Fate of food nitrogen in marine copepods

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ABSTRACT: The stable isotope of nitrogen, ^{15}N, was used to evaluate the nitrogen accumulation efficiency of copepods feeding on natural plankton assemblages from coastal waters over the period March to November 1998. Although allowances have to be made for selective feeding, the clearance rates calculated from the accumulation of ^{15}N were considerably lower than the rate of removal of the particles labeled with [15]N. The percentage of accumulation/removal varied from 9 to 75%. In 3 out of 5 experiments, the copepods released dissolved nitrogen from their food rather than accumulating it. It appears that, at least on some occasions, copepods may transfer much of the particulate organic nitrogen to the dissolved form, and thus short-circuit the food chain from phytoplankton to carnivores.

KEY WORDS: Copepods · Nitrogen · [15]N tracer

INTRODUCTION

In aquatic systems, mesozooplankton play an important role in nutrient cycles. They not only regenerate nutrients (Lehman 1980, Paffenhöfer & Gardner 1984), but also control nutrient regeneration in the system through grazing on protozoa that are also nutrient regenerators (Glibert et al. 1992, Miller et al. 1995). Consequently, mesozooplankton strongly influence primary production, although their production is also limited by available food quality and quantity (Checkley 1980, Kiørboe 1989, Jónasdóttir 1994).

'The primary driving force of all animals is the necessity of finding the right kind of food and enough of it' (Elton 1927). Mesozooplankton ingest food for growth, maintenance and reproduction. However, not all the nutritional content of food can be used for these purposes, i.e. some foods, including certain algae, are hardly digested and many are ejected as feces (Van Donk et al. 1997). Additionally, before entry to the digestive tract, some fractions of food are lost to the surrounding water by 'sloppy feeding' (Lampert 1978, Roy et al. 1989). Therefore, it is important to evaluate

the amount of food that is actually incorporated into the mesozooplankton.

In a marine ecosystem, one of the key nutrients limiting biological production is nitrogen (Ryther & Dunstan 1971, Checkley 1985), and the representative mesozooplankton in most marine environments are calanoid copepods. To estimate the nitrogen flux from prey to mesozooplankton, the stable isotope ^{15}N is a useful tool, allowing nitrogen flow to be directly traced. In this study, we applied a ^{15}N tracer technique to address the nitrogen flow from food to copepods, and evaluated the role of copepods in the marine nitrogen cycle.

MATERIALS AND METHODS

Incubation experiments. Surface seawater was sampled at a station (water depth, 13 m: 43° 01′ N, 144° 52′ E) in Akkeshi Bay, located on the eastern Pacific coast of Hokkaido, Japan. The bay has about a 12 km wide opening, but due to a line of shallow ridges and islands the bay is quite well isolated from the outer ocean. Annual net primary production in this bay is estimated to be about 300 g C m^{-2}, rather higher than in other bays of similar latitude (Motoda et al. 1977).

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Table 1. Dates and conditions of experiments and NH_4^+ regeneration rate by copepods. Values in parentheses represent range

Date	Incubation temperature (°C)	Incubation time (h)	Species	Copepods added Dry weight (mg l^{-1})	C/N ratio (atom/atom)	NH_4^+ regeneration rate nmol (mg dry wt)$^{-1}$ h^{-1}	n
12 Mar	2	12	*Acartia* spp.	0.314–0.449	5.9	18(8-23)	3
28 May	10	12	*Acartia* spp.	0.504–0.580	5.5	70(40-100)	3
2 Jul	13	12	*Acartia* spp.	0.549–0.675	5.2	46(28-69)	3
27 Aug	18	6	*Pseudocalanus* sp.	0.273–0.338	5.2	67(36-99)	2
12 Nov	9	12	*Paracalanus* sp.	0.448–0.472	4.6	31(29-34)	2

The spring and fall blooms consist of large diatoms (Taguchi et al. 1994). Copepods dominate the meso-zooplankton in the Bay (Motoda et al. 1977).

