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Net and gross incorporation of nitrogen by marine copepods fed on ^{15}N-labelled diatoms: Methodology and trophic studies

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Abstract

The stable isotope of nitrogen (^{15}N) and an appropriate three-compartment model were used in two 24-h lasting feeding experiments to trace the flow of N through the copepod *Acartia discaudata* and *Calanus helgolandicus* fed on ^{15}N-labelled *Skeletonema costatum* and *Thalassiosira weissflogii*, respectively. Details of the labelling technique and principles of the computation of N transport rates are given. At the end of a single 24-h feeding period only about one third of the total amount of N ingested by *A. discaudata* was incorporated into the copepod's body N; we refer to this rate as net incorporation. Most of the N ingested was lost as ammonium (48% of total N ingested), followed by losses in the form of eggs+fecal pellets (13%) and dissolved organic N (DON, 9%). The sum of net incorporation and the latter losses is defined as gross incorporation. Net incorporation by *C. helgolandicus* and N losses did not vary over time during a 24 h lasting time-series feeding experiment. On average, 79% of total N ingested was actually incorporated by the copepod whereas mean N losses as ammonium, eggs+fecal pellets represented only 12 and 9%, respectively. After a 24-h feeding period only 2% of N ingested was lost as DON. Inspection of individual DON pathways showed that both *A. discaudata* and *C. helgolandicus* highly contributed to total DON production via direct excretion (79 and 64%, respectively). The remaining DON appearing in the DON pool was derived from phytoplankton via direct release and/or indirect release (copepod 'sloppy feeding').

Keywords: Copepods; Nitrogen cycling; N gross incorporation; N net incorporation; ^{15}N tracer

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1. Introduction

N cycling driven by phytoplankton metabolism has been extensively studied using the stable isotope ^{15}N (Dugdale and Goering, 1967; Glibert et al., 1982; Bronk and Glibert, 1991; Slawyk and Raimbault, 1995). This isotope has also been used to trace various N fluxes generated by zooplankton metabolism and has provided further information on how phytoplanktonic N is transformed into zooplanktonic N and into dissolved inorganic N (DIN) and organic N (DON) excreted by zooplankton (O'Neil et al., 1996; Hasegawa et al., 2000a, b, c). Working on natural plankton assemblages and on copepods (*Acartia* sp.), Hasegawa et al. (2000a, b, c; 2001a, b) quantified ^{15}N accumulation efficiencies and determined the significance of plankton grazing in DON release. However, the fate of ^{15}N-labelled food ingested by copepods has not yet been studied in detail. To fully assess the role of copepods in N cycling it is essential to quantify the amount of N that is actually incorporated into copepods as well as the amount of N lost as ammonium (NH_4^+), DON, eggs and fecal pellets. Furthermore, it is important to determine the contribution of copepods through direct excretion and/or 'sloppy feeding' (Lampert, 1978) to total DON production.

To address these questions, we have used ^{15}N isotopic labelling in two feeding experiments to trace N transport between *Skeletonema costatum* and *Acartia discaudata* and between *Thalassiosira weissflogii* and *Calanus helgolandicus* as well as transport between the two copepods and their surrounding medium. The latter species were particularly abundant in our study areas. *A. discaudata* dominates, simultaneously with *S. costatum*, summer plankton assemblages in Arcachon Bay (Vincent 2002; Vincent et al., 2002). *Calanus* is representative of high-latitude pelagic ecosystems and encountered in the Western English Channel during spring blooms of diatoms (e.g. *Thalassiosira* sp.; Sournia and Birrien, 1995). Our specific objectives are (1) to define the significance of N fluxes involved in copepod feeding, (2) to determine the amount of N incorporated in copepods (net N incorporation), (3) to estimate the amount of N lost to the ambient medium but that passed through the copepods, (4) to evaluate gross incorporation of N in copepods (net N incorporation + N losses), and (5) to estimate the contribution of diatoms (via direct release and/or 'sloppy feeding') and copepods (via direct excretion) to DON production. The results demonstrate that our ^{15}N approach is suitable for studies on copepod ecology and provides new insights into the role played by copepods in N cycling.

