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Incorporation of nitrogen from N_2 fixation into amino acids of zooplankton

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Abstract

Eurytemora affinis (Copepoda) were fed ^{15}N-labeled *Rhodomonas salina* (Cryptophyta) or ^{15}N-labeled *Nodularia spumigena* (Cyanobacteria) in excess under controlled laboratory conditions. Zooplankton collected from the Baltic Sea were fed natural phytoplankton amended with ^{15}N-labeled *N. spumigena*. We quantified the direct incorporation of ^{15}N tracer from N_2-fixing *N. spumigena* (diazotroph nitrogen) and ammonium-utilizing *R. salina* into the amino acid nitrogen (AA-N) of zooplankton using complementary gas chromatography–combustion–isotope ratio mass spectrometry, gas chromatography–mass spectrometry, and elemental analysis–isotope ratio mass spectrometry approaches. Specific and mass-specific TN and AA-N incorporation rates of the ^{15}N tracers were calculated for zooplankton. Highest incorporation of ^{15}N was found in field zooplankton relying on *N. spumigena* and in *E. affinis* relying on *R. salina*. Lowest incorporation was found in *E. affinis* relying on *N. spumigena*. Decreasing specific and mass-specific rates during field experiments possibly were due to food shortage, whereas decreasing rates in *E. affinis* grazing on *R. salina* were more likely due to satiation. Specific and mass-specific rates were consistently low in *E. affinis* when exposed to *N. spumigena*, suggesting that these animals were reluctant to feed on *N. spumigena*. Essential isoleucine received most of the diazotroph nitrogen in field zooplankton, while nonessential amino acids received most ^{15}N tracer in *E. affinis*. *N. spumigena* was clearly an important amino acid nitrogen source for Baltic Sea zooplankton.

Global climate change and pronounced temperature increases in the oceans may affect the abundance of nitrogen-fixing organisms (diazotrophs) in the world's oceans, leading to N_2 fixation as an increasingly important component of oceanic primary production (Hutchins et al. 2007; Pearl and Huisman 2008). Cyanobacteria and other diazotrophs dominate warm, oligotrophic waters where the assimilation of atmospheric nitrogen is often the major source of new nitrogen for biological production (Karl et al. 1997; Tyrell 1999). Although a diverse community of N_2-fixing organisms are active in the ocean (Montoya et al. 2004; Moisander et al. 2010), we lack a good understanding of the fate of the newly fixed nitrogen in the ocean (Mulholland 2007). The movement of new nitrogen through the pelagic food web is a critical aspect of marine secondary production and one that may be especially sensitive to changes in cyanobacterial abundances associated with climate change (Hutchins et al. 2007; Pearl and Huisman 2008).

Unlike blooms of other primary producers such as diatoms and dinoflagellates, cyanobacterial blooms are generally thought to enter the food web via the microbial loop following lysis or remineralization of diazotroph particulate organic matter (Sellner 1997). Direct grazing of diazotrophs via zooplankton appears to be uncommon; however, some copepod species are known to graze on the filamentous cyanobacteria *Trichodesmium* and *Nodularia* (O'Neil et al. 1996; Koski et al. 2002). The factors regulating rates of diazotroph consumption are not well understood (O'Neil et al. 1996; Eberl and Carpenter 2007), although a number of factors, including toxicity, large size, and poor nutritional quality, tend to deter direct grazing by zooplankton (O'Neil 1999; Koski et al. 2002). In contrast to measurements of grazing rate, the nitrogen isotopic composition of zooplankton in regions with high diazotroph abundance implies substantial transfer of diazotroph nitrogen into the food web (Loick et al. 2007; Landrum et al. 2011). This is also confirmed by molecule-specific stable nitrogen isotope measurements in amino acids (AAs) by which diazotroph nitrogen was identified in essential and nonessential AAs of zooplankton from the tropical North Atlantic (McClelland et al. 2003). Although cyanobacteria are known to have a variety of negative effects on marine zooplankton, including reduced feeding, reduced egg production, and lethal effects due to toxicity, cyanobacteria may increase copepod fecundity by enhancing the nitrogen ration available to the animals (Schmidt and Jónasdóttir 1997; Koski et al. 2006). Food quality is often quantified in terms of lipid rather than nitrogen content, leading to classification of cyanobacteria as poor food sources (Porter and Orcutt 1980) despite their high protein content (Vargas et al. 1998).

Here we use a combination of laboratory and field studies to test the hypothesis that diazotrophic cyanobacteria are an important nitrogen source that supports amino acid metabolism in zooplankton. Specifically, we carried

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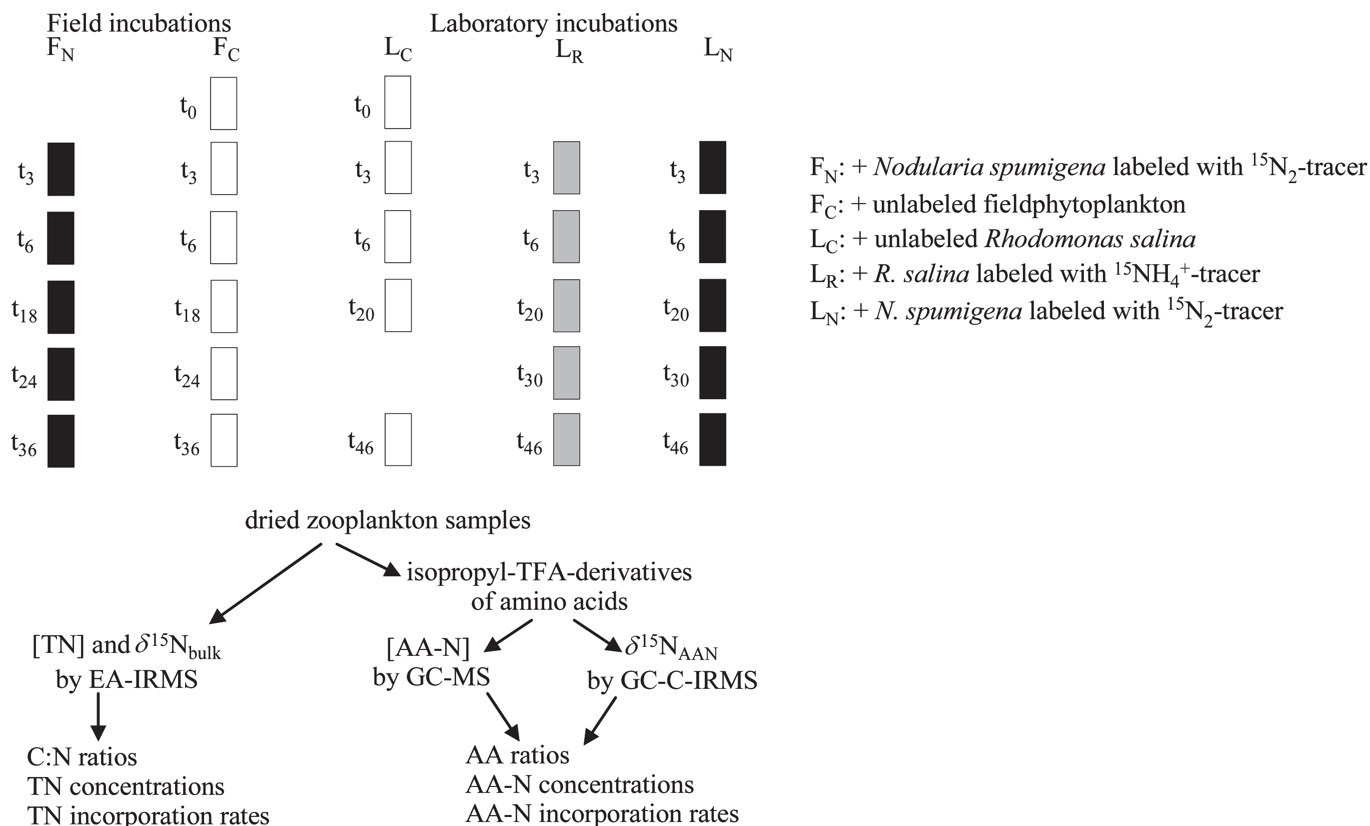

Fig. 1. Schematic description of the field and laboratory incubations and methodological processing of zooplankton samples after the experiments.

out laboratory experiments to measure the incorporation of $^{15}N_2$- and $^{15}N\text{-}NH_4^+$–labeled diet by a single copepod species. We used field-collected zooplankton from the Baltic Sea to quantify the rates of incorporation of diazotroph nitrogen into animal AAs. To our knowledge, these are the first direct measurements of the transfer of diazotroph nitrogen into amino acid nitrogen (AA-N) of mesozooplankton.

