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Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur

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Use of stable isotope ratios to trace pathways of organic matter among consumers requires knowledge of the isotopic shift between diet and consumer. Variation in trophic shift among consumers can be substantial. For data from the published literature and supplementary original data (excluding fluid-feeding consumers), the mean isotopic shift for C was $+0.5 \pm 0.13$‰ rather than 0.0‰, as commonly assumed. The shift for C was higher for consumers analyzed as muscle ($+1.3 \pm 0.30$‰) than for consumers analyzed whole ($+0.3 \pm 0.14$‰). Among consumers analyzed whole, the trophic shift for C was lower for consumers acidified prior to analysis (-0.2 ± 0.21‰) than for unacidified samples ($+0.5 \pm 0.17$‰). For N, trophic shift was lower for consumers raised on invertebrate diets ($+1.4 \pm 0.21$‰) than for consumers raised on other high-protein diets ($+3.3 \pm 0.26$‰) and was intermediate for consumers raised on plant and algal diets ($+2.2 \pm 0.30$‰). The trophic shift for S differed between high-protein ($+2.0 \pm 0.65$‰) and low-protein diets (-0.5 ± 0.56‰). Thus, methods of analysis and dietary differences can affect trophic shift for consumers; the utility of stable isotope methods can be improved if this information is incorporated into studies of trophic relationships. Although few studies of stable isotope ratios have considered variation in the trophic shift, such variation is important because small errors in estimates of trophic shift can result in large errors in estimates of the contribution of sources to consumers or in estimates of trophic position.

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Stable isotope ratios of carbon (C), nitrogen (N), and sulfur (S) have been used extensively to trace pathways of organic matter among consumers (Peterson and Howarth 1987, Hesslein et al. 1992), and are reported here in standard δ-notation with units of ‰ (ratios for C, N, and S are reported as $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively; Peterson and Fry 1987). Early laboratory studies showed that for C, isotope ratios of consumers usually are similar to isotope ratios of their diets (DeNiro and Epstein 1978), but that consumers typically are enriched in ^{15}N relative to their diets (DeNiro and Epstein 1981a, Minagawa and Wada 1984). For the early studies by DeNiro and Epstein (1978, 1981a), the mean trophic shift for C ($\Delta\delta^{13}C$; Δ denotes the change in isotope ratio between diet and consumer) was about +1‰, and the mean $\Delta\delta^{15}N$ was about +3‰. Although there have been few controlled studies of trophic shift for S, it has been assumed that the shift for S is negligible (Peterson and Howarth 1987, Hesslein et al. 1992). Hydrogen (H) isotope ratios have not been used routinely to study trophic relationships, but have been used to study migratory patterns of birds (Hobson

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and Wassenaar 1997). Although the shift in H isotope ratio between diet and consumer is small relative to variation in the environment (Estep and Dabrowski 1980, Macko et al. 1983), H isotope ratios of consumers are affected by factors other than diet (DeNiro and Epstein 1981b).

For cases in which there are two potential food sources, each with a distinct isotope ratio, estimates of the proportionate contribution of C from each food source to the growth of a consumer have been based on a two-source mixing model, as follows:

$$k_{carbon} = 1 - \frac{\delta^{13}C_A - \delta^{13}C_{consumer} + \Delta\delta^{13}C}{\delta^{13}C_A - \delta^{13}C_B} \quad (1)$$

where $\delta^{13}C_A$ and $\delta^{13}C_B$ are the isotope ratios of potential food sources, $\delta^{13}C_{consumer}$ is the isotope ratio of the consumer, $\Delta\delta^{13}C$ is the trophic shift for C (typically assumed to be 0.0‰), and k is the proportionate contribution of source A to the growth of the consumer. Estimates of $k_{nitrogen}$ or k_{sulfur}, the proportionate contribution of N or S in one of two potential food sources, are calculated similarly. Where there are three potential food sources, proportionate contributions have been estimated following the assumptions of a three-source mixing model (Peterson and Howarth 1987, Phillips 2001).

Proportionate contributions of food sources have been estimated from $\delta^{15}N$, but more commonly the large trophic shift for N has been used to estimate trophic position (λ), as follows:

$$\lambda = \left(\frac{\delta^{15}N_{consumer} - \delta^{15}N_{base}}{\Delta\delta^{15}N}\right) + 2 \quad (2)$$

where $\delta^{15}N_{base}$ is the nitrogen isotope ratio of the base of the food chain (i.e. primary producers). Also, $\lambda = 1$ for primary producers, $\lambda = 2$ for strict herbivores and, for trophic positions above 2, non-integer values of λ reflect feeding at more than one trophic level (as in Vander Zanden et al. 1997).

It has been widely accepted that the average values for $\Delta\delta^{13}C$ and $\Delta\delta^{34}S$ are near zero and that the average value of $\Delta\delta^{15}N$ is near $+3$‰ (Peterson and Fry 1987). Estimates of trophic shift for individual consumers, however, are quite variable. Uncertainty in $\Delta\delta^{13}C$ or $\Delta\delta^{34}S$ can cause errors in estimates of partitioning of food sources, and uncertainty in $\Delta\delta^{15}N$ can contribute to error in estimates of sources of N or in estimates of trophic position (McCutchan 1999, Vander Zanden and Rasmussen 2001). Thus, if trophic shift is not well quantified, the reliability of stable isotope methods for tracing trophic pathways of C, N, and S may be low. Better estimates of trophic shift will improve both the accuracy and precision of estimates of trophic dependence and trophic position from stable isotope ratios. Furthermore, if variation in trophic shift is not considered, conclusions drawn from studies of stable isotope studies may not reflect uncertainty in estimates of food sources or trophic position. The purposes of this paper are to use data from the published literature and selected new data on trophic shifts for C, N, and S isotopes by consumers raised under controlled conditions 1) to test previous assumptions regarding trophic shifts for C, N, and S, and 2) to demonstrate the effect of errors in estimates of trophic shift on estimates of the proportionate contribution of sources to consumers or trophic position of consumers.

Basis for the isotopic shift between diet and consumer

Ratios of stable isotopes can change between diet and consumer due to differential digestion or fractionation during assimilation and metabolic processes. Metabolic fractionation also may cause isotope ratios of different tissues to vary substantially within individual consumers (DeNiro and Epstein 1981a, Hobson and Clark 1992). Additionally, classes of compounds (e.g. lipids) from a single consumer may differ considerably in stable isotope ratio from other compounds (Focken and Becker 1998). Consequently, trophic shift can vary according to the tissue or compound chosen for isotope analysis and also because species and individuals differ in their biochemical composition. C, N, and S differ considerably in shift of ratios from diet to consumer and in the heterogeneity of ratios among tissues or classes of compounds within organisms.

