Doherty-Bone et al. 2018; Dunoyer et al. 2014). Many of these impacts have been attributed to particularly detrimental invaders like red swamp crayfish (*Procambarus clarkii*; Nentwig et al. 2018). But are different invasive crayfish species equally harmful or are they functionally equivalent (Hubbell 2005)? Evidence that crayfish invaders take up similar or different roles in food webs compared to established species is equivocal (Ercoli et al. 2014; Jackson et al. 2014; Larson et al. 2017), and species-specific data on diet and TP are presently lacking for many crayfish species.

In this study, we aimed at exploring the trophic interactions of marbled and spiny-cheek crayfish by combining an experimental approach with field work in invaded lakes: (1) experiments were conducted in the laboratory to determine food choice under controlled conditions for both species, and (2) stable isotope data from field samples were analyzed to investigate both trophic niche breadth and diet composition of the two species, including the question if marbled crayfish affect sympatric spiny-cheek crayfish. We hypothesized that marbled crayfish occupy lower TPs than spiny-cheek crayfish, and feed mainly on macrophytes and allochthonous detritus similar to its progenitor in its natural environment. Furthermore, we expected trophic niche segregation between sympatric populations of marbled crayfish and spiny-cheek crayfish. We also expected that individual TP depend on crayfish body size.

## Methods

### Study sites and sampling

In total, five lakes were sampled in Germany; spiny-cheek crayfish have established populations in these five lakes and marbled crayfish recently established in three of them, in sympatry with the spiny-cheek crayfish. In June 2016, we captured spiny-cheek crayfish and marbled crayfish living in sympatry in lake Moosweiher, and spiny-cheek crayfish living in allopatry in lake Silbersee; both gravel pit lakes are located in Baden-Württemberg, Germany. In May 2017, we captured spiny-cheek crayfish and marbled

crayfish living in sympatry in the natural lake Krumme Lanke, Berlin, Germany. Marbled crayfish have been reported from lake Moosweiher since 2009, and first anecdotal evidence for marbled crayfish in lakes Krumme Lanke and Meitzer See exists since 2009 and 2016, respectively. Crayfish were hand-collected in the littoral at different accessible sampling spots with head lamps after sunset. In lakes Moosweiher and Silbersee, we also used traps baited with dogfood (type "PIRAT", 610 × 315 × 250 mm, mesh width 40 × 10 mm, Rapurosvo, Parainen, Finland). Shallow to moderately deep spots were favored due to their higher accessibility. We measured the crayfish to the nearest millimeter with a sliding caliper and sexed them afterwards. Crayfish and all other samples used for stable isotope analyses were put on ice during field sampling and frozen in the lab at -20 °C. Crayfish length was measured as carapace length (CL) from the tip of the rostrum to the posterior edge of the carapace.

We collected allochthonous detritus (decaying leaves) and macrophytes in the littoral at least in four locations within each lake. The most common invertebrates were collected either with a surber net (500 µm mesh size) or by hand from different substrates (macrophytes, stones, woody debris). We caught fish by electrofishing and pelagic multi-mesh gillnet (Germany: 30 m × 1.5 m; twelve panels each 2.5 m long with mesh-sizes 5, 6.25, 8, 10, 12.5, 16, 19.5, 24, 29, 35, 43 and 55 mm; Lundgrens Fiskredskapsfabrik, Stockholm, Sweden). For each lake, we measured total phosphorous (µg/l) from a water sample at 1 m depth and determined the Secchi depth (m). The lakes' characteristics are given in Table 1.

Lakes Moosweiher, Silbersee and Krumme Lanke are small, mesotrophic and dimictic lakes (see Chucholl and Pfeiffer 2010; Vogt et al. 2018 for more detailed descriptions of lake Moosweiher). Alder (*Alnus glutinosa*), oak (*Quercus robur*), pine (*Pinus sylvestris*), and beach (*Fagus sylvatica*) trees grow along the shorelines of lakes Moosweiher and Krumme Lanke interspersed by open bathing areas. Lake Silbersee has wider bathing areas and less canopy cover. The littoral of both lakes Moosweiher and Krumme Lanke is mainly covered with macrophytes (e.g. *Potamogeton* sp. and *Elodea canadensis*) and coarse woody debris on sediments of coarse gravel and some soft-bottomed areas. In lake Silbersee, the littoral consisted mainly of macrophytes and open, gravel sediments. In all lakes, we found Eurasian

**Table 1** Location, physical and chemical characteristics of the sampled lakes invaded by marbled (M) and/or spiny-cheek (S) crayfish

| Lake               | Species | Lat. (DD) | Long. (DD) | Altitude (m) | Surface Area (ha) | Depth <sub>max</sub> (m) | Secchi (m) | TP (µg/l) |
|--------------------|---------|-----------|------------|--------------|-------------------|--------------------------|------------|-----------|
| Moosweiher         | M + S   | 48.030679 | 7.804152   | 216          | 7.6               | 8.0                      | 7.6        | 17        |
| Silbersee          | S       | 48.061923 | 7.817354   | 202          | 4.0               | 14.0                     | 6.1        | 14        |
| Krumme Lanke       | M + S   | 52.452050 | 13.232418  | 38           | 15.4              | 6.6                      | 3.6        | 12        |
| Meitzer See        | M + S   | 52.569557 | 9.788003   | 36           | 19.6              | 23.5                     | 4.5        | 3         |
| Steinwedeler Teich | S       | 52.400005 | 10.000238  | 55           | 11.0              | 9.1                      | 3.0        | 7         |

perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), pike (*Esox lucius*) and eel (*Anguilla anguilla*). Lake Moosweiher and Silbersee were also invaded by very abundant pumpkinseed sunfish (*Lepomis gibbosus*). Lakes Moosweiher and Krumme Lanke were inhabited by European catfish (*Silurus glanis*).

#### Animal maintenance

The crayfish were transferred to a climate chamber (17 °C, 14 h light: 10 h dark) and housed in single-individual tanks (300 × 200 × 200 mm). Tanks were equipped with air-driven sponge filters, gravel and PVC-pipes for shelter. Half a ring of commercial crayfish food (Crabs Natural, sera, Heinsberg, Germany) was fed to each crayfish daily. Water was changed once a week (ca. 75% fresh tap water).

The protocol and procedures employed were ethically reviewed and approved by the Landesamt für Gesundheit und Soziales (LAGeSo, State Office for Health and Social Affairs), Berlin, Germany. All experiments were performed in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

#### Food-choice experiments

Food choice was compared between marbled crayfish and spiny-cheek crayfish (males and females) according to Haddaway et al. (2012). All individuals were used in two experiments where they could choose between mobile and non-mobile food items. For mobile food items, we tested  $n = 10$  marbled crayfish (mean ± SD, CL = 33 mm ± 6) and  $n = 14$  spiny-

cheek crayfish (CL = 36 mm ± 4; 3 females: 11 males); for non-mobile food items, we tested  $n = 13$  marbled crayfish (CL = 33 mm ± 5) and  $n = 18$  spiny-cheek crayfish (CL = 34 mm ± 4; 5 females, 13 males). Species body size distributions were similar for both trials (Kolmogorov–Smirnov test, mobile food items:  $D = 0.457$ ,  $p = 0.175$ ; non-mobile food items:  $D = 0.399$ ,  $p = 0.155$ ). In each trial, four food items (either mobile or non-mobile) were presented to an individual crayfish. The four mobile food items used in the experiments were: amphipods, *Dikerogammarus villosus*; hard-shelled snails, *Bithynia tentaculata*; soft-shelled snails *Physidae*; bloodworms, Chironomidae larvae. The four non-mobile food items were: macrophytes, *Potamogeton* sp.; decaying oak leaves, *Quercus robur*; dead roach, *Rutilus rutilus*; small freshwater mussels, *Dreissena* sp. All food items were collected on the premises of our institute (oak leaves, *Quercus robur*) and the adjoining lake Müggelsee (roach, amphipods, snails, mussels and macrophytes), except for bloodworms, which were bought alive in a local pet store. For this food-choice experiment, naïve marbled crayfish from laboratory stocks were compared with spiny-cheek crayfish from lake Müggelsee.