Methods

Laboratory experiments—We assessed the influence of food quality on nitrogen incorporation rates through grazing experiments with the copepod *Eurytemora affinis*. We used two different food types of different nutritional quality, which we expected to yield a range of incorporation rates. To test the incorporation of diazotroph nitrogen, Food Type I consisted of *Nodularia spumigena*, which has been described as poor food for zooplankton (Porter and Orcutt 1980). Food Type II consisted of *Rhodomonas salina*, which is routinely used as monodiet for zooplankton cultures. Control incubations were run with unlabeled *R. salina* as reference for the calculations as well as crosscheck that no tracer was spilled or affected the overall nitrogen content in the animals.

N. spumigena and *R. salina* cultures were labeled isotopically prior to the start of the grazing experiments by growth on $^{15}N_2$ or $^{15}NH_4^+$, respectively, as a sole nitrogen source. Cultures of *N. spumigena* were labeled for 2 weeks, while *R. salina* required only 48 h of incubation to acquire sufficient ^{15}N for our experiments to get a clear signal well above natural abundance levels in the grazers. The laboratory experiments were performed in three arrays of five 30-liter polypropylene incubation containers at 18°C and under dim illumination (Fig. 1).

Unlabeled *R. salina* cells were added to one group of containers, which served as experimental controls (L_C). A second set of containers received $^{15}N_2$-labeled *N. spumigena*, which was pre-filtered through a 10-μm Nitex mesh to remove detritus particles (L_N), and a third setup received $^{15}NH_4^+$-labeled *R. salina* (L_R) (Table 1; Fig. 1). The containers were initially filled with filtered (0.8 μm) seawater, and food was added to saturating levels (> 600 μg C L^{-1}) (Barthel 1983) at the start of the experiment and again after 24 h of incubation (Table 1).

Samples of particulate organic matter (POM) were collected from each container at the beginning and end of the incubations to monitor the concentration and isotopic composition of the food available during each grazing experiment (Table 1). Adult *E. affinis* used in our experiments were taken from a culture established from adults collected in the southern Baltic Sea and reared for three generations. The copepod culture was not fed for the 2 d prior to our experiment, resulting in suboptimal food conditions of 368 ± 64 μg carbon (C) L^{-1} prior to the experiment. Animal density in the experiments was

Table 1. Experimental conditions during the laboratory (L_C, L_R, L_N) and field (F_C, F_N) experiments. All treatments were done in single replications. Initial food concentrations are averages from six incubation containers per treatment at time point t_0, final food concentrations at t_x are averages from five incubation containers per treatment at five different time points t_3, t_6, t_{18}, t_{24}, and t_{36} during field experiments, and t_3, t_6, t_{20}, t_{30}, and t_{46} during laboratory experiments. Sal. = salinity, Temp. = temperature.

Treatment	Tracer	Sal.	Temp. (°C)	Starvation time (h)	Grazer	Diet	Initial food (μg C L^{-1})	Final food at t_x (μg C L^{-1})	C : N diet	C : N grazer
L_C	None	8	15	48	*E. affinis*	*R. salina*	650±173	533±55	6.7	5.2
L_R	^{15}N-NH_4^+	8	15	48	*E. affinis*	*R. salina*	876±75	774±97	6.8	4.5
L_N	$^{15}N_2$	8	15	48	*E. affinis*	*N. spumigena*	959±122	876±183	6.3	4.3
F_C	None	8	19.8	18	Field zooplankton	Field phytoplankton	402±39	124±39	8.2	5.3
F_N	$^{15}N_2$	8	19.8	18	Field zooplankton	Field phytoplankton and *N. spumigena*	1584±159	19±11	7.5(6.5*)	5.3

* C : N of *N. spumigena* culture.

~ 200 ind L^{-1}, which is in the upper range for natural *E. affinis* abundances in the Baltic, where maximum abundances can exceed 350 ind L^{-1}, or an average dry weight (dry wt) of 0.6 mg L^{-1} (Feike and Heerkloss 2009). A qualitative screening of the animals during the experiments by light microscope showed that the mortality rate was negligible. Over a 46-h time course, we collected samples at six time points (0, 3, 6, 20, 30, and 46 h) to assess zooplankton abundance and isotopic composition (Fig. 1). Animals were isolated by gentle filtration through 100-μm Nitex mesh and held in filtered seawater amended with unlabeled *R. salina* for half an hour to allow clearance of ^{15}N-labeled fecal pellets from their guts. The animals were then transferred to 10-μm Nitex mesh, dried at 60°C for 48 h, then stored over desiccant for later gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS), gas chromatography–mass spectrometry (GC-MS), and elemental analysis–isotope ratio mass spectrometry (EA-IRMS).

Field experiments—We also carried out experiments with field-collected zooplankton during the R/V *Poseidon* cruise 371 in the Baltic Sea in August 2008. Our grazing experiments allowed us to follow the transfer of diazotroph nitrogen into total nitrogen (TN) as well as essential and nonessential amino acid nitrogen (AA-N) of zooplankton. Animals were presented with two different food types, either the natural phytoplankton community that served as a control (Field$_{\text{Control}}$ = F_C) or phytoplankton amended with $^{15}N_2$-labeled *N. spumigena* (Field$_{Nodularia}$ = F_N) (Table 1; Fig. 1).

Plankton for the grazing experiments were collected at Sta. AB Boje (54°52′N, 13°50′E) on 22 August 2008. Net phytoplankton were collected in multiple surface hauls with a 10-μm net (30-cm net diameter) and resuspended in surface water. Zooplankton were collected with a conical WP2 net of 200-μm mesh size (circular 0.25-m^2 opening) that was towed vertically at a rate of 0.5 m s^{-1} through the upper 20 m of the water column. Animals were immediately transferred into two 100-liter containers filled with surface seawater. After 15 h, living animals were collected using a light trap and transferred to another 100-liter container filled with filtered seawater. This water was well mixed, and 2-liter aliquots were transferred into each of the ten 25-liter experimental containers for a final zooplankton concentration of 0.8 mg dry wt L^{-1}, which is comparable to the average value for natural mesozooplankton dry mass in the Arkona Sea in August (L. Postel pers. comm.). At the start of the experiment, animals were transferred into 25-liter polycarbonate carboys two-thirds filled with 0.8-μm-filtered surface water amended with either 200 mL of a suspension of net phytoplankton (> 10 μm) containing 95 μg chlorophyll *a* (Chl *a*) L^{-1} for the control. For the F_N experiments, 385 mL of an *N. spumigena* culture containing 295 μg Chl *a* L^{-1} was added. The *Nodularia* culture was pre-labeled by incubation with $^{15}N_2$ for 48 h prior to the experiments. The experimental containers were completely filled with filtered seawater after addition of the zooplankton and phytoplankton.

Zooplankton samples were collected at five time points (3, 6, 18, 24, and 36 h) by passing the entire contents of one container, including any detritus or sedimented phytoplankton, through a 200-μm net with a filtering cod end. The animals were then transferred into fresh filtered seawater to allow gut clearance, while the filtrate was used to determine final food concentration. Seston from gut clearance was quantified on pre-weighed, pre-combusted (450°C for 2 h) 4.5-mm glass fiber (GF) filters. Animals were not fed during this period, and some ^{15}N-labeled undigested food may have remained in the gut. After 30 min, the animals were collected on 10-μm-mesh pieces and then dried at 60°C for 48 h for later EA-MS, GC-C-IRMS, and GC-MS analysis.