2. Materials and methods

2.1. Phytoplankton ^{15}N labelling

S. costatum and *T. weissflogii* were maintained in monospecific and axenic conditions in a f/2 medium

Table 1
Experimental conditions of feeding and DON excretion experiments carried out with two copepods fed on ^{15}N-labelled diatoms

Copepod/diatom		*A. discaudata*/*S. costatum*	*C. helgolandicus*/*T. weissflogii*
Copepod sampling site		Arcachon Bay (Southwestern Atlantic)	Western English Channel (Brittany coast)
Rearing period	Duration (month)	1–2	
	Temperature (°C)	15	
	Light:dark cycle (h)	12:12	
Feeding exp.	Carboy volume (L)	6.1	1.2
	Number of copepods	70	24
	Male:female ratio[a]	2:5	1:5
	Food density (x 10^3 cell ml^{-1})[b]	11.5±0.2	3.2±0.2
	Food concentration (μg N ml^{-1})	0.13	0.27
	Incubation duration (h)	24	6–12–18–24
	Temperature (°C)	15	15
	Light:dark cycle (h)	12:12	Dark
DON excretion exp.	Carboy volume (L)	7	1.2
	Number of copepods	150	24
	Male:female[a]	2:5	1:5
	Incubation (h)	24	24
	Temperature (°C)	15	15
	Light:dark cycle (h)	12:12	Dark

[a] Same ratio as for ^{15}N analyses.
[b] Mean±SD, equivalent to maximum ingestion rates determined in preliminary experiments.

(Guillard and Ryther, 1962) at 20 °C under a photon flux density of 123 μmol photons m^{-2} s^{-1} with a 12: 12-h light: dark cycle. Cells in exponential growth phase were inoculated into f/2 medium spiked with $Na^{15}NO_3$ (NO_3^- sole N source; 99% ^{15}N) and then grown in batch-mode for 6 to 10 days. This ensured sufficient ^{15}N isotopic excess enrichment (up to 37.5%) and uniform ^{15}N labelling of the cells (Hitchcock, 1983).

2.2. Feeding experiments

Experimental conditions are summarized in Table 1. Adult stages of *A. discaudata* and *C. helgolandicus* were collected by net tows (200-μm mesh size) and reared for 1–2 months under laboratory conditions. Only CVI stages were used in our experiments to ensure that N incorporation into copepods only contributed to egg production and not to somatic growth.

Experiments with *A. discaudata* (CVI stage) were carried out with two glass carboys equipped with 63-μm mesh chambers. The inner chambers prevented grazing on eggs by the incubated adults (e.g. Bonnet et al., 2004) and coprophagy (Paffenhöfer and Knowles, 1979) which both could lead to underestimate egg and fecal pellet production.

A control and an incubation carboy were filled with 0.2-μm filtered seawater and ^{15}N-labelled cells of *S. costatum* were added. The copepods were rinsed with 0.2-μm filtered seawater and placed inside the chamber of the incubation carboy at a population density and a male:female ratio similar to the one encountered in Arcachon Bay.

C. helgolandicus was used to perform a time series experiment. Adult stages (CVI) were first acclimated in 0.2-μm filtered seawater in the dark for 1 h to allow gut content evacuation. Cells of ^{15}N-labelled *T. weissflogii* were concentrated on a 0.4-μm polycarbonate membrane (Nuclepore), rinsed with 0.2-μm filtered seawater and then re-suspended in twenty-four polycarbonate bottles filled with 0.2-μm filtered seawater. Copepods were added individually to half of these bottles (3 for each time point) at a given density and male:female ratio (Table 1). They were fed for one day and sampled at regular intervals. The other half consisted of control bottles (3 for each time point) containing *T. weissflogii* but no copepods which were sampled at the same time intervals as for bottles with copepods. Contrary to the experiment with *A. discaudata*, the *C. helgolandicus* time series experiment was carried out in the dark to prevent as much as possible uptake of $^{14}NO_3^-$ and hence, to minimize changes in ^{15}N at.% enrichment of phytoplankton during feeding (Hasegawa et al., 2001a).

At the end of each feeding period, triplicate 500-ml samples from the control were filtered on 25-mm Whatman GF/F pre-combusted (4 h, 450 °C) glass-fibre filters to recover phytoplanktonic particulate organic N (PON). PON as well as NH_4^+ and DON in the filtrates were analysed for ^{15}N content according to Slawyk and Raimbault (1995) and Raimbault et al. (1999). The copepods in the incubation carboy were concentrated on 63-μm (*A. discaudata*) and 200 μm (*C. helgolandicus*) mesh sieve, and rinsed with 0.2-μm filtered seawater. Mortality was assessed by direct observation under a binocular microscope. Living individuals (20 and 8 in triplicate for *A. discaudata* and *C. helgolandicus*, respectively) were sorted by sex (same male:female ratio as the one applied to initiate feeding), placed on pre-combusted 25-mm GF/C glass-fibre filters and dried at 60 °C for later isotopic analyses. Remaining seawater was sampled and analysed as for controls. For the *C. helgolandicus* experiment, the total amount of fecal pellets and eggs produced was collected on a 63-μm mesh sieve, re-suspended in 0.2-μm filtered seawater and dried at 60 °C on pre-combusted 25-mm GF/C glass-fibre filters. The filters were treated as described above.