For each trial, one individual crayfish was transferred to the experimental tank (400 × 400 × 200 mm) containing sand as a substrate, a PVC-pipe as shelter and tap water up to 150 mm height. Then, 0.3 g (wet weight) of each food item was spread on the sandy areas of the tank and the crayfish were left for 24 h. The next day, the crayfish were removed, and food remains were collected and weighed again. The food items were dabbed with tissues prior to weighing in order to remove superficial water.

Differences between species in their food-choice preferences (i.e. proportions of consumed food) were tested using beta-regression models. Beta-regressions are commonly used for dealing with values distributed within the standard unit interval (0, 1) and following a beta distribution, like proportions (Cribari-Neto and Zeileis 2010). Since beta-regressions cannot handle true zeros and ones, these were transformed by adding or subtracting 0.001. We used the R package ‘betareg’ and the *betareg* function (link = “logit”; Cribari-Neto, Zeileis 2010). Additionally, we tested whether food choice differs between species by including the interaction term with food type. We also tested for sex-specific differences in spiny-cheek crayfish. The precision parameter ( $\Phi$ ) indicates the goodness of fit of the model: the larger it is, the smaller is the variance of the response variable.

### Stable isotope samples

We caught and processed marbled crayfish ( $n = 14$ ) and spiny-cheek crayfish ( $n = 10$ ) in lake Moosweher, spiny-cheek crayfish ( $n = 20$ ) in lake Silbersee, and marbled crayfish ( $n = 24$ ) and spiny-cheek crayfish ( $n = 16$ ) in lake Krumme Lanke for stable isotope analysis. We prepared the crayfish muscle tissue of the abdomen. For fish samples, we used muscle tissue from the lateral area in front of the dorsal fin; and for benthic invertebrates, we used whole organisms which were pooled to achieve 2–6 samples for each group, depending on the availability of the species in our samples; macrophytes and decaying leaves were washed with distilled water and pooled to 3–5 samples. All samples were dried for 24 h at 60 °C in aluminum trays. The samples were homogenized with mortar and pestle, and then weighed at 1 mg for each sample of animal tissue, 4 mg for macrophytes, and 5 mg for detritus. Samples were wrapped in tin cups and analyzed for carbon and nitrogen isotopic ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and elemental content (%C and %N) by the University of California, Davis Stable Isotope Facility, USA, using a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The resulting delta values refer to Vienna PeeDee Belemnite carbon and air nitrogen as calibration standards. Long-term standard deviations provided by UC Davis are 0.2‰ for  $\delta^{13}\text{C}$  and 0.3‰ for  $\delta^{15}\text{N}$ . We report stable isotope values in standard delta notation:  $\delta^H X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$ ,

where X is the element, H is the relative isotopic mass of the heavier isotope, and R is the ratio of the heavy-to-light isotope in the sample and standard. We then lipid-corrected  $\delta^{13}\text{C}$ -values of macrophytes, allochthonous detritus and invertebrates mathematically according to Post et al. (2007).

We included additional stable isotope data from a newly reported marbled crayfish population in the gravel pit lake Meitzer See, Lower Saxony, Germany. The sample of lake Meitzer See included marbled crayfish ( $n = 5$ ) and spiny-cheek crayfish ( $n = 2$ ). Sample processing followed similar procedures as described above (please see Trudeau 2018 for details). In addition, we included data of a reference gravel pit lake inhabiting only spiny-cheek crayfish ( $n = 7$ ; lake Steinwedeler Teich). The samples from these two lakes were collected in September and October of 2016 using baited traps.

### Isotopic niches and mixing models

#### *Trophic position of populations*

We used Bayesian models from package *tRophicPosition* in R (version 3.5.1.) to calculate the median and mode of TPs for each crayfish population with stable isotope data ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ). The model applies Markov Chain Monte Carlo Simulations for stable isotope data to estimate TP (see Quezada-Romegialli et al. 2018). We used the full two baselines model that incorporates the factor  $\alpha$  proposed by Post (2002), accounting for differences between littoral and pelagic food webs within lakes (Vadeboncoeur et al. 2002). We also calculated comparisons of the posterior samples of TPs and  $\alpha$  (from Post 2002) between lakes (Table 4).

We used littoral (snails: *Bithynia tentaculata*, *Lymnea* sp.) and pelagic (mussels: *Dreissena* sp., *Corbicula fluminea*, *Unio* sp.) primary consumers as baseline organisms for the Bayesian models. Filter-feeding mussels and herbivorous snails are longer-lived primary consumers that are often recommended to use as baseline organisms, as they integrate the highly variable isotopic signals from primary producers from pelagic and littoral food webs over longer time scales (Post 2002). The estimated TP of the baseline organisms was set to  $\lambda = 2$ .

Calculations of TPs are known to be sensitive to trophic discrimination factors (TDFs) which can in

turn vary between species and food types (Caut et al. 2009), thus the choice of TDF is critical. However, estimating TPs of omnivores like crayfish cannot be based upon TDF derived from experiments with single food items. Omnivores like crayfish pose a problem, thus we here decided to use the TDF proposed by Post (2002) that are commonly used in stable isotope ecology ( $\Delta^{15}\text{N} = 3.4 \pm 0.98 \text{ ‰}$ ,  $\Delta^{13}\text{C} = 0.39 \pm 1.3 \text{ ‰}$ ). In addition, we calculated TPs with TDF from McCutchan et al. (2003) and non-Bayesian TPs (Post 2002) to check for sensitivity (Table 5).

The crayfish sampled for stable isotope analyses were tested for size differences between populations (lakes) using Kolmogorov–Smirnov tests. The null hypothesis was that the samples were drawn from the same distribution. We excluded the spiny-cheek crayfish sample from lake Meitzer See due to small sample size ( $n = 2$ ). We then used the implemented pairwise comparison of posterior TP estimates from the Bayesian model of the *tRophicPosition* package in R (Quezada-Romegialli et al. 2018).

#### Trophic niche breadth

All stable isotope values were baseline-corrected because primary resources can be highly variable between sites (Vander Zanden and Rasmussen 1999). The nitrogen isotopes were expressed as TP according to Minagawa and Wada (1984). Since the Bayesian framework from package *tRophicPosition* has no individual model implemented, we calculated TP for each individual separately (1) following Post (2002) as:

$$\text{TP}_i = \lambda + (\delta^{15}\text{N}_i - [\delta^{15}\text{N}_{\text{littoral}} \times \alpha + \delta^{15}\text{N}_{\text{pelagic}} \times (1 - \alpha)]) / \Delta^{15}\text{N} \quad (1)$$

Here,  $\delta^{15}\text{N}_i$  is the measured  $\delta^{15}\text{N}$ -value of the individual crayfish for which TP should be calculated.  $\delta^{15}\text{N}_{\text{littoral}}$  and  $\delta^{15}\text{N}_{\text{pelagic}}$  are the measured  $\delta^{15}\text{N}$ -values of the baseline organisms chosen to represent littoral and pelagic food webs of each lake as described above. The  $\lambda$  represents the estimated TP of baseline primary consumers ( $\lambda = 2$ ). The TDF for the calculations was set to  $\Delta^{15}\text{N} = 3.4\text{‰}$ . The contribution of littoral food webs ( $\alpha$ ) to the consumer signature was estimated using carbon isotopes ( $\delta^{13}\text{C}$ ; see Post 2002).

