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Interspecific and nutrient-dependent variations in stable isotope fractionation: experimental studies simulating pelagic multitrophic systems

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Abstract Stable isotope signatures of primary producers display high inter- and intraspecific variation. This is assigned to species-specific differences in isotope fractionation and variable abiotic conditions, e.g., temperature, and nutrient and light availability. As consumers reflect the isotopic signature of their food source, such variations have direct impacts on the ecological interpretation of stable isotope data. To elucidate the variability of isotope fractionation at the primary producer level and the transfer of the signal through food webs, we used a standardised marine tri-trophic system in which the primary producers were manipulated while the two consumer levels were kept constant. These manipulations were (1) different algal species grown under identical conditions to address interspecific variability and (2) a single algal species cultivated under different nutrient regimes to address nutrient-dependent variability. Our experiments resulted in strong interspecific variation between different algal species (*Thalassiosira weissflogii*, *Dunaliella salina*, and *Rhodomonas salina*) and nutrient-dependent shifts in stable isotope signatures in response to nutrient limitation of *R. salina*. The trophic enrichment in ^{15}N and ^{13}C of primary and secondary consumers (nauplii of *Acartia tonsa* and larval herring) showed strong deviations from the postulated degree of 1.0‰ enrichment in $\delta^{13}\text{C}$ and 3.4‰ enrichment in $\delta^{15}\text{N}$.

Surprisingly, nauplii of *A. tonsa* tended to keep “isotopic homeostasis” in terms of $\delta^{15}\text{N}$, a pattern not described in the literature so far. Our results suggest that the diets’ nutritional composition and food quality as well as the stoichiometric needs of consumers significantly affect the degree of trophic enrichment and that these mechanisms must be considered in ecological studies, especially when lower trophic levels, where variability is highest, are concerned.

Keywords Food quality · Homeostasis · *Clupea harengus* · Trophic interactions · Food-web studies

Introduction

Stable isotopes have been considered a major tool in ecology for decades, and the use of natural ratios of the stable carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) isotopes for the reconstruction of food sources and trophic interactions have gained considerable importance in terrestrial and aquatic environments (Peterson and Fry 1987; Ponsard and Ardit 2000). Due to a stepwise enrichment of heavier isotopes from one trophic level to the next, natural isotope ratios can be used to trace consumer diets and to gain a better understanding of food-web interactions (Vander Zanden and Rasmussen 2001; Post 2002). This stepwise enrichment is assumed to be about 1.0‰ for $\delta^{13}\text{C}$ and 3.4‰ for $\delta^{15}\text{N}$ between trophic levels (Minangawa and Wada 1984; Peterson and Fry 1987). However, this assumption has been criticised, arguing that it disregards the variation in trophic fractionation, especially at lower trophic levels (Vander Zanden and Rasmussen 2001).

To date, it is known that the degree of enrichment can vary between species and, furthermore, that within species,

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the environmental, physiological and nutritional conditions can alter the isotopic compositions of primary producers (Fry 1996; Burckhardt et al. 1999; Needoba et al. 2003). Although there is an increasing knowledge of fractionation processes in autotrophs and indication that the actual degree of trophic enrichment in consumers can exceed by far the postulated degree of enrichment (Macko et al. 1983), the transfer of these signals to higher trophic levels has been the subject of only a few experimental studies (Schlechtriem et al. 2004; Haubert et al. 2005; Tamelander et al. 2006). Variation in trophic enrichment is assumed to be related to variations in stable isotope composition of food sources, metabolic processes (Ponsard and Averbuch 1999; Gorokhova and Hansson 1999), inter- and intraspecific differences (Vander Zanden and Rasmussen 2001; Post 2002), the C/N ratio of the food source as well as food availability (Adams and Sterner 2000; Vanderklift and Ponsard 2003). However, there is still a lot of debate in stable isotope ecology concerning the forcing factors of enrichment processes.

The present study aimed to elucidate the variability of isotope fractionation at the primary producer level and to trace enrichment processes through a tri-trophic food chain by experimental investigations on (1) interspecific variations of stable isotope signatures between different algal species, (2) nutrient-dependent variations of algae in relation to nutrient regimes and (3) the transfer of the primary producers' signal to primary (copepods) and secondary (larval herring) consumers.

Materials and methods

Algal cultures and growth

Stock culture of the diatom *Thalassiosira weissflogii*, the green alga *Dunaliella salina* and the cryptophyte *Rhodomonas salina* were cultivated in f/2 media following Guillard and Ryther (1962). For the experimental trials, we cultivated algae at 17°C under a light/dark cycle of 16/8 h in enriched natural seawater amended with nutrients at different levels (for nutrient details, see description in the section on "Interspecific 1 + 2" and "Limitation 1 + 2"). The entire water used for cultivation during the experiments was taken from the Baltic Sea at the beginning of February to guarantee winter nutrient concentrations. Subsequently, the water was 0.2- μm sterile filtered and stored cold and dark until use.

Prior to the experiments, a cell number versus in vivo fluorescence relationship was set up for each alga culture using flow cytometry. This calibration was used in the following for the daily determination of algal concentrations using in vivo fluorescence measurements (Turner,

10-AU-005-CE) at an excitation wavelength of 436 nm and an emission wavelength of 680 nm. Algae cultures were ventilated with an aeration system to ensure a sufficient CO_2 supply.

Acartia tonsa cultures

Prior to the experiments, eggs of the calanoid copepod *Acartia tonsa* were produced in 200-l cylindrical tanks. Copepods were cultivated at 18°C at a 12/12 light cycle. Copepods were fed on a mixture of the algae *R. salina* and *D. salina* and the flagellate *Oxhyrris spec.* Eggs were siphoned from the bottom of the tanks daily and stored in seawater at 4°C for later use. No eggs older than 3 months were used in the experiments. Hatch rate was around 20%.

Fish-larvae cultures

Larval herring were obtained by means of artificial fertilisation. Female fish were strip spawned on glass plates. Eggs were incubated in a flow-through system using 4 μm prefiltered natural seawater at 13°C. Larvae hatched after 11 days and were transferred to cylindrical 200-l stock tanks. The stock tanks were operated as a flow-trough system and supplied with prefiltered water. The egg incubation and the hatching procedures were conducted twice with a time gap of 10 days in-between in order to use larvae from the first trial for Interspecific 1 and Limitation 1 and the second trial for Interspecific 2 and Limitation 2.

Interspecific experiments

Algae

Cultures of *T. weissflogii*, *D. salina* and *R. salina* were cultivated in natural seawater amended with nutrients to final concentrations of 30 $\mu\text{mol l}^{-1}$ nitrogen, 2 $\mu\text{mol l}^{-1}$ phosphorous and, in case of the diatom *T. weissflogii* 80 $\mu\text{mol l}^{-1}$ silicate. To ensure constant food qualities and quantities, new cultures of each algal species were inoculated every day, and the amount of cells at the beginning was kept constant for each species (250 cells ml^{-1} for *R. salina*, 400 cells ml^{-1} for *D. salina* and 3 cells ml^{-1} for *T. weissflogii*). The different amounts inoculated were in relation to the different biovolumes of the algae (383 μm^3 for *R. salina*, 24 μm^3 for *D. salina* and 3,192 μm^3 for *T. weissflogii*) to keep the biovolumes for each algae species constant. The cultures were harvested at mean densities of 360,000, 880,000 and 47,000 cells ml^{-1} (*R. salina*, *D. salina* and *T. weissflogii*, respectively) after 7 days. These

were the maximum attainable densities during the predefined exponential growth phase under the given light and nutrient regimes. Isotope composition of each algal batch culture at the end of the growth period was measured. For each experimental run (Interspecific 1 + 2) the batch-to-batch variation of algae isotope composition was calculated from the mean value [\pm standard deviation (SD)] of all batch cultures (13–15 batches per treatment) used as food sources for primary consumers over the duration of the entire experimental period.