Samplings were done on 9 March, 25 May, 29 June, 24 August and 10 November 1998. The water was screened through a 94 µm mesh net to remove large zooplankton and poured into a 20 l carboy to which $^{15}NH_4^+$ (99.0 atom% ^{15}N) was added to a final concentration of about 0.5 µm. The samples were incubated for 2 to 3 d on a 15:9 h light/dark cycle to label the POM with ^{15}N. The incubation temperature was controlled by immersion in running seawater which was 2 to 3°C above ambient temperature.

Copepods were collected in the morning (on the day of experiments) with vertical tows (bottom to surface) of a 330 µm mesh net with a 0.45 m diameter opening. After the tow, the contents of the cod end were diluted with surface seawater. In the shore laboratory, copepods were sorted under a dissecting microscope, rinsed with filtered seawater (GF/F) and stored in filtered seawater (GF/F) for 3 h. Copepods used in these experiments were at late copepodite stages of the species dominating the mesozooplankton at the sampling time.

The pre-incubated seawater was divided into six to eight 1 l polycarbonate bottles. Cold NH_4^+ (final conc. ca 8 µM) and PO_4^{3-} (final conc. ca 1 µM) were added to each bottle to minimize the variability of availability of nutrients for each experiment. Copepods were added (feeding) to half of the bottles, the remainder acted as the control. Experimental conditions are listed in Table 1. One bottle from each treatment was taken for analysis at time zero.

At the beginning of the experiment, the content of the time zero bottle was screened with 193 µm mesh net to remove copepods (screened only for the feeding bottles and not the control bottles) and then a portion (600 to 800 ml) was filtered through a Whatman GF/F filter (47 mm). The GF/F filtrates were frozen in 10 ml test tubes (for later NH_4^+ analysis). The filter with particulate organic matter (POM) was frozen until analysis for its nitrogen contents and its isotopic ratio.

Copepods were collected on a 12 µm Nuclepore filter (25 mm), rinsed with filtered seawater (GF/F), transferred to a tin capsule and frozen until analysis for dry weight, nitrogen content and nitrogen isotope ratio. For chlorophyll a (chl *a*) analysis, a subsample (50 to 100 ml) was filtered through a GF/F filter (25 mm) and the filter was frozen for later analysis. The GF/F filters were pre-combusted at 450°C for 3 h and all bottles were acid washed and rinsed thoroughly with distilled water prior to use. Vacuum-filtrations were done at <50 mm Hg to minimize disruption of cells. Samples were incubated in the dark and the bottles were gently shaken at 0.5 to 2 h intervals to keep the contents well mixed. At the end of the experiment, identical sampling treatments for each incubation bottle were performed.

Chemical and isotope analysis. The nitrogen isotope ratio and nitrogen content in particulate organic nitrogen (PON) and copepods were analyzed using a continuous flow mass spectrometer (Tracermass, Europa Scientific) equipped with a CN analyzer (Roboprep-CN, Europa Scientific) (Kanda et al. 1998). The percentage of ^{15}N-nitrogen to total (^{15}N plus ^{14}N) is denoted as atom% of nitrogen. The copepods were lyophilized and dry weights were determined on a Cahn microbalance (model 29) prior to analysis. Sodium L-glutamate monohydrate (0.366 atom% ^{15}N) and glycine (1.66 to 4.51 atom% ^{15}N) were used as the standard samples. The standard deviation for atom% of L-glutamate monohydrate (1 µmol N) was below 0.0012 (n = 6). NH_4^+ concentrations were determined with a Technicon autoanalyzer (Strickland & Parsons 1972). Chl *a* was determined by the fluorometric method of Strickland & Parsons (1972) as modified by Suzuki & Ishimaru (1990) using a Turner Designs fluorometer.

