Hydrobiologia
https://doi.org/10.1007/s10750-024-05513-6

PRIMARY RESEARCH PAPER

Species-specific trophic discrimination factors can reduce the uncertainty of stable isotope analyses

Lukáš Veselý · Paride Balzani · Phillip J. Haubrock · Miloš Buřič · Mael Glon · Fabio Ercoli · Timo J. Ruokonen · Martin J. Kainz · Heikki Hämäläinen · Antonín Kouba

Received: 18 August 2023 / Revised: 15 February 2024 / Accepted: 29 February 2024

Abstract Stable isotope analysis has been broadly used to study food webs, but often relies on inaccurate assumptions of trophic isotopic discriminations, which could lead to misinterpretation of obtained results. While many taxa exhibit similar trophic discrimination factors (TDFs), crayfish, exhibit omnivorous feeding strategies, yet TDFs are missing. In this study, we determined TDFs and tissue biokinetic parameters of the marbled crayfish *Procambarus virginalis* as a model species. Moreover, we compared commonly used TDFs and those determined from this study and applied them across species and ecosystems as a first attempt to compare the effect of species-specific TDFs in Bayesian trophic mixing models. Our results revealed differences between the TDFs of different tissues and biokinetic parameters of crayfish. Our result also revealed TDFs differences between crayfish relying mostly on plants versus those relying on an animal diet. We found differences of TDF suitability among species and ecosystems, highlighting the need for specific TDFs for different crayfish species. This study improves our understanding and the need for species-calibrated TDFs for robust statistical analysis of stable isotope data. Our approach is widely applicable across taxa and ecosystems to

Handling editor: Lee B. Kats

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10750-024-05513-6.

L. Veselý (✉) · P. Balzani · P. J. Haubrock · M. Buřič · A. Kouba
Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in České Budějovice, Zátiší 728/II, 38925 Vodňany, Czech Republic
e-mail: veselyl@frov.jcu.cz

L. Veselý · M. J. Kainz
WasserCluster Lunz – Biologische Station, Dr. Carl Kupelwieser Promenade 5, 3293 Lunz am See, Austria

P. J. Haubrock
Department of River Ecology and Conservation, Senckenberg Research Institute and Natural History Museum Frankfurt, Clamecystr. 12, 63571 Gelnhausen, Germany

P. J. Haubrock
CAMB, Center for Applied Mathematics and Bioinformatics, Gulf University for Science and Technology, Hawally, Kuwait

M. Glon
Department of Evolution, Ecology and Organismal Biology, The Ohio State University, 318 W. 12th Avenue, 300 Aronoff Laboratory, Columbus, OH 43210, USA

F. Ercoli · H. Hämäläinen
Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, 40014 Jyvaskyla, Finland

Published online: 17 April 2024

reduce the bias introduced by using unspecific TDFs in Bayesian mixing models.

Keywords *Procambarus virginalis* · Stable isotopes · Carbon · Nitrogen · Bayesian mixing models · Trophic discrimination factors · Food web · Biokinetic

Introduction

Stable isotope analyses (SIA) provide an essential step toward a better understanding of trophic ecology, yet their statistical analysis relies on assumptions which, if inaccurate, can lead to biases in their interpretation (Nielsen et al., 2018). One of the most important problems related to the analysis of food source utilization when applying SIA is the implementation of trophic discrimination factors (TDFs; Post, 2002; Bond & Diamond, 2011) which represent the isotopic enrichment between two trophic levels (i.e., from the food source to the consumer's tissue; Martínez del Rio et al., 2009; Nielsen et al., 2018).

The most common way to measure TDFs is the use of diet-switching experiments. In these experiments, a group of individuals belonging to one species are initially fed by a single diet to reach stable isotope equilibrium in their tissues (Boecklen et al., 2011). Then, animals are fed by another diet and are continuously sampled to reveal both TDF and isotopic turnover rate and half-life (Hesslein et al., 1993; Fry, 2007). Isotopic metabolic turnover rates provide the time when a tissue reaches an isotopic equilibrium from a new food source (i.e., the time required by a given tissue to completely reflect the isotopic composition of the new food source) and the isotopic half-life (i.e., the half-time for metabolic replacement of a given isotope in a given tissue). Both parameters are strongly linked to internal biokinetic processes, which are tissue and species dependent (Fry, 2007; Caut et al., 2009). Furthermore, TDFs should be considered as a difference among the stable isotope values of the food source and the consumer's tissue at equilibrium (Hesslein et al., 1993; Glon et al., 2016). The Bayesian mixing models, used to reconstruct a consumer's diet, can account for the uncertainty associated with multiple food sources and TDFs, which slightly decreases biases in data interpretation (Moore & Semmens, 2008; Stock & Semmens, 2016). However, accurate TDFs still remain crucial elements for reliable Bayesian mixing models outputs (Nielsen et al., 2018).

Concomitantly, the accuracy of SIA depends on correctly chosen putative food sources of the consumer (collected at the right time and place) and the use of appropriate TDFs (Fry, 2007). The former mostly relies on the knowledge about the consumer species' biology and the ability to collect all putative food sources during sampling. The second factor can be determined in controlled laboratory settings, but it may not completely mirror natural conditions because of numerous environmental factors. Additionally, laboratory studies typically only consider TDFs from single food sources, whereas in natural settings, consumers rely on multiple sources (Bondar et al., 2005; Carolan et al., 2012; Jussila et al., 2015; Glon et al., 2016; Mazumder et al., 2018). However, obtaining TDFs to decrease bias of stable isotope mixing models' outputs remains the best option, underlining the need to develop an approach allowing researchers to test the performance of TDFs under different conditions encountered in nature.

Generally, TDFs can be influenced by many variables such as temperature, taxonomic group, tissue analyzed, and/or food source quality (McCutchan Jr et al., 2003; Fry, 2007; Martínez del Rio et al., 2009). For example, Canseco et al. (2021) found that increasing temperatures led to a decrease of nitrogen TDFs in Teleostei fish. Similarly, Madigan et al. (2012) found differences in stable isotope values and metabolic turnover rate among white muscle and liver in Bluefin tuna [*Thunnus thynnus* (Linnaeus, 1758)]. Additionally, Malpica-Cruz et al. (2012) found differences in TDF among tissues in leopard shark (*Triakis semifasciata* Girard, 1855). In addition, consumers that rely on either plant or animal diets can have different TDFs, making it particularly difficult to obtain correct TDFs for omnivorous taxa with varying ratios of herbivory and carnivory (Vander Zanden & Rasmussen, 2001; Del Rio & Wolf, 2005; Caut et al.,

F. Ercoli
Centre for Limnology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 5D, 51006 Tartu, Estonia

T. J. Ruokonen
Natural Resources Institute Finland, Survontie 9 A, 40500 Jyvaskyla, Finland

2009). Brauns et al. (2018) suggested that food source C:N:P ratio together with consumer-resource differences in C:N:P ratio might be a good predictor for TDFs, because the type of utilized food and food source quality might affect the range of TDFs (Bond & Diamond, 2011). In the case of low food source quality (i.e., nutritional value as determined by its biochemical composition and the compounds required for physiological processes such as somatic growth, survival, and fecundity; Müller-Navarra et al., 2000), organisms with the capability of de novo fatty acids synthesis might produce such compounds from precursors (Arts et al., 2009). Organisms without such ability will support critical organs and tissues, while others might be depleted. In addition, starvation causes the catabolism of internal reserves (Haubert et al., 2005). All these processes affect stable isotope values as well as metabolic turnover rates and finally affect measured TDFs, while concomitantly, biokinetic parameters such as isotope half-lives, metabolic turnover rates, and TDFs can vary enormously among tissues (Tieszen et al., 1983; Fry, 2007; Arts et al., 2009; Vander Zanden et al., 2015). Such variation may be due to different rates of anabolism and catabolism among consumer tissues, which reflects their different metabolic activities (Martínez del Rio et al., 2009; Boecklen et al., 2011). Despite these differences, muscle is the most commonly used tissue for SIA involving crayfish, and other tissues remain understudied (Yokoyama et al., 2005; Stenroth et al., 2006; Mazumder et al., 2018; Viozzi et al., 2021). Therefore, a mechanistic understanding of biotic and abiotic factors and their combination influencing TDFs is needed.

Crayfish (Decapoda: Astacidea) are a diverse and prominent taxonomic group in freshwater ecosystems occupying all continents except for Antarctica (Kozák et al., 2015). Crayfish are omnivores with a central position in ecosystems (Grey & Jackson, 2012; Ercoli et al., 2014; Lipták et al., 2019; Veselý et al., 2020), with large feeding plasticity including detritus, algae, macrophytes, zoobenthos, dead fish, and conspecifics (Twardochleb et al., 2013) as well as diverse terrestrial sources (Grey & Jackson, 2012). Their food preferences might further change during ontogeny (Momot, 1995; Veselý et al., 2020), while mediating nutrient and energy flows within and among ecosystems by feeding on species belonging to both lower and higher trophic levels (Grey & Jackson, 2012; Lipták et al., 2019).