Further, we corrected  $\delta^{13}\text{C}$  values of the crayfish for differences in basal resources following Olsson et al. (2009):

$$\delta^{13}\text{C}_{\text{corr}_i} = \frac{\delta^{13}\text{C}_i - \delta^{13}\text{C}_{\text{meaninv}}}{\delta^{13}\text{C}_{\text{maxinv}} - \delta^{13}\text{C}_{\text{mininv}}} \quad (2)$$

The corrected carbon isotope value ( $\delta^{13}\text{C}_{\text{corr}_i}$ ) for each consumer  $i$  was calculated from the individual carbon isotopic ratio ( $\delta^{13}\text{C}_i$ ), the mean carbon isotopic ratio of all sampled invertebrates ( $\delta^{13}\text{C}_{\text{meaninv}}$ ), which represent potential food items, and the carbon range of the invertebrates  $\text{CR}_{\text{inv}}$  used to calculate the baseline of TPs (i.e. the primary consumers).

We then calculated sample-size corrected standard ellipse areas (SEAc) in the isotopic space of individual TPs and  $\delta^{13}\text{C}_{\text{corr}_i}$  with the package SIBER in R for each population. We compared trophic niches of marbled crayfish and spiny-cheek crayfish within and among lakes in the biplots and calculated SEAc overlap for the crayfish populations in sympatry (lakes Moosweiher and Krumme Lanke) and the allopatric spiny-cheek crayfish reference populations of lake Silbersee with spiny-cheek crayfish from lake Moosweiher (Jackson et al. 2011). Also, we looked at individual TP in relation to CL of individual crayfish with linear regression models.

#### Stable isotope mixing models

Diet of crayfish was estimated using Bayesian inference. Bayesian mixing models are commonly used to estimate the proportion of different food items in consumers' diets based on stable isotope analysis (Phillips et al. 2014). Models were run for both marbled and spiny-cheek crayfish in the R package 'MixSIAR: Mixing Models for Stable Isotope Analysis in R' (MixSIAR; Parnell et al. 2013; Stock et al. 2018). The potential food items was grouped into six different food categories (sources) based on sampled communities in each lake: (1) detritus (decomposing leaves,  $n = 5$ ); (2) macrophytes ( $n = 3-5$ ); (3) benthic invertebrates including gammarids, Heptageniidae, Chironomids, Odonata and *Asellus aquaticus* ( $n = 3-26$ ); (4) mussels including *Dreissena* sp., Asian clam *Corbicula fluminea* and *Unio* sp. ( $n = 4-9$ ); (5) gastropods including *Bithynia tentaculata*, *Lymnaea* sp. and *Planorbarius corneus* ( $n = 2-7$ ); and (6) fish (*Perca fluviatilis*, as the species was dominant in the

different lakes). Since no TDF measurements on marbled crayfish exist, we incorporated the TDFs from laboratory-controlled experimental measurements of *F. rusticus* published in Glon et al. (2016) into our mixing models and allocated them to comparable food types. For plant-based food items (i.e. macrophytes and detritus) we used  $\Delta^{15}\text{N} = 3.35\text{‰} \pm 2.77$  and  $\Delta^{13}\text{C} = 1.57\text{‰} \pm 2.08$ , and for animal food items (i.e. fish and all invertebrates) we used  $\Delta^{15}\text{N} = 1.20\text{‰} \pm 2.50$  and  $\Delta^{13}\text{C} = 0.80\text{‰} \pm 1.05$ .

Initially, for all MixSIAR models, the Markov Chain Monte Carlo (MCMC) parameters were set as follows: chain length = 1,000,000, burn = 500,000, thin = 500, chains = 3. These parameters correspond to the “very long” run provided by the *run\_model* function of the MixSIAR package. Convergence of each model was evaluated by using the Gelman-Rubin and Geweke diagnostic tests (see package R ‘MixSIAR’). Since diagnostic tests were not satisfying for marbled crayfish models, we used the “extreme” run (chain length = 3,000,000, burn = 1 500,000, thin = 500, chains = 3). MixSIAR offers a statistical framework in which knowledge on trophic ecology of consumers can be included via informative prior distributions (Stock et al. 2018). We used average standardized and transformed proportions from the food-choice experiments with informative priors as follows: 0.01, 0.358, 1, 0.853, 0.583 and 0.883 for detritus, macrophytes, fish, arthropods, snails and mussels, respectively. Informative priors were used a posteriori for combining food items and exploring the diet of both crayfish species (Stock et al. 2018). The lake identity was added as a random factor in the models. Proportions of food resources were additively combined to obtain the total plant-based tissue proportions (macrophytes and detritus), invertebrate proportions (arthropods, mussels and snails) and fish proportions. The proportions of resources assimilated by crayfish reported are the median of the Bayesian simulations.

## Results

### Food choice

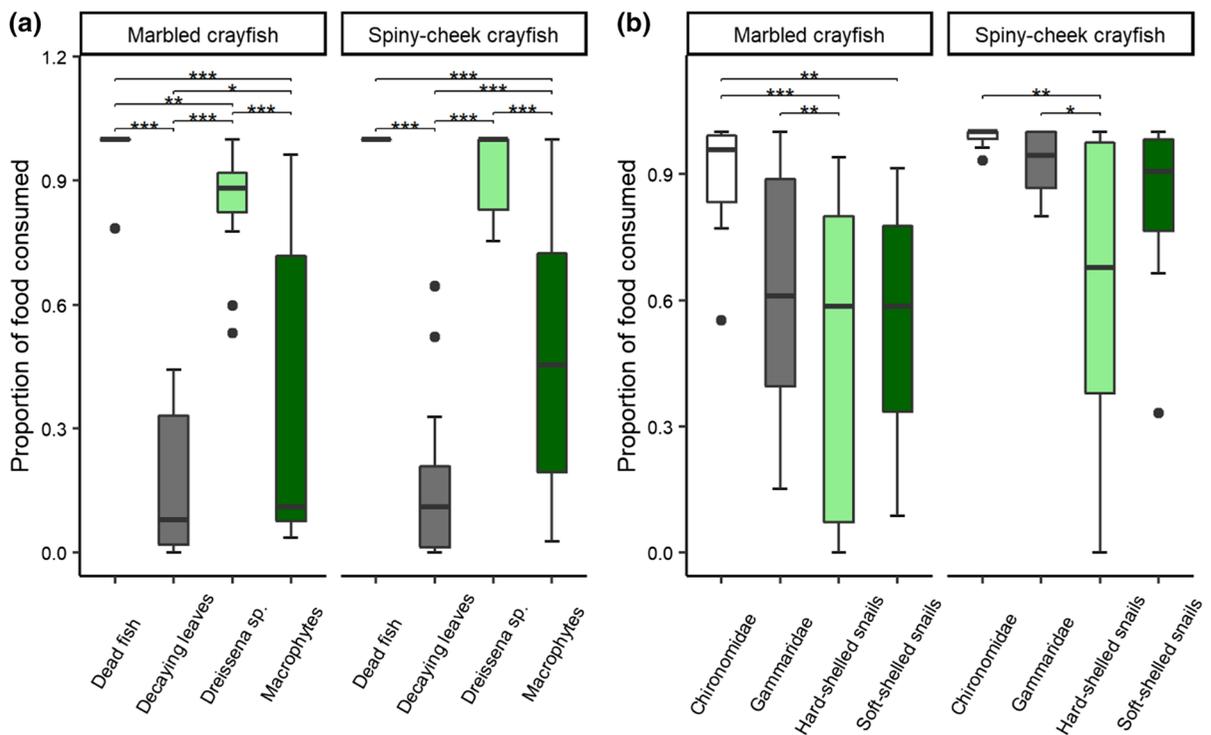
There was no difference in the proportion of consumed food items between spiny-cheek crayfish and marbled crayfish for most food items besides *Dreissena* mussels (non-mobile) and soft-shelled snails (mobile).