Calculations. Nitrogen flow mediated by copepods is schematically presented in Fig. 1. When copepods eat food tagged with ^{15}N, a part of the food ^{15}N is taken up into the copepods' body, but some fractions may be lost into surrounding water as dissolved or particulate forms. Some portion of food ^{15}N is excreted as dissolved (mainly NH_4^+; Corner & Newell 1967) or partic-

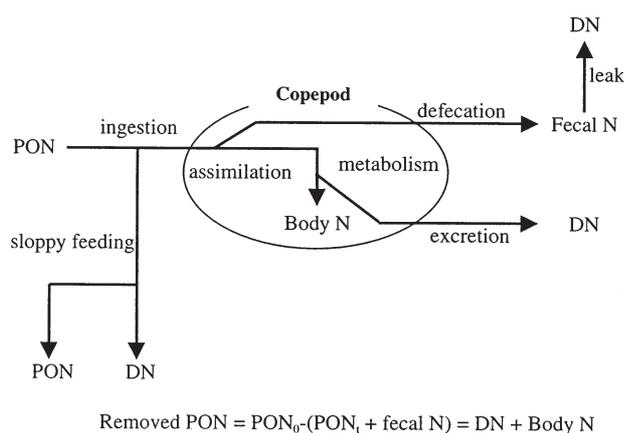Removed PON = $PON_0 - (PON_t + \text{fecal N}) = DN + \text{Body N}$

Fig. 1. Schematic representation of the nitrogen flux driven by copepods. PON: particulate organic nitrogen, DN: dissolved nitrogen, Body N: body nitrogen of copepod, Fecal N: fecal pellet nitrogen, PON_0 and PON_t: PON at the beginning and the end of incubation, respectively

ulate (fecal pellets) forms. The sum of ^{15}N accumulation in copepods and ^{15}N loss of dissolved forms is equal to the ^{15}N removal from the particulate fraction by copepods. In this study, we define percent success in accumulation (PSA) as the percentage of the accumulation into the body of the removal.

We evaluated the possible difference between ^{15}N accumulation into copepods and the PO^{15}N removal by copepods by calculating 2 independent weight-specific net clearance rate [ml (mg dry wt)$^{-1}$ h^{-1}] measurements. The clearance rate for accumulation is derived from Burns & Rigler (1967) with ^{15}N substituted for ^{32}P and with a correction for excess ^{15}N concentration because of a longer incubation period (6 to 12 h), and denoted by F:

$$F = \frac{C_t \cdot N_{\text{cope}} \cdot V}{^{15}N_{\text{PON}} \cdot W \cdot t} \tag{1}$$

where N_{cope} is concentration of copepods-N (μm); C_t is excess ^{15}N atom% of copepods at the end of incubation (excess ^{15}N atom% is ^{15}N atom% in each compartment minus ^{15}N atom% in copepods at time zero); W is dry weight of the copepods added (mg); t is the duration of incubation (h). V is volume (ml) of incubation; $^{15}N_{\text{PON}}$ is average excess ^{15}N concentration of PON during incubation period in each bottle. Then,

$$\overline{^{15}N_{\text{PON}}} = \frac{P_{ft} \cdot N_{ft} - P_{f0} \cdot N_{f0}}{\ln(P_{ft} \cdot N_{ft})/(P_{f0} \cdot N_{f0})} \tag{2}$$

where P_{f0} and P_{ft} are the excess ^{15}N (atom%) in PON at the beginning and the end of the incubation in the feeding bottles, respectively; N_{f0} and N_{ft} are the concentrations of PON (μM) at the beginning and the end of the incubation in the feeding bottles, respectively.

The clearance rate for removal is based on Frost (1972) but we used excess ^{15}N concentration of PON as a substitute for cell concentrations. It is denoted by F'.

$$P_{ct} \cdot N_{ct} = P_{c0} \cdot N_{c0} \cdot e^{\mu \cdot t} \tag{3}$$

$$P_{ft} \cdot N_{ft} = P_{f0} \cdot N_{f0} \cdot e^{(\mu - g) \cdot t} \tag{4}$$

$$F' = V \cdot g/W \tag{5}$$

where P_{c0} and P_{ct} are excess ^{15}N (atom %) in PON at the beginning and the end of incubation in control bottles, respectively; N_{c0} and N_{ct} are concentrations of PON (μM) at the beginning and the end of the incubation in control bottles, respectively. μ is the constant for change of excess ^{15}N concentration in PON and g is the grazing coefficient. We also estimate clearance rates by chl a concentration, denoted by F'', and the manner of calculation is same as for excess ^{15}N concentration of PON.