POM samples were collected from each container at the beginning and end of each grazing experiment to monitor the concentrations of food particle and tracers (Table 1). The initial POM concentrations were measured by filtering 1 liter of sample water onto pre-combusted (450°C for 2 h) 2.5-mm GF filters before the animals were inserted. For the POM end values, the entire filtrate from the 200-μm zooplankton filtration was concentrated by first pouring it through a 10-μm Nitex mesh and then filtering it onto 10-μm Nitex filters. All samples were dried at 60°C and stored for analysis in the laboratory onshore.

Sample preparation and analysis—GF filters containing POM samples were wrapped in tin capsules for EA-IRMS analysis. POM samples from the end of our incubations were removed from the Nitex disks and weighed into tin capsules. This approach may have led to an underestimate of final POM concentrations.

Dried zooplankton samples were homogenized and weighed, then subsamples of 1 to 2 mg were used for the total δ^{15}N and TN concentration measurements by continuous-flow isotope mass spectrometry using a Flash EA 1112 elemental analyzer coupled to a Finnigan Delta Plus mass spectrometer via a Conflow II open split interface (Loick et al. 2007). All isotope abundances are expressed in δ notation as per mil deviations from the ^{15}N : ^{14}N ratio ($R_{reference}$) in atmospheric N_2:

$$\delta^{15}\mathrm{N} = \left(\frac{R_{sample}}{R_{reference}} - 1\right) \times 1000 \qquad (1)$$

with an overall analytical precision of 0.1‰.

For the analysis of AA concentrations and stable nitrogen isotope composition, subsamples of 10 to 30 mg of dry weight were prepared for GC-MS and GC-C-IRMS analysis by acid hydrolysis followed by derivatization to trifluoro-acetylated (TFA) isopropyl AA esters (Hofmann et al. 2003). During the initial hydrolysis, glutamine and asparagine are converted to glutamic and aspartic acids, respectively. As a result, our measurements of the concentration and δ^{15}N of glutamic and aspartic acids include contributions from glutamine and asparagines and we will refer to these mixtures as Glx and Asx, respectively. AA concentrations include hydrolyzable AAs from proteins as well as from free AA pools. For AA concentration measurements, isopropyl-TFA-derivates were separated by gas chromatography on an HP 6890 gas chromatograph (Hewlett Packard) equipped with a 5% phenyl column (BPX-5, 30 m × 0.32, 0.25 μm, Scientific Glass Engineering) and an HP 5973 mass selective detector. For quantification we used an internal standard (*trans*-4-(aminomethyl)-cyclohexanecarboxylic acid, Fluka) which was added to the samples before hydrolysis. The GC temperature program for each run was as follows: Initially 50°C, ramp at 15°C min^{-1} to 100°C, dwell for 10 min; ramp to 130°C at 3°C min^{-1}, ramp to 175°C at 2°C min^{-1}, dwell for 5 min; ramp to 220°C at 10°C min^{-1}; ramp to 300°C at 30°C min^{-1}, and dwell for 5 min. In all, 13 individual AAs were quantified, including the essential AAs threonine (Thr), valine (Val), serine (Ser), leucine (Leu), isoleucine (Ile), methionine (Met), phenylalanine (Phe), and lysine (Lys). The nonessential AAs quantified included alanine (Ala), glycine (Gly), proline (Pro), aspartic acid and asparagine (Asx), and glutamic acid and glutamine (Glx). We measured AA concentrations in all 14 laboratory samples but in only five field samples due to lack of material. Field samples included samples t_6 and t_{36} from the tracer setup F_N and samples t_6, t_{24}, and t_{36} from the control setup F_C.

AA-N concentrations were calculated from total AA concentrations. We assumed only one rather than two nitrogen atoms for Glx as well as for Asx. The sum of all measured AA-N was termed total amino acid nitrogen (TAA-N).

We measured AA-N stable isotopes by GC-C-IRMS using the same GC temperature program as for the GC-MS measurements. All GC-C-IRMS samples were analyzed twice, yielding good results for nine AAs including Ala, Gly, Leu, Ile, Pro, Asx, Glx, Phe, and Lys. The precision of our isotopic measurements varied among experiments, but the standard deviation of replicate measurements typically was on the order of 1‰ to 2‰.

Rate calculations—This study allowed us to calculate the movement of ^{15}N-tracers from a specific food source (*N. spumigena* and *R. salina*) into TN and AA-N of different zooplankton. The incorporation of nitrogen (N) from food into the TN pool of zooplankton can be expressed as transport rate V (h^{-1}) or as specific rate based upon the TN concentration of the sample ρ (μg N [100 μg N]$^{-1}$ h^{-1}). We calculated incorporation rates for the nonessential AAs Glx, Asx, Ala, Gly, and Pro, as well as for the essential AAs Lys, Leu, Ile, and Phe (Guillaume 1997) using the mass balance approach of Montoya et al. (1996):

$$\mathrm{V}(\mathrm{T}^{-1}) = \left(\frac{1}{\Delta t}\right)\left(\frac{A_{zoo_{tx}} - A_{zoo_{t0}}}{A_{phyto_{t0}} - A_{zoo_{t0}}}\right) \qquad (2)$$

where V represents the specific rate of nitrogen incorporation of ^{15}N from phytoplankton by zooplankton in the experiment, Δt is the duration of the experiment, and A is the absolute abundance of ^{15}N (atom%) from TN stable isotope measurements in zooplankton (zoo) and phytoplankton or cyanobacteria (phyto). The subscript t_0 indicates values at the start of the incubations, and t_x represents the following sampling points after 3, 6, 20, 30,

and 46 h for the laboratory experiments and for 3, 6, 18, 24, and 36 h for the field experiments. The starting ^{15}N values for phytoplankton were corrected for isotope dilution through grazing by using the exponential average of the starting and ending atom% enrichment of the phytoplankton pool after Glibert et al. (1982). The mass-specific rate of N incorporation ρ (mol N mg^{-1} T^{-1}) is then:

$$\rho(\text{mol N mg}^{-1}\ \text{T}^{-1}) = \text{V} \times \text{TN}_{\text{zoo}_{\text{tx}}} \quad (3)$$

where $\text{TN}_{\text{zoo}_{\text{tx}}}$ is the concentration of TN in zooplankton at time point t_x. We normalized our rates to animal nitrogen content (in μg N [100 μg N] h^{-1}) for easier comparison to prior studies (Cowie and Hedges 1992; Kleppel et al. 1998).

We calculated the flux of nitrogen into individual AAs by comparing the isotopic composition of individual AAs in zooplankton with that of the available total food:

$$\text{V}(\text{T}^{-1}) = \left(\frac{1}{\Delta \text{t}}\right)\left(\frac{\text{A}_{\text{amino acid}_{\text{tx}}} - \text{A}_{\text{amino acid}_{\text{t0}}}}{\text{A}_{\text{phyto}_{\text{t0}}} - \text{A}_{\text{amino acid}_{\text{t0}}}}\right) \quad (4)$$

We did not use the isotopic composition of individual AAs in the phytoplankton as the source term in our calculation because AA-N can be translocated via intracellular transamination processes inside the cells. Our approach does not allow us to distinguish whether individual AAs acquired their label through incorporation of intact AAs directly from the food or via internal reprocessing of ingested AAs. We then estimated the mass-specific rate of nitrogen incorporation into individual AAs as:

$$\rho(\text{mol N mg}^{-1}\ \text{T}^{-1}) = \text{V} \times \text{TN}_{\text{amino acid}_{\text{tx}}} \quad (5)$$

where $\text{TN}_{\text{amino acid}_{\text{tx}}}$ represents the concentration of nitrogen in a specific zooplankton AA at time point t_x. We calculated the specific AA-N incorporation rates (V) for all time points during L_R, L_N, and F_N. Mass-specific incorporation rates (ρ) of ^{15}N into AA-N were calculated for all sampling points from tracer setups L_N and L_R but only for t_6 and t_{36} from setup F_N due to a lack of sample material at the other sampling points.