Carbon – Plants often contain a high proportion of complex carbohydrates, such as cellulose and lignin, that are poorly digested and differ in isotope ratio from the rest of the diet. Thus, differential digestion of plants by consumers may cause a shift in $\delta^{13}C$ from diet to consumer. For example, because lignin tends to be depleted in ^{13}C relative to bulk organic matter from plants (Benner et al. 1987, Wedin et al. 1995), $\Delta\delta^{13}C$ should be greater among animals raised on vascular plant diets than among animals raised on other diets. Also, the trophic shift for C should be greater for omnivores, which consume some vascular plants or plant detritus, than for carnivores.

Food assimilated by consumers contributes to growth through anabolic pathways or is lost through respiration or excretion. Over the lifetime of a consumer, a large fraction of assimilated material is ultimately lost through respiration and excretion (Humphreys 1979). Because respiratory CO_2 is isotopically lighter than assimilated carbon (DeNiro and Epstein 1978, Checkley and Entzeroth 1985), animals should be slightly enriched in ^{13}C relative to their diets, and trophic shift should be greatest among animals with the highest rates of respiration relative to growth.

Given that relative respiration generally is higher for homeotherms than for poikilotherms (Humphreys 1979), $\Delta\delta^{13}C$ should be higher for birds and mammals than for fish and invertebrates. Also, because urea and uric acid contain carbon but ammonia does not, the form of excreted waste may affect trophic shift, especially if rates of excretion are high, but this possibility has been little studied.

Lipids usually are depleted in ^{13}C relative to protein and carbohydrates by fractionation during the oxidation of pyruvate to acetyl coenzyme A (DeNiro and Epstein 1977, Focken and Becker 1998). Thus, $\delta^{13}C$ is likely to be lower for samples with high lipid content than for samples with low lipid content. $\delta^{13}C$ should be lower for animals analyzed whole than for animals analyzed as muscle tissue, which is low in lipid. For samples analyzed as muscle tissue, $\delta^{13}C$ should be lower for untreated samples than for samples treated by solvent extraction (Focken and Becker 1998) to remove lipids.

Among terrestrial animals, carbonates appear to be derived largely from respiratory CO_2 and usually are slightly depleted in ^{13}C relative to dietary C; for aquatic consumers, carbonate in shell or bone is derived from dissolved inorganic carbon from the environment and is generally enriched in ^{13}C relative to the diet (McConnaughey et al. 1997). Acidification of samples to remove carbonates should result in lower estimates of $\Delta\delta^{13}C$ for whole organisms, especially for aquatic consumers, but analytical variability can be higher when samples are acidified (Bunn et al. 1995, Bosley and Wainright 1999).

Nitrogen – Excreted nitrogen typically is depleted in ^{15}N relative to a consumer's diet (DeNiro and Epstein 1981a) or tissues (Checkley and Miller 1989). Thus, the trophic shift for N should be greatest among animals raised on diets having the highest N content (i.e. the highest rates of N excretion relative to assimilation of N). Because the ratio of excreted to assimilated N is infinitely high during periods of starvation, $\Delta\delta^{15}N$ also is high for starved animals (Scrimgeour et al. 1995). The form of nitrogenous waste (ammonia, urea, uric acid) excreted by a consumer may affect trophic shift for N, but the current understanding of the mechanisms that regulate $\Delta\delta^{15}N$ is insufficient to make specific predictions of the effect of the form of nitrogenous waste on $\Delta\delta^{15}N$.

Sulfur – Although there is little or no fractionation associated with the incorporation of S-containing amino acids into animal tissue, S isotope fractionation may be considerable for the oxidation of organic S or for other processes (Mekhtiyeva et al. 1976). Organic S in animal tissue is derived from organic S in the diet, but inorganic S from the environment also contributes to the total S pool of an animal and to its sulfur isotope ratio. Isotope ratios of organic and inorganic S within individual plants may differ by more than 5‰ (Mekhtiyeva et al. 1976). Thus, consumers feeding on diets rich in inorganic S or consumers with large pools of inorganic S may have S isotope ratios different from their diets.

Change in diet – Isotope ratios of C, N, or S for consumers may change gradually in response to changes in diet. For example, Fry and Arnold (1982) found that shrimp approached isotopic equilibrium with a new diet only after their mass had quadrupled. Because the rate of turnover for some tissues is very slow, estimates of trophic shift from diet-switching studies may be influenced by the isotope ratio of the initial diet even after a consumer has been maintained for a long period of time on the same diet.

Methods

Values of trophic shift for C, N, and S were compiled from published studies of consumers raised on controlled diets. Estimates of trophic shift were obtained only for consumers raised on constant diets, or, if diets were switched, for consumers that more than quadrupled in mass between the dietary switch and isotopic analysis. All data are for consumers analyzed whole or as muscle tissue. Because consumers raised on composite diets (e.g. a mixture of detritus and zooplankton) may consume one food item selectively, only data for consumers raised on a single type of food are considered here. In a similar manner, samples of muscle may not represent the actual diet of parasites feeding on body fluids (Pinnegar et al. 2001) and samples of bulk plant material may not represent the actual diets of insects that feed on plant fluids (e.g. aphids); data for fluid-feeding consumers are included in order to test for the possibility that trophic shift differs between fluid-feeders and other consumers. Data available only in graphical form were converted to numerical form with the image analysis program NIH Image (http://rsb.info.nih.gov/nih-image/). Average analytical precision for the studies from the literature was similar to precision for the data produced by new studies, as described below.

We also provide new estimates of trophic shift for seven species; these data were collected to provide estimates of trophic shift for taxa studied by the authors and by M. Camara. Five species of consumers were fed ad libitum on controlled diets in the laboratory. Buckeye butterflies (*Junonea coenia*) were fed either prepared food (Camara 1997), canker-root (*Kickxia elatine*), or English plantain (*Plantago lanceolata*). Rainbow trout (*Onchorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) were raised on commercial trout food; trout were fed once or twice daily and uneaten food was removed after 15–20 minutes. Aphids (*Periphyllus* sp.) and scale bugs (Diaspididae)

Table 1. Trophic shift (mean ± SE) for consumers raised in this study. (P) indicates pooled sample; * indicates a statistical outlier that was excluded from means presented in the text and in Table 2 and 3.