The ingestion rate I [nmol N (mg dry wt) $^{-1}$ h^{-1}] is calculated by the following equation:

$$I = N_{\text{ave}} \times F \tag{6}$$

where N_{ave} is average concentration of PON (μM) during the incubation period of each bottle and calculated as

$$N_{\text{ave}} = (N_{ft} - N_{f0})/\ln(N_{ft}/N_{f0}) \tag{7}$$

The excretion of NH_4^+ by copepods during the incubation was estimated from the difference in the change of NH_4^+ concentration between control and feeding bottles. The difference in NH_4^+ uptake by phytoplankton between feeding and control bottles was corrected assuming that NH_4^+ uptake is proportional to the concentration of chl a. The average concentration of chl a in each bottle was calculated in the same manner as PON. In the July experiment, NH_4^+ concentrations in control bottles increased during the incubation. Thus, the excretion rates of NH_4^+ in this month were estimated from the difference in the change of NH_4^+ concentration between control and feeding bottles without the correction.

RESULTS

Amounts and composition of PON

Changes in the quantity of the particulate fraction during pre-incubation are shown in Table 2. PON in our samples consisted of phytoplankton, micrograzers, bacteria and detritus. Except for the 12 March experiment, which contained more than 10 μg l^{-1} of chl a, initial chl a concentrations in the samples were 2.1 to 3.8 μg l^{-1}, while concentrations of PON were in a rather

Table 2. Duration of pre-incubation, concentration of chl *a*, PON and PON/chl *a* ratio before and after pre-incubation and ^{15}N atom% in PON at the end of pre-incubation

Date	Duration (d)		Chl *a* (μg l^{-1})	PON (μM)	PON/chl *a* (μmol/μg)	^{15}N atom% in PON
12 Mar	3	Before	12	4.8	0.40	
		After	14	5.5	0.39	6.34
28 May	3	Before	2.1	3.0	1.5	
		After	4.0	3.5	0.88	7.23
2 Jul	3	Before	3.8	4.2	1.1	
		After	5.5	4.1	0.75	5.76
27 Aug	3	Before	3.6	3.0	0.85	
		After	3.4	3.6	1.1	7.39
12 Nov	2	Before	2.6	2.6	1.0	
		After	3.9	3.0	0.76	3.50

narrow range (2.6 to 4.8 µM). During 2 to 3 d of pre-incubation, the increase in chl *a* concentrations was generally much higher than that in PON, resulting in a decrease of the the PON/chl *a* ratio. The ratio of PON (µmol) to chl *a* (µg) after the pre-incubation (same as the beginning of feeding experiments, Table 2) ranged from 0.39 to 1.1. These low values suggest a large contribution of phytoplankton-N to the total PON (Mc-Carthy & Nevins 1986). In all cases, significant ^{15}N transfer into the PON fraction was observed (Table 2).

Change of ^{15}N atom% in PON during feeding experiments

In both control and feeding bottles, ^{15}N atom% in the particulate fraction decreased during incubation possibly due to uptake of ^{14}NH$_4^+$ by phytoplankton (Fig. 2). However, the release of ^{15}N-nitrogen from PO^{15}N
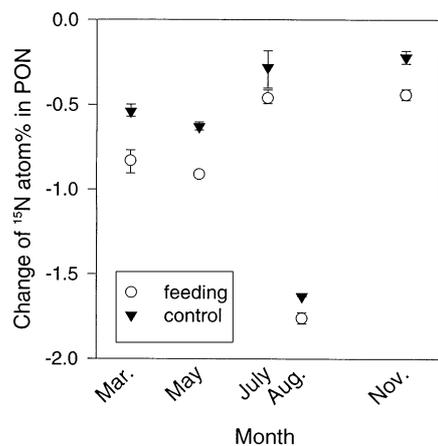
Fig. 2. Change of ^{15}N atom% in PON during incubation period in feeding and control bottle. Error bars represent the range

through cell lysis of phytoplankton or excretion by microzooplankton also resulted in a decrease of ^{15}N atom% in PON. In controls, decrease in ^{15}N atom% ranged from 0.22 to 1.6 and a large decrease in the August experiment indicated a high ^{14}NH$_4^+$ uptake rate into the particulate fraction in this month. In feeding experiments, the decrease in ^{15}N atom% ranged from 0.44 to 1.8 (Fig. 2). These decreases are significantly larger (*t*-test, p < 0.05, but in August, p < 0.1) in feeding bottles than in controls in all the experiments, and the differences ranged between 0.10 to 0.37 atom%.