Despite the importance of crayfish in aquatic food webs, specific TDFs have only been established for a small number of them (Table 1), whereas the few previous studies that focused on crayfish biokinetics have shown that the isotopic incorporation rate and TDFs vary greatly across species (Carolan et al., 2012; Jussila et al., 2015; Glon et al., 2016; Mazumder et al., 2018). The uncertainty of trophic interactions of crayfish is a continuing problem despite attempts to unravel them by established methods such as gut content or SIA (Whitledge & Rabeni, 1997; Rudnick & Resh, 2005). To date, most SIA studies on crayfish have relied on general TDFs, like those estimated by

Table 1 Comparison of trophic discrimination factors (TDFs) in muscle tissue from crayfish and other taxa for reference

References	Species	Diet	TDF		Life stage	Temperature (°C)
			C	N		
Vander Zanden & Rasmussen (2001)	Fish	Animal	0.47 ± 1.23	3.23 ± 0.41	NA	NA
		Plant	0.4 ± 0.28	2.4 ± 0.42		
Glon et al., (2016)	*Faxonius rusticus* and *F. virilis*	Animal	0.8 ± 0.99	1.2 ± 1.26	Juvenile crayfish	21
		Plant	1.57 ± 0.98	2.94 ± 1.12		
Carolan et al., (2012	*Cherax destructor*	Mixture diet	3.1 ± 0.5	1.5 ± 1	Adult	25 ± 1
Mazumder et al., (2018)	*C. destructor*	Mixture diet	2.22 ± 0.15	1.67 ± 0.66	Adult	22 ± 1
Present study	*Procambarus virginalis*	Animal	3.87 ± 3.07	− 1.94 ± 2.33	Sub-adult	20 ± 0.2
		Plant	8.13 ± 0.79	2 ± 1.46		
Brauns et al (2018)	Freshwater invertebrates	Mixture diet	1.84 ± 2.14	0.63 ± 1.89	NA	18

TDFs are presented as mean ± standard deviation (SD), combining data sourced from the literature with findings from the current study

Vander Zanden & Rasmussen (2001). However, the growing interest in the trophic ecology of crayfish, particularly of non-native species (Nilsson et al., 2012; Marufu et al., 2018; Veselý et al., 2021), highlights the urgent need to determine species-specific TDFs to increase the accuracy of applied models and ultimately minimize the effect of biased analyses. Hence, to compare the suitability of specific TDFs in Bayesian models across species and ecosystems, we used the marbled crayfish *Procambarus virginalis* Lyko, 2017 as a model organism and analyzed the biokinetics and TDFs of its different tissues. We then compared the performance of the obtained TDFs with the TDFs reported for other crayfish species and ecosystems available in the literature. We hypothesized that (i) biokinetic parameters (metabolic turnover rate, isotopic value of tissue at the equilibrium with the diet, and isotopic half-life [i.e., the time when a given tissue reaches 50% equilibrium with a new diet]) will vary across tissues and diets of marbled crayfish, (ii) TDFs will be species-specific, and (iii) TDFs will vary intraspecifically across localities.

Materials and methods

Study design

This study was divided into two parts. The first, experimental part, dealt with establishing new TDFs for marbled crayfish under controlled conditions and investigating the effect of food type on biokinetic parameters in different crayfish tissues. In the second part, commonly used TDFs as well as crayfish-specific TDFs from the literature and TDFs obtained from our experiment were collected and applied to different species and ecosystems to assess their suitability.

Controlled feeding experiment

Our experiment was conducted between April and September of 2020 at the Research Institute of Fish Culture and Hydrobiology in Vodňany, FFPW USB, Czech Republic. No specific permissions were required for the locations and activities involved in this study. As an experimental animal, we used marbled crayfish *Procambarus virginalis*. This originally North American species has been popular in the pet trade (Lipták et al., 2023) and later established numerous wild populations elsewhere. Especially European populations are numerous and span from Sweden and Estonia in the north to Sardinia and Malta in the south, as well as the Netherlands and France in the west to Ukraine in the east (Kouba et al., 2014; Marmorkrebs.org). Nowadays, this crayfish is considered an invasive crayfish of European Union concern (Veselý et al., 2021).

To identify new and robust TDFs for this species and to determine the time needed for specific tissues to incorporate the isotopic signal of new diets, we started the experimental trial with juveniles in the 3rd developmental stage (i.e., when the species becomes independent from the mother and begins exogenous feeding; Kouba et al., 2021). We first held experimental crayfish for 90 days in a 100 l aquarium with overly abundant shelters to minimize cannibalism and fed them exclusively with the sewage worm [*Tubifex tubifex* (Müller, 1774)] to equalize their stable isotope values. At the end of this period, we randomly sampled six individuals to be used as a baseline in further calculations.

For the actual experiment, we divided the crayfish equally into three 100 l aquaria (23 individuals each) and fed them with three different single diets for 12 weeks as follows: carrots (*Daucus carota* L.), catfish (*Silurus glanis* Linnaeus, 1758) dorsal muscle, and sewage worms as a control. To keep sources' stable isotopes values (Table S1) constant during the experiment, each diet was previously prepared and frozen at −20 °C to be only defrosted 1 h before feeding. Thus, the very same diet was used throughout the experiment. Crayfish were always fed ad libitum to avoid cannibalism and to maximize tissue assimilation. To further minimize aggression and cannibalism, we placed three fired clay bricks (6.5 × 28.5 × 13.5 cm), each with 39 cross holes (26 and 13 holes with a profile of 1 × 3 cm and 1 × 1 cm, respectively) in each aquarium to serve as shelter (Veselý et al., 2017). After 1 month, three additional shelters with greater dimensions (29 × 14 × 6.5 cm) with a 1 × 3 cm hole profile were added as individuals grew (Kouba et al., 2011). The water temperature was set to 20 ± 0.2 °C with a light:dark regime of 12L:12D. Every 3 weeks, we measured carapace length (CL) and weighed all crayfish to the nearest 0.1 mm and 0.1 g, respectively (Table S1). At these occasions, four specimens per group were randomly

selected and euthanized by short-term freezing at − 20 °C, followed by dissection for specific tissues (muscle, carapace—only hard shell, gill, and hepatopancreas). Additionally, three samples of each presented diet were collected, and all samples were stored frozen at − 20 °C until further processing.

Stable isotope analysis (SIA)

Stable isotopes of carbon and nitrogen were analyzed at the University of Jyväskylä, Finland, using a Carlo Erba Flash EA1112 elemental analyzer connected to a Thermo Finnigan DELTAplus Advantage continuous flow stable isotope-ratio mass spectrometry (CF-IRMS). For this, all collected samples were dried at 40 °C for 48 h and then homogenized. Results are expressed using the standard δ notation as parts per thousand (‰) difference from international standards: $\delta^{13}C$ or $\delta^{15}N = [((R_{sample}/R_{standard}) - 1) \times 1000]$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios, and the standards are Vienna Pee Dee belemnite (for carbon) and atmospheric N_2 (for nitrogen). Dried pike (*Esox lucius*, Linnaeus, 1758) white muscle (animals), and birch (*Betula* sp.) leaves (plants) were used as internal working standards, and two replicates were run repeatedly after every five samples in each sequence. Standard deviations within reference samples in each sequence were less than 0.08‰ for carbon and 0.15‰ for nitrogen in pike samples and less than 0.08‰ for carbon and 0.19‰ for nitrogen in birch leaf samples.

Data analysis

Following the approach described by Glon et al. (2016), we estimated $\delta^{13}C$, $\delta^{15}N$, and the metabolic rate of each crayfish tissue at equilibrium (δ_f) with their respective diets, using the growth-based model of Hesslein et al. (1993) based on Nonlinear Least Squares method:

$$\delta_t = \delta_f + (\delta_i - \delta_f)e^{-(k+m)t} \quad (1)$$

where δ_t is the $\delta^{13}C$ or $\delta^{15}N$ value of specific crayfish tissues at time t. Consequently, δ_f is the estimated $\delta^{13}C$ or $\delta^{15}N$ of crayfish tissues when reaching an equilibrium with their new diet, while δ_i is the initial $\delta^{13}C$ or $\delta^{15}N$ of the tissue, m the metabolic turnover rate, and k the growth rate, that in turn can be calculated as

$$k = \frac{\ln\left(\frac{W_t}{W_0}\right)}{t}, \quad (2)$$

where W_t is the weight of the individual crayfish at the time of the sampling (t), and W_0 the initial weight. We used this equation as it allowed us to separate the relative contributions of crayfish growth and metabolic tissue replacement to our isotope values, i.e., to quantify the half-life of stable isotopes in each tissue (Fry & Arnold, 1982).

