Journal of Great Lakes Research 41 (2015) 926–929

Contents lists available at ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

Notes

Effects of gut content on $\delta^{15}N$, $\delta^{13}C$ and C:N of the macroinvertebrate *Mysis diluviana*

Peter T. Euclide [1], Jason D. Stockwell *

University of Vermont, Rubenstein Ecosystem Science Laboratory, 3 College Street, Burlington, VT 05401, USA

ARTICLE INFO

Article history:
Received 11 December 2014
Accepted 22 April 2015
Available online 10 June 2015

Communicated by Michael Sierszen

Index words:
Gut evacuation
Stable isotopes
Mysis diluviana

ABSTRACT

Stable isotopes have become a popular method for studying aquatic food webs. Lack of laboratory studies and standardized methods, however, may limit the application and interpretation of stable isotope analysis. One procedure applied inconsistently among stable isotope studies for whole body and bulk invertebrate samples is gut evacuation after capture. We evaluated the importance of gut evacuation prior to stable isotope analysis in the omnivorous macroinvertebrate *Mysis diluviana*. *Mysis* ranging from 9 to 20 mm were fed a diet of *Artemia* for 58 days in the laboratory. Following this acclimation period, *Mysis* with empty guts, full guts of *Artemia*, and full guts of green algae (*Scenedesmus obliquus*) were processed for stable isotope analysis of $\delta^{15}N$, $\delta^{13}C$ and C:N. We found no significant difference among gut content treatments for any of these metrics. We conclude that gut evacuation is an unnecessary step in stable isotope analysis of *Mysis* in the size range we investigated.

Introduction

Over the past 30 years, stable isotope analysis (SIA) has been applied increasingly by ecologists as a tool to study food web dynamics (Mittermayr et al., 2014; Stockwell et al., 2014), trophic structure (Post, 2002), food source partitioning (Phillips and Gregg, 2003) and migration (Fleming et al., 1993; Fry, 1981; Hobson, 1999). As application of SIA has increased, many researchers have called for increased laboratory experiments to test analysis assumptions (del Rio et al., 2009), verify field observations (del Rio et al., 2009; Gannes et al., 1997), and standardize sample preparation (Smyntek et al., 2007). Isotope fractionation can vary extensively within a single species (Grey et al., 2004) and among tissue types (Pinnegar and Polunin, 1999; Tieszen et al., 1983), adding variation to analytical results (Peterson and Fry, 1987). Thus, experimentally testing turnover rates, tissue specific isotope composition and isotope assimilation are important steps to reduce variability (Gannes et al., 1997). Sample preparation techniques such as acidification, lipid extraction, and gut evacuation have been used to lower variability of observed isotope composition with inconsistent success (Feuchtmayr and Grey, 2003; Post et al., 2007). However, the variable use of procedures among studies makes comparisons of data and meta-analyses difficult (Smyntek et al., 2007).

Gut evacuation prior to SIA processing is one procedure that has produced conflicting results in the literature. When the sample organism is small and whole body samples are used, rather than tissue samples, one concern is that gut content at the time of capture might bias stable isotope values. Thus, gut evacuation procedures have been applied to account for this possible bias and have often been applied to macroinvertebrate and zooplankton SIA (France, 1995; Grey et al., 2001). To allow passage of gut contents prior to SIA analyses, individuals are incubated in filtered water for an extended period of time (usually 24 h; Schmidt et al. (2003)). Past studies, however, show mixed results. Feuchtmayr and Grey (2003) found that gut evacuation significantly changed isotope composition of bulk copepod samples, reducing error on estimates of $\delta^{15}N$ and $\delta^{13}C$ by >3.0‰, while gut clearance was unimportant for *Daphnia* and reduced error by <1.0‰. Smyntek et al. (2007) found no difference between bulk samples of zooplankton with and without evacuated guts. The simplicity of gut evacuation methods has led to continual recommendation of gut evacuation procedures even by researchers who found evidence suggesting that gut contents have no effect on observed isotope composition (Feuchtmayr and Grey, 2003; Smyntek et al., 2007). In studies where organisms die during capture or where immediate processing is necessary, however, gut evacuation can be a difficult or unfeasible step. Thus determining if gut content affects observed stable isotope composition is important for standardizing SIA procedures.