Ammonium regeneration rate

Weight-specific ammonium regeneration rate by copepods ranged from 18 to 70 nmol (mg dry wt)$^{-1}$ h^{-1} (Table 1). The rate was lowest in March and comparable in May and August (Table 1). These rates were significantly correlated with incubation temperature (p < 0.05, r^2 = 0.50, excluding May).

Clearance rates obtained from 3 distinct methods, ingestion rate, and PSA

Weight-specific net clearance rate *F* estimated by ^{15}N accumulation into copepods after Burns & Rigler (1967) ranged from 1.2 to 35 ml (mg dry wt)$^{-1}$ h^{-1} (Fig. 3). However, *F'* estimated by ^{15}N removal from particle fraction based on Frost (1972) ranged from 13 to 47 ml (mg dry wt)$^{-1}$ h^{-1} and *F''* estimated by the changes of chl *a* concentrations ranged from 12 to 32 ml (mg dry wt)$^{-1}$ h^{-1} (Fig. 3). A similar seasonal trend, i.e. rate increased from March to August and decreased in November, was observed in the above 3 dif-

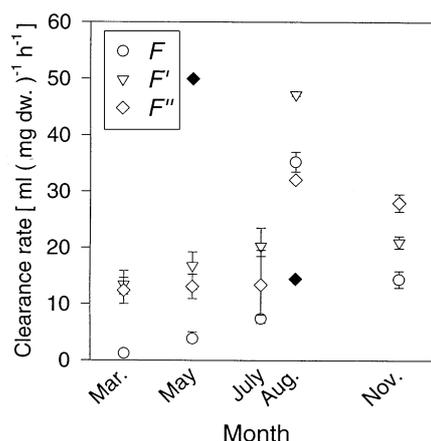Fig. 3. Seasonal change of clearance rate estimated by ^{15}N accumulation into copepods (F), ^{15}N removal from particulate organic nitrogen fraction (F') and chl a removal (F''). Error bars represent the range. For F'', (◆) deviated from (○,▽,◇) and were dealt with as exceptions
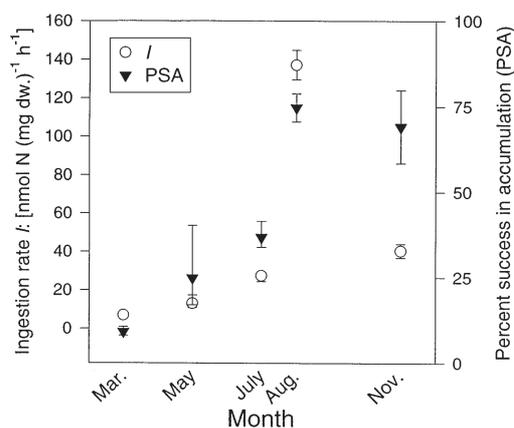

Fig. 4. Seasonal change of ingestion rate (I) and percent success in accumulation (PSA). Error bars represent the range

ferent estimates of clearance rates, but F was always lower than F'.

The ingestion rate ranged from 7.0 to 140 nmol N (mg dry wt)$^{-1}$ h^{-1} (Fig. 4). While food concentration (PON) was highest in March (Table 2), the ingestion rate was lowest (Fig. 4). Percent success in accumulation (PSA) ranged from 9.1 to 75% (Fig. 4), i.e., copepods lost 25 to 91% of their removal from PON as dissolved nitrogen (DN).

DISCUSSION

The 5 feeding experiments in different seasons presented here suggest that the fate of food nitrogen in copepods is different from season to season. Most of the food nitrogen was released as DN in spring, whereas (in August and November) most was retained in the copepod's body. Here, we discuss possible mechanisms which might affect the fate of food nitrogen and the roles of DN release by copepods in coastal environments.