For each tissue, we further calculated the TDF ($\Delta^{13}C$ and $\Delta^{15}N$), which reflects the difference between the crayfish δ values at the time they reach equilibrium (δ_f) and that of their diet (δ_d) as

$$\Delta = \delta_f - \delta_d. \quad (3)$$

We also estimated stable isotopes half-life, following the growth-based model of Hesslein et al. (1993) modified by Glon et al. (2016), by setting δ_t equal to the mid-point between the measured δ_i and the model estimate δ_f and then solving for t:

$$t = \frac{\ln\left(\frac{\delta_t - \delta_f}{\delta_i - \delta_f}\right)}{-(k+m)}, \quad (4)$$

where δ_t, δ_f, k, and m are the parameters mentioned above.

Finally, for each tissue, we ran generalized linear models (GLMs, with Gaussian distribution) with $\delta^{13}C$ or $\delta^{15}N$ as response variable and diet, time and their interaction as predictors. Tukey's post hoc tests were subsequently applied to test for significant differences among the treatments.

Comparison of TDF performance

To compare the obtained TDFs from our study to those already known, we collected information from previously published studies on crayfish. Using a series of keywords (i.e., trophic discrimination factor, fractionation factor, crayfish, biokinetic) in Google Scholar, we identified only three suitable studies that dealt with TDFs in crayfish (Table 1). Additionally, for both $\delta^{13}C$ and $\delta^{15}N$, we added commonly used (general) TDFs from Vander Zanden & Rasmussen

(2001), the calculated mean value of all TDFs found (i.e., those from the literature as well as those estimated by our study) and the study of Brauns et al. (2018), suggesting C:N:P ratio as a good predictor of TDFs.

To test and compare our TDF performance, we collected stable isotope data from four species: the marbled crayfish, the spiny-cheek crayfish [*Faxonius limosus* (Rafinesque, 1817)] the signal crayfish [*Pacifastacus leniusculus* (Dana, 1852)], and the noble crayfish [*Astacus astacus* (Linnaeus, 1758)] across seven localities of four European countries (Czech Republic, Slovakia, Hungary, and Finland; Table 2).

Using the collected TDFs from the literature (six values; Table 1), we ran Bayesian models using the `MixSIAR` R package (Stock et al., 2018), employing different TDFs for each site/species (Fig. 1). Specifically, in the case of this study, the single consumer-resource data obtained from a given locality were loaded into the Bayesian model with a single TDF obtained from literature/experiment. Mentioned single consumer-resource data from a given locality were then also separately loaded with other TDFs in single Bayesian models. Thus, we generated seven independent Bayesian models with the same single consumer-resource data from a given locality but with

Table 2 Localities and crayfish species used for TDF comparison

References	Location	Country	Ecosystem type	Crayfish species	Putative food sources
Veselý et al., (2021)	Barát stream	Hungary	Lotic	*Faxonius limosus* and *Procambarus virginalis*	Detritus, Macrophytes, Algae, Zoobenthos
Lipták et al., (2019)	Leopoldov gravel pit	Slovakia	Lentic	*P. virginalis*	Allochthonous detritus, Autochthonous detritus, Macrophytes, Algae, Zoobenthos
Veselý et al., (2020)	Nýrsko reservoir	Czech Republic	Lentic	*Astacus astacus*	Detritus, Macrophytes, Algae, Zoobenthos, Crayfish, Zooplankton
Ercoli et al., (2014)	Kallajärvi Lake	Finland	Lentic	*A. astacus*	Detritus, Macrophytes, Periphyton, Zoobenthos
	Suurimieloo Lake	Finland	Lentic	*A. astacus*	
	Syväjärvi Lake	Finland	Lentic	*P. leniusculus*	
	Karikkoselkä Lake	Finland	Lentic	*P. leniusculus*	

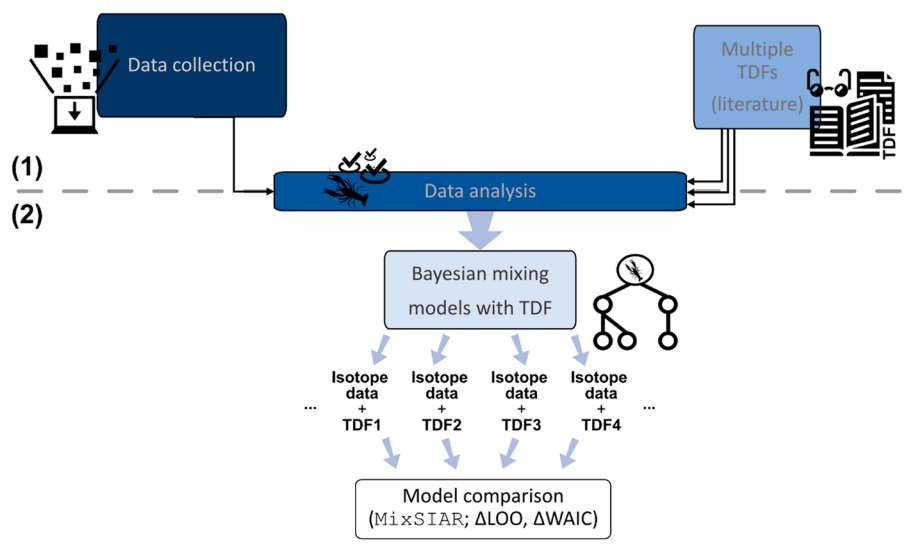

Fig. 1 Scheme of TDF performance comparison

different TDFs. Then, we compared these models (using the *compare_models* function) using Leave-one-out cross-validation (LOO) and widely applicable information criterion (WAIC; Stock & Semmens, 2016). Both methods estimate pointwise out-of-sample prediction accuracy from a fitted Bayesian model using the log-likelihood evaluated at the posterior simulations of the parameter value. Significant differences among models were assumed when ΔLOO or ΔWAIC was > 0.5. If LOO and WAIC provided differing results, we considered LOO a more robust and appropriate method due to the model weight based on Akaike information criterion (AIC).

To test if the consumers' isotope values fell within the resource isotope space, we applied the approach of Smith et al. (2013), using the trophic niche to validate our models. For this, we plotted the mean isotopic signatures of all sources enriched by the TDF together with the isotopic signatures of all individuals of the consumer. If the whole consumer's niche fell within the polygon area defined by the sources, then the model was considered valid, while an intersection would indicate the opposite (see Table S3).

Results

Controlled feeding experiment

For all four tissues, there was a significant effect of time, diet, and the interaction of the two factors on $\delta^{13}C$ and $\delta^{15}N$ (Figs. 1, 2; Supplementary Figs. S1, S2; Table 3). With the exception of carbon stable isotopes in hepatopancreas in animals fed by catfish diet, all combinations of diet and tissues reached an equilibrium in both isotopes (Table 4).

Crayfish fed with carrots had the most depleted values of $\delta^{13}C$ and $\delta^{15}N$ compared to crayfish provided with the other diets, regardless of the tissue (Figs. 2, 3). Hepatopancreas was the most depleted tissue in terms of $\delta^{13}C$, while the most enriched tissue was the carapace. Carapace was the most depleted tissue for $\delta^{15}N$, while the most enriched was muscle.

The estimated mean half-life of $\delta^{13}C$ differed among diets and tissues (Table 2). The estimated mean half-life of $\delta^{13}C$ in muscle and hepatopancreas was longer for carrot than for catfish diet. Carapace and gill tissues showed an opposite trend, with crayfish fed with carrots incorporating carbon from the new diet faster than crayfish provided with catfish. In muscle, carapace, and hepatopancreas, the estimated mean half-life of $\delta^{15}N$ was shorter in crayfish fed with carrots than in those fed with catfish. On the other hand, gills from crayfish fed with carrots required a longer time to incorporate nitrogen from the new diet than crayfish fed with catfish. However, it should be noted that, for some diet and tissue combinations, the metabolic turnover rate was not at equilibrium (see Table 4). Thus, although the computed values are reported for comparisons, the isotopic half-life is less reliable compared to other estimations.