Consumer	Diet	Trophic shift, mean ± SE (n)		
		$\Delta\delta^{13}C$	$\Delta\delta^{15}N$	$\Delta\delta^{34}S$
Buckeye adults	Artificial diet	−0.7 ± 0.02 (3)	+3.1 ± 0.64 (3)	+1.8 ± 0.36 (9)
Buckeye adults	Canker-root	+0.4 ± 0.57 (3)	+2.6 ± 1.58 (3)	−0.7 ± 0.57 (10)
Buckeye adults	Plantago	−1.8 ± 0.24 (3)	+5.4 ± 1.33 (3)	+6.9 ± 1.19 (8)*
Buckeye pupae	Canker-root	−2.1 ± 0.24 (4)	+0.9 ± 0.41 (4)	−1.5 ± 0.32 (8)
Buckeye pupae	Plantago	−2.7 ± 0.22 (4)	+3.6 ± 0.48 (4)	+7.3 ± 0.67 (10)*
Tent caterpillar	Choke cherry	+0.4 ± 0.26 (8)	+0.8 ± 0.43 (8)	−0.4 ± 0.02 (13)
Tiger moth caterpillar	Cottonwood	+0.2 (P)	+1.4 (P)	−3.2 (P)
Aphids	Thai dragon	+0.6 (P)	−0.8 (P)	−0.5 (P)
Scale bugs	Thai dragon	+1.0 (P)	−2.1 (P)	–
Rainbow trout	Trout chow	+1.9 ± 0.51 (4)	+3.2 ± 0.20 (4)	+4.0 ± 0.09 (6)
Brook trout	Trout chow	+3.3 ± 0.29 (8)	+3.8 ± 0.17 (8)	+1.6 ± 0.36 (8)

were raised on a single Thai dragon pepper plant (*Capsicum frutescens*). Tent caterpillars (*Malacosoma* sp.) were collected from a single chokecherry (*Prunus melanocarpus*) and tiger moth caterpillars (Arctiidae) were collected from a single cottonwood tree (*Populus deltoides*); the trees were assumed to be the sole sources of food for the caterpillars. Consumers to be analyzed were held without food for 24 h to allow for gut clearance, rinsed with deionized water, and freeze dried. Samples of muscle tissue were taken from trout, freeze dried, and ground. Other consumers and their freeze-dried foods were ground whole and analyzed for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ on a Micromass Optima isotope ratio mass spectrometer, which was operated in conjunction with a Carlo Erba elemental analyzer. Standard errors for replicate analytical standards averaged 0.05‰.

For data from the literature and the supplementary data, the Student's t-test was used to test for the effects of differences in diet, consumer, environment, and method of analysis on trophic shift; the Tukey-Kramer HSD (Honestly significant difference) procedure was used to test for differences among consumers raised on plant diets, invertebrate diets, and other high-protein diets. To test the effect of change in diet on trophic shift for C, estimates for consumers raised on constant diets were compared with those switched from enriched to more depleted diets or from depleted to more enriched diets by the Tukey-Kramer HSD procedure. Linear regression was used to test for a relationship between $\Delta\delta^{34}S$ and $\Delta\delta^{15}N$. All statistical analyses were performed with JMP Version 5 (SAS Institute Inc., Cary, NC, USA) on a Macintosh computer.

Results

New estimates of trophic shift (Table 1) were within the range of other published values (Appendix 1), except that some values for $\Delta\delta^{34}S$ were more extreme than previously reported values. $\delta^{13}C$ for consumers typically was slightly above dietary $\delta^{13}C$, but negative values of $\Delta\delta^{13}C$ were not uncommon and some values of $\Delta\delta^{13}C$ were greater than +3‰ (Fig. 1). For all available data, $\Delta\delta^{13}C$ averaged +0.4 ± 0.12‰ (mean ± SE). Scale bugs, aphids, and a small number of consumers from published studies were depleted in ^{15}N relative to their diets but, for most consumers, $\Delta\delta^{15}N$ was between 0 and +4‰ (Fig. 1). $\Delta\delta^{15}N$ averaged +2.0 ± 0.20‰ (mean ± SE); one estimate of $\Delta\delta^{15}N$ for *Artemia* (DeNiro and Epstein 1981a) was significantly higher than other estimates (Student's t-test; $t = 4.40$, $p < 0.001$) and was not included in the mean. $\delta^{34}S$ of most consumers was within 2‰ of their diets; about half of the consumers were depleted in ^{34}S relative to their diets and $\Delta\delta^{34}S$ for some consumers approached +4‰ (Fig. 1). $\Delta\delta^{34}S$ averaged +0.4 ± 0.52‰ (mean ± SE); estimates of $\Delta\delta^{34}S$ for *Junonea coenia* raised on English plantain were significantly higher than other estimates (Student's t-test; $t = 4.69$, $p < 0.001$) and were not included in the mean.

Because samples of muscle or bulk tissue may not represent the actual diets of fluid-feeding consumers (e.g. aphids or internal parasites), estimates of trophic

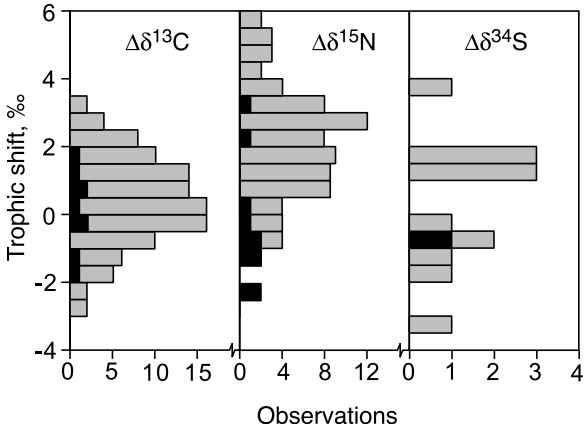

Fig. 1. Histograms for estimates of trophic shift for C, N, and S. Fluid-feeding consumers are indicated by dark bars. Statistical outliers (see Table 1 and Appendix 1) are not included.

shift were compared between fluid-feeding consumers and other consumers. There was no significant difference in $\Delta\delta^{13}C$ or $\Delta\delta^{34}S$ between fluid-feeding consumers and other consumers, but $\Delta\delta^{15}N$ was lower for fluid feeding consumers than for other consumers (Table 2, Fig. 1). For this reason, estimates of trophic shift for fluid-feeding consumers from this study and from the literature are not included in the comparisons presented in Table 3 (below). Excluding the data for fluid-feeding consumers, $\Delta\delta^{13}C$ averaged $+0.5 \pm 0.13$‰ (mean $\pm$ SE). $\Delta\delta^{15}N$ averaged $+2.3 \pm 0.18$‰ (mean $\pm$ SE), and $\Delta\delta^{34}S$ averaged $+0.5 \pm 0.56$‰ (mean $\pm$ SE). Mean values of trophic shift for C and N were significantly greater than zero (Student's t-test; for C, $t = 3.54$, $p < 0.001$; for N, $t = 12.5$, $p < 0.001$) but the mean for $\Delta\delta^{34}S$ was not ($t = 0.88$, $p = 0.20$).