2.3. Copepod DON excretion experiments

Adult stages of *A. discaudata* and *C. helgolandicus* were enriched with ^{15}N by feeding them respectively on ^{15}N-labelled *S. costatum* and *T. weissflogii* for one day at the same food density as for the feeding experiments (Table 1). Living ^{15}N-labelled individuals were then collected on a 200-μm mesh sieve, rinsed with 0.2-μm filtered seawater and sorted under a binocular microscope. Only healthy individuals (e.g. actively swimming) were transferred to three enclosures filled with 0.2-μm filtered seawater. No algal food was added to avoid possible ^{15}N recycling via copepod $^{15}NH_4^+$ excretion and subsequent $^{15}NH_4^+$ uptake and $DO^{15}N$ release by phytoplankton. At the end of incubation, copepods and <GF/F filtrates were sampled for isotopic analyses as described above. All samples were in triplicate.

2.4. Chemical and isotopic measurements

Ammonium concentrations were determined in duplicate 100-ml samples according to Koroleff (1969). Analyses of ^{15}N isotopic abundance and total N content in particulate and dissolved compartments were performed on a continuous flow mass spectrometer (INTEGRA-CN, Sercon). Measurement precision (i.e. standard deviation, $n=10$) of natural abundance of a

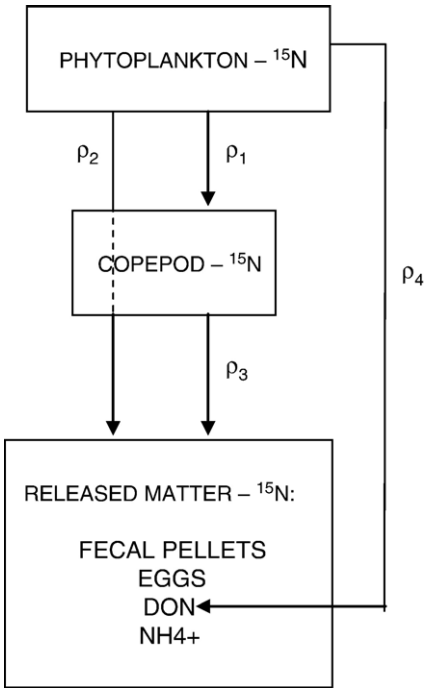

Fig. 1. Three-compartment model of nitrogen (N) cycling driven by copepods fed on ^{15}N-labelled phytoplankton. Arrows represent transport of ^{15}N-labelled nitrogen between source pools (phytoplankton, copepod) and target pools (copepod, released matter); see also in Table 2. Depicted are phytoplankton N net incorporation by copepods (ρ_1), phytoplankton N loss by copepods as particulate organic nitrogen (fecal pellets, eggs), dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (NH_4^+) (ρ_2), production of N as fecal pellets and eggs and excretion of N as DON and NH_4^+ by copepods (ρ_3), and DON release by phytoplankton through direct release and/or 'sloppy feeding' (ρ_4). Gross incorporation by copepods equals $\rho_1+\rho_2$. For rate calculations and units, see Materials and methods section.

glycine standard (0.3675 atom% ^{15}N) containing 2 μmol N was 0.0003.

2.5. Significance of N fluxes and rate calculations

At the end of a feeding period excess amounts of tracer are expected to be found in three main pools (Fig. 1): phytoplankton, copepods and released matter (RM) derived from copepods and phytoplankton. Transport of N from ^{15}N-labelled source pools into initially unlabelled target pools (Table 2) were calculated using an equation based on general principles of tracer methodology (Sheppard, 1962):

$$\rho = \left\{ \frac{R_T}{(\overline{R}_S\ T\ n_{\mathrm{ind}})} \right\} [\mathrm{N}]_T \qquad (1)$$

where R_T and $\overline{R}_s$ (average over the course of experiment) are respectively the ^{15}N at.% excess enrichments in the target and source pool, T the time (h), n_{ind} the number of copepods per enclosure and $[\mathrm{N}]_T$ the final concentration of N in the target pool. ρ has units of μg N ind^{-1} h^{-1}.

N transport rates (ρ) depicted in Fig. 1 are defined as follows: ρ_1 is the net incorporation rate of N by copepods without specifying if the N incorporated corresponds to N temporarily retained in the gut and/or assimilated into body compounds, ρ_2 represents the rate of phytoplanktonic N that passed through the copepods but was ultimately lost in the form of dissolved (inorganic and organic) and particulate organic matter, $\rho_1+\rho_2$ equals the gross incorporation rate of N, ρ_3 corresponds to the rate of N excretion of dissolved (inorganic and organic) and particulate organic matter by copepods, and ρ_4 is the rate of total DON release by phytoplankton via direct and/or copepod-mediated release ('sloppy feeding'; Lampert, 1978). Note that to calculate this latter rate the variable n_{ind} in Eq. (1) (number of copepods) is superfluous; DON release has then units of μg N volume^{-1} d^{-1}. Furthermore, in rate calculations for ρ_3 and ρ_4 we assumed that the ^{15}N enrichment in the source pool of copepods and phytoplankton is close to the average ^{15}N enrichment of total particulate N in copepods and phytoplankton, respectively (Slawyk et al., 1998).