We conducted a laboratory feeding experiment to test the null hypothesis that gut contents do not affect the observed stable isotope composition of whole organism samples of *Mysis diluviana* (*Mysis*). *Mysis* is an aquatic macroinvertebrate common to deep oligotrophic North American lakes (Carpenter et al., 1974). *Mysis* feeds omnivorously in both benthic and pelagic regions of lakes (Johannsson et al., 2001). The diet and behavior of *Mysis* spp. have been studied with SIA techniques previously, but sample preparation varies among studies (Gorokhova and Hansson, 1999; Johannsson et al., 2001; Ogonowski

* Corresponding author. Tel.: +1 802 859 3095.
E-mail addresses: peter.euclide@uvm.edu (P.T. Euclide), jason.stockwell@uvm.edu (J.D. Stockwell).
[1] Tel.: +1 802 859 3095.

http://dx.doi.org/10.1016/j.jglr.2015.05.002
0380-1330/

et al., 2013; Sierszen et al., 2011). Testing if gut contents affect the isotope composition of *Mysis* is an essential step to standardize preparation techniques for SIA and to interpret food web relationships.

Methods

Live *Mysis* were collected from Lake Champlain (44° 16′ 972″ N, 073° 19′ 448″ W) at night on 20 November 2013 using a benthic sled with a 1000-μm mesh net and 250-μm mesh cod end. *Mysis* were transported live to the Rubenstein Ecosystem Science Laboratory in Burlington, VT, in coolers filled with unfiltered, chilled lake water. Within 12 h of capture *Mysis* were divided into five separate 38-l tanks filled with aerated filtered lake water and placed in a Conviron CMP 4030 environmental chamber set to 4 °C in constant dark. The initial number of *Mysis* per tank was estimated at >200 individuals per tank except tank 3 which had 40 individuals. Any remaining *Mysis* (~500 individuals) were left in the cooler, and placed in a walk-in refrigerator outfitted with red lights at 4 °C. The plan was to use these *Mysis* as replacement individuals for dead *Mysis* in experimental tanks. However, *Mysis* in all tanks experienced high mortality (40–95%) regardless of density during the acclimation period. Rather than replace dead individuals with *Mysis* from the cooler, we used the cooler as an additional replicate allowing for statistical assessment of bias due to differences in environmental conditions between the environmental chamber and the refrigerator.

To standardize tissue isotope composition, *Mysis* were fed a daily ration of 30–40 ml filtered and rinsed live *Artemia* grown at a density of 1.0 g of cysts/l for 58 days leading up to the experiment. *Artemia* has been shown to be a sufficient food for *Mysis* spp. in captivity (Gorokhova, 1998). To test whether isotope composition of *Mysis* had completely turned over at the time of the experiment, we sampled 2 pre-experimental *Mysis* from each tank on day 41 of the 58-day acclimation period. These individuals were processed immediately to provide a two-point comparison of isotope composition to evaluate isotope turnover rates in *Mysis* (Gorokhova and Hansson, 1999; Johannsson et al., 2001). The only exception was tank 3, where high mortality precluded sampling of pre-experimental and experimental *Mysis*. Each day, any dead *Mysis* were siphoned from each of the five tanks in the environmental chamber and the single cooler in the refrigerator and the number of deaths per tank was recorded. Partial water changes for each tank were made every 3–6 days for the duration of the study.