For studies in which multiple consumers were raised on a given diet and animals were analyzed individually, the ratio of the mean variance within groups (i.e. consumers of the same species raised on a single diet) to the variance of group means was 0.21 for $\Delta\delta^{13}C$, 0.25 for $\Delta\delta^{15}N$, and 0.04 for $\Delta\delta^{34}S$. Thus, variation in trophic shift among individuals of a given species raised on a given diet and under similar conditions is small compared to variation among means for diets or consumer species.

Carbon – Estimates of $\Delta\delta^{13}C$ for consumers other than fluid-feeding consumers ranged from -2.7‰ to $+3.4$‰, but $\Delta\delta^{13}C$ did not vary significantly by type of diet or consumer (Table 3). Trophic shift for C did vary, however, based on methods of sample preparation (Table 3). $\Delta\delta^{13}C$ was higher for consumers ana-

Table 2. Mean ($\pm$ SE) estimates of trophic shift for C, N, and S using all available data. Results of the Student's t-test are given for each comparison. Statistically significant differences ($p < 0.05$) are indicated by *.

	$\Delta\delta^{13}C$		$\Delta\delta^{15}N$		$\Delta\delta^{34}S$	
Consumer	Trophic shift	t-test	Trophic shift	t-test	Trophic shift	t-test
All animals	$+0.4 \pm 0.12$ (111)		$+2.0 \pm 0.20$ (83)		$+0.4 \pm 0.52$ (13)	
Feeding mode						
Fluid-feeding	$+0.2 \pm 0.37$ (9)	$t = 0.58$;	-0.4 ± 0.57 (10)	$t = 4.92$;	-0.5 (1)	$t = 0.51$;
Other consumers	$+0.5 \pm 0.13$ (102)	$p = 0.56$	$+2.2 \pm 0.18$ (73)	$p < 0.001$*	$+0.5 \pm 0.56$ (12)	$p = 0.62$

Table 3. Mean ($\pm$ SE) estimates of trophic shift for C, N, and S; estimates for fluid-feeding consumers are excluded. Results of the Student's t-test are given for each comparison. Statistically significant differences ($p < 0.05$) are indicated by *. High-protein diets include animal and microbial diets; low-protein diets include plant and algal diets.

	$\Delta\delta^{13}C$		$\Delta\delta^{15}N$		$\Delta\delta^{34}S$	
Consumer	Trophic shift	t-test	Trophic shift	t-test	Trophic shift	t-test
All animals	$+0.5 \pm 0.13$ (102)		$+2.3 \pm 0.18$ (73)		$+0.5 \pm 0.56$ (12)	
Diet type						
Vascular plants	$+0.4 \pm 0.28$ (34)	$t = 0.39$;	$+2.4 \pm 0.42$ (19)	$t = 0.34$;	-0.9 ± 0.61 (6)	$t = 3.83$;
All other diets	$+0.5 \pm 0.14$ (68)	$p = 0.70$	$+2.2 \pm 0.20$ (54)	$p = 0.73$	$+1.9 \pm 0.42$ (6)	$p = 0.003$*
Protein content						
High	$+0.6 \pm 0.16$ (44)	$t = 1.10$;	$+2.4 \pm 0.22$ (38)	$t = 0.61$;	$+1.9 \pm 0.51$ (5)	$t = 2.80$;
Low	$+0.5 \pm 0.19$ (58)	$p = 0.27$	$+2.2 \pm 0.30$ (35)	$p = 054$	-0.5 ± 0.65 (7)	$p = 0.019$*
Metabolism						
Poikilotherms	$+0.4 \pm 0.14$ (91)	$t = 1.13$;	$+2.3 \pm 0.20$ (65)	$t = 0.45$;	$+0.5 \pm 0.56$ (12)	–
Homeotherms	$+0.9 \pm 0.37$ (11)	$p = 0.26$	$+2.0 \pm 0.38$ (8)	$p = 0.66$	–	
Nitrogenous waste						
Ammonia	$+0.4 \pm 0.18$ (49)	$t = 0.71$;	$+2.3 \pm 0.28$ (32)	$t = 0.14$;	$+1.9 \pm 0.51$ (5)	$t = 2.80$;
Urea/uric acid	$+0.5 \pm 0.19$ (53)	$p = 0.48$	$+2.3 \pm 0.24$ (41)	$p = 0.89$	-0.5 ± 0.65 (7)	$p = 0.019$*
Environment						
Aquatic	$+0.4 \pm 0.17$ (50)	$t = 0.58$; $p = 0.56$	$+2.3 \pm 0.28$ (33)	$t = 0.12$; $p = 0.90$	$+1.9 \pm 0.51$ (5)	$t = 2.80$; $p = 0.019$*
Terrestrial	$+0.5 \pm 0.19$ (52)		$+2.3 \pm 0.24$ (40)		-0.5 ± 0.65 (7)	
Analysis						
Whole organism	$+0.3 \pm 0.14$ (84)	$t = 2.93$;	$+2.1 \pm 0.21$ (58)	$t = 1.92$;	-0.5 ± 0.65 (7)	$t = 2.80$;
Muscle	$+1.3 \pm 0.30$ (18)	$p = 0.004$*	$+2.9 \pm 0.32$ (15)	$p = 0.090$	$+1.9 \pm 0.51$ (5)	$p = 0.019$*
Lipid removal (muscle)						
Lipid removed	$+1.8 \pm 0.29$ (5)	$t = 1.17$;	$+3.2 \pm 0.43$ (3)	$t = 0.46$;	–	–
No treatment	$+1.1 \pm 0.35$ (13)	$p = 0.26$	$+2.8 \pm 0.40$ (12)	$p = 065$	$+1.9 \pm 0.51$ (5)	
Acidification (whole)						
No treatment	$+0.5 \pm 0.17$ (62)	$t = 2.11$;	$+2.4 \pm 0.24$ (36)	$t = 2.82$;	-0.8 ± 0.81 (5)	$t = 0.64$;
Acidified	-0.2 ± 0.21 (22)	$p = 0.038$*	$+1.1 \pm 0.29$ (15)	$p = 0.007$*	$+0.2 \pm 1.25$ (2)	$p = 0.55$

lyzed as muscle tissue than for whole organisms. For samples analyzed as muscle, $\Delta\delta^{13}C$ was higher for samples treated to remove lipids than for untreated samples, but not significantly so. Among consumers analyzed whole, $\Delta\delta^{13}C$ was significantly higher for unacidified samples than for acidified samples.