Results

The laboratory experiments were conducted with a single copepod species, *E. affinis*, while the field experiments involved a diverse zooplankton community that was dominated by calanoid copepods of the genera *Centropages*, *Acartia*, *Pseudocalanus*, *Temora*, and cladocerans belonging to the genera *Evadne* and *Bosmina*. No large diazotrophs (> 10 μm) were observed in the natural phytoplankton, which mainly contained the Bacillariophyceae *Coscinodiscus* spp., *Chaetoceros* spp., and the Dinophycea *Ceratium* spp. In the field control F_C, the initial particulate organic carbon (POC) concentration was 402 ± 39 μg C L^{-1} and in the *Nodularia*-amended treatment F_N the POC concentration was 1584 ± 159 μg C L^{-1} (Table 1). Animals were not refed during field experiments and the average final POC concentrations from all time points of 124 ± 39 μg C L^{-1} in F_C and 19 ± 11 μg C L^{-1} in F_N reflect significant loss of phytoplankton material through grazing in all containers.

In the laboratory experiments with *R. salina* and *N. spumigena*, the POC concentration was approximately constant at levels above 600 μg C L^{-1} (Table 1). This was achieved by food replenishment after 24 h and intended avoidance of food limitation.

Seston collected from the filtered seawater in which the field zooplankton were allowed to clear their guts contained on average 4% nitrogen and 26% carbon (± 0.3, n = 10) and had C : N ratios of 7.9 ± 0.9 (n = 10). It made up in average 39% of TN and 58% of total carbon in zooplankton. The average δ^{15}N value of seston from F_N ranged from 99‰ to 156‰ and was always greater than the δ^{15}N of the zooplankton. It reflected the amount of ^{15}N tracer ingested from *N. spumigena* that was not assimilated by zooplankton. Seston from the unlabeled control had average δ^{15}N values of 5.1 ± 0.5‰.

C : N ratios, TN, and TAA-N concentrations of zooplankton—C : N ratios in zooplankton remained stable during all incubations and ranged from 4.2 to 5.5 during both field and laboratory studies, with some variation among treatments (Fig. 2a). The TN concentration ranged from 95.2 to 108.3 μg N (mg dry wt)$^{-1}$ in all samples (Fig. 2b).

Large increases in AA-N concentrations were apparent in the limited data set for field zooplankton, while AA-N concentrations stayed constant in L_C and L_R and tended to decrease in L_N (r^2 = 0.68, p = 0.08) (Fig. 2c). The AA-N concentrations from field animals increased from 33 μg N (mg dry wt)$^{-1}$ to 82 μg N (mg dry wt)$^{-1}$ in F_N and from 26 μg N (mg dry wt)$^{-1}$ to 51 μg N (mg dry wt)$^{-1}$ in F_C. These AA-N values correspond to total amino acid (TAA) values of 281 and 227 μg (mg dry wt)$^{-1}$ in F_N and F_C, respectively, at t_6 and to 711 and 443 μg (mg dry wt)$^{-1}$ in F_N and F_C, respectively, at t_{36}. TAA concentrations during the laboratory experiments were on average 418 ± 23 μg (mg dry wt)$^{-1}$ (n = 15) with a significantly lower value of 357 μg (mg dry wt)$^{-1}$ in L_N after 46 h (Fig. 2c).

Individual AA-N concentrations—We saw little variation in the concentration of individual AAs in the laboratory experiments (Fig. 3). In contrast AA-N from field animals was low at the beginning of the experiments and increased twofold in F_C and tripled in F_N after 36 h (Fig. 3). The relative abundance of individual AAs was generally similar in field and laboratory zooplankton. The AAs present in the highest concentrations included Glx-N, Lys-N, Asx-N, and Gly-N, while the lowest AA-N concentrations were found for Ile-N, Phe-N, and Met-N (Fig. 3). Ala was found in higher concentrations in *E. affinis* than in field zooplankton (average 6.7 ± 0.5 μg N [100 μg N]$^{-1}$ vs. average 4.0 ± 1.8 μg [100 μg N]$^{-1}$). Ser and Thr were partly not detectable in some of the animals.

^{15}N enrichment—The labeling of algae with ^{15}N tracer in the treatments L_R, L_N, and F_N substantially increased the δ^{15}N values of *R. salina* and *N. spumigena* above the natural δ^{15}N values of −5.3 ± 0.7‰ in *R. salina* and of −1.7‰ in

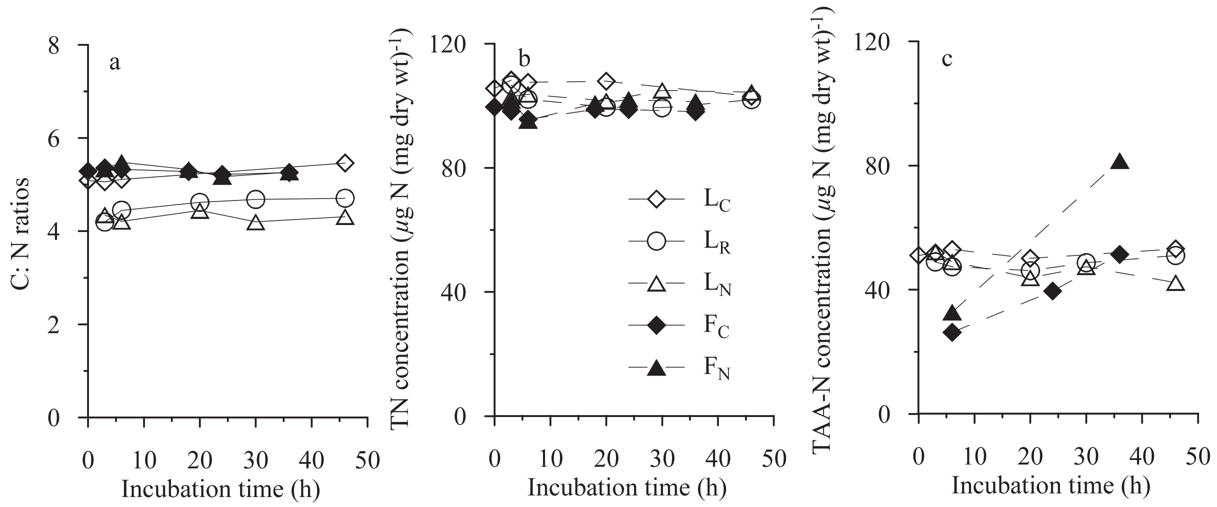

Fig. 2. (a) C : N ratios, (b) TN concentrations (in μg N [mg dry wt]$^{-1}$), and (c) TAA-N concentrations (in μg N [mg dry wt]$^{-1}$) of *E. affinis* in L_C, L_R, and L_N and of zooplankton in F_C and F_N.