To assess the effects of gut content on individual *Mysis* SIA results we measured the isotope composition of *Mysis* from three different gut content treatments: guts full of *Artemia*, guts full of green algae, and *Mysis* with fully evacuated guts. Treatments were applied to tanks consecutively over a 3-day period following the 58-day acclimation period. Because dietary changes are reflected in tissue isotope composition on the order of months (Gorokhova and Hansson, 1999), we assumed that carryover effects of our treatment procedure over three consecutive days were negligible. We randomly sampled 1 to 2 *Mysis* ≥10 mm from tanks 1 and 2 (due to high mortality) and 6 to 10 *Mysis* ≥10 mm from tanks 4 and 5, and the cooler. No *Mysis* from tank 3 were available because of high mortality. Sampled *Mysis* were examined and photographed using an Olympus SZS9 dissecting scope and Infinity1 camera to ensure all samples met treatment requirements of full or empty guts. Photographed individuals were measured to the nearest millimeter from the tip of the rostrum to the base of the telson using Image-Pro Insight, version 8.0 (MediaCybernetics), and then frozen at −20 °C in individual 2-ml test tubes. On Day 1 of the treatments, *Mysis* were collected with *Artemia*-filled guts using the above procedure. Remaining *Mysis* in each tank were then netted and moved, without mixing *Mysis* among tanks, to five new tanks containing fresh de-chlorinated water at 4 °C and starved for 24 h to allow time for complete gut evacuation (Chipps, 1998). On Day 2, *Mysis* with evacuated guts from original tanks 1, 2, 4 and 5 and the cooler were sampled using the same procedure as above. Following the sampling of *Mysis* with evacuated guts, remaining *Mysis* from tanks 1, 2, 4 and 5 and the cooler were fed green algae (*Scenedesmus obliquus*), left to feed *ad libitum* for 24 h, and then sampled on Day 3 using the same procedure as above. Bulk samples of *Artemia* and green algae were collected into 2 ml test tubes and dried for analysis of diet source at the time of the experiment.

Within two months of the conclusion of the experiment, *Mysis* were thawed for processing; several pleopods were removed from all selected individuals and re-frozen for genetic analysis for a different study. The remaining bodies along with samples of green algae and *Artemia* were dried in a drying oven at 60 °C for 72 h. Whole dried individual *Mysis* and three laboratory replicates of each source food were milled using a mortar and pestle into a fine powder, then 0.71–0.89 mg of each individual *Mysis* and bulk *Artemia* tissue and 2.4–2.5 mg of green algae were added to 3.5 × 5 mm tin capsules (Costech Analytical Technologies) for SIA.

Samples were analyzed for relative abundance of stable carbon and nitrogen isotopes at the University of Wyoming Stable Isotope Facility using continuous flow settings on a Costech 4010 Elemental Analyzer coupled to a Thermo Delta Plus XP IRMS. Results are expressed in parts per thousand (‰) and denoted as δ calculated according to the equation:

$$\delta X = \left[\left(\frac{R_{Sample}}{R_{standard}}\right) - 1\right] * 1000$$

where X is ^{15}N or ^{13}C, R is the corresponding ratio ^{15}N:^{14}N or ^{13}C:^{12}C, and the standard was Vienna Pee Dee belemnite (VPDB) for carbon and atmospheric N_2 for nitrogen. For quality control, 36-UWSIF-Glutamic 1, 39-UWSIF-Glutamic 2, and UWSIF01 Liver were used for measurements of standard quality assurance (n = 2 per 24 samples). The standard deviations for $\delta^{13}C$ and $\delta^{15}N$ of 36-UWSIF-Glutamic 1 and 39-UWSIF-Glutamic 2 were ±0.2 and ±0.05‰, and ±0.1 and ±0.1‰, respectively, and the standard deviations of UWSIF01 Liver were ±0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$.

We used two-way analysis of variance (ANOVA) and Chi-Square tests to identify potential differences in length and fecundity among treatment groups and tanks. Differences among *Artemia*-filled-, empty, and green algae-filled gut treatments were tested using ANOVA and an indicator variable test with gut content as the principal variable and length and tank as covariates. All statistical analyses were conducted in R version 3.1.0 (R core team, 2014) with {FSA} package (Ogle and D.H., NA. FSA: Fisheries Stock Analysis. R package version 0.4.17.).

Results

Mysis ranged from 9 to 20 mm, with a mean length (±SD) of 15.0 ± 2.71 mm. Approximately half (47%) of the *Mysis* analyzed were fecund (Table 1). Length and proportion fecundity did not vary among different gut content treatments (ANOVA $p > 0.05$ and Chi-Square $p > 0.05$ respectively). Additionally, experimental tank had no effect on $\delta^{15}N$, $\delta^{13}C$ or C:N ($p > 0.10$ for all), indicating that any difference in experimental environment or sampling among gut contents and tanks did not impact isotope composition.