Consumers

The copepods used for the experiments were reared in filtered seawater from the egg to the fifth naupliar stage at densities of 1,500 ind. l⁻¹ in 5-l plastic bags at 17°C. Copepods were fed first 48 h after adding the eggs to the rearing bags, assuming 2 days for the development from the egg to the first-feeding second naupliar stage. Copepods were harvested after 7 days of cultivation reaching the fifth naupliar stage. Isotope composition of each copepod culture was measured at the end of the feeding period; culture-to-culture variation (\pm SD) was calculated from the mean of all copepod cultures harvested during the experiments (9–11 batches per treatment). Three new copepod bags were started at each day of the feeding experiment to ensure a constant food quality for herring larvae as secondary consumers. Interspecific 1 was started using 3-days' posthatch herring larvae, which had no feeding experience. The rest of the larvae in the stock tanks were fed on *Brachionus plicatilis* from day 4. Interspecific 2 was started using 7 days post hatch larvae.

Two hundred herring larvae each were transferred to 20 l tanks filled with GF/F filtered seawater. For each treatment, one herring larvae tank was used. Larvae were fed daily around midday with copepods reared with the three different algal diets. Approximately 50% of the water was replaced daily. Water was changed before feeding to ensure that the majority of prey organisms remained in the experimental containers for a maximum of 24 h. This was

essential to avoid alterations of body composition and a change in isotope signatures of the copepods due to starvation and hence to assure constant food quality over the trial period. Isotope composition of individual or pooled larvae (one to four larvae per sample) was measured at the end of the feeding period; larvae-to-larvae variation was calculated as the mean (\pm SD) of larvae samples harvested at the end of the experiments (three to ten samples per treatment). For more details on the experimental set-up of the Interspecific experiments, see Tables 1 and 2.

Limitation experiments

Algae

The cryptophyte *R. salina* was cultivated in enriched seawater, under phosphorous limitation and under nitrogen limitation. The first treatment simulated a nonlimited bloom situation and consisted of f/2-enriched seawater (f/2 in the following), as described by Guillard and Ryther (1962); the other two treatments simulated decaying bloom situations under N or P limitation. The algae of the two limitation treatments were enriched with just one macronutrient (no P or N addition; -P and -N in the following) and could therefore only utilise the natural P or N sources contained in the seawater at the moment of filtration.

To ensure constant food quality, new cultures of each of the treatments were inoculated every day with roughly 0.2×10^6 cells ml⁻¹ for the -N treatment and 0.3 for the -P and f/2 treatments. Algae were harvested at densities of approximately 0.5, 1.0, and 1.3×10^6 cells ml⁻¹ (-N, -P and f/2, respectively) after the predefined growth phase of 6 days for -N and 7 days for f/2 and -P. These were the maximum densities possible to culture with the natural N or P sources contained in the seawater. For all treatments, algae were fed to the primary consumers at a concentration of 1.0-mg carbon per litre copepod culture medium d⁻¹. Concurrent with the Interspecific experiments, samples for isotope composition of algae batch cultures were sampled at the end of the growth period. For each experimental run

Table 1 Experimental details of the Interspecific and the Limitation experiments

Experimental run	Temperature (°C)	Age of herring larvae (d ⁻¹) at start	Copepod diet (mg algal carbon l ⁻¹ d ⁻¹)	Herring larvae diet (copepods l ⁻¹)	Days of copepod feeding	Days of herring larvae feeding
Interspecific 1	17	3	1.0	1	5	5 and 8
Interspecific 2	17	7	1.0	1	5	5 and 8
Limitation 1	15	4	1.0	1	5	5
Limitation 2	15	9	1.0	1	5	5

Table 2 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of algae and consumers during the Interspecific and the Limitation experiments' mean values \pm standard deviation (SD) and the number (*n*) of samples per treatment are given

Interspecific	$\delta^{15}\text{N}$						$\delta^{13}\text{C}$					
	Algae		Acartia		Clupea		Algae		Acartia		Clupea	
	5-day	8-day	5-day	8-day	5-day	8-day	5-day	8-day	5-day	8-day	5-day	8-day
<i>Rhodomonas</i> (1)	-0.7 \pm 2.0 (n = 15)	1.7 \pm 0.6 (n = 11)	12.1 \pm 0.4 (n = 10)	12.4 \pm 0.6 (n = 5)	-24.3 \pm 2.0 (n = 15)	-19.0 \pm 1.7 (n = 11)	-21.0 \pm 0.3 (n = 10)	-21.2 \pm 0.1 (n = 5)				
<i>Rhodomonas</i> (2)	1.5 \pm 1.6 (n = 13)	3.7 \pm 0.6 (n = 9)	12.0 \pm 2.3 (n = 7)	11.1 \pm 2.3 (n = 11)	-26.1 \pm 2.4 (n = 13)	-19.5 \pm 1.1 (n = 9)	-20.5 \pm 1.7 (n = 7)	-21.3 \pm 0.9 (n = 11)				
<i>Dunaliella</i> (1)	1.7 \pm 1.8 (n = 15)	2.2 \pm 1.8 (n = 11)	12.1 \pm 0.3 (n = 10)	12.1 \pm 0.6 (n = 6)	-14.0 \pm 1.3 (n = 15)	-16.1 \pm 2.3 (n = 11)	-21.2 \pm 0.3 (n = 10)	-21.0 \pm 0.6 (n = 6)				
<i>Dunaliella</i> (2)	3.8 \pm 1.4 (n = 13)	3.6 \pm 0.5 (n = 9)	12.4 \pm 2.3 (n = 6)	-	-14.2 \pm 1.2 (n = 13)	-13.9 \pm 1.0 (n = 9)	-20.1 \pm 1.5 (n = 6)	-				
<i>Thalassiosira</i> (1)	4.0 \pm 1.2 (n = 15)	2.5 \pm 3.3 (n = 11)	12.3 \pm 0.6 (n = 10)	12.4 \pm 0.1 (n = 3)	-20.9 \pm 1.4 (n = 15)	-15.9 \pm 1.9 (n = 11)	-20.9 \pm 0.4 (n = 10)	-21.3 \pm 0.2 (n = 3)				
<i>Thalassiosira</i> (2)	5.8 \pm 1.8 (n = 14)	3.4 \pm 0.3 (n = 9)	12.1 \pm 2.4 (n = 5)	10.4 \pm 2.1 (n = 9)	-21.3 \pm 0.9 (n = 14)	-14.6 \pm 0.7 (n = 9)	-20.8 \pm 1.7 (n = 5)	-21.4 \pm 1.4 (n = 9)				
Limitation												
					Algae	Acartia	Clupea	Clupea	Acartia	Algae	Acartia	Clupea
f/2 (1)	-2.0 \pm 1.1 (n = 9)	1.6 \pm 0.2 (n = 5)	11.7 \pm 0.5 (n = 5)	11.7 \pm 0.5 (n = 5)	-11.3 \pm 0.9 (n = 9)	-11.9 \pm 0.3 (n = 5)	-19.9 \pm 1.0 (n = 5)	-19.9 \pm 1.0 (n = 5)				
f/2 (2)	-0.7 \pm 0.9 (n = 11)	3.5 \pm 1.2 (n = 6)	7.4 \pm 0.9 (n = 3)	7.4 \pm 0.9 (n = 3)	-12.1 \pm 2.7 (n = 11)	-13.2 \pm 1.1 (n = 6)	-17.8 \pm 1.3 (n = 3)	-17.8 \pm 1.3 (n = 3)				
-N (1)	1.3 \pm 2.3 (n = 7)	2.8 \pm 0.9 (n = 5)	12.1 \pm 0.2 (n = 5)	12.1 \pm 0.2 (n = 5)	-21.7 \pm 2.2 (n = 7)	-19.4 \pm 0.5 (n = 5)	-21.1 \pm 0.4 (n = 5)	-21.1 \pm 0.4 (n = 5)				
-N (2)	2.6 \pm 0.9 (n = 11)	6.0 \pm 1.1 (n = 6)	8.6 \pm 0.5 (n = 4)	8.6 \pm 0.5 (n = 4)	-23.8 \pm 2.8 (n = 11)	-19.0 \pm 1.6 (n = 6)	-21.6 \pm 1.1 (n = 4)	-21.6 \pm 1.1 (n = 4)				
-P (1)	0.1 \pm 2.2 (n = 7)	3.4 \pm 0.7 (n = 5)	12.3 \pm 0.3 (n = 6)	12.3 \pm 0.3 (n = 6)	-15.3 \pm 2.8 (n = 7)	-15.4 \pm 0.9 (n = 5)	-21.0 \pm 0.3 (n = 6)	-21.0 \pm 0.3 (n = 6)				
-P (2)	-1.8 \pm 0.3 (n = 12)	3.9 \pm 0.7 (n = 6)	7.1 \pm 0.5 (n = 3)	7.1 \pm 0.5 (n = 3)	-22.4 \pm 2.3 (n = 12)	-17.8 \pm 2.4 (n = 6)	-22.4 \pm 1.0 (n = 3)	-22.4 \pm 1.0 (n = 3)				

(Limitation 1 + 2), the batch-to-batch variation of algae isotope composition was calculated from the mean value (\pm SD) of all batch cultures (7–12 batches per treatment).