Regarding the calculation of DON loss rates (ρ_2), DON excretion by copepods (ρ_3) and total DON release by phytoplankton (ρ_4), an additional problem arises from the fact that ^{15}N-labelled DON recovered in the total DON pool (see Fig. 1) may stem from both DO^{15}N in phytoplankton and copepods. Direct phytoplankton DON release was measured according to Slawyk et al. (2000) by incubating *T. weissflogii* (not done with *S. costatum*) without its corresponding grazer. DON

Table 2
^{15}N-labelled source and target pools used to calculate N transport rates depicted in Fig. 1

Transport rate (mass ind^{-1} h^{-1})	ρ_1	ρ_2	ρ_3	ρ_4
Source	Phytoplankton	Phytoplankton	Copepod	Phytoplankton
Target	Copepod	RM [a]	RM	DON

[a] RM (=Released Matter) designates three possible target pools: fecal pellets+eggs, DON and NH_4^+.

excretion by *A. discaudata* and *C. helgolandicus* (ρ_3) was obtained from incubations without food addition. To calculate DON loss rates (ρ_2) we estimated the amount of ^{15}N-labelled DON in the target pool due to copepods from the rates of DON excretion of unfed copepods (ρ_3, see above). In this latter case we assumed that unfed copepods excreted at least as much DON as fed ones. Total DON released by phytoplankton via direct release and/or 'sloppy feeding' (ρ_4) was computed by subtracting the amount of ^{15}N-labelled DON stemmed from unfed copepods from the total amount of ^{15}N in the DON pool.

3. Results

^{15}N at.% excess enrichment in particulate and dissolved pools sampled at the end of various feeding periods ranged from 0.1 to 16.0 and from 1.9 to 46.4 for the *S. costatum–A. discaudata* and the *T. weissflogii–C. helgolandicus* experiment, respectively (Table 3). Corresponding total N contents ranged from 25.5 to 1445.9 and from 7.4 to 414.2 μg carboy^{-1}, respectively.

Results from a ^{15}N inventory of the *S. costatum–A. discaudata* experiment are shown in Table 4. The total amount of ^{15}N tracer recovered after 24 h of feeding was 89.9%. Almost all of the tracer initially added was accounted for in the N food pool (81.5%) whereas only 2.3 and 6.1% were found in copepods and released matter (RM), respectively. Total amounts of ^{15}N label recovered during the *T. weissflogii–C. helgolandicus* time-series experiment, obtained by adding up ^{15}N of individual N pools, ranged from 89.5 (T18) to 104.5% (T6) of tracer added with a mean (±SD) of 96.5±6.7% (Fig. 2). The percentage of tracer added in phytoplankton decreased from 100 (T0) to 46.4 (T24) whereas it increased for the same period from 0 to 31.3 and from 0 to 15.2 in copepods and released matter (RM), respectively.

Table 4
Isotopic mass balance for *S. costatum–A. discaudata* experiment: amount of ^{15}N label (mean±SD) added at the beginning of the experiment and recovered after a 24-h feeding period

		^{15}N (μg carboy^{-1})[a]	% of tracer added
^{15}N added as	Food (*S. costatum*)	83.4±2.0	
^{15}N label recovered in			
	Food (*S. costatum*)	67.9±3.1	81.5
	Copepods (*A. discaudata*)	1.9±0.4	2.3
	RM[b]	5.0	6.1
Total tracer recovered		74.8	89.9

[a] Carboy volume=6.1 L.
[b] RM = released matter (NH_4^++DON+eggs+fecal pellets), see Fig. 1.

Table 3
Final ^{15}N atom percent excess enrichments and N contents in samples recovered from particulate and dissolved pools from two feeding experiments

	Pools	at.% excess	N content (μg carboy^{-1})[a]
Particulate	Phytoplankton		
	S. costatum	16.0±0.7	424.6±5.6
	T. weissflogii	31.1–37.5	124.4–244.2
	Copepods		
	A. discaudata	7.3±0.1	26.9±5.6
	C. helgolandicus	3.3–12.0	250.8–414.2
	Fecal pellets+eggs		
	A. discaudata	3.6[b]	25.5[b]
	C. helgolandicus	5.5–28.5	7.4–17.7
Dissolved	NH_4^+		
	A. discaudata	1.6±0.4	175.5±24.9
	C. helgolandicus	9.8–46.4	7.6–30.2
	DON		
	A. discaudata	0.1[c]	1445.9[c]
	C. helgolandicus	1.9–3.5	178.6–226.9

Values from the *S. costatum–A. discaudata* experiment correspond to means (±SD) obtained after a single 24-h feeding period whereas those from the *T. weissflogii–C. helgolandicus* experiment correspond to ranges obtained from a 6–24-h lasting time-series experiment. For volumes filtered and number of copepods per sample see Materials and methods section.
[a] Carboy volume=1.2 L and 6.1 L for *C. helgolandicus* and *A. discaudata*, respectively.
[b] Single measurement on total amount of fecal pellets and eggs.
[c] Single measurement.