Trophic discrimination factors also differed among diets and tissues. TDFs of $\delta^{15}N$ for all tissues were higher in crayfish fed with carrots than in those fed with catfish. Similarly, $\delta^{13}C$ TDFs were higher in all tissues of crayfish fed with carrots except for gills, which showed a higher TDF in crayfish fed with catfish. A consistent $\delta^{13}C$ TDF pattern was found among tissues regardless of the diet, with the lowest value in the hepatopancreas, followed by gill, muscle, and carapace samples. Such a consistent pattern was not found for $\delta^{15}N$ TDF (Table 4). Anyway, it should be noted that for some tissues other than muscle, for some diet treatments, the isotopic value did not reach equilibrium (see Table 4). For these cases, we reported the computed TDFs for comparison with other studies, but these TDFs should be taken with caution, since they are less reliable.

Comparison of TDF performance

The applied models identified differences in the performance of the different TDFs across studies (Table 5). In the Barát stream occupied by the spiny-cheek crayfish and/or marbled crayfish, a cluster of TDFs suggested by Glon et al. (2016) and Vander Zanden & Rasmussen (2001) were significantly more accurate than others. In the Leopoldov gravel pit lake occupied by marbled crayfish, the TDFs suggested by Glon et al. (2016) were significantly more accurate than others. On the other hand, in the Nýrsko reservoir, which was occupied by noble crayfish, TDFs reported by Vander Zanden & Rasmussen (2001) performed significantly better than others. Interestingly, there were no significant differences in performance among TDFs for the Finnish lakes, which were occupied by the noble and signal crayfish.

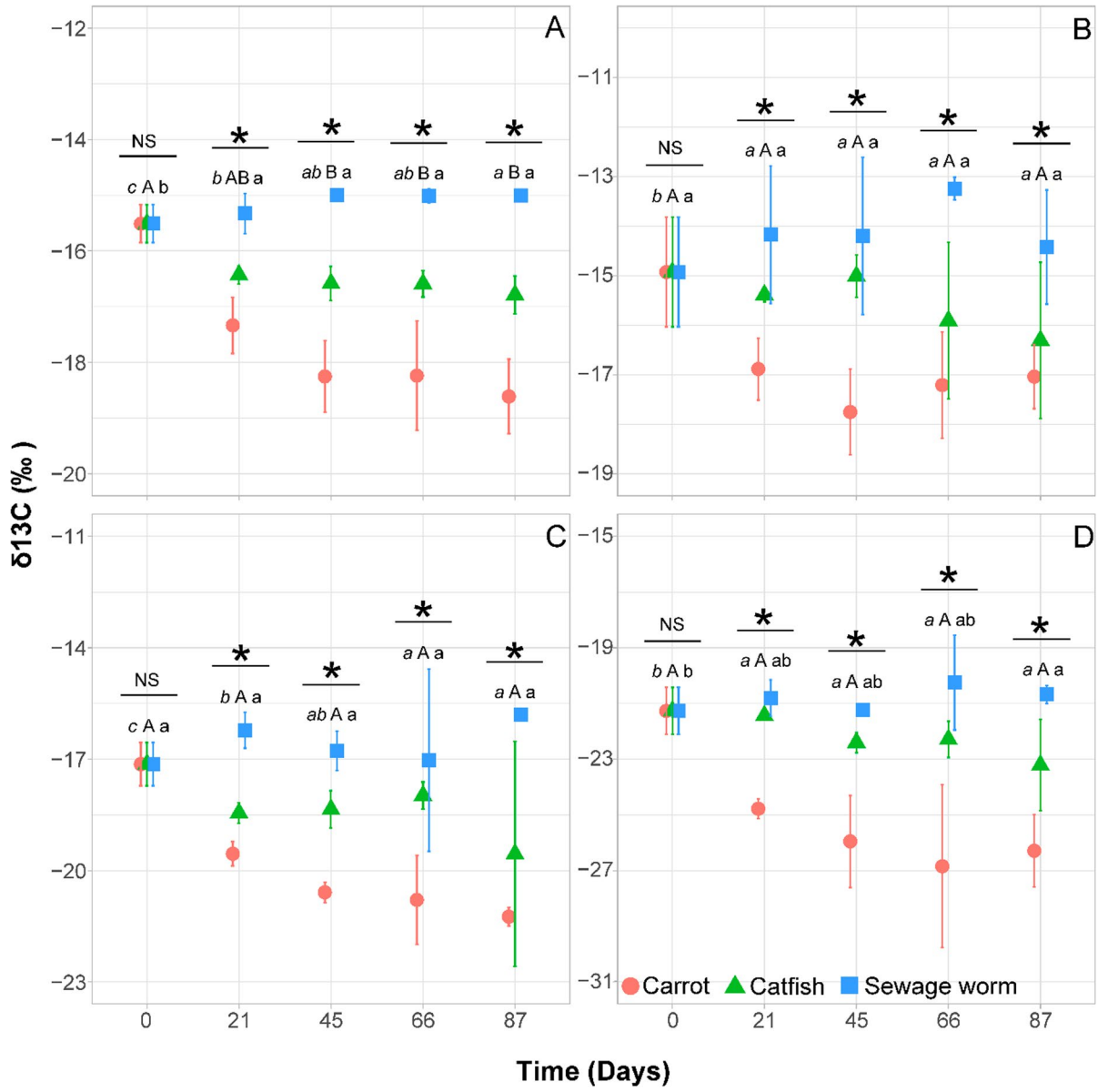

Fig. 2 Mean ± SD of carbon ($\delta^{13}C$) of crayfish tissues over time. Please note the variable scales on *y*-axes. Different letters denote significant ($P < 0.05$) differences between time at a given diet (carrot = small letters in italic, catfish = capital letters, sewage worm = small letter) and tissue. Significant differences between diet within each time are marked by asterisks, non-significant ones by 'NS'. **A** muscle, **B** carapace, **C** gill, **D** hepatopancreases

The overlap between model-based simulations of mixing regions of food sources and their consumers provides a generally similar picture as with the above model comparison method of the relative performance of the TDFs. However, both method outcomes provided a slightly different picture (Supplementary Figs. S3–S10, Table S3): In the marbled and spiny-cheek crayfish from Barát stream, consumers were within the polygons of their food sources in each model with TDF of Vander Zanden & Rasmussen (2001) and Brauns et al. (2018) (Supplementary Figs. S3, S5, Table S3). Similarly, situation was in the Nýrsko reservoir, where models with TDF of Vander Zanden & Rasmussen (2001) and Brauns et al. (2018)

Table 3 Summary of the most parsimonious model for nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) in different tissues

	df	$\delta^{15}N$		$\delta^{13}C$	
		F	*P* value	*F*	*P* value
Muscle					
Diet	2	140.561	**< 0.001**	47.171	**< 0.001**
Time	1	20.998	**< 0.001**	35.097	**< 0.001**
Diet × time	2	54.615	**< 0.001**	12.271	**< 0.001**
Carapace					
Diet	2	25.575	**< 0.001**	25.328	**< 0.001**
Time	1	12.464	**< 0.001**	4.088	**0.047**
Diet × time	2	19.253	**< 0.001**	4.942	**0.011**
Gill					
Diet	2	223.289	**< 0.001**	44.998	**< 0.001**
Time	1	21.073	**< 0.001**	16.745	**< 0.001**
Diet × time	2	87.800	**< 0.001**	12.237	**< 0.001**
Hepatopancreases					
Diet	2	128.906	**< 0.001**	54.79	**< 0.001**
Time	1	12.415	**< 0.001**	20.692	**< 0.001**
Diet × time	2	46.053	**< 0.001**	12.834	**< 0.001**

Significant values ($P < 0.05$) in bold

df degrees of freedom

and Carolan were most suitable (Supplementary Fig. S6, Table S3). The simulation of the mixing region for the Leopoldov gravel pit lake suggested that the TDFs proposed by Brauns et al. (2018) was the most suitable for marbled crayfish (Supplementary Fig. S4, Table S3). An opposite situation was found for Finnish lakes. In Karikoselkä and Syväjärvi Lake, occupied by signal crayfish, none TDF can be consider as a suitable, only Brauns et al. (2018) resulted in the consumer falling within the source polygons with exception of one specimen in both lakes. On the other hand, in Suurimieloo Lake, occupied by noble crayfish, TDF suggested by Brauns et al. (2018) was considered suitable. In Kallajärvi Lake, inhabited by noble crayfish, TDF suggested by Vander Zanden & Rasmussen (2001) and Brauns et al. (2018) consider as a suitable, resulted in the consumer falling within the source polygons.