Consumers

The copepods used for the Limitation experiments were reared in the same way as for the Interspecific experiments. In order to guarantee the algal mineral limitation in the copepod rearing bags also, copepods were reared in artificial, N- and P-free seawater (hw-Marinemix, <http://www.hw-wiegandt.de>). Again, the first feeding of the second naupliar stages of the copepods started 48 h after adding the eggs to the incubation chambers. Similar to the Interspecific experiments, three new copepod bags were started each day, copepod cultures were measured at the end of the feeding period and culture-to-culture variation (\pm SD) was calculated (five to six batches per treatment).

Limitation 1 was started using 4 days' posthatch larvae, which had no feeding experience. The rest of the larvae in the stock tanks were fed on *B. plicatilis* from day 4. Limitation 2 was started using 9 days' posthatch larvae.

Twenty larvae each were transferred to 1-l glass beakers filled with GF/F filtered seawater. The three treatments (-P, -N, f/2) were replicated 10 times in Limitation 1 and three times in Limitation 2. More than 80% of the water was replaced daily. Water was changed before feeding to ensure that the vast majority of uneaten prey organisms were in the experimental container for a maximum of 24 h to avoid alterations of the body composition of the copepods due to starvation. Isotope composition of single or pooled larvae (1–4 larvae per sample) was measured at the end of the feeding period; larvae-to-larvae variation is given as the mean (\pm SD) of larvae at the end of the experiments (three to six samples per treatment). For more details on the experimental set-up of the Limitation experiments see Tables 1 and 2.

Stable isotope analysis and fractionation

According to cell densities, the amount of each alga species needed for stable isotope analysis was calculated, and the biovolume of the amount of algae sampled each day was kept constant. Thereafter, the algae were filtered on precombusted GF/F filters. For stable isotope analysis of *A. tonsa*, a total of 1,000 nauplii was sampled each day; and for herring larvae, several individuals of newly hatched larvae were pooled (two to three individuals per sample). For the 3–10 days' posthatch larvae, individual larvae were measured. Samples for stable isotope analysis of

algae, copepod nauplii and herring larvae were all transferred to tin caps (HEKAtech) and dried in an oven at 50°C.

Stable isotope analysis of the samples was performed by using an isotope ratio mass spectrometer (ThermoFinnigan EA 1110 CHNS). During measurements, the mass spectrometer determines the ratio of stable isotope ¹³C/¹²C and ¹⁵N/¹⁴N. Isotopic compositions are expressed by the δ notation, which are parts per thousand deviations from a standard.

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

- R = heavy/light isotope
- δX > 0 = enriched in heavy isotope
- δX < 0 = depleted in heavy isotope or enriched in light isotope

An acetanilide standard with δ¹⁵N of -2.56‰ ± 0.53 (SD) and δ¹³C of -30.45‰ ± 0.79 (SD) was measured after every sixth samples during mass spectrometry. Trophic fractionation of stable isotopes is described in terms of the difference in delta (δ) values between food source (A) and consumer (B) using the Δ notation, where Δ = δ_B - δ_A. A positive Δ value indicates a relatively greater concentration of the heavier isotope in B. Food source isotope composition was calculated as the average of algal diets' isotope composition of batch cultures over the experimental period and the copepods' isotope composition at the day of sampling. To account for variability in isotope signatures between *A. tonsa* cultures, mean isotope fractionation per culture and SDs are given. Isotope composition of *A. tonsa* as food source for herring larvae was calculated as the average of *A. tonsa*

signatures during the course of the feeding period of larvae; mean isotope fractionation per larvae and SD are given to account for variability between larvae.

Statistical analysis

To test for significant differences in δ¹⁵N and δ¹³C values of algae and consumers during the Interspecific and the Limitation experiments, two-factorial analysis of variance (ANOVA) with the independent factors experiment run (F1) and food source (F2) and a Tukey's HSD test were performed using STATISTICA. Relationships between diet stoichiometry and trophic fractionation as well as between diet-stable isotope signatures and trophic fractionation are based on linear regressions between the mean C:N, δ¹⁵N, Δδ¹⁵N, δ¹³C, Δδ¹³C value calculated for every treatment. To test the significance of the linear regressions, GraphPad Prism (GraphPad Software Inc.) was used.

Results

Interspecific experiments

Isotope composition of algae

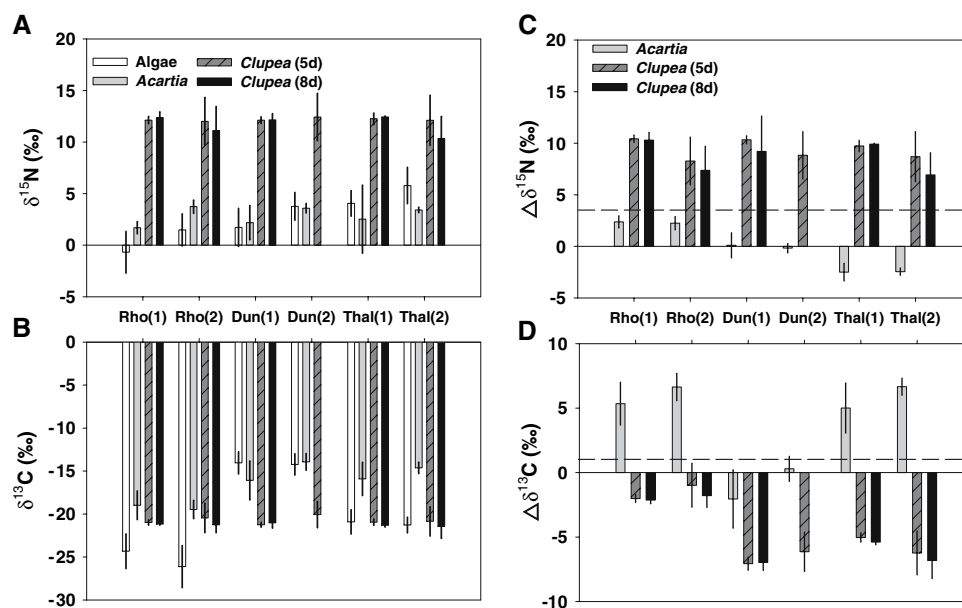
During the experiments, the δ¹⁵N showed significant variations between the different algae and between Interspecific 1 and 2 (for P values, see Table 3). The δ¹⁵N values ranged from -0.7‰ ± 2.0-4.0‰ ± 1.2 (Interspecific 1) to 1.5‰ ± 1.6-5.8‰ ± 1.8 (Interspecific 2) for *R. salina* and *T. weissflogii*, respectively (Fig. 1a) (values give

Table 3 δ¹⁵N and δ¹³C values of algae and consumers during the Interspecific and the Limitation experiments

δ ¹⁵ N	Interspecific				Limitation		
	Algae	<i>Acartia</i>	<i>Clupea</i>		Algae	<i>Acartia</i>	<i>Clupea</i>
			5-day	8-day			
Experiment run	0.000	0.001	0.935	-	0.959	0.000	0.000
Food source	0.000	0.094	0.941	0.355	0.000	0.000	0.004
Experiment run × food source	0.779	0.506	0.854	-	0.064	0.007	0.004
δ ¹³ C	Algae	<i>Acartia</i>	<i>Clupea</i>		Algae	<i>Acartia</i>	<i>Clupea</i>
			5-day	8-day			
	Experiment run	0.0337	0.020	0.074	-	0.019	0.034
Food source	0.0000	0.000	0.720	0.866	0.000	0.000	0.000
Experiment run × food source	0.1423	0.034	0.459	-	0.028	0.067	0.001