Nitrogen – For consumers other than fluid-feeding consumers (and excluding the one statistical outlier), $\Delta\delta^{15}N$ averaged $+2.0‰$ and ranged from $-0.8‰$ to $+5.9‰$. Trophic shift for N was higher for carnivores and other consumers with high-protein diets (vertebrates, microbes, and animal-based prepared diets) than for consumers with plant or algal diets (including plant-based prepared diets), but not significantly so. However, $\Delta\delta^{15}N$ was significantly lower for consumers raised on invertebrate diets than for consumers raised on other high-protein diets (Fig. 2). $\Delta\delta^{15}N$ was significantly higher for unacidified samples than for acidified samples but was not affected by any of the other factors considered here (Table 3).

Sulfur – The number of estimates of $\Delta\delta^{34}S$ is small, and variation among these was considerable. Most of the values for $\Delta\delta^{34}S$ were between $-2.5‰$ and $+2.5‰$, but $\Delta\delta^{34}S$ ranged from $-3.2‰$ to $+4.0‰$ and averaged $+0.5‰$ (excluding fluid-feeding consumers and statistical outliers). $\Delta\delta^{34}S$ was significantly higher for consumers raised on diets other than vascular plants and for consumers raised on high-protein diets (Table 3). $\Delta\delta^{34}S$ also was higher for animals that excrete nitrogenous wastes primarily as ammonia, for animals from aquatic environments, and for animals analyzed as muscle.

Change in diet – For C, the mean trophic shift was slightly higher for consumers that were switched from enriched diets to more depleted diets than for consumers raised on constant diets (Fig. 3). Likewise, the mean trophic shift was lower for consumers switched from depleted to more enriched diets than for consumers raised on constant diets. These differences, however, were not significant (Tukey-Kramer HSD; $q^* = 2.38,\ p > 0.05$).

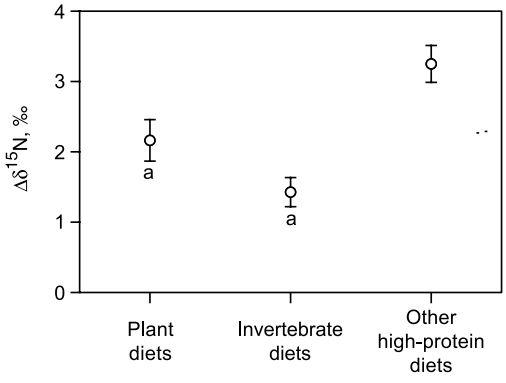

Fig. 2. $\Delta\delta^{15}N$ (mean $\pm$ SE) for three categories of diets. Plant diets include vascular plants, algae, and plant-derived prepared diets; other high-protein diets include vertebrate diets, microbial diets, and animal-derived prepared diets. Means for categories with the same letter are not significantly different (Tukey-Kramer HSD; $q^* = 2.39,\ p < 0.05$).

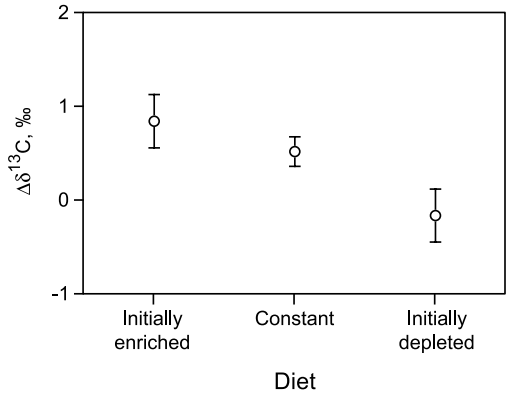

Fig. 3. Mean ($\pm$ SE) estimates of $\Delta\delta^{13}C$ for consumers raised on constant and changing diets.

Discussion

For C, N, and S, much of the variation in trophic shift can be explained by differences in diet or method of sample preparation. Consequently, mean estimates of trophic shift for all consumers generally are not appropriate for use in field studies. Instead, estimates for field studies should reflect what is known about trophic shift among the consumers of interest and how such estimates are affected by the methods used to prepare samples for isotope analysis. Even when the best estimates of trophic shift are applied, remaining uncertainty in trophic shift can affect estimates of the proportionate contribution of food sources or estimates of trophic position.

Although there are relatively few estimates of trophic shift for fluid-feeding consumers, analyses presented here suggest that estimates of trophic shift for nitrogen differ systematically between fluid-feeding consumers and other consumers. Pinnegar et al. (2001) reported that blood may differ isotopically from muscle tissue and plant fluids also may differ isotopically from bulk plant material. Thus, estimates of trophic shift for fluid-feeding consumers probably should be based on isotopic differences between fluids and consumers, rather than on differences between bulk plant material or muscle and consumers.

Carbon – DeNiro and Epstein (1978) reported values of $\Delta\delta^{13}C$ ranging from $-1.5‰$ to $+2.7‰$ and a mean of about $+0.8‰$. In most subsequent stable isotope studies of trophic relationships, the assumption has been made that there is no isotopic shift for carbon or that there is a small increase (usually $+0.8$ to $+1.0‰$)

in $\delta^{13}C$ from one trophic level to the next. Although substantial trophic shift for carbon can occur under special conditions (e.g. for longer lived animals after final weight has been reached), and $\Delta\delta^{13}C$ may be above or below the mean for certain groups of consumers, the common assumptions of no trophic shift for C or a trophic shift of $+0.8$ to $+1$‰ for all consumers are inappropriate. The best estimate of $\Delta\delta^{13}C$ for consumers analyzed whole is $+0.3 \pm 0.14$‰; for consumers analyzed as muscle tissue, the best estimate is $+1.3 \pm 0.30$‰.

Although $\Delta\delta^{13}C$ was similar between homeotherms and poikilotherms and did not vary according to the type of diet, methods of sample preparation significantly affected estimates of $\Delta\delta^{13}C$. Because lipids tend to be depleted in ^{13}C relative to other tissues, estimates of $\Delta\delta^{13}C$ were higher for consumers analyzed as muscle than for consumers analyzed whole. Because small animals typically are ground whole prior to analysis, while muscle tissue usually is analyzed for larger animals, estimates of trophic shift may differ between small invertebrates and large vertebrates due to differences in methods of sample preparation, even if isotope ratios of whole organisms are similar. The results indicate that care must be taken when comparing data for samples prepared in different ways. Studies of diverse groups of organisms are possible, however, if assumptions about $\Delta\delta^{13}C$ are based on the method of sample preparation.