Of the non-mobile food items, dead fish were significantly preferred over the other food items followed by *Dreissena* mussels, macrophytes and decaying leaves ( $N = 124$ ;  $\Phi = 2.22 \pm 0.29$ ,  $p < 0.001$ ; Fig. 1). We found higher proportions of consumed *Dreissena* mussels (non-mobile) and soft-shelled snails (mobile) for spiny-cheek crayfish. The most preferred mobile food items for both species were chironomids and gammarids, whereas both soft- and hard-shelled snails were consumed less ( $N = 96$ ;  $\Phi = 1.32 \pm 0.18$ ,  $p < 0.001$ ). We did not find species-specific food preferences. There were almost no differences in the proportion of consumed food items between spiny-cheek crayfish sexes, except that females consumed significantly more macrophytes than males ( $N = 72$ ;  $\Phi = 3.02 \pm 0.58$ ,  $p < 0.01$ ).

### Trophic positions and niche space

Both crayfish species took up central positions in lake food webs (Fig. 2). TPs for marbled crayfish ranged from 2.91 in lakes Krumme Lanke and Meitzer to 3.20 in lake Moosweiher (Fig. 3, Table 2), and TPs of spiny-cheek crayfish ranged from 2.66 in lake Silbersee and 3.33 in lake Krumme Lanke. All crayfish populations relied on littoral resources with  $\alpha$  values ranging from 0.50 to 0.96 (Table 2). Comparisons of the posterior samples of TP and  $\alpha$  between lakes are given in Table 4. The core niche of marbled crayfish and spiny-cheek crayfish overlapped by 16.9% in lake Moosweiher and 10.5% in lake Krumme Lanke.

Except marbled crayfish from lake Moosweiher, which were significantly smaller than other populations (Kolmogorov–Smirnov test,  $D = 0.571$ ,  $p < 0.05$ ), crayfish did not significantly differ from each other in their size. There was a significantly negative relationship between size and TP in marbled crayfish ( $R^2 = 0.63$ ,  $p < 0.001$ ; Fig. 3) and spiny-cheek crayfish ( $R^2 = 0.76$ ,  $p < 0.001$ ) from lake Moosweiher. In this lake, very small marbled crayfish (< 23 mm) had distinctively high TPs; Fig. 4. In lake Silbersee, spiny-cheek crayfish exhibited a positive trend for the size-TP relationship ( $R^2 = 0.15$ ,  $p = 0.053$ ). In lake Krumme Lanke we did not find a relationship of crayfish size with TP in marbled crayfish ( $R^2 = -0.06$ ,  $p = 0.874$ ), but size and TP were positively correlated for spiny-cheek crayfish ( $R^2 = 0.13$ ,  $p < 0.05$ ). There was no significant relationship for the marbled crayfish from lake Meitzer



**Fig. 1** Non-mobile (a) and mobile (b) food items consumed by marbled and spiny-cheek crayfish in the food-choice experiments. Boxplots show medians with lower and upper quartiles, whiskers indicate the largest and smallest value still within the

1.5 interquartile range, outliers are denoted as black dots. Significant differences between food items are denoted by asterisks

See ( $R^2 = -0.31$ ,  $p = 0.845$ ), and a weak positive trend for spiny-cheek crayfish from Steinwedeler Teich ( $R^2 = 0.30$ ,  $p = 0.074$ ).

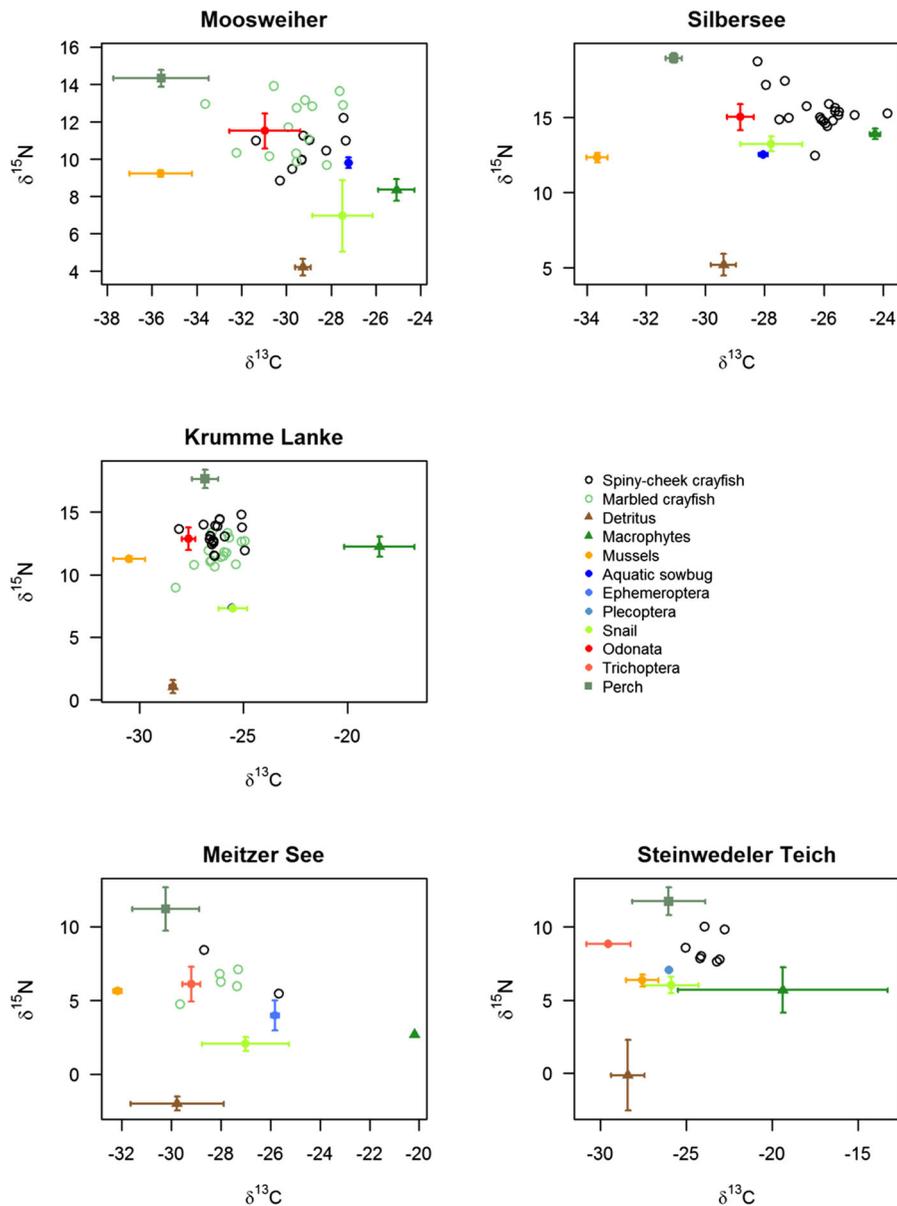
#### Diet estimates

The estimated contribution of different food items to spiny-cheek and marbled crayfish diets highlight that both species are generalist consumers (Table 3). In general—without considering the variability between lakes—the combined proportion of invertebrates was 0.62 (95% CI 0.27–0.88), the combined proportion of plants-based tissues was 0.21 (95% CI 0.04–0.29) and the proportion of dead fish was 0.16 (95% CI 0.01–0.24) in the diet of marbled crayfish. For spiny-cheek crayfish, invertebrates represented 0.54 (95% CI 0.26–0.77) of the diet, plants 0.31 (95% CI 0.11–0.54) and dead fish 0.14 (95% CI 0.02–0.20). Exploring lake-by-lake diet composition of combined food resources, we found consistency within and between species.