P values of the two-factorial analysis of variance (ANOVA) with the independent factors experiment run (F1) and food source (F2) and δ¹⁵N and δ¹³C as dependent variables. Eight-day-old herring from the Interspecific experiment were analysed by a one-factorial ANOVA using food source as the independent variable

Fig. 1 $\delta^{15}\text{N}$ (a), $\delta^{13}\text{C}$ (b), $\Delta\delta^{15}\text{N}$ (c) and $\Delta\delta^{13}\text{C}$ (d) of algae, *Acartia* and *Clupea* [mean \pm standard deviation (SD)] after 5 (5d) and 8 days (8d) of feeding during the first (1) and the second (2) Interspecific experiment. The diet basis was: *Rho* (*Rhodomonas salina*), *Dun* (*Dunaliella salina*), *Thal* (*Thalassiosira weissflogii*). Dashed lines indicate the postulated degree of 3.4‰ enrichment in $\delta^{15}\text{N}$ (c) and 1.0‰ enrichment in $\delta^{13}\text{C}$ and (d)



the mean per treatments and ± 1 SD). Different algal species showed significant variations in their $\delta^{13}\text{C}$ values between the experimental runs, ranging from $-14.0\text{‰} \pm 1.3$ to $-24.3\text{‰} \pm 2.0$ (Interspecific 1) and $-14.2\text{‰} \pm 1.2$ to $-26.1\text{‰} \pm 2.4$ (Interspecific 2) for *D. salina* and *R. salina*, respectively (Fig. 1b).

Isotope composition of consumers

During Interspecific 1, the $\delta^{15}\text{N}$ values of *A. tonsa* nauplii ranged from $1.7\text{‰} \pm 1.7$ (*R. salina* diet) to $2.5\text{‰} \pm 3.3$ (*T. weissflogii* diet) (Fig. 1a), but no significant variations could be observed depending on the food source. During Interspecific 2, the $\delta^{15}\text{N}$ values of nauplii were in mean 1–2‰ higher than during the first experiment, and the differences between experimental runs were significant. $\delta^{15}\text{N}$ ranged from $3.4\text{‰} \pm 0.3$ on *T. weissflogii* diet to $3.7\text{‰} \pm 0.6$ on *R. salina* diet. In terms of $\delta^{13}\text{C}$, nauplii showed significant variations between treatments and in the *D. salina* and *T. weissflogii* treatments also between the experimental runs. The $\delta^{13}\text{C}$ values differed significantly, ranging from $-15.9\text{‰} \pm 1.9$ (*T. weissflogii* diet) to $-19.0\text{‰} \pm 1.7$ (*R. salina* diet) during Interspecific 1 and $-13.9\text{‰} \pm 1.0$ (*D. salina* diet) to $-19.5\text{‰} \pm 1.1$ (*R. salina* diet) during Interspecific 2 (Fig. 1b).

The initial $\delta^{15}\text{N}$ of herring yolk-sac larvae right after hatch (start) was similar during both experiments, with values of $12.6\text{‰} \pm 0.3$ (Interspecific 1) and $11.8\text{‰} \pm 1.7$ (Interspecific 2). In terms of $\delta^{13}\text{C}$, the values between both experiments showed a 1‰ difference, with values of $-22.4\text{‰} \pm 0.3$ (Interspecific 1) and $-21.4\text{‰} \pm 1.3$ (Interspecific 2).

After 5 days of feeding, herring larvae showed only slight changes in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ when compared with the initial values of the yolk-sac larvae. After 5 days of feeding, $\delta^{15}\text{N}$ of larvae showed no significant differences in relation to food sources and experimental runs with values ranging from $12.0\text{‰} \pm 2.3$ (Rho; Interspecific 2) to $12.4\text{‰} \pm 2.3$ (Dun; Interspecific 2) (Fig. 1a). $\delta^{13}\text{C}$ ranged from $-20.1\text{‰} \pm 1.5$ (Dun; Interspecific 2) to $-21.2\text{‰} \pm 0.3$ (Dun; Interspecific 1) and no significant differences between food sources and experimental runs were observed (Fig. 1b). This pattern changed only slightly after 8 days of feeding during Interspecific 2, where changes in $\delta^{15}\text{N}$ of larvae fed on *R. salina*- and *T. weissflogii*-based diet were about 1–2‰ depleted compared with larvae after 5 days of feeding. However, the differences were not significant (Fig. 1a).

Trophic fractionation

The enrichment of stable isotope signatures per trophic level showed a high variability, and no constant patterns could be observed. Significant variation in the $\Delta\delta^{15}\text{N}$ of *A. tonsa* nauplii was observed between food sources but not between experimental runs (for *P* values, see Table 4). Nauplii fed with *R. salina* showed $\Delta\delta^{15}\text{N}$ of $2.3\text{‰} \pm 0.6$ (Interspecific 1) and $2.2\text{‰} \pm 0.6$ (Interspecific 2) (Fig. 1c) and thus corresponded most to the empirical degree of enrichment of 3.4‰. When *D. salina* or *T. weissflogii* were the basis of the tri-trophic food chain, hardly any trophic enrichment—and in most cases, a depletion—was observed. Herring larvae as secondary consumers showed a much higher degree of enrichment than the 3.4‰ empirical

Table 4 $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ values of consumers during the Interspecific and Limitation experiments

$\Delta\delta^{15}\text{N}$	Interspecific			Limitation	
	<i>Acartia</i>	<i>Clupea</i>		<i>Acartia</i>	<i>Clupea</i>
		5-day	8-day		
Experiment run	0.194	0.000	–	0.000	0.000
Food source	0.000	0.779	0.065	0.000	0.000
Experiment run \times food source	0.749	0.565	–	0.061	0.130
$\Delta\delta^{13}\text{C}$	<i>Acartia</i>	<i>Clupea</i>		<i>Acartia</i>	<i>Clupea</i>
		5-day	8-day		
	Experiment run	0.000	0.416	–	0.000
Food source	0.000	0.000	0.000	0.000	0.000
Experiment run \times food source	0.578	0.008	–	0.001	0.000

P values of the two-factorial analysis of variance (ANOVA) with the independent factors experiment run (F1) and food source (F2) and $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ as dependent variables. Eight-day-old herring from the Interspecific experiment were analysed by a one-factorial ANOVA using food source as the independent variable

degree of ^{15}N enrichment, and the signals were very similar to the initial signatures of larvae. Significant differences in the $\Delta\delta^{15}\text{N}$ of herring larvae were only observed between experimental runs after 5 days of feeding.

An even stronger variability in trophic fractionation was observed for the $\Delta\delta^{13}\text{C}$ (Fig. 1d), where an enrichment of 1.0‰ could not be observed in any case. Significant variation in the $\Delta\delta^{13}\text{C}$ of *A. tonsa* nauplii was observed both between food sources and experimental runs. The $\Delta\delta^{13}\text{C}$ of *A. tonsa* nauplii on *R. salina*- and *T. weissflogii*-based diet showed the highest degree of ^{13}C enrichment of $6.7\% \pm 0.7$ (Thal) and $6.6\% \pm 1.1$ (Rho) during Interspecific 2. *A. tonsa* nauplii on *D. salina*-based diet showed a very low degree of ^{13}C enrichment of $0.3\% \pm 1.0$ (Interspecific 1) or even a depletion of $-2.0\% \pm 2.3$ (Interspecific 2). Herring larvae showed a strong depletion in ^{13}C , and significant variation in the $\Delta\delta^{13}\text{C}$ of larvae was found after 5 and 8 days of feeding. ^{13}C -depletion was highest when *D. salina* or *T. weissflogii* were the basis of the food chain. Significant differences in the $\Delta\delta^{15}\text{N}$ of herring larvae were only observed between experimental runs.