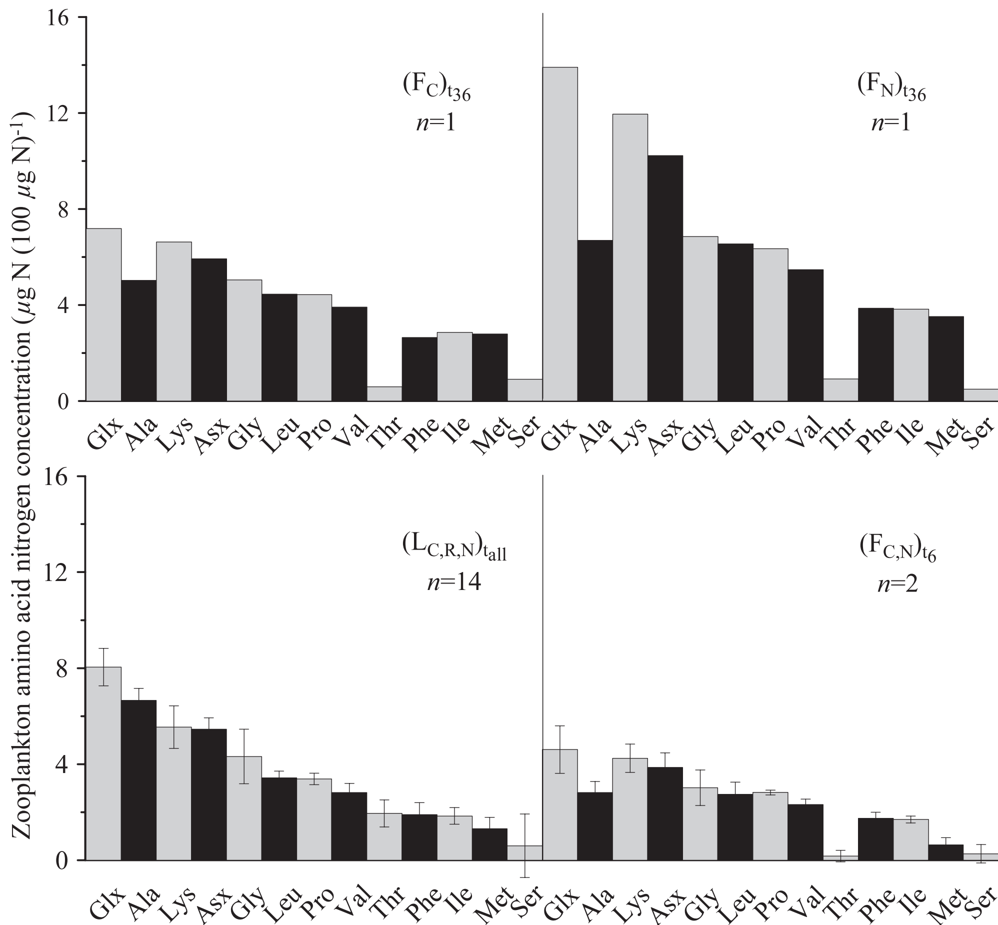

Fig. 3. AA-N concentrations (in μg N [100 μg N]$^{-1}$) of 13 AAs in zooplankton given as average from all time points of the laboratory incubations (L_C, L_R, L_N)$_{all}$, as average from both t_6 field incubations (F_C, F_N)$_{t6}$, and as single measurements from t_{36} for F_C and F_N, respectively. Glutamine and asparagine were converted to glutamic and aspartic acids during hydrolysis and are displaced as mixtures termed Glx and Asx, respectively.

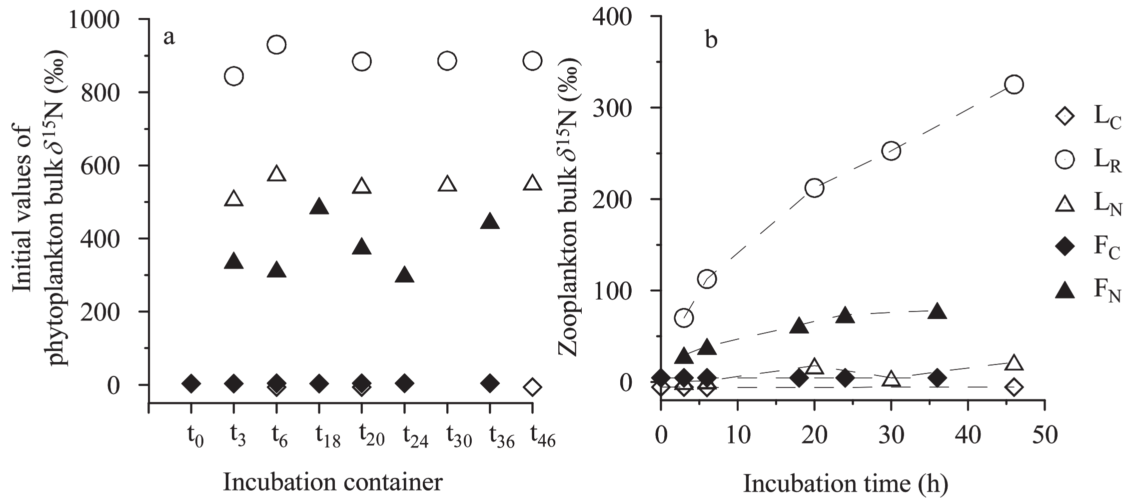

Fig. 4. (a) Initial bulk $\delta^{15}N$ values (in ‰) of the phytoplankton food from all treatments at the beginning of the experiments, (b) bulk $\delta^{15}N$ values (in ‰) of the zooplankton from all treatments during the experiments. Please note the different scales. The precision of the measurements was 0.1‰.

N. spumigena (Fig. 4a). When ^{15}N-labeled algae were fed to *E. affinis* in the laboratory, the $\delta^{15}N$ values of *E. affinis* increased significantly from -5.3 ± 0.7‰ to 325.34‰ ($r^2 = 0.93$; $p = 0.002$) in L_R and to 23.4‰ ($r^2 = 0.68$; $p = 0.04$) in L_N (Fig. 4b).

In the field experiments, ^{15}N-labeled *N. spumigena* had an average $\delta^{15}N$ value of 380 ± 84‰ and grazing on it significantly increased the $\delta^{15}N$ value of zooplankton from 4.4 ± 0.06‰ to 78‰ after 36 h ($r^2 = 0.86$; $p = 0.007$). In the control treatment, the $\delta^{15}N$ values of phytoplankton and zooplankton were similar with an average value of 4.7 ± 0.2‰ for phytoplankton and an average $\delta^{15}N$ value of 4.4 ± 0.06‰ for zooplankton.

The incorporation of ^{15}N was detected in nitrogen of all AAs, with AA-N $\delta^{15}N$ values increasing along with total $\delta^{15}N$ values. The maximum AA-N $\delta^{15}N$ values at the end of the experiments in L_R, L_N, and F_N, and the $\delta^{15}N$ values from the non-labeled controls L_C and F_C are shown in Fig. 5.

TN incorporation rates—A maximum specific TN incorporation rate of 0.03 h^{-1} occurred after 3 h in L_R and F_N, and both rates decreased to 0.01 h^{-1} and 0.008 h^{-1} after 46 and 36 h, respectively (Fig. 6). Specific TN incorporation in L_N was initially 0.005 h^{-1} and decreased to 0.0001 h^{-1} after 46 h. Rates in L_N were not significantly different from zero and have to be considered with care.

Mass-specific TN incorporation followed the same trend as the specific rates because nitrogen made up a relatively constant 10% of zooplankton dry weight (Fig. 6).

AA-N incorporation rates—The AA-N incorporation rates by *E. affinis* from *N. spumigena* were 10-fold lower than from *R. salina* with an average value of 0.0022 ±

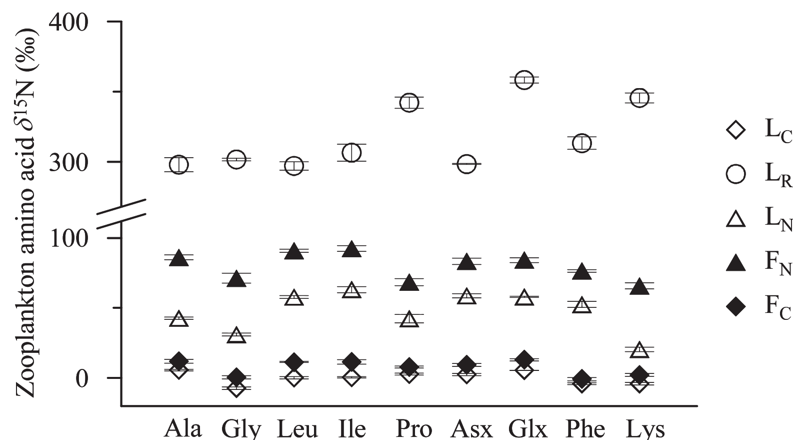

Fig. 5. Natural and labeled AA-N $\delta^{15}N$ values (in ‰) of the zooplankton from the non-tracer treatments L_C and F_C (given as average from all time points) and the ^{15}N-labeled treatments L_R, L_N, and F_N for the final time points (t_{46} and t_{36}, respectively). Glutamine and asparagine were converted to glutamic and aspartic acids during hydrolysis and are displaced as mixtures termed Glx and Asx, respectively.