The diet differences between lakes showed that spiny-cheek crayfish ingested more macrophyte tissues than marbled crayfish while the proportion of allochthonous detritus was higher for marbled crayfish. Estimated proportions of snails and mussels show that both crayfish species rely similarly on these food resources (Table 3). However, the food item most consumed by marbled crayfish was arthropods. This is particularly true in lakes Krumme Lanke and Moosweiher, while the spiny-crayfish in these lakes mostly consumed mussels.

#### Discussion

We reported rarely observed comparisons of sympatric populations of invasive spiny-cheek crayfish in natural systems after an over-invasion by marbled crayfish, allopatric populations of the over-invaded spiny-cheek crayfish and compared both with laboratory feeding experiments. We found that both species



**Fig. 2** Isotopic biplots ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of individual crayfish (open circles) in the studied lakes. Potential food organisms are depicted as means of stable isotope values ( $\pm$  SD). Benthic

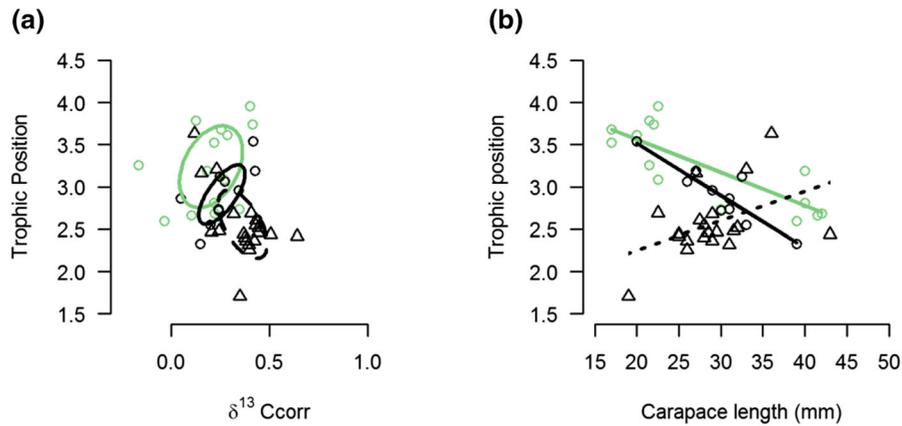
invertebrates are represented as circles, macrophytes and detritus as triangles, and fish (perch) as squares

did not differ in their food choice under controlled conditions where they preferentially fed on animal prey. The stable isotope analyses provided a long-term picture of crayfish feeding habits and showed that both species were highly plastic in their trophic niche, occupied different positions in the isotopic niche space and had a very generalist diet.

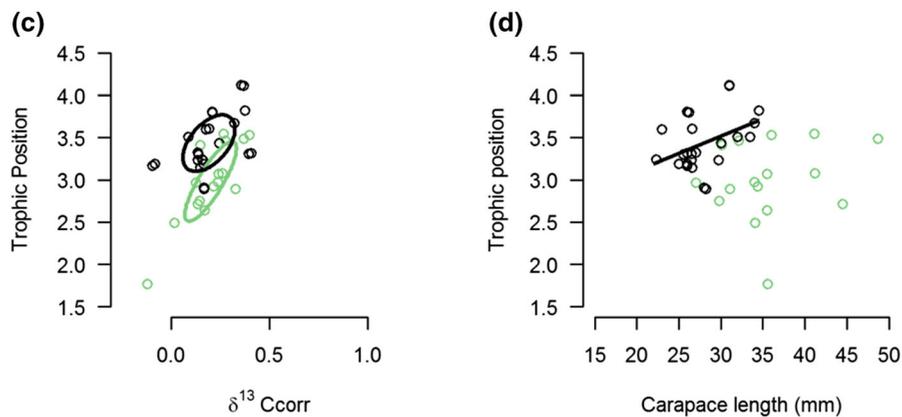
#### Food selection in controlled environment

Non-mobile food trials in the laboratory showed that both species prefer animal tissues, which is consistent with previous experimental studies on other species where crayfish preferred protein-rich food (Chucholl 2012; Correia 2003; Haddaway et al. 2012). In the mobile prey trials, both species chose chironomids and

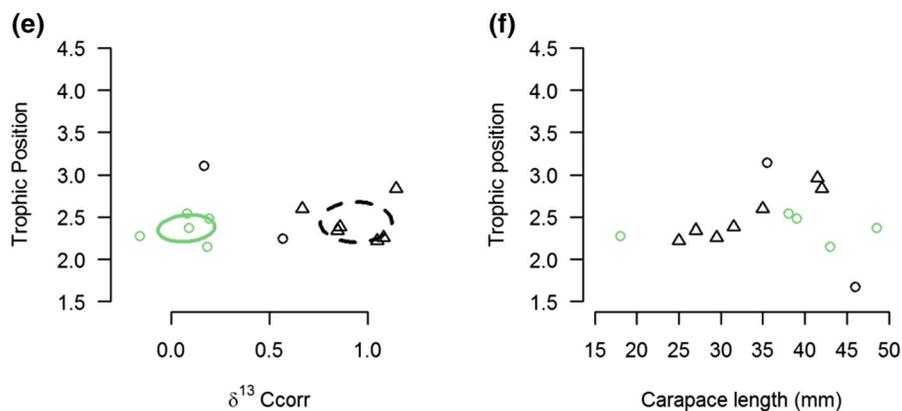
## Moosweiher &amp; Silbersee



## Krumme Lanke



## Meitzer See &amp; Steinwedeler Teich



gammarids, i.e. invertebrates with little defense against crayfish predators. As native populations of

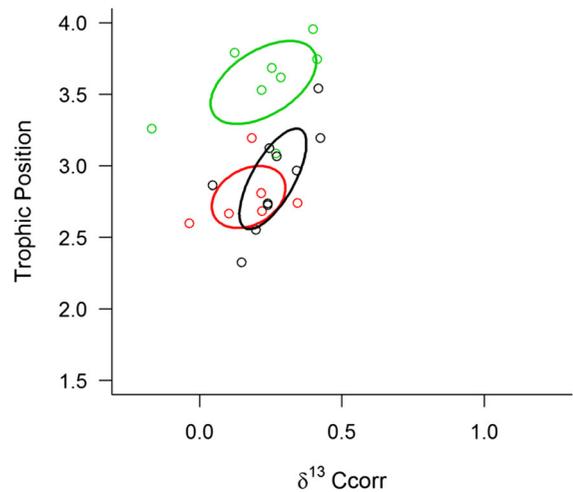
marbled crayfish do not exist, the best indicators of its potential trophic niche came from its progenitor