Limitation experiments

Isotope composition of algae

The $\delta^{15}\text{N}$ of *R. salina* grown under different nutrient regimes showed significant variations. $\delta^{15}\text{N}$ ranged from $-2.0\% \pm 1.1$ (f/2) to $1.3\% \pm 2.3$ (-N) during Limitation 1

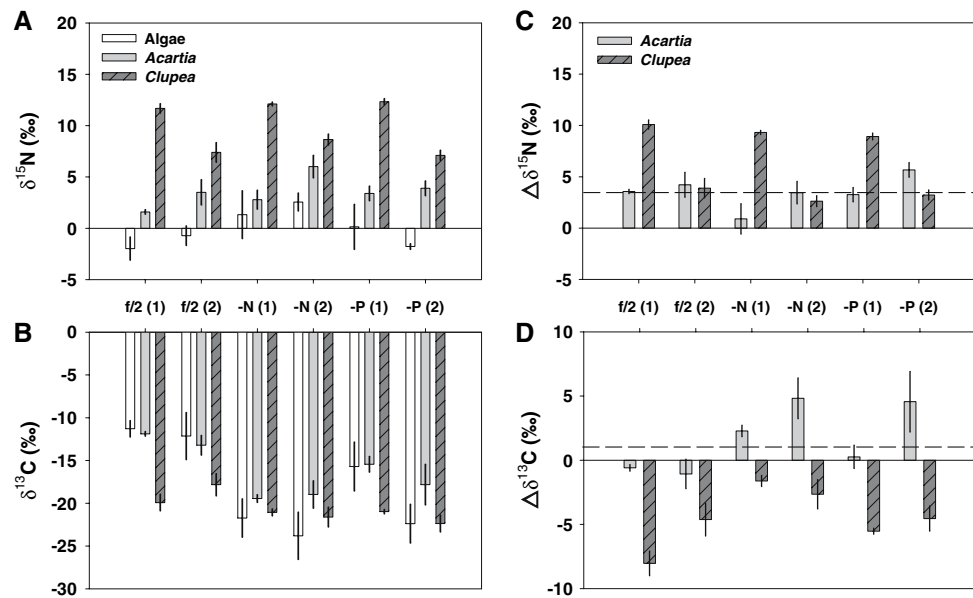
and $-1.8\% \pm 0.3$ (-P) to $2.6\% \pm 0.9$ (-N) during Limitation 2 (Fig. 2a; for *P* values, see Table 3). The $\delta^{13}\text{C}$ values between the different nutrient treatments showed significant variations in relation to the nutrient status, and the experimental runs ranging from $-21.7\% \pm 2.2$ (-N) to $-11.3\% \pm 0.9$ (f/2) during Limitation 1 and $-23.8\% \pm 2.8$ (-N) to $-12.1\% \pm 2.7$ (f/2) during Limitation 2 (Fig. 2b).

Isotope composition of consumers

The $\delta^{15}\text{N}$ values of *A. tonsa* nauplii showed significant differences in the $\delta^{15}\text{N}$ in relation to the growth conditions of their algal diets and between experimental runs (Fig. 2a; for *P* values, see Table 3). During Limitation 1, the $\delta^{15}\text{N}$ of nauplii ranged from $1.6\% \pm 0.2$ (f/2 diets) to $3.4\% \pm 0.7$ (-P diets). During Limitation 2, the $\delta^{15}\text{N}$ ranged from $3.5\% \pm 1.2$ (f/2 diets) to $6.0\% \pm 1.1$ (-N diets). In terms of $\delta^{13}\text{C}$, the nauplii showed significant variations between the different nutrient treatments and between Limitations 1 and 2. $\delta^{13}\text{C}$ values ranged from $-11.9\% \pm 0.3$ (f/2 diets) to $-19.4\% \pm 0.5$ (-N diets) (Limitation 1) and $-13.2\% \pm 1.1$ (f/2 diets) to $-19.0\% \pm 1.6$ (-N diets) (Limitation 2) (Fig. 2b). The initial $\delta^{15}\text{N}$ of herring yolk-sac larvae right after hatch (start) was the same as during Interspecific 1 and Interspecific 2, as larvae from the same trials were used for the experiments.

After 5 days of feeding, larvae showed only slight changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ when compared with the initial values of the yolk-sac larvae (Limitation 1). Their $\delta^{15}\text{N}$

Fig. 2 $\delta^{15}\text{N}$ (a), $\delta^{13}\text{C}$ (b), $\Delta\delta^{15}\text{N}$ (c) and $\Delta\delta^{13}\text{C}$ (d) of algae, *Acartia* and *Clupea* [mean \pm standard deviation (SD)] during the first (1) and the second (2) Limitation experiment. Diet basis was *Rhodomonas salina* grown under different nutrient regimes: f/2 (enriched seawater), -N (nitrogen limited), -P (phosphorous limited). Dashed lines indicate the postulated degree of 3.4‰ enrichment in $\delta^{15}\text{N}$ (c) and 1.0‰ enrichment in $\delta^{13}\text{C}$ (d)



varied significantly between food sources and experimental runs and ranged from $11.7\text{‰} \pm 0.5$ (f/2 diets) to $12.3\text{‰} \pm 0.3$ (-P diets) (Fig. 2a). During Limitation 2, herring larvae showed depletion in $\delta^{15}\text{N}$ in comparison with the initial $\delta^{15}\text{N}$ of yolk-sac larvae ranging from $7.1\text{‰} \pm 0.5$ (-P diets) to $8.6\text{‰} \pm 0.5$ (-N diets). $\delta^{13}\text{C}$ differed significantly between food sources and was slightly higher or lower than the initial $\delta^{13}\text{C}$, ranging from $-19.9\text{‰} \pm 1.0$ (f/2 diets) to $-21.1\text{‰} \pm 0.4$ (-N diets) (Limitation 1) and $-17.8\text{‰} \pm 1.3$ (f/2 diets) to $-22.4\text{‰} \pm 1.0$ (-P diets) (Limitation 2, Fig. 2b).

Trophic fractionation

Some Limitation experiment treatments corresponded well to the empirical degree of 3.4‰ enrichment per trophic level. $\Delta\delta^{15}\text{N}$ of *A. tonsa* nauplii and herring larvae varied significantly between food sources and experimental runs, ranging from $0.9\text{‰} \pm 1.5$ (-N, Limitation 1) to $5.7\text{‰} \pm 0.7$ (-P, Limitation 2) for *A. tonsa* (Fig. 2c; for *P* values, see Table 4) and $2.6\text{‰} \pm 0.5$ (-N, Limitation 2) to $10.1\text{‰} \pm 0.5$ (f/2, Limitation 1) for herring larvae. The trophic enrichment of heavier carbon isotopes in *A. tonsa* nauplii and herring larvae showed a significant variability depending on the food sources and experimental runs and contrasted the empirical degree of enrichment of 1.0‰. In cases where the tri-trophic food chain was based on limited diets (-N and -P diets), the $\Delta\delta^{13}\text{C}$ of nauplii showed a ^{13}C enrichment, whereas on f/2 diets a depletion was observed (Fig. 2d). $\Delta\delta^{13}\text{C}$ of herring larvae were consistently

negative, with the strongest degree of depletion when *R. salina* grown under full media (f/2) and under P-limited conditions were the basis of the food chain.

Diet stoichiometry versus trophic fractionation

During the Interspecific and the Limitation experiments, the relationship between the C:N of algae and the $\delta^{15}\text{N}$ of *A. tonsa* showed no significant relationship (Fig. 3a), whereas there was a negative relation between the C:N of the algae and the $\Delta\delta^{15}\text{N}$ of *A. tonsa* (Fig. 3b; $r^2 = 0.42$, $P = 0.019$).

In terms of ^{13}C , there was no significant relationship between the C:N of algae and the $\delta^{13}\text{C}$ of *A. tonsa*. In contrast, the relationship between C:N and $\Delta\delta^{13}\text{C}$ of *A. tonsa* showed a significant positive trend (Fig. 3d; $r^2 = 0.59$, $P = 0.004$).

Diet-stable isotope signatures versus trophic fractionation

When comparing the stable isotope signatures of algae and those of copepod nauplii, no regression was found between $\delta^{15}\text{N}$ of algae and $\delta^{15}\text{N}$ of *A. tonsa* (Fig. 4a). In contrast, a negative regression was found between $\delta^{15}\text{N}$ of algae and $\Delta\delta^{15}\text{N}$ of *A. tonsa* (Fig. 4b; $r^2 = 0.74$, $P = 0.0002$, $b = -0.92$).

