In situ ingestion rates of appendicularian tunicates in the Northeast Water Polynya (NE Greenland)

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ABSTRACT: We report *in situ* ingestion rates of individual oikopleurid appendicularians from the Northeast Water Polynya (NEW) determined by a modification of the gut pigment technique. Appendicularians were most abundant at ice edge stations and were rare at open water stations in the centre of the polynya. Gut passage time (GPT), which was determined in experiments onboard ship, was not related to body size (mean ± SD = 74 ± 23 min). Gut chlorophyll content (GCC) excluding phaeopigments (i.e. GCC = chlorophyll $a + b + c$), averaged 4.06 ng ind.$^{-1}$, with quartiles of 1.20 to 4.73 ng ind.$^{-1}$. There was no evidence of a diel feeding rhythm. GCC was higher and more variable at non-bloom (i.e. at chlorophyll concentrations <0.8 mg m^{-3}) than at bloom stations, and was inversely related to the relative proportion of chlorophyll in particles >5 μm in size. This suggests inhibition of ingestion rates at ice edge stations with diatom blooms. The ingestion rate (IR, ng chlorophyll ind.$^{-1}$ d^{-1}) of individual appendicularians in the NEW can be predicted by IR = 2.5 BL:BT$^{-0.41}$TL$^{2.14}$ (r^2 = 0.43) where BL:BT is the ratio of chlorophyll a in particles >5 μm in diameter to the total chlorophyll biomass, and TL (mm) is the trunk length.

KEY WORDS: Zooplankton · Appendicularian · Feeding · Polynya · Gut pigments

INTRODUCTION

Polynyas are mesoscale ice-free areas in the midst of ice-covered seas that serve as important foraging areas for large animals, including birds, seals, walruses, whales and man (Stirling 1980). Polynyas are created and maintained by physical factors such as winds, tides, upwelling or regional currents (Smith et al. 1990, Schneider & Budéus 1995). During spring and summer 1993, an international, multidisciplinary expedition to the Northeast Water Polynya (NEW) tested the hypothesis that opening of the polynya leads to the formation of a short food chain based upon diatom production while the under-ice food web is a complex microbial loop based upon very small producers (e.g. bacteria). Because 2 oikopleurid appendicularians, *Oikopleura vanhoeffeni* and *O. labradoriensis*, are particularly abundant in subarctic and arctic waters (Udvardy

1954, Grainger 1965, Buchanan & Browne 1981), and because appendicularian populations frequently have a particle removal capacity equivalent to that of all of the copepod species in the community combined (Knoechel & Steel-Flynn 1989, Buck & Newton 1995), we were interested in quantifying the individual and population ingestion rates of these animals in the NEW.

Appendicularians use a system of external and internal mucous filters to remove particles from seawater suspension. Gut contents and particle size retention efficiencies indicate that appendicularians retain a broad size range of particles, from submicrometre colloids to large diatoms (Deibel & Turner 1985, Deibel & Lee 1992, Urban et al. 1992, 1993). In previous studies of appendicularian ingestion, investigators have used proxy food, including latex spheres (Alldredge 1981, Deibel 1988) and radio-labeled algae (Knoechel & Steel-Flynn 1989). The gut pigment technique, based on determining the quantity of phytoplankton pigments in the gut and the turnover rate of gut contents during short experiments, is an alternative to artificial marker particles for determining *in situ* ingestion rates