**Fig. 3** The left column depicts the trophic niche of marbled crayfish and spiny-cheek crayfish. Marbled crayfish are displayed as green circles, while spiny-cheek crayfish co-occurring with marbled crayfish in lake Moosweiher (a), lake Krumme Lanke (c) and lake Meitzer See (e) are displayed as black circles. The allopatric spiny-cheek crayfish populations in the reference water bodies lake Silbersee (a), lake Steinwedeler Teich (e) are shown as black triangles. Trophic niche breadth is represented as Standard Ellipses Areas (SEAc). The right column (b, d, f) shows the relationship of trophic position and carapace length (in mm) of individual marbled and spiny-cheek crayfish of the corresponding lakes

species: the slough crayfish. This species can thrive under a wide range of biotic and abiotic conditions (Dorn and Volin 2009; Hendrix and Loftus 2000). In contrast to our assumptions based on published data from free-living slough crayfish, marbled crayfish and spiny-cheek crayfish showed low interest in plant tissues. The food choice in the laboratory reflects simple optimal foraging scenarios where easily accessible, energy-rich food is preferred (Charnov 1976). Under natural conditions, however, variations in both resource availability and predation risk additionally shape the diet of consumers (Anholt and Werner 1995), and thus the patterns observed in the laboratory might not hold true in the field.

Sympatric crayfish act as key species in food webs but differ in diets

In the invaded lakes, we found that individual crayfish occupied a large range of trophic positions from 1.7 to 4.1, meaning that they act as primary consumers or detritivores up to predators. This wide span of trophic functions puts both crayfish species in a key position for energy transfer between trophic levels (Alp et al. 2016; Kreps et al. 2016). The population niche breadth



**Fig. 4** Trophic niche of spiny-cheek crayfish (black circles), marbled crayfish < 23 mm (green circles) and marbled crayfish > 30 mm (red circles) in lake Moosweiher

we observed was mostly consistent between the lakes and between species except for lake Moosweiher. In contrast to the signal crayfish studied by Larson et al. (2017), TPs of sympatric populations were not generally higher for one species, but niche partitioning among species differed between lakes. Marbled crayfish in lake Krumme Lanke had lower TPs than sympatric spiny-cheek crayfish, whereas in lake Moosweiher marbled crayfish seemed to occupy higher TPs. These differences can vary in the course of a year, as species grow differently and food availability (as well as accessibility) changes. Generally, high trophic variability among populations is commonly observed in crayfish (Ercoli et al. 2014) and some sympatric species pairs even exhibit trophic differences (Larson et al. 2017). The core niches of sympatric marbled and spiny-cheek crayfish, however, only marginally overlapped. Similar results were

**Table 2** Summary of niche statistic (sample-size corrected Standard Ellipse Area (SEAc)), posterior trophic position estimates and posterior  $\alpha$  estimates (median and [95% credibility interval]) for marbled crayfish (M) and spiny-cheek crayfish (S) populations

| Lake           | SEAc |      | Trophic position  |                   | Littoral reliance ( $\alpha$ ) |                   |
|----------------|------|------|-------------------|-------------------|--------------------------------|-------------------|
|                | M    | S    | M                 | S                 | M                              | S                 |
| Moosweiher     | 0.24 | 0.10 | 3.20 [2.61, 3.81] | 2.87 [2.30, 3.51] | 0.68 [0.50, 0.88]              | 0.76 [0.59, 0.94] |
| Silbersee      | –    | 0.13 | –                 | 2.66 [2.42, 2.93] | –                              | 0.96 [0.82, 1.00] |
| Krumme Lanke   | 0.11 | 0.12 | 2.91 [2.59, 3.25] | 3.33 [3.04, 3.65] | 0.79 [0.61, 0.96]              | 0.75 [0.58, 0.93] |
| Meitzer See    | 0.09 | N/A  | 2.91 [2.11, 6.61] | 3.88 [2.12, 9.23] | 0.59 [0.12, 0.96]              | 0.50 [0.04, 0.97] |
| Steinwedeler T | –    | 0.18 | –                 | 2.72 [2.22, 4.10] | –                              | 0.71 [0.11, 0.99] |

**Table 3** Bayesian mixing model estimated proportions (median, 95% CI) of arthropods, mussels, detritus, fish, macrophytes and snails in diets of spiny-cheek crayfish and marbled crayfish in the five sampled lakes

| Lake               | Food item   | Spiny-cheek crayfish | Marbled crayfish    |
|--------------------|-------------|----------------------|---------------------|
| Global             | Arthropods  | 0.139 (0.009–0.438)  | 0.207 (0.015–0.547) |
|                    | Mussels     | 0.181 (0.032–0.422)  | 0.171 (0.023–0.427) |
|                    | Detritus    | 0.088 (0.005–0.306)  | 0.126 (0.008–0.366) |
|                    | Fish        | 0.141 (0.023–0.362)  | 0.166 (0.013–0.396) |
|                    | Macrophytes | 0.194 (0.054–0.402)  | 0.060 (0.003–0.264) |
|                    | Snails      | 0.163 (0.010–0.458)  | 0.177 (0.013–0.451) |
| Moosweiher         | Arthropods  | 0.119 (0.005–0.503)  | 0.329 (0.008–0.879) |
|                    | Mussels     | 0.171 (0.007–0.406)  | 0.099 (0.002–0.326) |
|                    | Detritus    | 0.124 (0.002–0.468)  | 0.089 (0.001–0.337) |
|                    | Fish        | 0.097 (0.005–0.281)  | 0.144 (0.001–0.415) |
|                    | Macrophytes | 0.172 (0.009–0.407)  | 0.049 (0.001–0.317) |
|                    | Snails      | 0.189 (0.005–0.529)  | 0.141 (0.002–0.463) |
| Silbersee          | Arthropods  | 0.102 (0.003–0.578)  | –                   |
|                    | Mussels     | 0.086 (0.003–0.253)  | –                   |
|                    | Detritus    | 0.064 (0.062–0.218)  | –                   |
|                    | Fish        | 0.058 (0.002–0.175)  | –                   |
|                    | Macrophytes | 0.389 (0.166–0.552)  | –                   |
|                    | Snails      | 0.155 (0.004–0.674)  | –                   |
| Krumme Lanke       | Arthropods  | 0.096 (0.004–0.389)  | 0.183 (0.006–0.548) |
|                    | Mussels     | 0.335 (0.036–0.595)  | 0.218 (0.015–0.463) |
|                    | Detritus    | 0.062 (0.002–0.273)  | 0.131 (0.003–0.364) |
|                    | Fish        | 0.234 (0.023–0.500)  | 0.161 (0.006–0.386) |
|                    | Macrophytes | 0.098 (0.008–0.228)  | 0.045 (0.001–0.153) |
|                    | Snails      | 0.101 (0.004–0.331)  | 0.193 (0.005–0.475) |
| Meitzer See        | Arthropods  | 0.152 (0.005–0.373)  | 0.168 (0.005–0.550) |
|                    | Mussels     | 0.154 (0.004–0.286)  | 0.206 (0.011–0.572) |
|                    | Detritus    | 0.056 (0.001–0.124)  | 0.122 (0.002–0.372) |
|                    | Fish        | 0.149 (0.003–0.263)  | 0.164 (0.003–0.376) |
|                    | Macrophytes | 0.144 (0.006–0.222)  | 0.056 (0.001–0.207) |
|                    | Snails      | 0.107 (0.002–0.216)  | 0.187 (0.004–0.482) |
| Steinwedeler Teich | Arthropods  | 0.122 (0.004–0.522)  | –                   |
|                    | Mussels     | 0.176 (0.006–0.555)  | –                   |
|                    | Detritus    | 0.064 (0.001–0.272)  | –                   |
|                    | Fish        | 0.136 (0.007–0.345)  | –                   |
|                    | Macrophytes | 0.175 (0.033–0.347)  | –                   |
|                    | Snails      | 0.184 (0.005–0.659)  | –                   |

found by Jackson et al. (2014) for four other crayfish invaders that all separated within isotopic niche space. Overall, we consider our findings for lakes Moosweiher and Krumme Lanke robust, but the sample size for lake Meitzer See was close to the minimum to calculate niche ellipses (Jackson et al. 2011). As both species exhibited niche partitioning and high trophic

plasticity, the impact of marbled crayfish invasions depends on the differences in resource use.