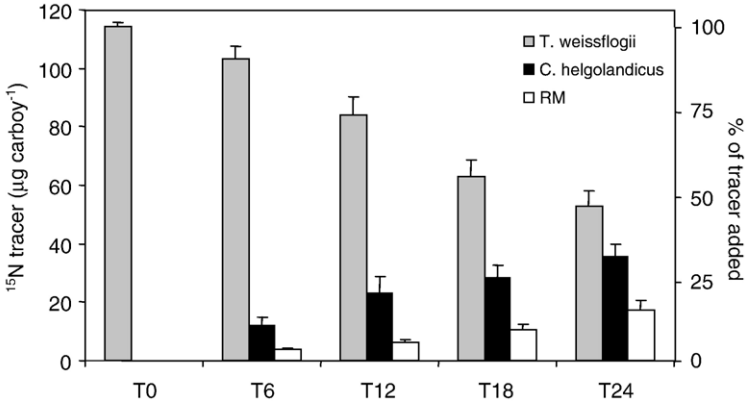

Fig. 2. Isotopic mass balance from the *T. weissflogii–C. helgolandicus* time-series experiment: amount (µg carboy^{-1}) of ^{15}N label added as phytoplankton at the beginning of the experiment (T0) and amount of ^{15}N label recovered in particulate and dissolved pools at 6-h intervals over a 24-h feeding period. RM stands for released matter (NH_4^++DON+fecal pellets+eggs; see Fig. 1).

Mean (±SD) net incorporation of *A. discaudata* during feeding was 0.007±0.002 µg N ind^{-1} h^{-1} which corresponded to 30.4% of gross incorporation and to a daily ration of 28.5% body N d^{-1} (Table 5). Mean loss rates ranged from 0.002 to 0.011 µg N ind^{-1} h^{-1}. Almost half of total phytoplanktonic N ingested (47.8% of gross incorporation) was lost as NH_4^+, followed by N losses as fecal pellets+eggs (13.0%) and DON (8.7%). Expressed as daily rations, the latter losses represented 44.7, 12.2 and 8.1% body N d^{-1}, respectively.

Net incorporation rates of *C. helgolandicus* did not significantly vary over time with a mean rate (±SD) of 0.221±0.021 µg N ind^{-1} h^{-1} (Table 6, Kruskal Wallis test, $p>0.4$). The same holds in respect of N losses: NH_4^+ along with fecal pellets+eggs showed mean rates (±SD) of 0.033±0.017 and 0.025±0.004 µg N ind^{-1} h^{-1}, respectively. N loss as DON (only estimated in the 24-h feeding sample) was on average (±SD) 0.005± 0.0002 µg N ind^{-1} h^{-1}. In terms of N accumulation efficiency, *C. helgolandicus* retained on average 79.1% of N ingested (% of gross incorporation), the remainder was essentially lost as NH_4^+ (11.6%) and fecal pellets+eggs (8.8%). In the 24-h sample only 1.8% of the total N ingested was lost as DON. Net incorporation by this copepod was equivalent to a mean daily ration of 34.2% body N d^{-1} whereas losses as NH_4^+ and fecal pellets+eggs represented only 5.1 and 3.9% body N d^{-1}, respectively.

Table 7 shows an attempt to partition DON fluxes with their corresponding rates during a 24-h feeding period. The bulk of DON produced resulted from copepod DON excretion: 79.1 and 64.3% of total for *A. discaudata* and *C. helgolandicus*, respectively. The remaining was essentially associated with phytoplankton via copepod mediated release ('sloppy feeding'): 20.9 and 35.7% for the *S. costatum–A. discaudata* and the *T. weissflogii–C. helgolandicus*, respectively. Direct DON release by *T. weissflogii* (not measured for *S. costatum*) represented only 8% of total DON production.

Table 5
Net and gross incorporation rates and loss rates of nitrogen (mean± SD) obtained from a 24-h experiment with *Acartia discaudata* fed on *Skeletonema costatum*

N transport	Transport rate (µg N ind^{-1} h^{-1})	% of gross incorporation
Net incorporation (1) (ρ_1) [a]	0.007±0.002 [28.5]	30.4
NH_4^+ loss (2) ($\rho_{2-NH_4^+}$)	0.011±0.003 [44.7]	47.8
FP+egg loss (3) ($\rho_{2-FP-egg}$)	0.003 [b] [12.2]	13.0
DON loss (4) (ρ_{2-DON})	0.002±0.0001 [8.1]	8.7
Gross incorporation $\sum$ (1–4)	0.023 [93.6]	

Values in brackets are daily rations (% body N d^{-1}) based on the average N content of 0.59 µg copepod^{-1}.
[a] ρ in parentheses refers to N transport shown in Fig. 1.
[b] single measurement on total amount of fecal pellets and eggs.