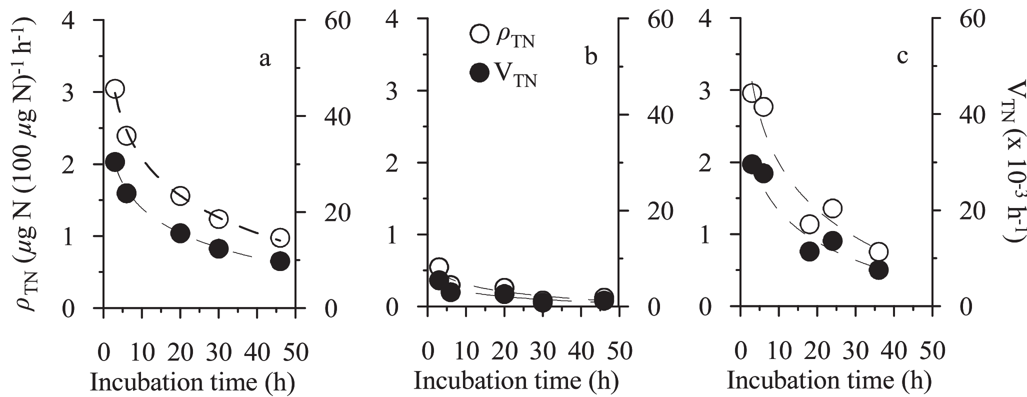

Fig. 6. Mass-specific TN incorporation rates (ρ_{TN}) in μg N (100 μg N)$^{-1}$ h^{-1} and nitrogen-specific TN incorporation rates (V_{TN}) per hour of zooplankton from (a) L_R, (b) L_N, and (c) F_N. Dotted lines indicate logarithmic decrease of TN incorporation rates. The error range associated with the mass-specific and the specific TN rates was 0.09 and 0.009, respectively.

0.0011 h^{-1} (Fig. 7). In the other treatments (L_R, F_N), the incorporation rates were initially high and decreased with time. The range of specific incorporation rates was similar in L_R and F_N, with clear differences among individual AAs. In treatment L_R, specific AA-N incorporation rates of 0.043 $\pm$ 0.001 h^{-1} were high for Ala and Glx, intermediate for Lys, Leu, Gly, Pro, Ile, and Phe (0.030 $\pm$ 0.004 h^{-1}), and low for nitrogen moving into Asx (0.015 h^{-1}; Fig. 7). In the field (F_N), zooplankton incorporated diazotroph nitrogen most intensively into Ile with a rate of 0.040 h^{-1}, while intermediate rates of 0.028 $\pm$ 0.003 h^{-1} were measured for Glx, Ala, Lys, Leu, Gly, Asx, and Phe. Pro, with a low rate of 0.018 h^{-1}, received the least nitrogen in field zooplankton (Fig. 7).

The limited data set for mass-specific rates in F_N showed considerably lower rates for Glx and Ala compared to L_R and only clearly higher rates for Asx (Fig. 8).

Discussion

Through a combination of laboratory and field experiments, we demonstrate that N_2-fixing cyanobacteria can be a direct source of nitrogen supporting the AA metabolism of zooplankton. The trophic transfer of this nitrogen likely depends, however, on the species composition of zooplankton, which has strong implications for the incorporation of essential and nonessential AAs into the body nitrogen of copepods.

The assimilation rate of food particles in copepods depends on a variety of factors, including the concentration, quality, size spectrum, nutritional quality of the food, as well as the feeding history and nutritional state of the animals (Mauchline 1998). Assimilation is described as the intestinal absorption of a molecule from digested food into a metabolic pool after food ingestion and before defecation (Mayzaud and Conover 1988). Incorporation means that an isotopic tracer like ^{15}N is followed after its assimilation from a labeled food into a biochemical end product like an AA (Roman 1991).

In our study, we found high incorporation rates of nitrogen from *R. salina* by *E. affinis* in the laboratory, but obtained contrasting results regarding the incorporation of diazotroph nitrogen of *N. spumigena* by *E. affinis* and a mixed-zooplankton assemblage. The high incorporation of TN and AA-N from *R. salina* is in accordance with its adequate size and nutritional quality for copepod growth (Klein Breteler et al. 1982). The low incorporation of nitrogen from *N. spumigena* by *E. affinis* in contrast to the mixed assemblage of copepods and cladocerans might reflect the reluctance of these species to feed on this cyanobacterium at high rates. Low feeding rates have been observed in earlier investigations, and the avoidance has been related to different abilities to feed size selectively on filaments or to discriminate against toxic strains among zooplankton (Sellner 1997). The size of *N. spumigena*, however (filament length: 100–200 μm), appeared generally suitable for the ingestion by *E. affinis* in our experiments and toxicity as the factor determining feeding acceptance is contradicted by other studies (Koski et al. 2002). The incubation time may also have been too short to stimulate feeding or to allow full physiological acclimation of digestive enzymes to the new food (Hassett and Landry 1988; Mayzaud et al. 1998). The reason for the low incorporation rates in *E. affinis* remains unresolved, as the study was not designed to evaluate the feeding behavior.

Despite the absence of evidence for *N. spumigena* assimilation by *E. affinis*, the detection of ^{15}N in the mixed-zooplankton assemblage emphasizes the direct utilization of diazotroph nitrogen by zooplankton. The ^{15}N incorporation rates were potentially overestimated, as the chosen time for the complete evacuation of gut contents defecation was short. However, the increase in ^{15}N with time despite the complete clearance of food, and the recovery of excreted ^{15}N in the filtered seawater suggest that the bodily ^{15}N was indeed incorporated. This clearly shows that *N. spumigena* was a source of AA-N for zooplankton in the Baltic Sea and strengthens earlier hypotheses that cyanobacteria supplement AAs to the diet of copepods (Schmidt and Jónasdóttir 1997; O'Neil 1999).

The initially high mass-specific TN incorporation rates around 3.0 μg N (100 μg N)$^{-1}$ from L_R and F_N in our study compare well with high rates of 1.2–3.25 μg N (mg dry wt)$^{-1}$ reported for *Acartia* spp. and *Pseudocalanus* spp. from the eastern Pacific coast of Hokkaido, Japan

Fig. 7. Specific nitrogen incorporation V ($\times$ 10^{-3} h^{-1}) in the AAs Glx, Ala, Lys, Leu, Gly, Pro, Ile, Asx, and Phe of zooplankton from treatments L_R, L_N, and F_N. The error range associated with the specific nitrogen incorporation into AA-N was 0.0002.

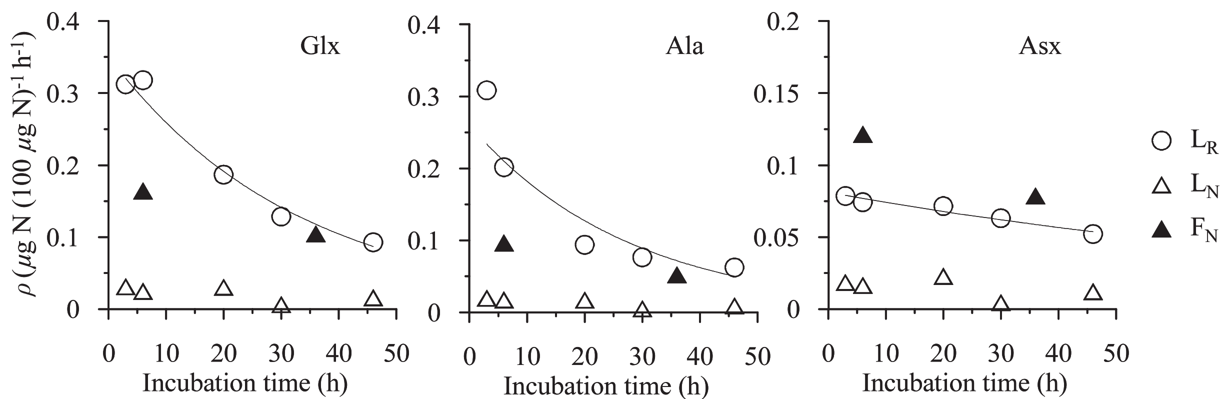

Fig. 8. Zooplankton mass-specific AA-N incorporation rates (ρ) in μg N (100 μg N)$^{-1}$ h^{-1} into the amino acids Glx, Ala, and Asx, in L_R, L_N, and F_N. Please note the different scales. The error range associated with the mass-specific AA-N rates was 0.0006.

(Hasegawa et al. 2001), for *Acartia discaudata* and *Calanus helgolandicus* feeding on diatoms during a laboratory study (Vincent et al. 2007), and for *Macrosetella gracilis* feeding on *Trichodesmium thiebautii* from the tropical North Atlantic (O'Neil et al. 1996). The exponential decrease of our rates compared to stable rates in Vincent et al. (2007) can be explained by methodology because animals were unsaturated with food when entering our experiments. The lower final rates between 0.8 and 1.0 μg N (100 μg N)$^{-1}$ in treatments L_R and F_N may point to a lower basic incorporation rate of nitrogen in brackish *E. affinis* and Baltic Sea zooplankton compared to the marine species. Copepods need AAs for growth and for osmoregulation (Goolish and Burton 1989) and lower basic TN mass-specific incorporation rates therefore may be related to lower growth rates or to lower requirements of AAs as osmotic solute.