The relationship between $\delta^{13}\text{C}$ of algae and $\delta^{13}\text{C}$ of *A. tonsa* showed a positive trend (Fig. 4c; $r^2 = 0.76$, $P = 0.0002$, $b = 0.44$), while a significant negative trend

Fig. 3 Relationship between the C:N ratio of algae and $\delta^{15}\text{N}$ of *Acartia* (a), the C:N ratio of algae and $\Delta\delta^{15}\text{N}$ of *Acartia* (b), the C:N ratio of algae and $\delta^{13}\text{C}$ of *Acartia* (c) and the C:N ratio of algae and $\Delta\delta^{13}\text{C}$ of *Acartia* (d). Open circles indicate mean values per treatment during the Interspecific experiments, and closed circles indicate mean values per treatment during the Limitation experiments

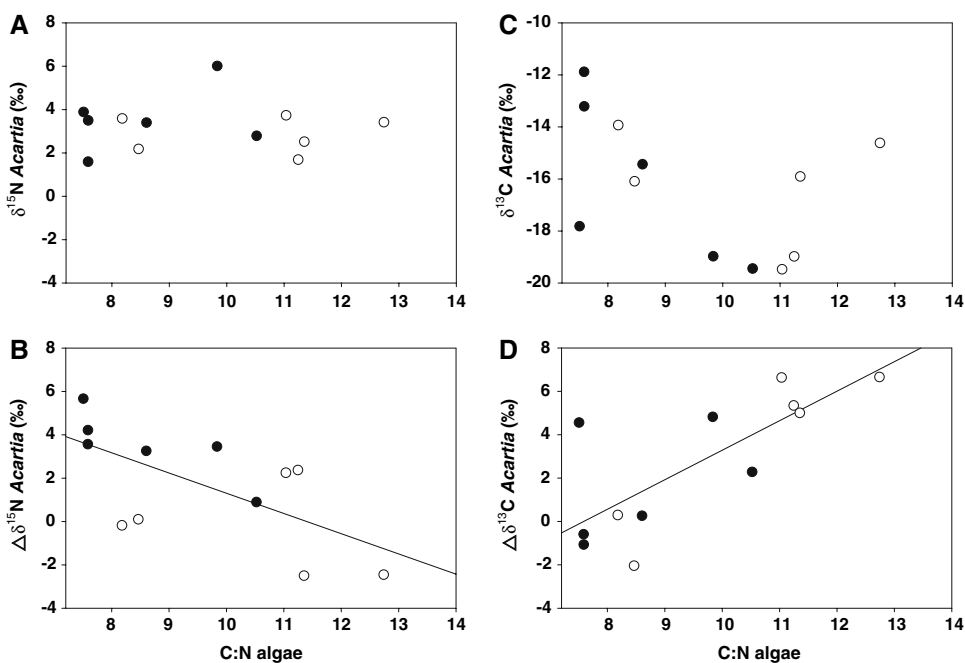
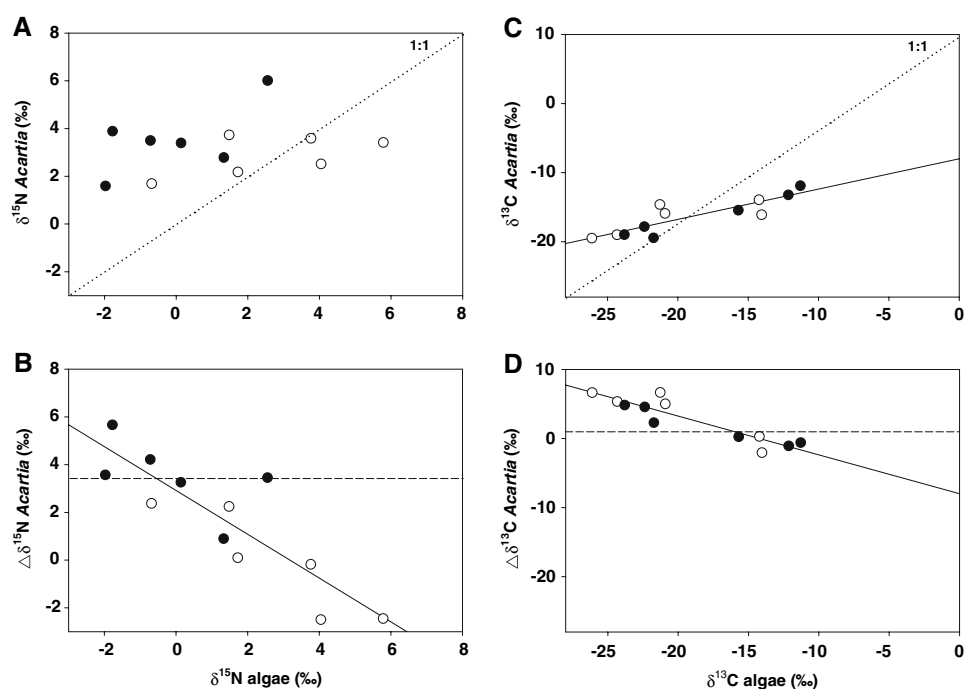


Fig. 4 Relationships between the $\delta^{15}\text{N}$ of algae and $\delta^{15}\text{N}$ of *Acartia* (a), the $\delta^{15}\text{N}$ of algae and $\Delta\delta^{15}\text{N}$ of *Acartia* (b), the $\delta^{13}\text{C}$ of algae and $\delta^{13}\text{C}$ of *Acartia* (c) and the $\delta^{13}\text{C}$ of algae and $\Delta\delta^{13}\text{C}$ of *Acartia* (d). Open circles indicate mean values per treatment during the Interspecific experiments and closed circles indicate mean values per treatment during the Limitation experiments. Dotted lines indicate the line of equality (a, c); dashed lines indicate the postulated degree of 1.0‰ enrichment in $\delta^{13}\text{C}$ and 3.4‰ enrichment in $\delta^{15}\text{N}$ (b, d)



was found for $\delta^{13}\text{C}$ of algae and $\Delta\delta^{13}\text{C}$ of *A. tonsa* (Fig. 4d; $r^2 = 0.83$, $P = 0.001$, $b = -0.56$).

Discussion

Inter- and intraspecific variability as well as the impact of abiotic conditions on enrichment processes on the primary producer (Fry 1996; Burkhardt et al. 1999; Needoba et al.

2003) and the consumer level (Macko et al. 1983; Vander Zanden and Rasmussen 2001) have gained considerable importance in stable isotope ecology only recently. Therefore, Gannes et al. (1997) encouraged experimental ecologist to study enrichment processes in detail under laboratory conditions, and Vander Zanden and Rasmussen (2001) urged for a more detailed consideration of stable isotope fractionation processes when interpreting stable isotope data from the field. Our study aimed at elucidating

such interspecific and nutrient-dependent variability of isotopic fractionation and trophic enrichment in an artificial food web by using a tri-trophic experimental approach.

Nutrient-dependent variation of algae

The Limitation experiment aimed at elucidating nutrient-dependent differences in isotope fractionation of the cryptophyte *R. salina* in relation to nutrient conditions. Cryptophytes are generally considered to be more competitive than other algae when light intensities are low and nutrient concentrations high (Gervais 1997). Depending on nutrient availability of the alga, the $\delta^{15}\text{N}$ showed large nutrient-dependent variation showing high fractionation under full media and low fractionation under nutrient-limited conditions. Our results are in good correspondence with Waser et al. (1998), who reported low $\delta^{15}\text{N}$ for algae grown under N-sufficient conditions. Nutrient-limited conditions therefore do not allow a strong discrimination against the heavier isotope ^{15}N by microalgae, a pattern that is underpinned by our data on nitrogen-limited treatments showing high degrees of ^{15}N enrichment, i.e., a weaker degree of fractionation compared with nutrient-sufficient situations. Furthermore, the elevated $\delta^{15}\text{N}$ of *R. salina* grown under nutrient-limited conditions might not only result from isotopic fractionation during nitrogen uptake alone, but could also be related to the fact that phytoplankton excrete dissolved organic nitrogen (DON) with a low ^{15}N content (Montoya and McCarthy 1995), thus increasing their $\delta^{15}\text{N}$.