If the isotopic difference between two food sources is small and the estimate of $\Delta\delta^{13}C$ is incorrect, the estimate of the relative importance of the two sources to growing consumers will be biased. For example, if two food sources differing in $\delta^{13}C$ by 4‰ are available to a consumer and the consumer's $\delta^{13}C$ is midway between these values, a two-source mixing model (Eq. (1)) predicts that the consumer is equally dependent on the two end members if there is no trophic shift for carbon. If a shift of $+0.3$‰ is assumed, the estimated importance of the lighter (more negative) end member is 57.5%. If a shift of $+1.1$‰ is assumed, the estimated importance of the lighter end member rises to 77.8%. Thus, where isotopic differences between end members are small, estimates of source apportionment for individual consumers or taxa are very sensitive to differences in assumptions about trophic shift.

Nitrogen – The shift in $\delta^{15}N$ between diet and consumer usually is assumed to be $+2.6$ to $+3.4$‰ (DeNiro and Epstein 1981a, Minagawa and Wada 1984, Owens 1987), but the mean calculated here is $+2.3 \pm 0.18$‰. Although differences in $\Delta\delta^{15}N$ between high-protein and low-protein diets have been reported in previous reviews (McCutchan 1999, Vander Zanden and Rasmussen 2001, Post 2002), a significant difference was not found here. $\Delta\delta^{15}N$ was significantly lower for consumers raised on invertebrate diets ($+1.4 \pm 0.20$‰) than for those raised on other high-protein diets (vertebrates, microbes, and animal-based prepared diets; $+3.3 \pm 0.26$‰) but $\Delta\delta^{15}N$ or consumers raised on plant or algal diets (including plant-based prepared diets; $+2.2 \pm 0.30$) did not differ significantly from consumers raised on invertebrate diets. Because $\Delta\delta^{15}N$ varies between invertebrate diets and other high-protein diets, the mean is influenced by the proportion of animals in the sample population raised on each type of diet. A mean value, if applied to all consumers, would underestimate trophic position for animals feeding on invertebrates and overestimate trophic position for animals feeding on vertebrates (i.e. many top predators). A mean value may be appropriate, however, for detritivores or omnivores that consume mixtures of plant material and microbial or animal material.

The results of this study and of studies reviewed by Owens (1987) show that $\Delta\delta^{15}N$ is higher for some predators than for primary consumers, but recent studies (Webb et al. 1998, Adams and Sterner 2000) found that $\Delta\delta^{15}N$ increased with the C:N ratio of the diet. Although these conclusions appear contradictory, the protein content of diets used in the two recent studies was low compared to diets classified here as high-protein diets. Webb et al. (1998) and Adams and Sterner (2000) both concluded that the high trophic shift for N associated with diets of low quality (i.e. very low protein content) may have resulted from internal recycling of N, which occurs in starving animals (Gannes et al. 1997). Thus, it is possible that $\Delta\delta^{15}N$ is high when dietary N either exceeds or is well below requirements for optimal growth, and that $\Delta\delta^{15}N$ is low when dietary N is near the requirements for optimal growth.

If $\delta^{15}N$ data are used to determine nitrogen sources or trophic position for consumers, the uncertainty associated with $\Delta\delta^{15}N$ (SD = 1.75‰ for plant diets, SD = 0.88 for invertebrate diets, and SD = 1.17 for other predators) cannot be ignored when isotopic differences between the potential sources of nitrogen are small. Because the variance in trophic shift is compounded across each trophic transfer, the absolute uncertainty in estimates of trophic position for individual consumers will be higher for top predators than for consumers feeding at a lower trophic position, even if mean estimates of trophic position for large groups of consumers are quite accurate. Because $\Delta\delta^{15}N$ differs between invertebrate diets and vertebrate diets, accurate estimation of trophic position for top predators depends on knowledge of the types of trophic transfers separating primary producers from top predators. Even if estimates of trophic position are normalized to primary consumers (Vander Zanden and Rasmussen 1999, Post 2002), uncertainty in estimates of trophic position for top predators may be high unless the number of invertebrate versus vertebrate transfers is known.

Sulfur – Previous analyses of sulfur isotope ratios for animals raised on controlled diets (Peterson and Howarth 1987) suggested that $\Delta\delta^{34}S$ generally is small. Even when new estimates are considered, the mean

estimate of trophic shift for S ($+0.5 \pm 0.56$‰) is not significantly different from zero. Estimates of $\Delta\delta^{34}S$ from the current study, however, are more variable than previous estimates. For the entire data set excluding fluid-feeding consumers and statistical outliers, $\Delta\delta^{34}S$ differed by diet, environment, and mode of excretion; these differences in $\Delta\delta^{34}S$ were not predicted. Some of the differences probably are an artifact of small sample size, but the correlation between $\Delta\delta^{15}N$ and $\Delta\delta^{34}S$ (Fig. 4) provides additional evidence that $\Delta\delta^{34}S$ is affected by protein content of the diet. Consequently, $\delta^{34}S$ could be useful for estimating trophic position, but estimates of food sources and estimates of trophic position based on $\delta^{34}S$ will require correction for the difference in trophic shift among different types of diets.

Categories of consumers and diets are not independent of one another. For example, vascular plant diets usually are low in protein and consumers analyzed whole tend to have low-protein diets. Although protein content of the diet is probably an important factor affecting $\Delta\delta^{34}S$, this relationship appears to be nonlinear and it is not possible to determine from this data set whether other factors also are important. Even with the additional estimates presented here, further work will be required to develop a thorough understanding of the factors that control trophic shift for S.

Change in diet – Data from studies of consumers switched from one diet to another suggest that rates of turnover vary among pools of C (Fig. 3), although the effect of changing diets on estimates of $\Delta\delta^{13}C$ was small (< 0.5‰) and not significant. These data also suggest that, for some tissues, it can take longer for a consumer to reach isotopic equilibrium than to quadruple in mass. Comparable data for $\Delta\delta^{15}N$ or $\Delta\delta^{34}S$ do not exist, but it must be assumed that, in general, isotope ratios of consumers do not necessarily reflect their recent diet. The effects of tissue turnover on the isotopic mass balance of consumers can, however, be incorporated into estimates of proportional contribution of food sources (McCutchan and Lewis 2000, 2002, Harvey et al. 2002).

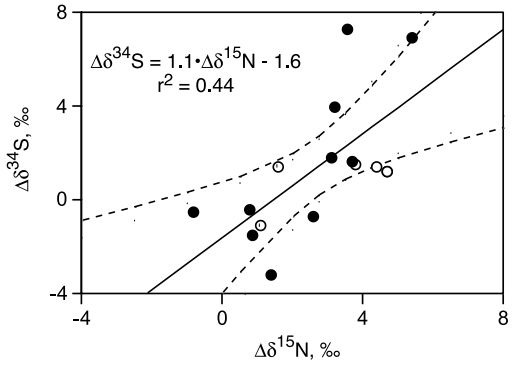

Fig. 4. Relationship between the trophic shift for S and the trophic shift for N. Open circles represent data from previous studies and solid circles represent data for this study.