4. Discussion

Rates of ingestion, excretion, fecal pellets and egg production were often measured individually (e.g. Mauchline, 1998). The isotopic method presented here allows to measure these rates simultaneously by

Table 6
Net and gross incorporation rates and loss rates of nitrogen (mean±SD) obtained from a time-series experiment with *C. helgolandicus* fed on *T. weissflogii*

N transport (μg N ind^{-1} h^{-1})	T6	T12	T18	T24	Mean (T6–T24)
Net incorporation (ρ_1)[a]	0.229±0.042 (82.0)	0.246±0.059 (84.6)	0.212±0.025 (78.9)	0.198±0.023 (71.0)	0.221±0.021 (79.1) [34.2]
NH_4^+ loss ($\rho_{2-NH_4^+}$)	0.020±0.004 (7.1)	0.021±0.005 (7.1)	0.033±0.017 (12.1)	0.056±0.017 (20.1)	0.033±0.017 (11.6) [5.1]
FP+egg loss ($\rho_{2-FP-egg}$)	0.030±0.006 (10.9)	0.024±0.002 (8.3)	0.024±0.004 (9.0)	0.020±0.001 (7.1)	0.025±0.004 (8.8) [3.9]
DON loss (ρ_{2-DON})	nm[b]	nm	nm	0.005±0.0002 (1.8) [0.8]	
Gross incorporation $\sum$ (1–4)	0.279±0.04	0.291±0.06	0.269±0.04	0.279±0.04	0.280±0.045 [43.4]

Values in parentheses are in % of gross incorporation. Values in brackets are daily rations (% body N d^{-1}) based on the average N content of 15.5 μg copepod^{-1}.
[a] ρ in parentheses refers to N transport shown in Fig. 1.
[b] Not measured.

inspecting in the same sample, food, copepods and their released metabolic products for N content and ^{15}N excess enrichment. Data from literature on copepod metabolism such as NH_4^+ excretion (Gaudy et al., 1996; 2000; Miller and Glibert, 1998; Ikeda et al., 2001), fecal pellet production (Butler and Dam, 1994) and egg production (Cervetto et al., 1993; Rey-Rassat et al., 2002; Dam and Lopes, 2003) are generally based on direct chemical measurements and therefore are not comparable with our N loss data although both reflect the same process. It should be emphasized again that phytoplankton is the N source pool in our N loss measurements. In direct chemical measurements of their metabolic activity, copepods themselves are the N

Table 7
Contribution of phytoplankton DON release, 'sloppy feeding' and copepod DON excretion to total daily DON production (mean±SD)

	1	2	3	4	5
	Phytoplankton release[a]	Copepod excretion[b]	Phytoplankton+sloppy feeding[c]	Sloppy feeding (3−1)	Total release (2+3)
A. discaudata/S. costatum[d]					
μg N carboy^{-1} d^{-1}	nm[e]	14.99±0.28	3.95±0.21	–	18.94
μg N ind^{-1} d^{-1}		0.10±0.001			
% total release		79.1	20.9		
C. helgolandicus/T. weissflogii[f]					
μg N carboy^{-1} d^{-1}	1.99±0.04	15.96±0.66	8.86±0.39	6.87±0.35	24.82±0.83
μg N ind^{-1} d^{-1}		0.67±0.03			
% total release	8.0	64.3	35.7	27.7	

[a] Measured in controls (without copepods).
[b] Obtained from incubations with non fed animals (ρ_3, Fig. 1).
[c] Corresponds to total DON release by phytoplankton (ρ_4, see in Materials and methods) via direct release and 'sloppy feeding' corrected for DO^{15}N contribution of copepods estimated from DON excretion of unfed animals.
[d] 150 individuals in 7 L.
[e] nm = not measured.
[f] 24 individuals in 1.2 L.

source pool (see Fig. 1, Table 2). In other words, N loss data are referring to phytoplankton N whereas data obtained from direct chemical measurements are referring to the copepods' N.

With regard to our experimental protocol, isotopic enrichment and total N content of inspected pools were well above the lower detection limit of our mass spectrometer. This indicates that the initial ^{15}N abundance in *S. costatum* and *T. weissflogii*, the amount of copepods, fecal pellets and eggs sampled at the end of each incubation interval were suitable for the purpose of N transport rate measurements. Only fecal pellets and eggs had to be pooled to ensure sufficient N content.