Effect of the heterogeneity of labeling in the cell

The feeding experiments in this study reflect natural conditions to some extent, although composition of the particulate fraction might change during pre-incubation (Table 2). ^{15}N labeling of food might not be uniform in the 2 to 3 d duration of pre-incubation. However, Kanda & Hattori (1988) showed that regardless of species and growth phases, NH$_4^+$ which was taken up by phytoplankton entered into higher molecular weight (HMW) compounds within 30 min in the cell. It was also reported that most of the DIN taken up was rapidly (4 to 5 h) synthesized into HMW compounds in various field observations (Wheeler et al. 1982, Glibert & McCarthy 1984, Kanda et al. 1988). Further, the labeling time (2 to 68 h) of the alga *Stichococcus* with ^{14}C-bicarbonate did not have a detectable effect on estimating the assimilation rate of *Daphnia pulex* (Lampert 1977). Therefore, we judged that our 2 to 3 d pre-incubation time was long enough for the ^{15}N labels in the phytoplankton cell to be representative of the cell's nitrogen.

Difference in ^{15}N atom% in PON between feeding and control bottles

In all feeding experiments, ^{15}N atom% of PON decreased more in feeding bottles than in control ones. One possible explanation of the above difference is the copepods' ejection of fecal pellets originating from the food ingested before feeding experiments. However, this might have minor effects on ^{15}N atom% decrease because the copepods used were kept starved for >3 h in filtered seawater prior to the feeding experiments (Dagg & Grill 1980).

Another possibility is selective feeding on high ^{15}N atom% particles by copepods. Clearance rates estimated from PO^{15}N (F') and chl a (F'') removal were almost the same (Fig. 3), and this suggested that we primarily detected copepods' grazing on ^{15}N-labeled phytoplankton. Although we did not examine what were the high ^{15}N atom% particles, selective feeding on phytoplankton probably explains the difference in ^{15}N atom% decrease.

Possible mechanisms for the lower PSA

In our calculation, F was always lower than F' (Fig. 3) and the discrepancy between those 2 rates strongly suggests that copepods release a part of removed $PO^{15}N$ as DN. Copping & Lorenzen (1980) reported partitioning of food carbon in *Calanus pacificus*, i.e., 53% to body, 19 to DOC, 23 to DIC, and 4.4 to fecal pellet. If food nitrogen is assimilated more efficiently than food carbon (Landry et al. 1984, Cowie & Hedges 1996), then their result was consistent with 75% (August) and 69% (November) of PSA in this study. However, it is difficult to explain the lower PSA from 9.1 to 37% in the other 3 experiments. There are 3 possible pathways of ^{15}N-labeled DN release by copepods, i.e. exudation from fecal pellets, excretion such as NH_4^+, and 'sloppy feeding' (Fig. 1).

Mechanisms contributing to the considerable DN release by copepods in the spring experiments could not be determined from our data. However, nitrogen exudation from fecal pellets would not explain such high DN release. Defecation of ingested organic matter by zooplankton was reported to be some 5 to 40% of their food (Conover 1968). Jumars et al. (1989) concluded that most organic matter was lost from fecal pellets within minutes after pellet release assuming that the peritrophic membrane of the pellet was completely permeable. Previous experimental data did not agree with this hypothesis (Lampitt et al. 1990, Strom et al. 1997). DON release from fecal pellets might require mechanical breakage including breakage by copepods (Lampitt et al. 1990, Strom et al. 1997). Even if this enhancement is added, only up to 15% of a fecal pellet was released as dissolved matter within 28 h (Lampitt et al. 1990). These studies suggest that DN exudation from fecal pellets are of minor importance for the observed lower PSA in these experiments.