Large variations in $\delta^{13}\text{C}$ also occurred between nutrient-sufficient and nutrient-depleted algae. The high $\delta^{13}\text{C}$ of *R. salina* grown under nutrient-sufficient conditions corresponded well with the literature on nutrient-dependent carbon isotope fractionation (Fry and Wainright 1991; Burkhardt et al. 1999). At times when nutrients are in excess and growth rates are high, microalgae show enrichment in heavy carbon isotopes. Interestingly, when comparing the stable isotope signatures of *R. salina* during the Limitation with those during the Interspecific experiments, it became obvious that the *R. salina* cultures during the Interspecific experiments were most likely nitrogen limited. This supports our assumption that the $\delta^{13}\text{C}$ depletion of *R. salina* in the Interspecific experiments results from reduced growth rates under limiting conditions.

Interspecific variation in isotope fractionation of algae

During the Interspecific experiments, the different algae showed considerable species-specific variations in $\delta^{15}\text{N}$

and $\delta^{13}\text{C}$. Evidence for a taxon-specific effect was first shown by Montoya and McCarthy (1995), who reported differences in isotope fractionation between diatoms and flagellates. They found lower ^{15}N fractionation for flagellates (1–3‰) when compared with the diatoms *Skeletonema costatum* and *T. weissflogii* (9–12‰) and speculated that the ability of a cell to swim may enhance fractionation by preventing small-scale, local nutrient limitation. This is supported by our findings of strong ^{15}N depletion in the flagellates *D. salina* and *R. salina* when compared with the diatom *T. weissflogii*. Not only cell motility may play an important role in determining the degree of isotope fractionation, but factors such as cell size or different metabolic demands may, also. In this context, it can also be speculated that a higher cell-surface to volume ratio of the diatom frustule might decrease the efficiency of nutrient uptake and thus might lead to lower degrees of isotope fractionation (i.e. higher enrichment levels). Taxon-specific variations in $\delta^{15}\text{N}$ have also been reported lately for lake phytoplankton by Vuorio et al. (2006). However, their study showed a strong enrichment of flagellated crysophytes (single-celled *Mallomonas caudatum* and colonies of *Synura* spp.), whereas Needoba et al. (2003) found no relationship between isotope fractionation and organism group. Thus, the ability to move seems to be only one factor influencing the degree of ^{15}N fractionation by phytoplankton, and characteristics such as colony type, cell shape and environmental conditions (Vuorio et al. 2006) can additionally affect ^{15}N -fractionation pattern in algae cells.

In terms of ^{13}C , however, both flagellates showed contrasting degrees of fractionation, with *Dunaliella* spp. showing a lower discrimination against heavy carbon isotopes when compared with *R. salina*. Carbon isotope fractionation during photosynthesis is known to change strongly depending on algal growth rates and the carbon sources used (Burkhardt et al. 1999). In general, cells using CO_2 are expected to have lower $\delta^{13}\text{C}$ values than cells using bicarbonate, and at high growth rates, a lower degree of fractionation leading to high $\delta^{13}\text{C}$ is expected (Fry 1996). In our study, the different algae species were grown under equal nutrient and light conditions. As optimum growth conditions vary strongly between species, it seems likely that one algal species used in our experiment experienced better growth conditions than the other.

In addition to interspecific variability, we also found considerable batch-to-batch variability in isotope composition of algae. It can be assumed that the batch-to-batch variability was most likely related to inevitable changes in the biochemical composition of stock cultures used for the daily inoculation of the batch cultures as well as irregularities in the aeration systems, which might have led to changing CO_2 supply during the growth periods.

Trophic enrichment in the tri-trophic food chain

During the food-web experiments, the enrichment of ^{15}N and ^{13}C per trophic level showed considerable variations from the degree of enrichment postulated in stable isotope ecology. We found no consistent enrichment patterns in trophic fractionation, as both enrichment and depletion in heavier carbon and nitrogen isotopes relative to the consumers' food sources were observed. Usually, variations in stable isotope signatures on the primary producer level are assumed as being transferred to higher trophic levels. However, the factors influencing the trophic enrichment are not sufficiently clear as yet. Recent studies reported substantial variation in consumer enrichment, thus doubting the general concept of a stepwise trophic enrichment (Oelbermann and Scheu 2002; Vanderklift and Ponsard 2003; Gaye-Siessegger et al. 2004). The authors' pointed to misleading data interpretation by neglecting nutritional, physiological and ecological background information.

One possible explanation for the deviation from the theoretical framework of trophic enrichment might be related to a lag between the isotopic turnover time of consumers (Hesslein et al. 1993; Herzka and Holt 2000), leading to disequilibrium between food and consumer. Such effects might be visible in herring larvae, which may still carry the isotopic signal of their mothers. It seems likely that the duration of the experiment was not long enough to allow the herrings to be in isotopic equilibrium with their food. This is in accordance with Schlechtriem et al. (2004), who showed that after 6 days of feeding, carp larvae contained relatively high amounts of initial material, reflecting the isotopic signature of their mothers. Interestingly, the feeding experience of herring larvae did not show a strong effect on their isotope composition, as a constant maternal signal was observed in first-feeding larvae and those who had feeding experience prior to the experiments. Only during Limitation 2 did older larvae with feeding experience show a good correspondence to the theoretical degree of 3.4‰ $\Delta\delta^{15}\text{N}$ enrichment. This could be evidence for an inefficient consumption and assimilation of copepod nauplii by herring larvae in most treatments. Recent literature reported that 1 week of feeding would be an adequate period for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of consumers converging towards equilibrium (Klein Breteler et al. 2002; Olive et al. 2003), but this clearly depends on the consumers body mass and turnover rates. Therefore, we assume that *A. tonsa* nauplii, with high turnover rates at the onset of naupliar growth and an increase in body mass of ~100% from the egg to the fifth naupliar stage (Mauchline 1998), reached the isotopic equilibrium after 1 week of feeding. This assumption was based on the expectation that food sources of *A. tonsa* nauplii would show almost constant isotope composition. However, isotope composition

of algae varied on a day-to-day basis, and this might be one reason for the differences between the trophic fractionation observed within our study and the theoretical framework. The pattern observed within our laboratory study provides important information on the transfer of primary producers' signals onto higher trophic levels in the field. Recent studies have pointed to large discrepancies between the isotope composition of food sources and isotope fractionation in consumers (Grey et al. 2000; Santer et al. 2006). As phytoplankton exhibit large and quick variability in their isotope composition in the field (Vuorio et al. 2006), such deviations from the expected degree of trophic enrichment in consumers could be related to considerable variability in primary producers.

A common pattern observed in stable isotope studies is that isotope signatures of consumers tend to increase in conditions of nutritional stress and starvation (Adams and Sterner 2000; Vanderklift and Ponsard 2003; Olive et al. 2003). We could not detect an increase in $\delta^{15}\text{N}$ with decreasing food quality, but we found a clear negative trend in trophic ^{15}N enrichment ($\Delta\delta^{15}\text{N}$) in copepods with increasing C:N ratio (i.e. decreasing food quality for copepods). Our data are in good correspondence with earlier studies, which reported low ^{15}N enrichment in consumers feeding on low-quality food or on diets with a low nitrogen content (Oelbermann and Scheu 2002; Haubert et al. 2005). These findings might be related to homeostatic efforts of consumers, similar to the homeostasis of consumers in respect to their body nutrient composition. As under nutrient-limited conditions algae depend on the use of the entire nutrient pool and thus depend on the uptake of a high proportion of heavy isotopes, their signatures tend to increase with decreasing nutrient availability. Provided that food availability and food quality are low, similar mechanisms can be assumed for consumers. However, if food is available in excess but food quality decreases, consumers obviously tend to fractionate more strongly against heavy nitrogen isotopes in order to keep homeostasis. As shown with our data, this resulted in almost constant $\delta^{15}\text{N}$ signatures of consumers no matter how low food quality was. This pattern is contradictory to the assumption of 3.4‰ enrichment in $\delta^{15}\text{N}$ of consumers per trophic level that has widely been used in stable isotope ecology, although several studies stressed the flaws of the general concept, especially as far as lower trophic levels are concerned (Gannes et al. 1997; Vander Zanden and Rasmussen 2001). Today it is known that trophic fractionation of $\Delta\delta^{15}\text{N}$ is far more variable than commonly assumed and that variability is highest in detritivores and herbivores at the base of the food web (Vander Zanden and Rasmussen 2001; Vanderklift and Ponsard 2003).