Our mixing models showed that spiny-cheek crayfish consumed higher proportions of macrophytes, and marbled crayfish fed more on allochthonous detritus. Even small differences in resource utilization or predation yield important implications on the community and ecosystem functioning (Jackson et al.

2014). On the one hand, macrophytes and macrophyte-dependent organisms might be more affected by spiny-cheek crayfish. Invasive crayfish have been shown to severely reduce macrophyte stands and inhibit their growth (Baldrige and Lodge 2014; van der Wal et al. 2013). On the other hand, marbled crayfish might modify ecosystem functioning by potentially accelerating litter decomposition. Such effects have been found in highly invasive red swamp crayfish and signal crayfish, which both increased litter decomposition rates, compared to native species or spiny-cheek crayfish (Alp et al. 2016; Dunoyer et al. 2014). The density of crayfish or food organisms in the study lakes also affects these processes (Reynolds et al. 2013). Momot (1995) suggested that high crayfish densities force crayfish to utilize less favorable, but abundant resources like detritus or macrophytes. Accordingly, niche breadth and TPs are negatively correlated to crayfish abundance (Jackson et al. 2017; Kreps et al. 2016).

#### Carnivory in marbled crayfish

Mesocosm experiments and stable isotope studies showed that slough crayfish can have a large impact on gastropods, but generally are associated with feeding on leaf litter and macrophytes, being detritivores or herbivores (Sargeant et al. 2010; VanArman 2011). In contrast to its progenitor, our mixing models and food-choice trials indicated that marbled crayfish rely less on macrophytes and detritus, but more on animal prey. However, scavenging on dead fish was of minor importance in the field compared to food-choice trials, probably because fish carcasses are relatively rare there. But both crayfish studied here were comparatively predatory with up to 67% of invertebrates in their diet. For comparison, northern clearwater crayfish (*F. propinquus*) and rusty crayfish (*F. rusticus*) consumed about 42% of animal material, from which fish comprised approximately 12% (Taylor and Soucek 2010).

Invertebrate prey was generally preferred in the populations we studied. High degrees of invertivory can be problematic when taxa of conservation concern like dragonflies are negatively affected; but also less conspicuous taxa like leeches, mayflies and caddisflies can be suppressed by invasive crayfish (Mathers et al. 2016). Both species similarly consumed snails and mussels, but marbled crayfish had a stronger

preference for arthropods like e.g. dragonflies and waterlice. In conjunction with the elevated grazing of spiny-cheek crayfish on macrophytes, the associated invertebrates could be put under additional pressure when marbled crayfish invade. Especially, native shredders might be affected by marbled crayfish as they prey on them and compete for detritus (Jackson et al. 2014). This impact has, for example, been found in rusty crayfish which reduce shredder biomass and detritus availability (Bobeldyk and Lamberti 2008).

A recent stable-isotope study on a population of naturalized marbled crayfish in Slovakia revealed that algae and detritus provide their main food resources, similar to the aforementioned results on slough crayfish (Lipták et al. 2019). While our results are consistent with Lipták et al. (2019) regarding the low importance of macrophytes and generally a wide range of ingested resources, the populations we studied were much more carnivorous. This disparity could arise from differences in crayfish and food density but could also stem from high trophic plasticity. Such high trophic plasticity is often found in successful invaders and especially crayfish.

#### High trophic plasticity: role of ontogeny and environment

Both species populations displayed high trophic plasticity either in their TP, littoral reliance, niche breadth or diet composition. The largest niche breadth was quantified for the marbled crayfish population living in Moosweiher, the population with the highest proportion of arthropods consumed by marbled crayfish. In this lake, very small individuals were sampled and showed higher trophic positions than the other size classes. Ontogenetic dietary shifts are well known in many crayfish species where smaller crayfish usually prey upon aquatic invertebrates while adults feed on plant tissues (Correia 2003; Parkyn et al. 2001; Stenroth et al. 2006; Taylor and Soucek 2010). These ontogenetic patterns of size and TP can, however, be reversed (e.g. Larson et al. 2017) and are linked to lake productivity (Jackson et al. 2017).

Generally, trophic niches of crayfish are shaped by lake size, trophic state (Post et al. 2000) and the existing habitats (Ruokonen et al. 2014). The gravel pit lakes Meitzer See and Steinwedeler See were more oligotrophic and larger in size than the other sampled lakes, which might explain why crayfish TPs were

lower in those systems. The niche differences could also be related to habitat segregation. We found some differences in reliance on littoral food sources in lake Moosweiher (and lake Meitzer See), where  $\alpha$  differed by about 9% between species; in lake Krumme Lanke, however, the difference was only 4%. In addition, higher proportions of typical littoral food items like snails and macrophytes were estimated by the mixing model for spiny-cheek crayfish of lakes Moosweiher and Meitzer See. The crayfish species could feed at different depths and access more profundal sources, as isotopic signals of crayfish differ along depth gradients (Ruokonen et al. 2012). Chucholl and Pfeiffer (2010) found that marbled crayfish in lake Moosweiher, for example, preferred shallow areas composed of mainly detritus, mud and wood; and spiny-cheek crayfish preferred more stony substrates. We cannot quantify the distribution of crayfish in the sampled lakes but can qualitatively confirm the findings from Chucholl and Pfeiffer (2010) based on our samplings at lakes Moosweiher and Krumme Lanke.

High trophic plasticity enables crayfish to modify their trophic niche when a functionally similar species is present (Jackson et al. 2016; Larson et al. 2017). Allopatric and sympatric spiny-cheek crayfish populations had a similar niche breadth, which fits the observations made by Jackson et al. (2014) on other co-occurring crayfish invaders. Yet, niche shifts do not necessarily follow over-invasion. Pacific rats (*Rattus exulans*), for example, retained their TP also in the presence of numerically and morphologically superior black rats (*Rattus rattus*; Russell et al. 2015). Data on additional allopatric and sympatric populations could support these observations and better quantify the impact on spiny-cheek trophic niches.

Trophic plasticity of crayfish is further driven by predation pressure (Hill and Lodge 1994), resource availability (Roth et al. 2006) and the availability of shelters, a key resource for crayfish (Martin and Moore 2007). The latter might play a particularly important role in over-invasions of crayfish (Hudina et al. 2011; James et al. 2016). Also, marbled crayfish have been shown to be more aggressive than spiny-cheek crayfish (Hossain et al. 2019; Linzmaier et al. 2018) and might have caused the observed niche differences through interference competition.