Conclusions

Stable isotope methods are among the most powerful tools for the study of trophic relationships in aquatic and terrestrial ecosystems and the utility of stable isotope methods has been increased through improved estimates of trophic shift. Although much of the observed variation in trophic shift among consumers is explained by differences in food quality or methods of sample preparation, factors not yet considered also may be important. More information on trophic shift, as suggested by Gannes et al. (1997), may help to determine which other factors are important, especially for S. Even for C and N, there are very few estimates of trophic shift for some groups of consumers (e.g. freshwater invertebrates). To date, this study provides the most comprehensive analysis of the factors that affect trophic shift in controlled diet studies, but additional research will be necessary before stable isotope methods can be used to their full potential.

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Appendix 1. Estimates of trophic shift (mean ± SD) from the literature. Numbers of consumers of each estimate are given in parentheses; where no number is given, estimates are for a single individual or samples were pooled prior to analysis. * indicates a statistical outlier that was excluded from means presented in the text and in Table 2 and 3.

Study	Consumer	Diet	Trophic shift, mean ± SE (n)		
			$\Delta\delta^{13}C$	$\Delta\delta^{15}N$	$\Delta\delta^{34}S$
Adams and Sterner 2000	*Daphnia magna*	Diatoms:			
		molar C:N = 7	+0.9	–	–
		molar C:N = 10	+3.0	–	–
		molar C:N = 11	+1.7	–	–
		molar C:N = 16	+2.8	–	–
		molar C:N = 22	+3.3	–	–
		molar C:N = 25	+5.5*	–	–
Bosley et al. 2002	*Pleuronectes americanus* (winter flounder)	Artemia			
		18°C	−0.3 ± 0.51 (5)	+2.0 ± 0.69 (5)	–
		13°C	+1.7 ± 0.27 (4)	+2.5 ± 0.17 (4)	–
DeNiro and Epstein 1978	*Artemia salina* (brine shrimp)	Algae	+1.3	+9.2	–
DeNiro and Epstein 1981a, b	*Caenorhabditis elegans* (nematode)	Bacteria	−0.8	+2.7	–
	Calliphora sp. (blowfly)	Horse meat	−0.4 ± 0.20 (3)	+1.4 ± 0.08 (3)	–
	Calliphora sp. (blowfly)	Pork	−0.6 ± 0.07 (4)	+1.8 ± 0.30 (3)	–
	Desmia sp. (moth)	Grape leaves	+2.6 ± 0.62 (4)	+4.2 ± 1.54 (3)	–
	Helix aspersa (snail)	Romaine lettuce	+1.1 ± 0.38 (6)	−0.4 ± 0.23 (3)	–
	Melanoplus sanguinipes (grasshopper)	Corn seedlings	+1.6 ± 0.15 (3)	+1.7 ± 0.28 (3)	–
	Melanoplus sanguinipes (grasshopper)	Wheat seedlings	+2.7 ± 0.70 (4)	−0.8 ± 0.17 (3)	–
	Mus musculus (mouse)	Prepared diet	−1.6	+2.8	–
	Musca sp. (housefly)	Horse meat	+0.5 ± 0.39 (4)	+4.6 ± 0.54 (2)	–
	Musca sp. (housefly)	Pork	−0.7 ± 0.07 (4)	+3.4 ± 0.45 (3)	–
	Oncopeltus sp. (milkweed bug)	Milkweed seeds	+0.3 ± 0.49 (4)	+2.8 ± 0.70 (4)	–
	Stitophilus grandarius (weevil)	Wheat seeds	−0.2	+5.9	–
	Stitophilus oryzae (weevil)	Wheat seeds	+1.0	+2.8	–
Dittel et al. 1997	*Penaeus vannamei* (shrimp)	Brine shrimp	+2.5	+0.9	–
	Penaeus vannamei (shrimp)	Zooplankton	+0.4	+2.7	–
Dittel et al. 2000	*Callinectes sapidus* (blue crab)	Brine shrimp	+1.0	+1.5	–
		Snail meat	+0.2	+0.8	–
		Crab meat	−0.1	+0.9	–
		Zooplankton	−0.1	+0.1	–
Doucett et al. 1999	*Nanocladius* sp. (chironomid)	Stonefly (parasitic)	+0.9 ± 0.67 (61)	+3.4 ± 0.50 (61)	–
Focken and Becker 1998	*Cyprinus carpio* (carp)	Prepared diet	−0.1 ± 0.51 (22)	–	–
	Oreochromis niloticus (tilapia)	Prepared diet	+0.9 ± 0.29 (44)	–	–
Fry and Arnold 1982	*Penaeus aztecus* (brown shrimp)	Brine shrimp	+0.9 ± 0.38 (13)	–	–
		Brine shrimp	+1.3 ± 0.32 (7)	–	–
		Shrimp	−0.7 ± 0.21 (15)	–	–
	Penaeus aztecus (brown shrimp)	Shrimp	−0.8 ± 0.21 (5)	–	–
	Penaeus aztecus (brown shrimp)	Squid	−0.7 ± 0.61 (5)	–	–
Harvey et al. 2002					–
Herzka and Holt 2000	*Sciaenops ocellatus* (red drum)	Artemia			
		28°C	+1.6 ± 0.50 (17)	+0.1 ± 0.80 (17)	–
		24°C	+1.1 ± 0.19 (4)	+1.0 ± 0.17 (4)	–
		Prepared diet	+0.9 ± 0.46 (20)	+1.7 ± 0.43 (20)	–
Hesslein et al. 1993	*Coregonus nasus* (broad whitefish)	Prepared diet	+2.0 ± 2.10 (28)	+3.8 ± 2.30 (33)	+1.5 ± 1.00 (6)

Appendix 1. (Continued).