Pre-experimental *Mysis* from day 41 of the acclimation period had lower $\delta^{13}C$ than experimental *Mysis* following the 58-day acclimation period ($F_{1,28} = 8.096$; $p < 0.01$), but similar $\delta^{15}N$ ($F_{1,28} = 0.06$; $p = 0.81$) and C:N ($F_{1,28} = 0.51$; $p = 0.48$). The difference in $\delta^{13}C$ and $\delta^{15}N$ between *Artemia* and *Mysis* with evacuated guts was −5.54‰ and 0.45‰, respectively. The fractionation of $\delta^{13}C$ with trophic level is <1‰ in most organisms (Peterson and Fry, 1987) while $\delta^{15}N$ fractionation in *Mysis mixta* is between 2.7 and 3.6‰ for (Gorokhova and Hansson, 1999). Therefore, we would expect fully turned over $\delta^{13}C$ of *Mysis* to be approximately equal to *Artemia* $\delta^{13}C$ and $\delta^{15}N$ to be >2.7‰ higher in *Mysis* than *Artemia* suggesting that neither isotope had completely turned over in *Mysis* at the time of the experiment.

Table 1
Mean (±SD) length (mm), $\delta^{15}N$, $\delta^{13}C$, and C:N of experimental *Mysis* and percent of individuals sampled that were fecund. Sample names relate to different sample groups of *Mysis* or collected source material.

Sample	n	Length (mm)	% Fecund	$\delta^{15}N$	$\delta^{13}C$	C:N
Pre-exp. *Mysis*	10	16.3 ± 2.21	0.50	13.2 ± 0.47	−28.9 ± 1.43	4.5 ± 0.55
Starved *Mysis*	23	14.6 ± 2.50	0.39	13.7 ± 0.72	−27.4 ± 1.15	4.3 ± 0.35
Algae *Mysis*	25	15.2 ± 2.81	0.52	13.5 ± 0.61	−27.5 ± 1.36	4.5 ± 0.39
Artemia Mysis	21	15.2 ± 2.86	0.52	13.4 ± 0.53	−27.3 ± 1.21	4.3 ± 0.33
Green Algae	3	X	X	−8.2 ± 0.06	−21.5 ± 0.06	21.2 ± 0.21
Artemia	3	X	X	13.3 ± 0.06	−21.8 ± 0.06	4.4 ± 0.06

All but three individuals used for stable isotope analysis were visually verified for the presence of gut contents, confirming that we were comparing individuals with full and empty guts (Fig. 1). Both $\delta^{15}N$ and $\delta^{13}C$ had a negative relationship with length ($r^2 = 0.16$, $F_{3,65} = 2.95$, $p = 0.01$ and $r^2 = 0.71$, $F_{3,65} = 158.54$, $p < 0.01$ respectively) while C:N showed no relationship with length ($r^2 = 0.08$, $F_{3,65} = 1.99$, $p = 0.15$; Fig. 2). Gut content had no effect on the $\delta^{15}N$, $\delta^{13}C$ or C:N composition of *Mysis* ($F_{2,65} = 1.35$, 1.08, and 2.52 respectively; $p > 0.05$ for all; Fig. 2).