Discussion

Over the last several decades, the use of SIA has been of paramount importance to ecologists interested in understanding food webs and trophic interactions (Post, 2002; Nielsen et al., 2018). However, limited availability or the use of incorrect TDFs remains important limitations for SIA (Nielsen et al., 2018; Stock et al., 2018). Establishing species- or tissue-specific TDFs using a laboratory experiment requires considerable effort, and laboratory-derived TDFs may not accurately reflect natural conditions (Bondar et al., 2005; Jussila et al., 2015; Glon et al., 2016). In this study, we demonstrated that biokinetics vary among tissues and diets of marbled crayfish, in accordance with our first hypothesis. Furthermore, this study revealed species-specific and ecosystem differences in the performance of TDFs, in accordance with our second and third hypotheses, respectively. Moreover, we propose a novel approach for assessing TDF suitability for a given consumer which may be widely applicable across consumers and ecosystems.

Controlled feeding experiment

No study to date has examined the growth of tissues or organs in crustaceans, which limits the application of the Hesslein model (Hesslein et al., 1993) that is based on known relationships between body size and growth rates, introducing biases into data interpretation. It is also well known that biokinetic parameters also strongly vary among species (Boecklen et al., 2011; Glon et al., 2016). Therefore, the assumption that isotope half-lives, metabolic turnover rates, or TDFs obtained from one species can be used for another one is misleading. In crustaceans, differences in biokinetic parameters between species can be found even when only the muscle tissue is considered. Suring & Wing (2009) estimated the isotope half-life ($\delta^{13}C$ and $\delta^{15}N$) of red rock lobster [*Jasus edwardsii* (Hutton, 1875)] muscle to be 147 days, whereas for a freshwater shrimp *Macrobrachium borellii* (Nobily, 1896) it was much shorter for both $\delta^{13}C$ and $\delta^{15}N$ (75 and 40 days, respectively; Viozzi et al., 2021). These results differ from Glon et al. (2016), who estimated isotope half-lives of two *Faxonius* species [*F. rusticus* (Girard, 1852) and *F. virilis* (Hagen, 1870)]. Their results for $\delta^{15}N$ (28–30 days) were similar to ours. On the other hand, the results for $\delta^{13}C$ were substantially longer (33–36 days) for both species compared to ours. Similarly, in the common yabby (*Cherax destructor* Clark, 1936), $\delta^{15}N$ isotope

Table 4 Model-estimated parameters of metabolic turnover rates and carbon and nitrogen signatures at equilibrium and mean half-lives of stable isotopes in days and trophic discrimination factor (TDFs; mean ± SD) for each tissue and diet combination

Isotope	Tissue	Diet	Metabolic turnover rate				Isotopic value of tissue in equilibrium				Mean half-life (days)	TDF
			Estimate	Low CI	High CI	*P* value	Estimate	Low CI	High CI	*P* value		
C	Muscle	D.c	0.04	0.03	0.11	< 0.01	− 18.54	− 19.16	− 18.05	< 0.01	15.27	8.13 ± 0.80
C	Carapace	D.c	0.09	− 0.11	0.31	0.24	− 17.27	− 17.84	− 16.68	< 0.01	6.22	9.41 ± 0.86
C	Gill	D.c	0.04	0.03	0.08	< 0.01	− 21.15	− 21.82	− 20.66	< 0.01	15.19	5.50 ± 0.89
C	Hepatopancreas	D.c	− 0.01	− 0.03	< 0.01	< 0.01	− 7.14	− 20.73	− 15.39	0.13	42.86	0.02 ± 2.26
C	Muscle	S.g	0.07	0.01	0.13	0.03	− 16.68	− 16.98	− 16.50	< 0.01	11.81	6.05 ± 0.31
C	Carapace	S.g	0.01	− 0.06	0.07	0.89	− 16.86	− 26.86	− 6.87	< 0.01	17.79	6.75 ± 1.49
C	Gill	S.g	− 0.01	− 0.02	< 0.01	0.25	− 13.80	− 27.10	− 0.50	0.04	45.45	5.92 ± 2.31
C	Hepatopancreas	S.g	− 0.01			< 0.01	− 42.36			0.02	41.02	− 0.03 ± 1.1
C	Muscle	T.t	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.70 ± 0.32
N	Muscle	D.c	0.02	0.01	0.04	0.03	6.84	5.48	7.54	< 0.01	29.44	2.00 ± 0.1
N	Carapace	D.c	0.00	< − 0.01	0.02	0.43	− 1.85	− 7.23	1.55	0.43	35.87	− 2.68 ± 1.39
N	Gill	D.c	0.01	< 0.01	0.03	0.03	3.17	0.59	4.63	0.03	47.58	− 0.15 ± 1.35
N	Hepatopancreas	D.c	− 0.01	− 0.02	− 0.01	< 0.01	16.56	12.33	21.88	< 0.01	25.64	− 1.00 ± 1.52
N	Muscle	S.g	− 0.01	− 0.03	0.01	0.15	9.04	6.79	11.28	< 0.01	39.49	− 2.93 ± 3.21
N	Carapace	S.g	− 0.01	− 0.01	< 0.01	< 0.01	− 1.81	− 12.19	8.57	0.72	44.65	− 5.761 ± 1.94
N	Gill	S.g	< − 0.00	− 0.01	< 0.01	< 0.01	16.17	7.75	24.58	< 0.01	19.89	− 2.54 ± 1.62
N	Hepatopancreas	S.g	− 0.01	− 0.03	0.01	0.28	6.61	− 0.18	13.49	0.06	44.48	− 2.50 ± 0.333
N	Muscle	T.t	NA	NA	NA	NA	NA	NA	NA	NA	NA	− 0.99 ± 1.47

Low and high CI denote upper and lower limits of confidential intervals

D.c carrot *Daucus carota*, *S.g* catfish *Silurus glanis*, *T.t* sewage worm *Tubifex tubifex*, *C* carbon, *N* nitrogen

Table 5 Comparison of Bayesian models with different trophic discrimination factor

TDF	ΔLOO	Weight	ΔWAIC	Weight
Barát stream, Hungary—marbled crayfish				
Vander Zanden & Rasmussen (2001)	**0.3**	0.262	**0.4**	0.267
Glon et al. (2016)	**0.0**	0.226	**0.0**	0.218
Carolan et al. (2012)	3.6	0.043	3.6	0.044
Mazumder et al. (2018)	1.5	0.124	1.5	0.126
Brauns et al. (2018)	**0.2**	0.238	1.9	0.103
Present study	1.8	0.107	**0.2**	0.241
Leopoldov gravel pit, Slovakia—marbled crayfish				
Vander Zanden & Rasmussen (2001)	1.7	0.121	0.9	0.168
Glon et al. (2016)	**0.0**	0.284	**0.0**	0.263
Carolan et al. (2012)	2.0	0.105	1.4	0.130
Mazumder et al. (2018)	**0.3**	0.245	0.5	0.205
Brauns et al. (2018)	1.1	0.164	1.1	0.152
Present study	2.5	0.081	2.3	0.083
Barát stream, Hungary—spiny-cheek crayfish				
Vander Zanden & Rasmussen (2001)	**0.2**	0.264	**0.3**	**0.256**
Glon et al. (2016)	**0.0**	**0.292**	**0.0**	0.298
Carolan et al. (2012)	2.6	0.080	2.8	0.073
Mazumder et al. (2018)	1.0	0.177	1.1	0.172
Brauns et al. (2018)	0.9	0.186	0.8	0.199
Present study	10.2	0.002	10.3	0.002
Karikkoselkäa lake, Finland—signal crayfish				
Vander Zanden & Rasmussen (2001)	1.6	0.091	1.8	0.084
Glon et al. (2016)	0.1	0.192	0.2	0.186
Carolan et al. (2012)	0.6	0.150	0.4	0.169
Mazumder et al. (2018)	0.0	0.202	0.0	0.206
Brauns et al. (2018)	0.2	0.183	0.4	0.169
Present study	0.2	0.183	0.2	0.186
Syväjärvi lake, Finland—signal crayfish				
Vander Zanden & Rasmussen (2001)	0.0	0.202	0.0	0.187
Glon et al. (2016)	0.5	0.157	0.3	0.161
Carolan et al. (2012)	0.7	0.142	0.2	0.169
Mazumder et al. (2018)	0.1	0.192	0.2	0.169
Brauns et al. (2018)	0.5	0.157	0.4	0.153
Current study	0.6	0.150	0.3	0.161
Suurimieloo lake, Finland—noble crayfish				
Vander Zanden & Rasmussen (2001)	0.2	0.161	0.2	0.160
Glon et al. (2016)	0.1	0.169	0.1	0.168
Carolan et al. (2012)	0.1	0.169	0.1	0.168
Mazumder et al. (2018)	0.3	0.153	0.3	0.152
Brauns et al. (2018)	0.0	0.178	0.0	0.176
Present study	0.1	0.169	0.0	0.176
Kallajärvi lake, Finland—noble crayfish				
Vander Zanden & Rasmussen (2001)	0.2	0.174	0.2	0.173
Glon et al. (2016)	0.1	0.183	0.1	0.182
Carolan et al. (2012)	0.4	0.158	0.3	0.164
Mazumder et al. (2018)	0.4	0.158	0.4	0.156