The specific TN incorporation rates were initially high but decreased strongly with time, which likely was due to increased food clearance following starvation typically observed in starved copepods (Runge 1980). The low AA concentrations of zooplankton from the Baltic suggest that animals must have been severely starved at the beginning of our field experiments and replenished their bodily N by feeding on *N. spumigena*. Their AA content contributed 30% to their dry weight, which was much lower than the average of 48% found in laboratory *E. affinis* or reported for marine copepods (Steinberg and Saba 2008). Such low AA content likely indicates starvation and a dietary shortage of protein that cause a continuous removal of nitrogen from the free AA pool (Mayzaud and Conover 1988). In our study, the nitrogen deficiency was apparently rapidly compensated by the intense feeding as indicated by the depletion of the initially very abundant food concentration and the associated increase in TAA concentration. After 36 h TAA levels increased to 50% of TN in F_C and even to 80% of TN when *N. spumigena* was amended (Fig. 2c), which is explained by the higher concentration with which this cyanobacterium was offered in experiments.

In contrast to field zooplankton, body TAA concentrations in *E. affinis* were already high at the beginning of the laboratory experiment. This is explained by the optimal food conditions in culture prior to the experiments, and a less severe reduction of food to only suboptimal but not starvation levels during experimental preconditioning. Although incorporation rates of nitrogen in treatment L_R were as high as in treatment F_N, TAA concentrations remained stable (Fig. 3). This suggests a saturation level for body nitrogen, which is independent of nitrogen turnover. Assimilated AAs may not have been used to build up proteins but rather may have been used to synthesize nonprotein molecules such as carbohydrates or lipids (Sargent and Henderson 1986; Lehninger 1987), or for energy production via the oxidation of their carbon skeletons in the citrate cycle (Mayzaud and Conover 1988). Large parts of the AAs also may have been deaminated and the nitrogen excreted without making use of α-keto-acids in the energy production pathways (Mayzaud and Conover 1988). *N. spumigena*-nitrogen was incorporated by *E. affinis* at a 7 times lower rate (0.0026 h^{-1}) than *R. salina*-nitrogen. AA-N concentrations in L_N treatments tended to decrease after 36 h (Fig. 2c). This indicates degradation of body protein due to starvation.

To our knowledge, these are the first direct measurements of the specific rate of nitrogen incorporation into zooplankton proteins. The preferential incorporation of one AA over another, such as Ile over Pro in field zooplankton and Glx over Asp in *E. affinis*, may reflect the animals' physiological attempt to maintain or restore an optimal AA balance. Animals obtain the AAs for protein synthesis and energy production from their internal free AA pool, which is replenished from food sources by gut absorption or, in case of starvation, from tissue degradation (Mayzaud and Conover 1988). We found clear differences in mass-specific incorporation rates between zooplankton from L_R and F_N for Glx, Ala, and Asx, but not for Ile (Fig. 8). All of these AAs showed clear, and at times large, differences in specific incorporation rate (Fig. 7), suggesting that specific rates provide a more sensitive index to the movement of nitrogen into zooplankton biomass.

E. affinis had the highest demand for the nonessential AAs Ala and Glx, which were assimilated at maximum rates of 0.044 and 0.042 h^{-1}, respectively, and the lowest demand for Asx as indicated by a low rate of 0.015 h^{-1} (Fig. 7). Glutamic acid, glutamine, alanine, and aspartic acid are among the most abundant AAs in zooplankton (Cowie and Hedges 1992) and their molar ratios were between 11 and 16 : 1 in our samples. Before proteins from tissues are catabolized, crustaceans selectively dispose of nonessential AAs in the internal free AA pool, and should first catabolize AAs such as Ala, Glx, and Asx that enter the citric acid cycle via α-ketoglutarate, oxaloacetate, or pyruvate (Mayzaud and Conover 1988). The intense incorporation of Ala and Glx that we observed may therefore reflect Glx and Ala depletion in the free AA pool due to short time starvation in *E. affinis*, which was not severe enough to cause degradation of body proteins. However, this does not explain why Asx-N incorporation was so low. According to Campbell (1973), most L-AAs can undergo transamination reactions either via glutamate-pyruvate transaminase (GPT) or via glutamate-oxaloacetate transaminase (GOT). Aspartic acid and asparagine are transaminated by GOT, while Ala and Glx are transaminated by GPT (Mayzaud and Conover 1988). The low incorporation of ^{15}N into Asx therefore may have been the result of a lower activity of GOT compared to GPT.

Ile-N showed the highest incorporation rate in field zooplankton, suggesting that this AA was most limiting among the nine AAs for which AA-N rates were measured. Ile is an essential AA for crustaceans (Guillaume 1997) and an important component of muscular tissue (Lehninger 1987). The high initial rates we observed suggest muscle anabolism after severe starvation, which is also consistent with the low overall protein concentrations in these animals. The lowest incorporation in field animals was found for Pro-N (0.018 h^{-1}). Pro is a nonessential AA in

crustaceans that can be synthesized from glutamic acid (Lehninger 1987), allowing the animals to meet their needs independently of incorporation.

Specific incorporation rates were 10-fold lower in L_N, but it is interesting to note that all three treatments showed similar patterns of high, middle, and low incorporation rates among the AAs measured. As noted earlier, these rate ranges may have been set by aminotransferase activities, though more data are needed to test this idea.

This study allowed us to explore the incorporation kinetics of dietary nitrogen into AA-N of laboratory-reared and field-collected zooplankton with similar nitrogen content. Our data suggest that protein depletion in zooplankton may enhance the incorporation of diazotroph nitrogen. Further studies concentrating on the incorporation of nitrogen from globally significant diazotrophs such as *Trichodesmium* into zooplankton AAs are necessary to support the data presented here.

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References

Barthel, K.-G. 1983. Food uptake and growth efficiency of *Eurytemora affinis* (Copepoda: Calanoida). Mar. Biol. **74:** 269–274, doi:10.1007/BF00403450

Campbell, J. W. 1973. Nitrogen excretion, p. 279–316. *In* C. L. Prosser [ed.], Comparative animal physiology. W. B. Sanders.

Cowie, G. L., and J. I. Hedges. 1992. Sources and reactivities of amino acids in a coastal marine environment. Limnol. Oceanogr. **37:** 703–724, doi:10.4319/lo.1992.37.4.0703

Eberl, R., and E. J. Carpenter. 2007. Association of the copepod *Macrosetella gracilis* with the cyanobacterium *Trichodesmium* spp. in the North Pacific Gyre. Mar. Ecol. Prog. Ser. **333:** 205–212, doi:10.3354/meps333205

Feike, M., and R. Heerkloss. 2009. Does *Eurytemora affinis* (Copepoda) control the population growth of *Keratella cochlearis* (Rotifera) in the brackish water Darß-Zingst Lagoon (southern Baltic Sea)? J. Plankton Res. **31:** 571–576, doi:10.1093/plankt/fbp004

Glibert, P. M., F. Lipschultz, J. J. McCarthy, and M. A. Altabet. 1982. Isotope dilution model of uptake and remineralization of ammonium by marine plankton. Limnol. Oceanogr. **27:** 639–650, doi:10.4319/lo.1982.27.4.0639

Goolish, E. M., and R. S. Burton. 1989. Energetics of osmoregulation in an intertidal copepod: Effects of anoxia and lipid reserves on the pattern of free amino accumulation. Funct. Ecol. **3:** 81–89, doi:10.2307/2389678

Guillaume, J. 1997. Protein and amino acids, p. 26–50. *In* L. R. D'Abramo, D. E. Conklin, and D. M. Akiyama [eds.], Crustacean nutrition. Advances in world aquaculture. World Aquaculture Society.