## Conclusions

Marbled crayfish invasions are symptomatic for a new wave of ornamental crayfish that compete with established invaders in Europe (Chucholl and Wendler 2017; Kouba et al. 2014). The literature on crayfish ecology suggests that most species are functionally equivalent, which is supported by our experimental results on food choice. Furthermore, stable isotope data suggest that spiny-cheek crayfish and marbled crayfish both represent keystone species in the food webs because of their TP and trophic plasticity. However, crayfish have been shown to partition niches when co-occurring with other species (Jackson et al. 2014; Larson et al. 2017). Our study supports these findings for marbled crayfish over-invading lakes inhabited by spiny-cheek crayfish, where they seem to partition their diet to some extent.

Niche partitioning probably explains their co-existence as long as food or shelter are not limiting in the invaded systems. So far, marbled crayfish are known to coexist in Europe with other invasive species (spiny-cheek crayfish and red swamp crayfish (Chucholl et al. 2012)) and in Madagascar with native crayfish species (Andriantsoa et al. 2019). Our results suggest that the addition of marbled crayfish brings new functions in ecosystems already invaded by spiny-cheek crayfish. However, the latter seemingly adapt to this new competitor. To our knowledge, marbled crayfish were recorded in the studied systems of lakes Moosweiher and Krumme Lanke in 2003 and 2009, respectively, and have yet maintained healthy stocks of both species.

The complex feeding ecology of crayfish can have varying and unexpected ecosystem consequences during invasions, leading to trophic cascades (Jackson et al. 2014). Marbled crayfish invasions can lead to increased consumption of allochthonous detritus and a wide range of invertebrates which entail several direct and indirect consequences for ecosystem functions. Our study provides information to assess important aspects of marbled crayfish and crayfish isotope ecology, which is needed in the face of ongoing management plans like for the European Union's "List of Invasive Alien Species of Union Concern".

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## Appendix

See Tables 4, 5 and Fig. 4.

**Table 4** Pairwise comparisons of posterior trophic positions and Post's (2002)  $\alpha$  (littoral reliance)

|                                    | Moos. S | Moos. M | Silber. S | Krumm. S | Krumm. M | Meitz. M | Stein. S |
|------------------------------------|---------|---------|-----------|----------|----------|----------|----------|
| <i>Trophic position</i>            |         |         |           |          |          |          |          |
| Moosweiher S                       | 0       | 0.792   | 0.236     | 0.917    | 0.544    | 0.757    | 0.536    |
| Moosweiher M                       | 0.208   | 0       | 0.045     | 0.666    | 0.183    | 0.668    | 0.355    |
| Silbersee S                        | 0.764   | 0.955   | 0         | 0.998    | 0.893    | 0.829    | 0.677    |
| Krumme Lanke S                     | 0.083   | 0.334   | 0.002     | 0        | 0.034    | 0.629    | 0.282    |
| Krumme Lanke M                     | 0.456   | 0.817   | 0.107     | 0.966    | 0        | 0.747    | 0.498    |
| Meitzer See M                      | 0.243   | 0.332   | 0.171     | 0.371    | 0.253    | 0        | 0.293    |
| Steinwedeler Teich S               | 0.464   | 0.645   | 0.323     | 0.718    | 0.502    | 0.707    | 0        |
| <i>Alpha (<math>\alpha</math>)</i> |         |         |           |          |          |          |          |
| Moosweiher S                       | 0       | 0.261   | 0.965     | 0.474    | 0.611    | 0.221    | 0.263    |
| Moosweiher M                       | 0.739   | 0       | 0.987     | 0.716    | 0.815    | 0.309    | 0.373    |
| Silbersee S                        | 0.035   | 0.013   | 0         | 0.032    | 0.054    | 0.039    | 0.033    |
| Krumme Lanke S                     | 0.526   | 0.284   | 0.968     | 0        | 0.628    | 0.229    | 0.262    |
| Krumme Lanke M                     | 0.389   | 0.185   | 0.946     | 0.372    | 0        | 0.194    | 0.216    |
| Meitzer See M                      | 0.779   | 0.691   | 0.961     | 0.771    | 0.806    | 0        | 0.577    |
| Steinwedeler Teich S               | 0.737   | 0.627   | 0.967     | 0.738    | 0.784    | 0.423    | 0        |

Each row gives the probability that species (S = spiny cheek crayfish, M = Marbled crayfish) from that lake have a posterior trophic position/alpha less than or equal to crayfish from the lake in the column

**Table 5** Posterior trophic position estimates and posterior alpha ( $\alpha$ ) estimates for marbled crayfish (M) and spiny-cheek crayfish (S) populations from Bayesian Models with two different sets of trophic discrimination factors (median and [95% credibility interval]) and parametric trophic position estimates ( $\pm$  standard deviation) according to Post (2002)

| Lake   | Trophic position  |                   | Alpha ( $\alpha$ ) |                   |
|--|-------------------|-------------------|--------------------|-------------------|
|  | M                 | S                 | M                  | S                 |
| Trophic positions Bayesian Model TDF = Post (2002)           |                   |                   |                    |                   |
| Moosweiher   | 3.21 [2.60, 3.83] | 2.89 [2.30, 3.52] | 0.68 [0.50, 0.88]  | 0.76 [0.59, 0.94] |
| Silbersee  | –                 | 2.67 [2.43, 2.93] | –                  | 0.96 [0.82, 1.00] |
| Krumme Lanke   | 2.91 [2.58, 3.25] | 3.33 [3.03, 3.63] | 0.79 [0.61, 0.96]  | 0.75 [0.58, 0.93] |
| Meitzer See  | 2.94 [2.11, 7.75] | 3.80 [2.12, 9.31] | 0.59 [0.12, 0.96]  | 0.50 [0.04, 0.97] |
| Steinwedler Teich  | –                 | 2.72 [2.22, 4.10] | –                  | 0.71 [0.11, 0.99] |
| Trophic positions Bayesian Model TDF = Mc Cutchan 2003       |                   |                   |                    |                   |
| Moosweiher   | 3.20 [2.64, 3.80] | 2.88 [2.30, 3.53] | 0.67 [0.49, 0.88]  | 0.76 [0.58, 0.95] |
| Silbersee  | –                 | 2.67 [2.43, 2.93] | –                  | 0.97 [0.81, 0.99] |
| Krumme Lanke   | 2.91 [2.58, 3.24] | 3.33 [3.05, 3.65] | 0.79 [0.62, 0.96]  | 0.75 [0.59, 0.93] |
| Meitzer See  | 2.90 [2.09, 7.50] | 3.90 [2.11, 9.33] | 0.58 [0.11, 0.97]  | 0.50 [0.04, 0.96] |
| Steinwedler Teich  | –                 | 2.73 [2.24, 4.19] | –                  | 0.72 [0.10, 0.99] |
| Mean of individual trophic positions (parametric; Post 2002) |                   |                   |                    |                   |
| Moosweiher   | 3.24 $\pm$ 0.48   | 2.91 $\pm$ 0.35   | –                  | –                 |
| Silbersee  | –                 | 2.56 $\pm$ 0.40   | –                  | –                 |
| Krumme Lanke   | 2.98 $\pm$ 0.47   | 3.44 $\pm$ 0.33   | –                  | –                 |
| Meitzer See  | 2.37 $\pm$ 0.16   | 2.41 $\pm$ 1.04   | –                  | –                 |
| Steinwedler Teich  | –                 | 2.52 $\pm$ 0.29   | –                  | –                 |

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