Table 5 (continued)

TDF	ΔLOO	Weight	ΔWAIC	Weight
Brauns et al. (2018)	0.7	0.136	0.7	0.134
Present study	0.0	0.192	0.0	0.191
Nýrsko reservoir, Czech Republic—noble crayfish				
Vander Zanden & Rasmussen (2001)	**0.0**	**0.960**	**0.0**	**0.958**
Glon et al. (2016)	6.8	0.032	6.7	0.034
Carolan et al. (2012)	12.7	0.002	12.7	0.002
Mazumder et al. (2018)	10.6	0.005	10.5	0.005
Brauns et al. (2018)	14.1	0.001	14.1	0.001
Present study	14.5	0.001	14.8	0.001

Leave-one-out cross-validation (ΔLOO) and widely applicable information criterion (ΔWAIC) denote differences among the models within a given ecosystem. Significant values (ΔLOO or ΔWAIC > 0.5) in bold. Weight = model weight based on Akaike criterion, ΔLOO differences among models in Leave-one-out cross-validation method, ΔWAIC = differences among models widely applicable information criterion method

half-life was estimated as 19 days, while for $\delta^{13}C$ it could not be established (Carolan et al., 2012). Such observed differences are not unequivocally species-specific but may be related to uncontrolled external biotic and abiotic factors such as temperature, salinity, ecosystem type, species origin, or ontogeny. For example, Barnes et al. (2007) reported that temperature can modify TDFs in European sea bass [*Dicentrarchus labrax* (Linnaeus, 1758)].

It is also known that TDFs vary depending on other factors, such as diet composition or species (Caut et al., 2009; Auerswald et al., 2010; Perga & Grey, 2010). Indeed, herbivores generally show higher TDFs than carnivores (Vander Zanden & Rasmussen, 2001; Del Rio & Wolf, 2005). Yet, few studies investigating the trophic ecology of crayfish have examined the isotopic incorporation and TDFs of different tissues (Stenroth et al., 2006; Mazumder et al., 2018). In line with this expectation and in accordance with the findings from Mazumder et al. (2018), we found lower TDFs in almost all tissues from crayfish fed with catfish compared to those fed with carrots. The only exception to this pattern was the $\delta^{13}C$ TDF for gills. Further, omnivorous animals, like crayfish, assimilate the isotopic signatures of their food sources differently depending on the proportion of plant and animal material ingested. Therefore, it is likely that TDFs of omnivores are intermediate between those of herbivores and carnivores (Bastos et al., 2017). As a result, it is crucial not to consider a single (averaged) TDF, but instead the potential TDF range or preferably species-specific TDFs whenever possible.

In their review, Caut et al. (2009) found that $\delta^{15}N$ TDFs ranged from 0.7 to 5.2‰ in freshwater invertebrates. More recent studies on $\delta^{15}N$ TDF of freshwater crayfish muscle found values ranging between 0.1 and 3.7‰ (Glon et al., 2016; Mazumder et al., 2018) or a mean value of 1.5‰ (Carolan et al., 2012). Viozzi et al. (2021) found $\delta^{15}N$ TDF in the muscle of *Macrobrachium borellii* ranging from 3.18 to 4.02‰. The current study found lower values for the $\delta^{15}N$ range, underlining the food source dependency. In our study, the muscle $\delta^{13}C$ TDF was higher compared to those reported in the literature (Mazumder et al., 2018: −1.4 to 2.3‰; Carolan et al., 2012: −1.1 ‰; Viozzi et al., 2021: −0.93 to 0.73‰), while our estimated hepatopancreas TDF ranged lower for both $\delta^{13}C$ and $\delta^{15}N$ when compared to those reported for a freshwater shrimp (from 0.34 to 1.06‰ and from 1.27 to 2.13‰, respectively; Viozzi et al., 2021). Our carapace TDF ranges were considerably higher for both $\delta^{13}C$ and $\delta^{15}N$ than those reported for *Cherax destructor* (Mazumder et al., 2018). These results suggest altogether a strong TDF species and tissue dependency.

Comparison of TDF performance

Stable isotope mixing models and estimates of trophic positions can be highly affected by the choices of

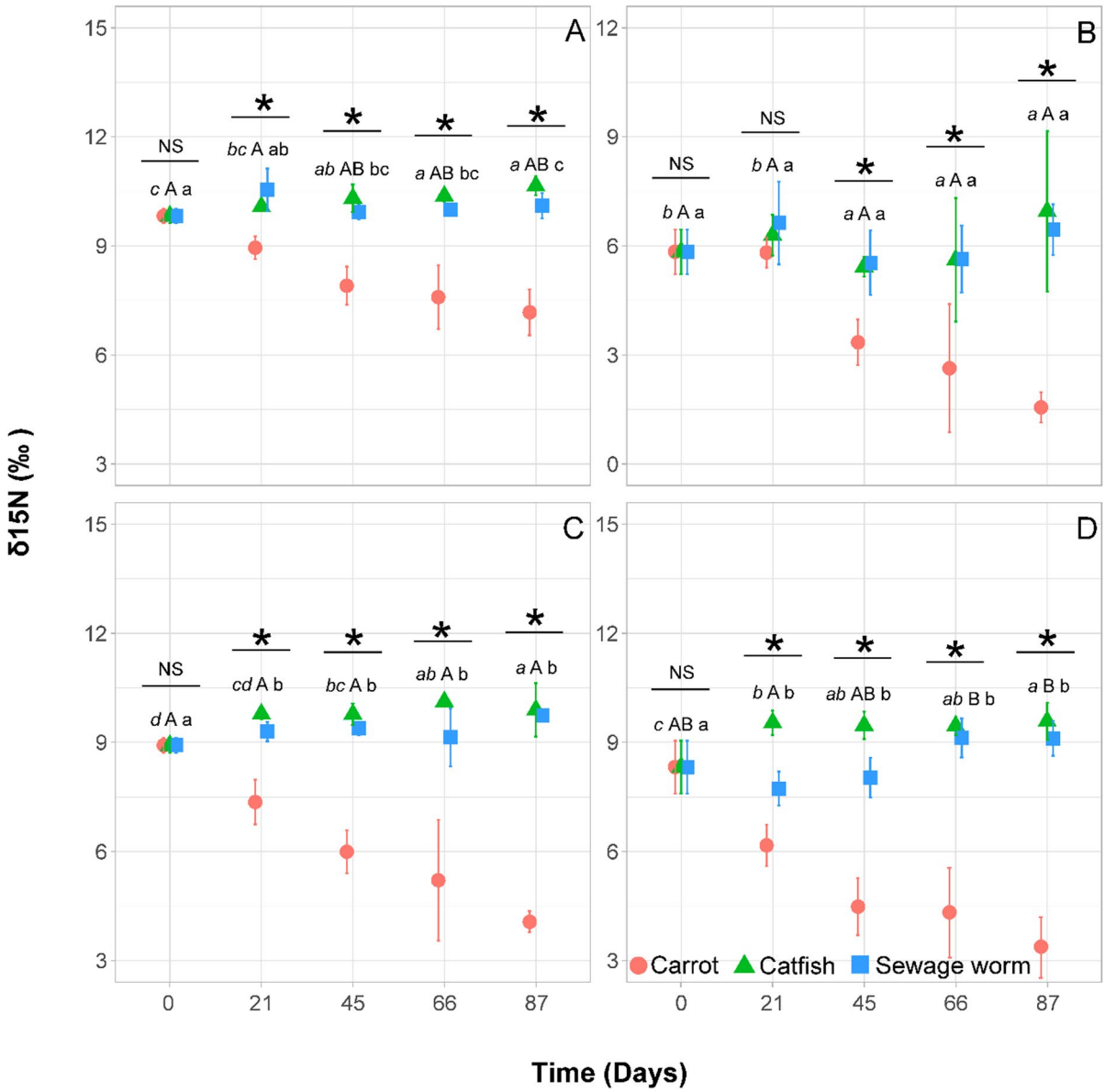

Fig. 3 Mean ± SD of nitrogen ($\delta^{15}N$) of crayfish tissues over time. Different letters denote significant ($P < 0.05$) differences between time at a given diet (carrot = small letters in italic, catfish = capital letters, sewage worm = small letter) and tissue. Significant differences between diet within each time are marked by asterisks, non-significant ones by 'NS'. **A** Muscle, **B** carapace, **C** gill, **D** hepatopancreases

TDFs, reflecting the taxonomic group and tissue type as well as the diet of the studied consumers (Healy et al., 2017). While previous studies reported specific TDFs for given species (fish, mammals), there is no consensus regarding which TDFs should be used for crayfish. Nonetheless, many previous studies have used the values of Vander Zanden & Rasmussen (2001). However, such an approach might be misleading, strongly affecting results due to species-specific TDF dependency. The versatility of observed TDFs and their variability among species examined arguably substantiates this need, especially when abiotic

factors (such as temperature, salinity, etc.) might have differential effects on carbon and nitrogen stable isotope fractionation in a given environment.