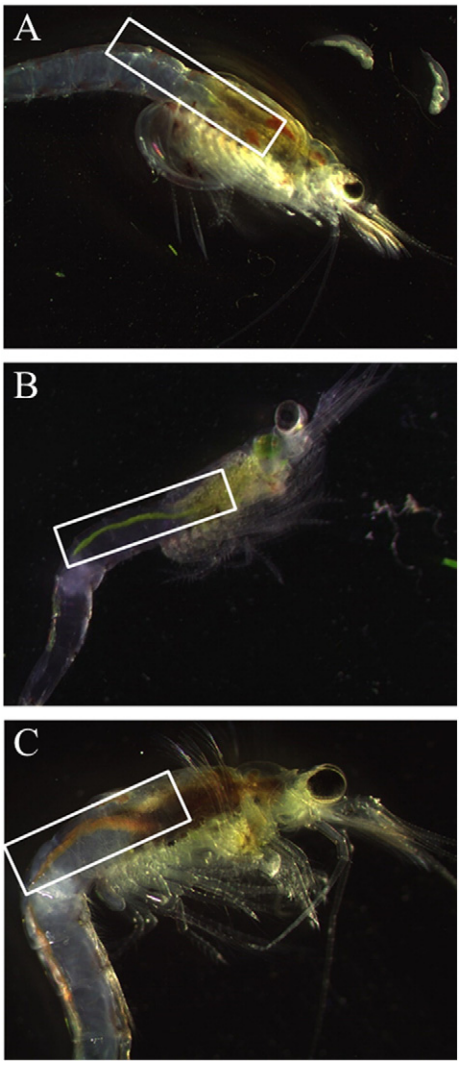

Fig. 1. Images of *Mysis* with guts empty (A), filled with green algae (B), and filled with *Artemia* (C). Guts are outlined with white boxes. Photographs were taken between 6.3 and 30.0× magnification with an Infinity1 camera mounted on an Olympus SZS9 dissecting microscope.

Discussion

Gut contents did not affect the observed isotope composition of 9–20 mm *Mysis* in our experiment. Therefore, we conclude that gut evacuation prior to processing samples for SIA is an unnecessary step to determine isotopic composition of field-caught *Mysis* in this size range. Our results are consistent with observations of other zooplankton where gut evacuation did not alter observed isotope composition of bulk samples (Feuchtmayr and Grey, 2003; Smyntek et al., 2007). Feuchtmayr and Grey (2003) had conflicting results where gut evacuation significantly changed isotope composition in copepods but not in *Daphnia*. They suggested that the ratio of gut mass to body mass ("relative gut mass") may impact the importance of gut content to observed isotope composition and explain why gut content may have altered

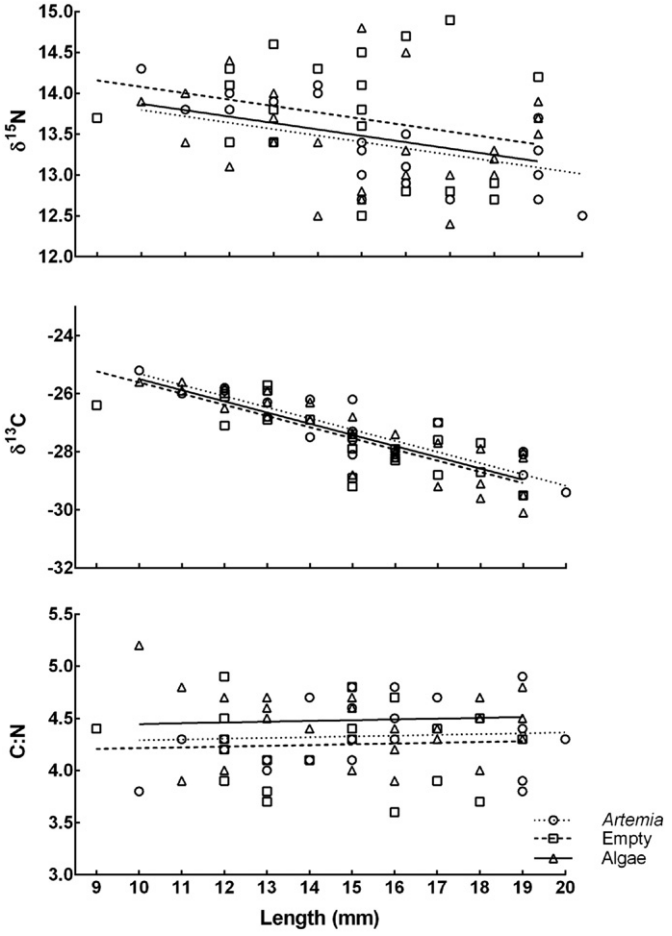

Fig. 2. Indicator variable regressions of $\delta^{13}C$, $\delta^{15}N$, and C:N as a function of body length for experimental *Mysis* with three different gut contents: green algae (Algae), *Artemia*, and nothing (Empty).

observed isotope composition of copepods (relative gut mass of ~20%) but not *Daphnia* (~5%; Feuchtmayr and Grey, 2003). Relative gut mass can be estimated from the gut passage time and feeding rate (Feuchtmayr and Grey, 2003). Using gut passage time (3.1 h) and feeding rates (0.12 g g^{-1} day^{-1}) of *Mysis* taken from Chipps (1998), we estimate the relative gut mass of a 5-mg *Mysis* (dry mass) to be between 1.0 and 1.7%, consistent with the hypothesis that gut content of organisms with relatively low gut mass should have little effect on observed isotope composition.