Similar to diet-specific trends in $\Delta\delta^{15}\text{N}$ of nauplii, the ^{13}C enrichment per trophic level showed a highly negative

relationship between the $\delta^{13}\text{C}$ of algae and $\Delta\delta^{13}\text{C}$ of *A. tonsa*. As with the $\delta^{15}\text{N}$ in copepods, the $\delta^{13}\text{C}$ of the copepods did not show a reaction on food quality, thus indicating that “isotopic homeostasis” also is apparent for carbon isotopes. The results show that more isotopically enriched diets lead to a high trophic fractionation and thus a low trophic enrichment in consumers, whereas depleted diets lead to a low fractionation and consequently a high enrichment per trophic level, which by far exceeds the postulated trophic enrichment of 1.0‰. However, Adams and Sterner (2000) and Vanderklift and Ponsard (2003) did not report on “isotopic homeostasis”, and we hypothesise that this might be related to lower food availabilities to consumers in their studies and therefore stronger constraints in terms of heavy isotope discrimination in comparison with ad libitum conditions in our experiments. However, the C:N ratio alone does not necessarily determine the quality of a food source but, rather, the entire biochemical composition. This is of considerable importance, as recent studies reported on different fractionation patterns depending on the lipid, protein and carbohydrate contents (Focken and Becker 1998; Tamelander et al. 2006), the digestibility (Aberle et al. 2005) and the microbial conditioning (Goedkoop et al. 2006) of the food items.

The aspect of “isotopic homeostasis” has not been described in the literature so far, and the regulatory mechanisms standing behind it are unknown. However, further evidence for isotopic homeostasis has been gained from a new set of experiments conducted in 2006 in which surprisingly similar results were achieved (Aberle et al., unpublished data). Future laboratory studies are needed to address the regulatory mechanisms of consumers and the potential benefit of keeping homeostasis. Furthermore, the active role of consumers in controlling fractionation processes needs to be investigated.

Overall, our results suggest that the nutritional composition of diets and the stoichiometric needs of consumers significantly affect the degree of enrichment per trophic level and that food quality aspects are of considerable importance when studying food-web relations based on stable isotope analyses in the field. More experiments under controlled laboratory conditions are needed to study heavy isotope regulatory processes and to confirm the tendency of “isotopic homeostasis” in consumers.

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References

- Aberle N, Hillebrand H, Grey J, Wiltshire KH (2005) Selectivity and competitive interactions between two benthic invertebrate grazers (*Asellus aquaticus* and *Potamopyrgus antipodarum*): an experimental study using ^{13}C - and ^{15}N -labelled diatoms. *Freshw Biol* 50:369–379
- Adams TS, Sterner RW (2000) The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limnol Oceanogr* 3:601–607
- Burkhardt S, Riebesell U, Zondervan I (1999) Effects of growth rate, CO_2 concentration, and cell size on the stable carbon isotope fractionation in marine phytoplankton. *Geochim Cosmochim Acta* 63:3729–3741
- Focken U, Becker K (1998) Metabolic fractionation of stable carbon isotopes: implications of different proximate compositions for studies of the aquatic food web using $\delta^{13}\text{C}$ data. *Oecologia* 115:337–343
- Fry B (1996) $^{13}\text{C}/^{12}\text{C}$ fractionation by marine diatoms. *Mar Ecol Prog Ser* 134:283–294
- Fry B, Wainright SC (1991) Diatom sources of ^{13}C -rich carbon in marine food webs. *Mar Ecol Prog Ser* 76:149–157
- Gannes L, O'Brien D, Martinez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78:1271–1279
- Gaye-Siessegger J, Focken U, Muetzel S, Abel H, Becker K (2004) Feeding level and individual metabolic rate affect $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in carp: implications for food web studies. *Oecologia* 138:175–183
- Gervais F (1997) Light-dependent growth, dark survival, and glucose uptake by cryptophytes isolated from a freshwater chemocline. *J Phycol* 33:18–25
- Goedkoop W, Akerblom N, Demandt MH (2006) Trophic fractionation of carbon and nitrogen stable isotopes in *Chironomus riparius* reared on food of aquatic and terrestrial origin. *Freshw Biol* 51:878–886
- 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
- Grey J, Jones RG, Sleep D (2000) Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. *Oecologia* 123:232–240
- Guillard RL, Ryther J (1962) Studies of marine planktonic diatoms. *Can J Microbiol* 8:229–239
- Haubert D, Langel R, Scheu S, Ruess L (2005) Effects of food quality, starvation and life stage on stable isotope fractionation in Collembola. *Pedobiologia* 49:229–237
- Herzka S, Holt G (2000) Changes in isotope composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: potential applications to settlement studies. *Can J Fish Aquat Sci* 57:137–147
- Hesslein RH, Hallard KA, Ramlal P (1993) Replacement of sulfur, carbon and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}\text{S}$, ^{13}C , and ^{15}N . *Can J Fish Aquat Sci* 50:2071–2076
- Klein Breteler WCM, Grice K, Schouten S, Kloosterhuis HT, Damste JSS (2002) Stable carbon isotope fractionation in the marine copepod *Temora longicornis*: unexpectedly low ^{13}C value of faecal pellets. *Mar Ecol Prog Ser* 240:195–204
- Macko SA, Estep MLF, Lee WY (1983) Stable hydrogen isotope analysis of food webs on laboratory and field populations of marine amphipods. *J Exp Mar Biol Ecol* 72:243–250
- Mauchline J (1998) The biology of calanoid copepods. Academic Press, San Diego, p 710

- Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and relation between $\delta^{15}\text{N}$ and animal age. *Geochim Cosmochim Acta* 48:1135–1140
- Montoya JP, McCarthy JJ (1995) Isotopic fractionation during nitrate uptake by phytoplankton grown in continuous culture. *J Plankton Res* 17:439–464
- Needoba J, Waser N, Harrison P, Calvert S (2003) Nitrogen isotope fractionation in 12 species of marine phytoplankton during growth on nitrate. *Mar Ecol Prog Ser* 255:81–91
- Oelbermann K, Scheu S (2002) Stable isotope enrichment ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality. *Oecologia* 130:337–344
- Olive PJW, Pinnegar JK, Polunin NVC, Richards G, Welch R (2003) Isotope trophic-step fractionation: a dynamic equilibrium model. *J Anim Ecol* 72:608–617
- Peterson B, Fry B (1987) Stable isotopes in ecosystem studies. *Ann Rev Ecol Syst* 18:293–320
- Ponsard S, Arditì R (2000) What can stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) tell about the food web of soil macro-invertebrates? *Ecology* 81:852–864
- Ponsard S, Averbuch P (1999) Should growing and adult animals fed on the same diet show different $\delta^{15}\text{N}$ values? *Rapid Commun Mass Spectr* 13:1305–1310
- Post D (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703–718
- Santer B, Sommerwerk N, Grey J (2006) Food niches of cyclopoid copepods in eutrophic Plußsee determined by stable isotope analysis. *Arch Hydrobiol* 167:301–316
- Schlechtriem C, Focken U, Becker K (2004) Stable isotopes as a tool for nutrient assimilation studies in larval fish feeding on live food. *Aquat Ecology* 38:93–100
- Tamelaender T, Soreide JE, Hop H, Carroll ML (2006) Fractionation of stable isotopes in the Arctic marine copepod *Calanus glacialis*: effects on the isotopic composition of marine particulate organic matter. *J Exp Mar Biol Ecol* 333:231–240
- Vander Zanden M, Rasmussen J (2001) Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: implications for aquatic food web studies. *Limnol Oceanogr* 46:2061–2066
- Vanderklift M, Ponsard S (2003) Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichment: a meta-analysis. *Oecologia* 136:169–182
- Vuorio K, Meili M, Sarvala J (2006) Taxon-specific variation in the stable isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of lake phytoplankton. *Freshw Biol* 51:807–822
- Waser NAD, Harrison PJ, Nielsen B, Calvert SE, Turpin DH (1998) Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. *Limnol Oceanogr* 43:215–224