Although crayfish are considered omnivorous (Momot, 1995), dietary differences can be observed among species, with some species preferring plant sources, while others tend to be more carnivorous (Veselý et al., 2021). For example, for marbled crayfish and spiny-cheek crayfish, both considerably herbivorous, the outputs of model comparison revealed the TDFs suggested by Glon et al. (2016) as the most suitable. In contrast, the TDFs reported by Vander Zanden & Rasmussen (2001) were most suitable for the noble crayfish, which relies more on high protein foods (Ackefors et al., 1992).Our results also suggested the latitude as a factor affecting TDFs. Yang et al. (2009, 2011) found differences in deuterium TDFs in high latitude terrestrial plants. Similarly, Sternberg & Ellsworth (2011) suggested that differences in oxygen TDFs cannot be explained by temperature variation but rather biochemical fractionalization. However, we are not aware of any study examining TDFs for animals across latitude. It should be noted, however, that we have a limited number of sites to support our evidence, and that such differences can be caused by missing food sources or spatial variability in the isotope values of the sources (Syvaranta et al., 2006) or strong seasonality in high latitudes (Vincent et al., 2008). The current version of the Bayesian mixing model is robust and accounts for the uncertainty associated with multiple sources, fractionation factors, and isotope signatures, which decrease biases in data interpretation (Moore & Semmens, 2008; Stock & Semmens, 2016). Thus, it is likely that another factor, such as latitude, which affects temperature and light regime at a given locality, may play a role.

Conclusions

In this study, we demonstrated that the accuracy of TDFs for crayfish strongly depends on the tissue, species, and site. Further studies on crayfish species from other genera and families under strictly controlled conditions are needed to better determine the most appropriate TDFs. More broadly, we proposed a new approach to identify specific TDFs for a given consumer. Our statistical approach cannot define new TDFs but it can be a guideline to find suitable TDFs for specific consumers. While we are aware that even such a methodological approach might have some limitations, we feel that this is currently the best solution for robust analysis of stable isotope data. We, therefore, recommend the application of our approach to other taxa and ecosystems to develop a broader understanding of TDFs.

Acknowledgements We thank Mikko Kiljunen for helping with sample preparation and analysis. This study was supported by the Czech Science Foundation (No. 19-04431S).

Author contributions LV designed the experiment and conducted statistical analysis. AK, MB, and LV conducted the experiment. LV, FE and TR analyzed samples. LV, PB, and PH wrote the first draft. MG did English proofreading and provide instruction about Hesslein model usage. All authors provided comments and additional text revisions.

Funding Open access publishing supported by the National Technical Library in Prague. This study was funded by Grantová Agentura České Republiky (Grant No. 19-04431S).

Data availability If the manuscript will be accepted for publication, data will be stored in ISOBANK which is a specialized online storage repository for isotopic data.

Declarations

Conflict of interest There is no conflicts of interest.

References

Ackefors, H., J. D. Castell, L. D. Boston, P. Räty & M. Svensson, 1992. Standard experimental diets for crustacean nutrition research. II. Growth and survival of juvenile crayfish *Astacus astacus* (Linné) fed diets containing various amounts of protein, carbohydrate and lipid. Aquaculture 104(3–4): 341–356.

Arts, M. T., M. T. Brett & M. Kainz, 2009. Lipids in Aquatic Ecosystems, Springer Science & Business Media, New York.

Auerswald, K., M. H. Wittmer, A. Zazzo, R. Schäufele & H. Schnyder, 2010. Biases in the analysis of stable isotope discrimination in food webs. Journal of Applied Ecology 47(4): 936–941.

Barnes, C., C. J. Sweeting, S. Jennings, J. T. Barry & N. V. Polunin, 2007. Effect of temperature and ration size on carbon and nitrogen stable isotope trophic fractionation. Functional Ecology 21(2): 356–362.

Bastos, R. F., F. Corrêa, K. O. Winemiller & A. M. Garcia, 2017. Are you what you eat? Effects of trophic discrimination factors on estimates of food assimilation and trophic position with a new estimation method. Ecological Indicators 75(2017): 234–241.

Boecklen, W. J., C. T. Yarnes, B. A. Cook & A. C. James, 2011. On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics 42: 411–440.

Bond, A. L. & A. W. Diamond, 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecological Applications 21(4): 1017–1023.

Bondar, C. A., K. Bottriell, K. Zeron & J. S. Richardson, 2005. Does trophic position of the omnivorous signal crayfish (*Pacifastacus leniusculus*) in a stream food web vary with life history stage or density? Canadian Journal of Fisheries and Aquatic Sciences 62(11): 2632–2639.

Brauns, M., I. G. Boëchat, A. P. C. de Carvalho, D. Graeber, B. Gücker, T. Mehner & D. von Schiller, 2018. Consumer-resource stoichiometry as a predictor of trophic discrimination ($\Delta^{13}C$, $\Delta^{15}N$) in aquatic invertebrates. Freshwater Biology 63(10): 1240–1249.

Canseco, J., E. Niklitschek & C. Harrod, 2021. Variability in $\delta^{13}C$ and $\delta^{15}N$ trophic discrimination factors for teleost fishes: a meta-analysis of temperature and dietary effects. Reviews in Fish Biology and Fisheries. https://doi.org/10.1007/s11160-021-09689-1.

Carolan, J. V., D. Mazumder, C. Dimovski, R. Diocares & J. Twining, 2012. Biokinetics and discrimination factors for $\delta^{13}C$ and $\delta^{15}N$ in the omnivorous freshwater crustacean, *Cherax destructor*. Marine and Freshwater Research 63(10): 878–886.

Caut, S., E. Angulo & F. Courchamp, 2009. Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology 46(2): 443–453.

Martinez del Rio, C. and Wolf, B.O. (2005) Mass-Balance Models for Animal Isotopic Ecology. In: Matthias Starck, J., and Wang, T., Eds., Physiological and Ecological Adaptations to Feeding in Vertebrates, Science Publishers, Inc., Enfield, Chapter 6, 141–174.

Ercoli, F., T. J. Ruokonen, H. Hämäläinen & R. I. Jones, 2014. Does the introduced signal crayfish occupy an equivalent trophic niche to the lost native noble crayfish in boreal lakes? Biological Invasions 16(10): 2025–2036.

Fry, B., 2007. Stable Isotope Ecology, Springer, New York.

Fry, B. & C. Arnold, 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia 54(2): 200–204.

Glon, M., E. R. Larson & K. Pangle, 2016. Comparison of ^{13}C and ^{15}N discrimination factors and turnover rates between congeneric crayfish *Orconectes rusticus* and *O. virilis* (Decapoda, Cambaridae). Hydrobiologia 768(1): 51–61.

Grey, J. & M. Jackson, 2012. 'Leaves and eats shoots': direct terrestrial feeding can supplement invasive red swamp crayfish in times of need. PLoS ONE 7(8): e42575.

Haubert, D., R. Langel, S. Scheu & L. Ruess, 2005. Effects of food quality, starvation and life stage on stable isotope fractionation in Collembola. Pedobiologia 49(3): 229–237.

Healy, K., S. B. Kelly, T. Guillerme, R. Inger, S. Bearhop & A. L. Jackson, 2017. Predicting trophic discrimination factor using Bayesian inference and phylogenetic, ecological and physiological data. DEsIR: Discrimination Estimation in R. PeerJ Preprints.

Hesslein, R. H., K. Hallard & P. Ramlal, 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Canadian Journal of Fisheries and Aquatic Sciences 50(10): 2071–2076.

Jussila, J., T. Ruokonen, J. Syväranta, H. Kokko, A. Vainikka, J. Makkonen & R. Kortet, 2015. It takes time to see the menu from the body: an experiment on stable isotope composition in freshwater crayfishes. Knowledge and Management of Aquatic Ecosystems. https://doi.org/10.1051/kmae/2015021.

Kouba, A., A. Petrusek & P. Kozák, 2014. Continental-wide distribution of crayfish species in Europe: update and maps. Knowledge and Management of Aquatic Ecosystems. https://doi.org/10.1051/kmae/2014007.

Kouba, A., M. Buřič, T. Policar & P. Kozák, 2011. Evaluation of body appendage injuries to juvenile signal crayfish (*Pacifastacus leniusculus*): relationships and consequences. Knowledge & Management Aquatic Ecosystem 401(2011): 04.