Both $\delta^{15}N$ and $\delta^{13}C$ of *Mysis* had a negative relationship with length. Typically, $\delta^{15}N$ increases and $\delta^{13}C$ decreases with *Mysis* size as relative carnivory increases (Branstrator et al., 2000; Johannsson et al., 2001). Because all *Mysis* in our study were fed a uniform diet of *Artemia* for 58 days, we expected *Mysis* to be at an equal trophic level regardless of size as tissue turnover rate of $\delta^{15}N$ has been estimated at 1 to 2 months (Gorokhova and Hansson, 1999; Johannsson et al., 2001). The turnover rate of $\delta^{13}C$ in *Mysis* is unknown, as complete turnover was undetectable in *Mysis* after 67 days (Johannsson et al., 2001). Comparisons of isotope composition between experimental *Mysis*, and both pre-experimental *Mysis* and *Artemia*, suggest that both $\delta^{15}N$ and $\delta^{13}C$ were still approaching equilibrium at the time of the experiment. Because *Mysis* were kept at a consistent 4 °C, turnover rates could have been slowed, lengthening the required time for total turnover of $\delta^{15}N$. Additionally, we observed high mortality rates in experimental tanks. If *Mysis* were stressed and suffering high mortality, they may not have eaten or grown as quickly as expected which would further decrease turnover rates. Because *Mysis* did not reach equilibrium with their food at the time of the experiment, we suggest that the negative relationship of $\delta^{15}N$ and $\delta^{13}C$ with length is the result of faster growth and turnover rates in smaller individuals compared to larger ones.

We conclude that gut evacuation is an unnecessary step in sample preparation for SIA of *Mysis* and can be excluded from future studies. Additionally, past studies using inconsistent gut evacuation practices should be comparable in this respect. Because our results contradict the findings of similar studies on other species, the importance of gut evacuation is likely species-specific. Consequently, our results should be applied to other species with caution.

Controlled laboratory studies of SIA have commonly been stated as an important step for the progress of isotope research (del Rio et al., 2009; Gannes et al., 1997). Though we have answered questions about gut evacuation procedures for *Mysis*, more controlled experiments testing effects of other potential sources of error in SIA such as lipid content, tissue type and turnover rate, and the interspecific variation of these sources, remain an important and fertile area of stable isotope research.

Acknowledgments

We extend our appreciation to Mike Sierszen, Sture Hansson, and two anonymous reviewers for their helpful comments on earlier drafts of the manuscript. We also thank Captain Steve Cluett and Deckhand Suz Ball of the R/V *Melosira* for their field expertise, Rachael DeWitt and Jake Calvitti for their help collecting *Mysis*, and Ellen Marsden for her assistance in the laboratory.

References

Branstrator, D., Cabana, G., Mazumder, A., Rasmussen, J., 2000. Measuring life-history omnivory in the opossum shrimp, *Mysis relicta*, with stable nitrogen isotopes. Limnol. Oceanogr. 45, 463–467.

Carpenter, G., Mansey, E., Watson, N., 1974. Abundance and life history of *Mysis relicta* in St. Lawrence. J. Fish. Res. Board Can. 31, 319–325.

Chipps, S., 1998. Temperature-dependent consumption and gut-residence time in the opossum shrimp *Mysis relicta*. J. Plankton Res. 20, 2401–2411. http://dx.doi.org/10.1093/plankt/20.12.2401.

Del Rio, C.M., Wolf, N., Carleton, S.A., Gannes, L.Z., 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol. Rev. Camb. Philos. Soc. 84, 91–111. http://dx.doi.org/10.1111/j.1469-185X.2008.00064.x.

Feuchtmayr, H., Grey, J., 2003. Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. Rapid Commun. Mass Spectrom. 2605–2610 http://dx.doi.org/10.1002/rcm.1227 (RCM 17).