Previous studies showed that copepods excrete a considerable amount of DN and on some occasions, the excretion exceeded the ingestion of nitrogen (Corner et al. 1976, Gardner & Paffenhöfer 1982). However, these studies did not show that copepods excreted their food nitrogen immediately as catabolite. Indeed, the excretion rate of NH_4^+ by copepods was lowest in March (Table 1) as was the PSA. Our estimations of NH_4^+ release by copepods may be biased and somewhat incorrect because other NH_4^+ regenerators were affected by copepods' activity (Glibert et al. 1992, Miller et al. 1995). However, NH_4^+ regeneration rates, estimated after Ikeda (1985), were also lowest in March (data not shown). Gardner & Paffenhöfer (1982) argued that endogenous metabolism is important for ammonium release. Further, adult females of *Acartia* spp. and *Centropages furcatus* showed a 12 h time lag between maximum ingestion rate and maximum excretion rate (Checkley et al. 1992).

If the excretion by copepods explains the lower PSA, copepods might have catabolized a considerable part of the food without any anabolism in the March, May and July experiments. The developmental stage and sex of the experimental copepods might explain this metabolism. Adult males need less food for growth and reproduction compared to adult females and younger copepodite stages. However, this might be the reason why the ingestion rates of adult males are commonly lower than those of adult females (Båmstedt et al. 1990, Klein Breteler et al. 1990). Thus, would be unexpected that adult males increase their catabolic rates.

If the ingested-amount-specific metabolic rate is not so different among sexes, developmental stages, and species, a possible mechanism contributing to lower PSA might be 'sloppy feeding'. In this case, it means that food environments for copepods in March, May and July were poor. In these months, copepods would have lost a significant part of the food nitrogen into the surrounding water. *Calanus pacificus* releases up to 24% of food carbon as DOC; however, this is not only caused by 'sloppy feeding' but also by 2 other mechanisms (Copping & Lorenzen 1980). Strom et al. (1997) suggested 'sloppy feeding' was of minor importance for DOC release for the same species. Although the size of copepods in our experiments (around 1 mm) and in the above (around 3 mm) may explain the difference in the DN release, available data do not support such high ratios of nitrogen loss (63 to 91%) through 'sloppy feeding' alone.

Consequently, such a low PSA cannot be explained solely by a single mechanism. However, there are few studies directly evaluating the fate of food compounds and these might not include the whole situation surrounding copepods. Our data suggested that copepods release DN through catabolism and 'sloppy feeding' at higher percentages than the traditional view.

Roles of DN release by copepods

Although we could not evaluate which mechanisms were important, copepods released a significant part of food-nitrogen as DN and this DN release was larger than accumulation into the body in 3 out of 5 experiments. This might be a new aspect of the effect of copepods within the marine nitrogen flux. Generally, copepods act as a direct link between primary production and higher trophic levels. However, in this study, copepods released a considerable part of their food nitrogen as DN without any production. It is suggested that at least on some occasions copepods transfer PON

mainly to dissolved nitrogen and cut off the food chain between phytoplankton to carnivores.

By packing small particles such as nanoplankton into large peritrophic membrane packages, i.e., fecal pellets, copepods contribute to the vertical transport of organic matter into the deep layers of the ocean (Lee & Wakeham 1987). Zooplankton defecate 5 to 40 % of ingested organic matter as feces (Conover 1968). However, in this study, copepods released, as DN, 0.3 to 10× more nitrogen than they accumulated into their bodies. In Akkeshi Bay, spring and fall blooms consisted of large diatoms (Motoda et al. 1977, Taguchi et al. 1994), and it is thought that the bloom of diatoms declines through sedimentation (Brussaard et al. 1995). If the lowest PSA prevails during blooms, copepods grazing on diatoms contribute to retention of nitrogen within the surface layer and less to vertical transport.

Recently, a number of studies revealed that some diatoms repressed egg production and hatching success of copepods owing to the lack of certain fatty acids (Jónasdóttir 1994, Kleppel & Burkart 1995) and unknown inhibitory compounds (Poulet et al. 1994, Ianora et al. 1995). In our March experiment, the dominant phytoplankton were diatoms such as *Thalassiosira anguste-lineata*, *T. gravida/T. rotula* and *T. nordenskioeldii* (Furuya pers. comm.) and copepods might often ingest these diatoms. However, the present study showed that a significant part of food-nitrogen was not accumulated in the copepods' body in March. This might be another mechanism that represses reproduction when copepods feed on diatoms.

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