Hasegawa, T., I. Koike, and H. Mukai. 2001. Fate of food nitrogen in marine copepods. Mar. Ecol. Prog. Ser. **210:** 167–174, doi:10.3354/meps210167

Hassett, R. P., and M. R. Landry. 1988. Short-term changes in feeding and digestion by the copepod *Calanus pacificus*. Mar. Biol. **99:** 63–74, doi:10.1007/BF00644978

Hofmann, D., M. Gehre, and K. Jung. 2003. Sample preparation techniques for the determination of natural $^{15}N/^{14}N$ variations in amino acids by gas chromatography combustion–isotope ratio mass spectrometry (GC-C-IRMS). Isotopes Environ. Health Stud. **39:** 233–244, doi:10.1080/1025601031000147630

Hutchins, D. A., F.-X. Fu, Y. Zhang, M. E. Warner, Y. Feng, K. Fortune, P. W. Bernhardt, and M. R. Mulholland. 2007. CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry. Limnol. Oceanogr. **52:** 1293–1304, doi:10.4319/lo.2007.52.4.1293

Karl, D., R. Letelier, L. Tupas, J. Dore, J. Christian, and D. Hebel. 1997. The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. Nature **388:** 533–538, doi:10.1038/41474

Klein Breteler, W. C. M., H. G. Fransz, and S. R. Gonzalez. 1982. Growth and development of four calanoid copepod species under experimental and natural conditions. Neth. J. Sea Res. **16:** 195–207, doi:10.1016/0077-7579(82)90030-8

Kleppel, G. S., C. A. Burkart, and L. Houchin. 1998. Nutrition and the regulation of egg production in the calanoid copepod *Acartia tonsa*. Limnol. Oceanogr. **43:** 1000–1007, doi:10.4319/lo.1998.43.5.1000

Koski, M., W. K. Breteler, N. Schogt, S. Gonzalez, and H. H. Jakobsen. 2006. Life-stage specific differences in exploitation of food mixtures: Diet mixing enhances copepod egg production but not juvenile development. J. Plankton Res. **28:** 919–936, doi:10.1093/plankt/fbl029

———, K. Schmidt, J. Engström-Öst, M. Viitasalo, S. Jónasdóttir, S. Repka, and K. Sivonenand. 2002. Calanoid copepods feed and produce eggs in the presence of toxic cyanobacteria *Nodularia spumigena*. Limnol. Oceanogr. **47:** 878–885, doi:10.4319/lo.2002.47.3.0878

Landrum, J. P., M. A. Altabet, and J. P. Montoya. 2011. Basin-scale distributions of stable nitrogen isotopes in the subtropical North Atlantic Ocean: Contribution of diazotroph nitrogen to particulate organic matter and mesozooplankton. Deep-Sea Res. I **5:** 615–625, doi:10.1016/j.dsr.2011.01.012

Lehninger, A. L. 1987. Prinzipien der Biochemie. De Gruyter, [Principles in biochemistry.]

Loick, N., J. W. Dippner, H. N. Doan, I. Liskow, and M. Voss. 2007. Pelagic nitrogen dynamics in the Vietnamese upwelling area according to stable nitrogen and carbon isotope data. Deep-Sea Res. I **54:** 596–607, doi:10.1016/j.dsr.2006.12.009

Mauchline, J. 1998. The biology of calanoid copepods. Academic Press.

Mayzaud, P., and R. J. Conover. 1988. O : N atomic ratio as a tool to describe zooplankton metabolism. Mar. Ecol. Prog. Ser. **45:** 289–302, doi:10.3354/meps045289

———, V. Tirelli, J. M. Bernard, and O. Roche-Mayzaud. 1998. The influence of food quality on the nutritional acclimation of the copepod *Acartia clausi*. J. Mar. Syst. **15:** 483–493, doi:10.1016/S0924-7963(97)00039-0

McClelland, J. W., C. M. Holl, and J. P. Montoya. 2003. Nitrogen sources to zooplankton in the Tropical North Atlantic: Stable isotope ratios of amino acids identify strong coupling to N_2-fixation. Deep-Sea Res. I **50:** 849–861, doi:10.1016/S0967-0637(03)00073-6

MOISANDER, P. H., AND OTHERS. 2010. Unicellular cyanobacterial distributions broaden the oceanic N_2 fixation domain. Science **327:** 1512–1514, doi:10.1126/science.1185468

MONTOYA, J. P., C. M. HOLL, J. P. ZEHR, A. HANSEN, T. A. VILLAREAL, AND D. G. CAPONE. 2004. High rates of N_2-fixation by unicellular diazotrophs in the oligotrophic Pacific. Nature **430:** 1027–1031, doi:10.1038/nature02824

———, M. VOSS, P. KAEHLER, AND D. G. CAPONE. 1996. A simple, high precision, high sensitivity tracer assay for dinitrogen fixation. Appl. Environ. Microbiol. **62:** 986–993.

MULHOLLAND, M. R. 2007. The fate of nitrogen fixed by diazotrophs in the ocean. Biogeosciences **4:** 37–51, doi:10.5194/bg-4-37-2007

O'NEIL, J. M. 1999. Grazer interactions with nitrogen-fixing marine cyanobacteria: Adaptation for N-acquisition? Bull. Inst. Oceanogr. **19:** 293–317.

———, P. M. METZLER, AND P. M. GLIBERT. 1996. Ingestion of $^{15}N_2$-labeled *Trichodesmium* spp. and ammonium regeneration by the harpacticoid copepod *Macrosetella gracilis*. Mar. Biol. **125:** 89–96, doi:10.1007/BF00350763

PEARL, H. W., AND J. HUISMAN. 2008. Climate. Blooms like it hot. Science **320:** 57–58, doi:10.1126/science.1155398

PORTER, K. G., AND J. D. J. ORCUTT. 1980. Nutritional adequacy, manageability, and toxicity as factors that determine the food quality of green and blue-green algae for *Daphnia*, p. 268–281. *In* W. C. Kerfoot [ed.], Evolution and ecology of zooplankton communities. Univ. Press of New England.

ROMAN, M. R. 1991. Pathways of carbon incorporation in marine copepods: Effects of developmental stage and food quantity. Limnol. Oceanogr. **36:** 796–807, doi:10.4319/lo.1991.36.4.0796

RUNGE, J. A. 1980. Effects of hunger and season on the feeding behavior of *Calanus pacificus*. Limnol. Oceanogr. **25:** 134–145, doi:10.4319/lo.1980.25.1.0134

SARGENT, J. R., AND R. J. HENDERSON. 1986. Lipids, p. 59–108. *In* E. D. S. Corner and S. C. M. O'Hara [eds.], The biological chemistry of marine copepods. Oxford Univ. Press.

SCHMIDT, K., AND S. JÓNASDÓTTIR. 1997. Nutritional quality of two cyanobacteria: How rich is 'poor' food? Mar. Ecol. Prog. Ser. **151:** 1–10, doi:10.3354/meps151001

SELLNER, K. G. 1997. Physiology, ecology, and toxic properties of marine cyanobacteria blooms. Limnol. Oceanogr. **42:** 1089–1104, doi:10.4319/lo.1997.42.5_part_2.1089

STEINBERG, D. K., AND G. K. SABA. 2008. Nitrogen consumption and metabolism in marine zooplankton, p. 1135–1196. *In* D. G. Capone, D. A. Bronk, M. R. Mulholland, and E. J. Carpenter [eds.], Nitrogen in the marine environment. Elsevier Academic Press.

TYRELL, T. 1999. The relative influences of nitrogen and phosphorus on oceanic primary production. Nature **400:** 525–531, doi:10.1038/22941

VARGAS, M. A., H. RODRíGUEZ, J. MORENO, H. OLIVARES, J. A. DEL CAMPO, J. RIVAS, AND M. G. GUERRERO. 1998. Biochemical composition and fatty acid content of filamentous nitrogen fixing cyanobacteria. J. Phycol. **34:** 812–817, doi:10.1046/j.1529-8817.1998.340812.x

VINCENT, D., AND OTHERS. 2007. Net and gross incorporation of nitrogen by marine copepods fed on ^{15}N-labeled diatoms: Methodology and trophic studies. J. Exp. Mar. Biol. Ecol. **352:** 295–305, doi:10.1016/j.jembe.2007.08.006

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