Study	Consumer	Diet	Trophic shift, mean ± SE (n)		
			$\Delta\delta^{13}C$	$\Delta\delta^{15}N$	$\Delta\delta^{34}S$
Hobson and Clark 1992	*Corturnix japonica* (quail)	Prepared diet	+1.1 ± 0.10 (5)	+1.0 ± 0.50 (5)	–
	Gallus gallus (chicken)	Prepared diet	+0.3 ± 0.20 (8)	+0.2 ± 0.30 (8)	–
	Larus delawarensis (gull)	Perch	+0.3 ± 0.10 (14)	+1.4 ± 0.40 (14)	–
Hobson et al. 1996	*Phagophilus groinlandiensis* (seal)	Herring	+1.3	+2.4	–
Macko et al. 1982	*Amphithoe valida* (amphipod)	Algae	−0.9	−0.7	–
	Amphithoe valida (amphipod)	Algae	−1.5	−0.2	–
	Amphithoe valida (amphipod)	Algal detritus	−0.1	−0.1	–
	Parahyale hawaiensis (amphipod)	Algae	−1.1	+2.3	–
	Parahyale hawaiensis (amphipod)	Algae	−1.3	+2.2	–
		Algal detritus	−0.1	+2.7	–
Minagawa and Wada 1984	*Artemia salina* (brine shrimp)	Yeast	–	+4.9 ± 0.50 (3)	–
	Lebistes sp. (guppy)	Fish food	–	+3.2 ± 1.10 (6)	–
	Mus musculus (mouse)	Yeast	–	+2.9 ± 0.60 (2)	–
Mitchell et al. 1993	*Sus scofra* (pig)	Prepared diet (C4)	±2.2 ± 0.44 (3)	–	–
		Prepared diet (C3)	+2.6 ± 1.14 (3)	–	–
Mizutani et al. 1991	*Phalacrocorax carbo* (cormorant)	Fish	+2.1	+2.4	–
Oelbermann and Sechu 2002	*Pardosa lagubris* (lycosid spider)	Drosophila	−0.4 ± 0.10 (2)	+2.1 ± 0.43 (2)	–
		Aphids	+1.4 ± 0.22 (2)	+1.5 ± 0.39 (2)	–
		Colembolans	0.0 ± 0.03 (2)	+2.5 ± 0.09 (2)	–
	Heteromurus nitidus (colembolan)	Yeast	+0.3 ± 0.26 (3)	+5.2 ± 0.95 (3)	–
	Drosophila melanogaster (fruit fly)	Banana	+1.6 ± 0.30 (3)	+3.3 ± 0.26 (3)	–
	Rhopalosiphum padi (aphid)	Wheat	+0.2 ± 0.09 (3)	−1.2 ± 0.35 (3)	–
Ostrom et al. 1997	*Hippodamia variegata* (ladybird beetle)	Aphids	−0.2 ± 0.10 (3)	+2.9 ± 0.30 (3)	
	Aphids	Sorghum	−1.1	0.0	
Petelle et al. 1979	*Apenteles* sp. (wasp)	Catalpa worm	+0.4	–	–
	Apenteles sp. (wasp)	Hornworm	+2.3	–	–
	Ceratomia catalpae (catalpaworm)	Catalpa	+0.9	–	–
	Epilachna varivestis (bean beetle)	Bean	−0.4	–	–
	Epilachna varivestis (bean beetle)	Eggplant	+0.7	–	–
	Manduca quinquemaculata (hornworm)	Tomato	−0.6	–	–
	Murgantia histrionica (harlequin bug)	Broccoli	+0.6	–	–
	Pseudaletia unipunctata (army worm)	Corn	+0.1	–	–
	Pseudaletia unipunctata (army worm)	Johnson grass	+1.2	–	–
Peterson and Howarth 1987	*Orchelimum fidicinium* (leaf hopper)	Spartina	−0.3	+1.1	−1.1
	Porthetria dispar (gypsy moth)	Plant diet	−1.4	+1.6	+1.4
	Salvelinus fontinalis (brook char; large)	Commercial diet	+0.8	+4.7	+1.2
	Salvelinus fontinalis (brook char; small)	Commercial diet	+2.0	+4.4	+1.4
Pinnegar et al. 2001	*Anilocra physodes* (isopod)	Boops boops (parasitic)	−0.1 ± 1.18 (6)	−0.3 ± 0.42 (6)	–
	Hysterothylaciu aduncum (nematode)	Whiting (parasitic)	+2.0 ± 0.94 (6)	−1.4 ± 0.80 (6)	–
	Lernaeocera brachialis (copepod)	Flounder (parasitic)	−1.6 ± 0.57 (3)	−0.8 ± 1.20 (3)	–
	Schistocephalus solidus (cestode)	Stickleback (parasitic)	−0.1 ± 0.23 (3)	−2.4 ± 0.41 (3)	–
Rosenfeld and Roff 1992	*Hydropsychidae* (caddisfly larva)	Trout chow	+0.1	–	–
	Catostomus commersoni (white sucker)	Tropical fish food	−0.1	–	–
	Notropis cornutus (common shiner)	Tropical fish food	−1.5	–	–
Roth and Hobson 2000	*Vulpes vulpes* (red fox)	Martin fox	+1.1 ± 0.36 (10)	3.3 ± 0.22 (10)	–
Rounick and Hicks 1985	*Onchorhynkus mykiss* (rainbow trout)	Trout pellets	+1.3 ± 0.50 (12)	–	–

Appendix 1. (Continued).

Study	Consumer	Diet	Trophic shift, mean ± SE (n)		
			$\Delta\delta^{13}C$	$\Delta\delta^{15}N$	$\Delta\delta^{34}S$
Scrimgeour et al. 1995	*Adalia bipunctata* (ladybird beetle; adult)	Aphids	–	+1.2	–
	Adalia bipunctata (ladybird beetle; larva)	Aphids	–	+0.5	–
	Amphorophora idaei (aphid)	Raspberry	–	+2.0	–
	Byturus tomentosus (raspberry beetle; larva)	Raspberry	–	+1.4	–
	Coccinella septempunctata (ladybird beetle; adult)	Aphids	–	+1.7	–
Stephenson et al. 1986	*Gammarus lawrencianus* (amphipod)	Prepared diet	+0.4	–	–
		Prepared diet	+1.8	–	–
		Prepared diet	+1.2	–	–
		Prepared diet	−1.5	–	–
		Kelp	+0.6	–	–
		Seagrass	−1.9	–	–
	Idotea baltica (isopod)	Prepared diet	+0.6	–	–
		Prepared diet	+1.2	–	–
		Prepared diet	+0.5	–	–
		Prepared diet	+1.1	–	–
		Prepared diet	−0.6	–	–
		Kelp	+0.7	–	–
		Seagrass	−2.7	–	–
Teeri and Schoeller 1979	*Tribolium castaneum* (flour beetle)	Corn	−2.7	–	–
		Prepared diet	+1.8	–	–
		Prepared diet	+3.0	–	–
		Prepared diet	+2.3	–	–
		Wheat	+2.4	–	–
Tieszen et al. 1983	*Meriones unguiculatus* (gerbil)	Corn	−0.3 ± 0.86 (9)	–	–
		Wheat	+0.5 ± 0.87 (3)	–	–
Webb et al. 1998	*Locusta migratoria* (locust)	Corn	+2.8 ± 2.16 (60)	+2.1 ± 1.99 (60)	–
		Wheat	−2.5 ± 1.08 (10)	+5.1 ± 0.92 (10)	–