Fleming, T., Nuñez, R., Sternberg, L., 1993. Seasonal changes in the diets of migrant and non-migrant nectarivorous bats as revealed by carbon stable isotope analysis. Oecologia 94, 72–75.

France, R., 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol. Oceanogr. 40, 1310–1313.

Fry, B., 1981. Natural stable carbon isotope tag traces Texas shrimp migrations. Fish. Bull. 79, 337–345.

Gannes, L.Z., O'Brien, D., Rio, C., 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78, 1271–1276.

Gorokhova, E., 1998. Exploring and modeling the growth dynamics of *Mysis mixta*. Ecol. Model. 110, 45–54.

Gorokhova, E., Hansson, S., 1999. An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. Can. J. Fish. Aquat. Sci. 56, 2203–2210. http://dx.doi.org/10.1139/cjfas-56-11-2203.

Grey, J., Jones, R., Sleep, D., 2001. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. Limnol. Oceanogr. 46, 505–513.

Grey, J., Kelly, A., Jones, R., 2004. High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. Limnol. Oceanogr. 49, 239–244.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120, 314–326. http://dx.doi.org/10.1007/s004420050865.

Johannsson, O.E., Leggett, M.F., Rudstam, L.G., Servos, M.R., Mohammadian, A.M., Gal, G., Dermott, R.M., Hesslein, R.H., 2001. Diet of *Mysis relicta* in Lake Ontario as revealed by stable isotope and gut content analysis. Can. J. Fish. Aquat. Sci. 58, 1975–1986. http://dx.doi.org/10.1139/cjfas-58-10-1975.

Mittermayr, A., Hansen, T., Sommer, U., 2014. Simultaneous analysis of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ ratios uncovers food web relationships and the trophic importance of epiphytes in an eelgrass *Zostera marina* community. Mar. Ecol. Prog. Ser. 497, 93–103. http://dx.doi.org/10.3354/meps10569.

Ogle, D.H., NA. FSA: Fisheries Stock Analysis. R package version 0.4.17.

Ogonowski, M., Duberg, J., Hansson, S., Gorokhova, E., 2013. Behavioral, ecological and genetic differentiation in an open environment—a study of a *Mysid* population in the Baltic Sea. PLoS ONE 8, e57210. http://dx.doi.org/10.1371/journal.pone.0057210.

Peterson, B., Fry, B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18, 293–320.

Phillips, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136, 261–269. http://dx.doi.org/10.1007/s00442-003-1218-3.

Pinnegar, J., Polunin, N., 1999. Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. Funct. Ecol. 225–231.

Post, D., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montaña, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179–189. http://dx.doi.org/10.1007/s00442-006-0630-x.

R: A Language and Environment for Statistical Computing. R Foundation For Statistical Computing, Vienna, Austria URL http://www.R-project.org/.

Schmidt, K., Atkinson, A., Stubing, D., McClelland, J., Montoya, J., Voss, M., 2003. Trophic relationships among Southern Ocean copepods and krill: some uses and limitations of a stable isotope approach. Limnol. Oceanogr. 48, 277–289.

Sierszen, M., Kelly, J., Corry, T., Scharold, J., Yurista, P., 2011. Benthic and pelagic contributions to *Mysis* nutrition across Lake Superior. Can. J. Fish. Aquat. Sci. 68, 1051–1063. http://dx.doi.org/10.1139/F2011-033.

Smyntek, P.M., Teece, M.A., Schulz, K.L., Thackeray, S.J., 2007. A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models. Limnol. Oceanogr. 52, 2135–2146. http://dx.doi.org/10.4319/lo.2007.52.5.2135.

Stockwell, J.D., Yule, D.L., Hrabik, T.R., Sierszen, M.E., Isaac, E.J., 2014. Habitat coupling in a large lake system: delivery of an energy subsidy by an offshore planktivore to the nearshore zone of Lake Superior. Freshw. Biol. 97, 1197–1212. http://dx.doi.org/10.1111/fwb.12340.

Tieszen, L., Boutton, T., Tesdahl, K., Slade, N., 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57, 32–37.