We evaluated our data for ^{15}N-mass balance, i.e. we checked if the amount of ^{15}N added as food (phytoplankton) at the beginning of the feeding experiment is recovered at the end in the particulate (phytoplankton, copepods, fecal pellets + eggs) and dissolved (NH_4^+, DON) fractions. Tracer inventories show that mass balance of ^{15}N label was virtually achieved for the *T. weissflogii–C. helgolandicus* experiment as on average 97% of ^{15}N was recovered (Fig. 2). Individual values of ^{15}N recovery were within the range of those published for ^{15}N inventories of phytoplankton uptake experiments (Bronk and Glibert, 1994; Slawyk and Raimbault, 1995) and zooplankton feeding experiments (Hasegawa et al., 2001a): 85–111% of ^{15}N initially added. Closer examination of the data reveals that final accounting of ^{15}N label was more difficult to achieve in samples incubated for >12 h. In those samples, ~10% of the ^{15}N added remained undiscovered at the end of the experiment. The same held with respect of the 24-h incubation with the *S. costatum–A. discaudata* experiment. Theoretically, longer grazing durations should result in more balanced label distributions between source and target pools, but they could create new problems such as copepod mortality and label recycling and increase bottle containment effects (Wilkerson and Dugdale, 1992; Slawyk and Raimbault, 1995). For example, incomplete ^{15}N recovery could be due to phytoplanktonic adherence onto the walls of the polycarbonate bottles and glass carboys (Wilkerson and Dugdale, 1992). However, this latter loss of $PO^{15}N$ in the sample would not change the ^{15}N atom% excess enrichment in the phytoplanktonic source pool and thus would not affect N transport rate calculations. According to our ^{15}N inventory obtained from the *C. helgolandicus–T. weissflogii* experiment (Fig. 2), feeding durations of ≤12 h and ^{15}N atom% excess enrichments in the food N of ≥30% seemed to be appropriate for optimal ^{15}N recovery.

We have introduced the terms 'net incorporation' and 'gross incorporation' in order to clearly distinguish between the amount of food N that is actually incorporated into the copepods for growth, maintenance and reproduction purposes and the total amount of food N ingested by the copepods including N fractions ultimately lost to the medium. Net incorporation rates expressed as percentage of gross incorporation (accumulation efficiency) are known to vary a lot, even for the same species. This can be seen from Hasegawa et al.'s (2001a) percent success in N accumulation values (PSA) obtained from *Acartia* sp. (9.1 to 45%) fed on natural assemblages. In our experiments, *A. discaudata* lost about two thirds of the food N ingested and recycled most of it as NH_4^+. On the contrary, *C. helgolandicus* retained more than two thirds of the food N ingested and constantly maintained high net incorporation rates during the entire feeding period, even though the N food: N copepod ratio in experimental samples was ~10 times lower for *C. helgolandicus* than for *A. discaudata*.

Under our experimental conditions with 24-hour grazing durations, *C. helgolandicus* and *A. discaudata* both primarily lost NH_4^+ (20 and 48% of gross incorporation, respectively). N from fecal pellets + eggs represented the second most important N loss (7 and 13% of total) followed by N losses as DON (2 and 9% of total). *A. discaudata* wasted high amounts of N since N losses (~70% of total, essentially as NH_4^+) largely exceeded N net incorporation (~30% of total). *C. helgolandicus* incorporated almost three times as much N as *A. discaudata* (~80 *vs.* 30% of total N) but lost much less N (29% of total N as NH_4^+, DON, fecal pellets + eggs) than *A. discaudata* (~70% of total N). Large differences among copepods in the % of N intake lost as NH_4^+ are apparently not rare. For instance, feeding on natural prey, *Acartia* sp. lost 25–91% of total N ingested as NH_4^+ (Hasegawa et al., 2000a; 2001a). Seeing that *C. helgolandicus* is much bigger in body size than *A. discaudata*, the former seemed to be less active than the latter, at least with regards to N metabolism (Ikeda et al., 2001). Differences in food quality, especially N cell content, may also explain the distinct pattern of N utilization of our two copepods. In general, the more prey is rich in N the more time copepods give to gut transit (Jones and Flynn, 2005; Mitra and Flynn, 2005). In our experiments, *T. weissflogii* (food for *C. helgolandicus*) has a eight times higher N cell content than *S. costatum* (food for *A. discaudata)* so that gut transit may have lasted longer for the former than for the latter.