Kouba, A., B. Lipták, J. Kubec, M. Bláha, L. Veselý, P. J. Haubrock, F. J. Oficialdegui, H. Niksirat, J. Patoka & M. Buřič, 2021. Survival, growth, and reproduction: comparison of marbled crayfish with four prominent crayfish invaders. Biology 10(5): 422.

Kozák, P., Z. Ďuriš, A. Petrusek, M. Buřič, I. Horká, A. Kouba, E. Kozubíková-Balcarová, T. Policar & K. Němečková, 2015. Crayfish Biology and Culture. University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Water. České Budějovice, Czech Republic.

Lipták, B., L. Veselý, F. Ercoli, M. Bláha, M. Buřič, T. Ruokonen & A. Kouba, 2019. Trophic role of marbled crayfish in a lentic freshwater ecosystem. Aquatic Invasions 14(2): 299–309.

Lipták, B., A. Kouba, J. Patoka, M. Paunović & P. Prokop, 2023. Biological invasions and invasive species in freshwaters: perception of the general public. Human Dimensions of Wildlife. 29(1), 48–63. https://doi.org/10.1080/10871209.2023.2177779.

Madigan, D. J., S. Y. Litvin, B. N. Popp, A. B. Carlisle, C. J. Farwell & B. A. Block, 2012. Tissue turnover rates and isotopic trophic discrimination factors in the endothermic

teleost, Pacific bluefin tuna (*Thunnus orientalis*). PLoS ONE 7(11): e49220.
Malpica-Cruz, L., S. Z. Herzka, O. Sosa-Nishizaki & J. P. Lazo, 2012. Tissue-specific isotope trophic discrimination factors and turnover rates in a marine elasmobranch: empirical and modeling results. Canadian Journal of Fisheries and Aquatic Sciences 69(3): 551–564.
Martínez del Rio, C., N. Wolf, S. A. Carleton & L. Z. Gannes, 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews 84(1): 91–111.
Marufu, L. T., T. Dalu, C. Phiri, M. Barson, R. Simango, B. Utete & T. Nhiwatiwa, 2018. The diet of an invasive crayfish, *Cherax quadricarinatus* (Von Martens, 1868), in Lake Kariba, inferred using stomach content and stable isotope analyses.
Mazumder, D., M. P. Johansen, B. Fry & E. Davis, 2018. Muscle and carapace tissue–diet isotope discrimination factors for the freshwater crayfish Cherax destructor. Marine and Freshwater Research 69(1): 56–65.
McCutchan, J. H., Jr., W. M. Lewis Jr., C. Kendall & C. C. McGrath, 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102(2): 378–390.
Momot, W. T., 1995. Redefining the role of crayfish in aquatic ecosystems. Reviews in Fisheries Science 3(1): 33–63.
Moore, J. W. & B. X. Semmens, 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11(5): 470–480.
Müller-Navarra, D. C., M. T. Brett, A. M. Liston & C. R. Goldman, 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403(6765): 74–77.
Nielsen, J. M., E. L. Clare, B. Hayden, M. T. Brett & P. Kratina, 2018. Diet tracing in ecology: method comparison and selection. Methods in Ecology and Evolution 9(2): 278–291.
Nilsson, E., C. Solomon, K. A. Wilson, T. V. Willis, B. Larget & M. Vander Zanden, 2012. Effects of an invasive crayfish on trophic relationships in north-temperate lake food webs. Freshwater Biology 57(1): 10–23.
Perga, M. E. & J. Grey, 2010. Laboratory measures of isotope discrimination factors: comments on Caut, Angulo & Courchamp (2008, 2009). Journal of Applied Ecology 47(4): 942–947.
Post, D. M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83(3): 703–718.
Rudnick, D. & V. Resh, 2005. Stable isotopes, mesocosms and gut content analysis demonstrate trophic differences in two invasive decapod crustacea. Freshwater Biology 50(8): 1323–1336.
Smith, J. A., D. Mazumder, I. M. Suthers & M. D. Taylor, 2013. To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. Methods in Ecology and Evolution 4(7): 612–618.
Stenroth, P., N. Holmqvist, P. Nyström, O. Berglund, P. Larsson & W. Granéli, 2006. Stable isotopes as an indicator of diet in omnivorous crayfish (*Pacifastacus leniusculus*): the influence of tissue, sample treatment, and season. Canadian Journal of Fisheries and Aquatic Sciences 63(4): 821–831.
Sternberg, L. & P. F. V. Ellsworth, 2011. Divergent biochemical fractionation, not convergent temperature, explains cellulose oxygen isotope enrichment across latitudes. PLoS ONE 6(11): e28040.
Stock, B. C. & B. X. Semmens, 2016. Unifying error structures in commonly used biotracer mixing models. Ecology 97(10): 2562–2569.
Stock, B. C., A. L. Jackson, E. J. Ward, A. C. Parnell, D. L. Phillips & B. X. Semmens, 2018. Analyzing mixing systems using a new generation of Bayesian tracer mixing models. PeerJ 6: e5096.
Suring, E. & S. R. Wing, 2009. Isotopic turnover rate and fractionation in multiple tissues of red rock lobster (*Jasus edwardsii*) and blue cod (*Parapercis colias*): consequences for ecological studies. Journal of Experimental Marine Biology and Ecology 370(1–2): 56–63.
Syvaranta, J., H. Hamalainen & R. I. Jones, 2006. Within-lake variability in carbon and nitrogen stable isotope signatures. Freshwater Biology 51(6): 1090–1102.
Tieszen, L. L., T. W. Boutton, K. G. Tesdahl & N. A. Slade, 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57(1–2): 32–37.
Twardochleb, L. A., J. D. Olden & E. R. Larson, 2013. A global meta-analysis of the ecological impacts of nonnative crayfish. Freshwater Science 32(4): 1367–1382.
Vander Zanden, M. & J. B. Rasmussen, 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography 46(8): 2061–2066.
Vander Zanden, M. J., M. K. Clayton, E. K. Moody, C. T. Solomon & B. C. Weidel, 2015. Stable isotope turnover and half-life in animal tissues: a literature synthesis. PLoS ONE 10(1): e0116182.
Veselý L., V. Hrbek, P. Kozák, M. Buřič, R. Sousa, & A. Kouba, 2017. Salinity tolerance of marbled crayfish *Procambarus fallax* f. *virginalis*. Knowledge & Management of Aquatic Ecosystems. 418: 21. https://doi.org/10.1051/kmae/2017014
Veselý, L., F. Ercoli, T. J. Ruokonen, M. Bláha, J. Kubec, M. Buřič, H. Hämäläinen & A. Kouba, 2020. The crayfish distribution, feeding plasticity, seasonal isotopic variation and trophic role across ontogeny and habitat in a canyon-shaped reservoir. Aquatic Ecology 54(4): 1169–1183.
Veselý, L., T. J. Ruokonen, A. Weiperth, J. Kubec, B. Szajbert, W. Guo, F. Ercoli, M. Bláha, M. Buřič, H. Hämäläinen & A. Kouba, 2021. Trophic niches of three sympatric invasive crayfish of EU concern. Hydrobiologia 848(3): 727–737.
Vincent, W. F., J. E. Hobbie & J. Laybourn-Parry, 2008. Introduction to the limnology of high-latitude lake and river ecosystems. In Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems. 1–24.
Viozzi, M. F., C. Martínez del Rio & V. Williner, 2021. Tissue-specific isotopic incorporation turnover rates and trophic discrimination factors in the freshwater shrimp *Macrobrachium borellii* (Crustacea: Decapoda: Palaemonidae). Zoological Studies 60: 32.

Whitledge, G. W. & C. F. Rabeni, 1997. Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. Canadian Journal of Fisheries and Aquatic Sciences 54(11): 2555–2563.

Yang, H., M. Pagani, D. E. Briggs, M. Equiza, R. Jagels, Q. Leng & B. A. LePage, 2009. Carbon and hydrogen isotope fractionation under continuous light: implications for paleoenvironmental interpretations of the high Arctic during Paleogene warming. Oecologia 160(3): 461–470.

Yang, H., W. Liu, Q. Leng, M. T. Hren & M. Pagani, 2011. Variation in n-alkane δD values from terrestrial plants at high latitude: implications for paleoclimate reconstruction. Organic Geochemistry 42(3): 283–288.

Yokoyama, H., A. Tamaki, K. Harada, K. Shimoda, K. Koyama & Y. Ishihi, 2005. Variability of diet-tissue isotopic fractionation in estuarine macrobenthos. Marine Ecology Progress Series 296: 115–128.