Our net N incorporation rates are not comparable with ingestion rates found in the literature which are traditionally measured by estimating prey (cells, Chl-a) disappearance expressed in terms of N (Frost, 1972;

Marin et al., 1986). Only our gross incorporation rates are comparable with ingestion rates since they represent the total amount of phytoplanktonic N that entered the copepods regardless of its fate (incorporated or finally lost). Mean gross incorporation rates obtained from *A. discaudata* and *C. helgolandicus* (0.023 and 0.280 μg N ind^{-1} h^{-1}, respectively) are within the same range as the ingestion rates of marine copepods obtained from laboratory and field studies (Abou Debs, 1984; O'Neil et al., 1996). Using the ^{15}N tracer, Hasegawa et al. (2001a) reported ingestion rates of 7 to 140 nmol N (mg dry weight)$^{-1}$ h^{-1} for copepods (*Acartia* sp. and *Pseudocalanus* sp.) fed on natural prey assemblages. Assuming that copepod N content was 10% of body dry weight (Båmstedt, 1986), our gross incorporation rates were 278.5 and 129.0 nmol N (mg dry weight)$^{-1}$ h^{-1} for *A. discaudata* and *C. helgolandicus*, respectively, which are close to the upper limit given by Hasegawa et al. (2001a).

N daily rations are further valuable units for deriving information on copepod nutrition. As for comparisons between our N incorporation rates and ingestion rates from other workers (see above), only daily rations derived from gross incorporation rates are comparable with corresponding literature data. Daily rations for *A. discaudata* (93.6% body N day^{-1}) and *C. helgolandicus* (43.3%) are congruent with literature data for both species (Deason, 1980; Gaudy, 1989; Hassett and Landry, 1990; Pagano and Saint-Jean, 1994). Although corresponding values for net incorporation are similar for both species (~30% body N d^{-1}), *A. discaudata* required >50% of its body N to meet its daily N losses (NH_4^+, fecal pellets and eggs, Table 5) while *C. helgolandicus* only needed ~10% to cover all its N losses (Table 6). As mentioned above, differences in food quality and body size probably explain differences in metabolic behaviour of the two copepod species.

However, we must bear in mind that our incubation conditions only approximated *in situ* conditions. Monospecific phytoplankton diet, unusual for omnivorous copepods, may have altered N net ingestion and N loss rates of our copepods. Copepods strongly respond to the quantity and nutritional quality of food by either increasing or decreasing ingestion rates (Mitra and Flynn, 2005). Therefore, absolute values of N incorporation and loss rates of our two copepod species should not be interpreted straightforwardly as species specific *per se*.

The ^{15}N tracer has been used extensively to trace release of DON derived from phytoplankton via direct release and/or microzooplankton grazing activity (Bronk and Glibert, 1991; Slawyk and Raimbault, 1995; Hasegawa et al., 2000a, b, c; 2001b). In this study we have attempted to partition the contribution of copepods and phytoplankton to DON production during a 24-h feeding period. Direct DON excretion by *A. discaudata* and *C. helgolandicus* was the major process in DON production (79.1 and 64.3% of total DON produced, respectively), although we do not know what kind of DON compound (amino-acids, urea) is actually excreted (Berman and Bronk, 2003; Frangoulis et al., 2005; Urban-Rich et al., 2006). Note that these estimates are based on the assumption that feeding animals excrete DON at the same rate as non-feeding ones. Data on phytoplanktonic DON production are generally allocated to 'sloppy feeding' and/or direct release (Bronk and Glibert, 1991; Hasegawa et al., 2000a, b, c; Bronk and Ward, 2005) but evaluations of the relative contribution of the two processes are scarce (Hasegawa et al., 2000a, b, c). Our data from the *T. weissflogii*−*C. helgolandicus* experiment show that DON derived from 'sloppy feeding' is about half the DON excretion by the copepods themselves, i.e. about one third of total DON production. On the other hand, direct phytoplankton release seemed to be of minor importance in DON supply. Data from natural plankton communities (Hasegawa et al., 2000a, b; 2001b), although obtained with different ^{15}N tracer methodologies, show similar trends: (1) the contribution of phytoplankton to DON production was the lowest among the examined plankton assemblages, (2) copepods actively contributed to DON accumulation through direct DON excretion, and (3) 'sloppy feeding' was an important mechanism responsible for DON release.

5. Conclusion

The ^{15}N approach we have described allows to qualify and to quantify N transfers during copepod feeding. The distinction between net and gross incorporation led to balance the copepod's gain against losses of N. We encourage workers to use ^{15}N-labelled substrates together with our model of N-transport calculations in copepod feeding experiments although *in situ* conditions of the trophic environment, especially quality and quantity of food offered, are at present, still difficult to replicate in the laboratory. One further effective approach would be to run feeding experiments with ^{13}C and ^{15}N-labelled food. Dual labelling work is in progress in our laboratory but is still hampered by the lack of a suitable procedure for the measurement of respiration using the ^{13}C isotope. Our approach combined with ^{13}C labelling would offer an attractive addition or alternative to available traditional methods for measurements on

zooplankton feeding and could significantly advance our understanding of C and N cycling driven by copepods in marine systems.

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