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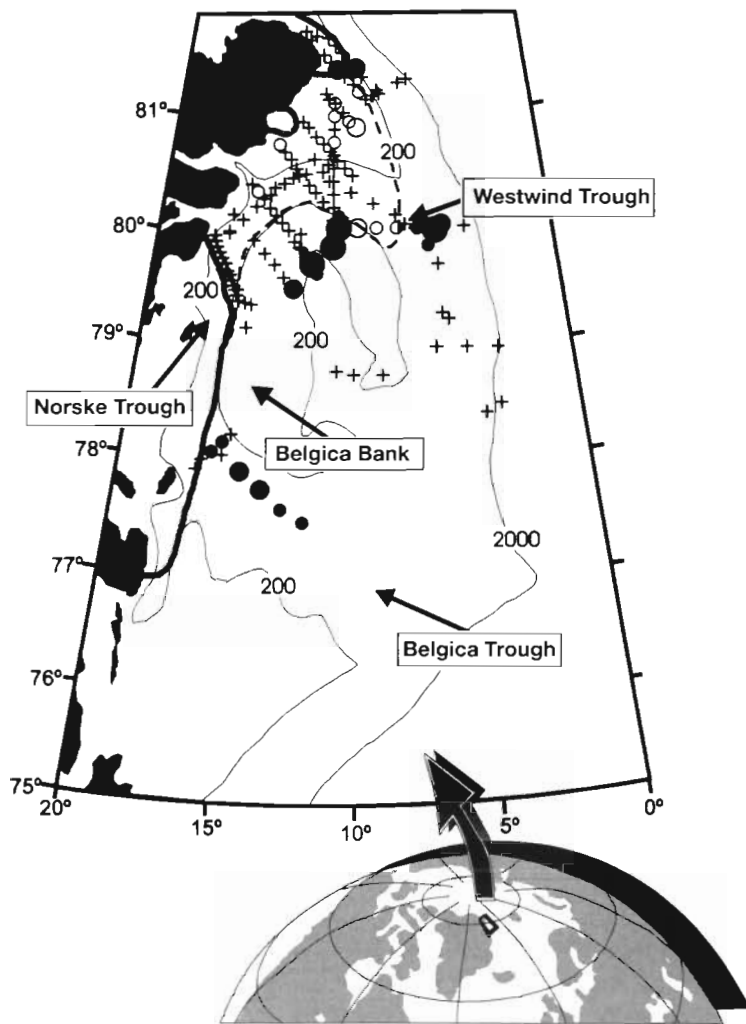
Fig. 1. Map of the study area of RV 'Polarstern' cruise ARK IX/3 showing the location of stations at which appendicularians were sampled for gut pigment analysis (●, ○) and those at which appendicularians were essentially absent (+). (○) Bloom stations at which the vertically averaged chlorophyll concentration (over the upper 50 m) was higher than 0.8 mg m⁻³, and (●) non-bloom stations at which the vertically averaged chlorophyll concentration was less than 0.8 mg m⁻³ The 3 different sizes of circles indicate the value of the gut pigment index 10^i (ng chl ind.⁻¹) for each station. 10^i is the normalized gut chlorophyll content (GCC) of a standard appendicularian 1 mm long. Small circles represent 10^i <1 ng ind.⁻¹, medium circles $1 < 10^i < 2$ ng ind.⁻¹, and large circles $10^i > 2$ ng ind.⁻¹. (- - -) Outer limit of open water (i.e. the open polynya water was located between this line and the coast) on 27 July 1993, during a time of maximum opening of the polynya (adapted from Pesant et al. 1996). (▬) Edges of the landfast ice of the Ob Bank Ice Barrier (northernmost), and the Norske Øer Ice Barrier (southernmost)

Cetta 1984, Madin & Purcell 1992, Madin & Kremer 1995) and copepods (Head 1986). To date, only 2 studies have considered *in situ* feeding by appendicularians determined using gut fluorescence information (Jansa 1977, Landry et al. 1994a,b). However, neither of these studies included the determination of gut passage time, so the derivation of ingestion rates was not possible.

Here we report individual ingestion rates of the predominant oikopleurid appendicularians in the NEW as measured by a modification of the gut pigment technique, including development of new methods to sample, maintain and conduct experiments on living appendicularians onboard ship. We aimed at describing the variability of ingestion rates of appendicularians in the NEW over large space and time scales (10s to several hundred km over 6 wk), and at determining the most likely physical and biological factors accounting for this variability.

METHODS

We report data collected during RV 'Polarstern' cruise ARK IX/3 to the NEW, between 25 June and 4 August 1993 (Fig. 1). Methods for collecting temperature, salinity and *in situ* fluorescence data are given in Hirche & Kattner (1994) and Budéus & Schneider (1995) and for collecting ice cover information in Hirche & Kattner (1994) and Pesant et al. (1996). Total phytoplankton pigments (i.e. the sum of chlorophyll and a-type phaeopigments) were determined on 2 size fractions (0.45 to 5 μm, and >5 μm) using a Turner 112 fluorometer fitted with a standard, broad-band filter set (Pesant et al. 1996). Phaeopigments were subtracted from total pigments to arrive at undegraded chlorophyll values, making the measurements of available phytoplankton pigments comparable to our measurements of gut chlorophyll content (see below).

Gut chlorophyll content (GCC). Appendicularians for gut chlorophyll analyses were collected using vertical hauls of a Bongo net frame with a 50 cm mouth opening, fitted with a net made of 200 μm mesh. The net was retrieved from 200 m depth to the surface at a velocity of ca 1 m s⁻¹. Periodic, vertically stratified tows indicated that most of the appendicularians were living within 50 m of the surface (Ashjian et al. 1995, 1997, Hirche et al. un-

of autotrophic food by pelagic tunicates (Madin & Kremer 1995). Rapid sample collection and analysis using this technique allows for large-scale mapping of ingestion rates which is not possible using other time-consuming approaches, which explains why the gut pigment technique has been used with salps (Madin &

publ.). Immediately after arrival on deck, the cod end contents were diluted with filtered seawater (Whatman GF/F filters) collected from ca 7 m depth. The diluted sample was taken quickly to a 0°C container laboratory below deck. Following removal of sub-samples for taxonomy (4% formaldehyde-seawater), appendicularians were removed one-at-a-time from the dilute cod end contents using a glass pipette. Each appendicularian was rinsed 3 times with filtered seawater, and deposited onto a glass well slide. After 5 to 18 individuals had been collected on slides depending on the station, each was identified and measured to the nearest 0.02 mm under red light using a Zeiss dissecting microscope. Our measurement of trunk length was defined as the distance between the mouth and the posterior edge of the stomach, not taking into account the gonads (Shiga 1976).

We did not sort the appendicularians by species. The 2 oikopleurid appendicularians known from the arctic are *Oikopleura vanhoeffeni* and *O. labradoriensis*, which are very similar and can only be distinguished from one another when alive. Because of time demands of the gut pigment work, we were not able to look through live samples onboard, although preliminary inspection in the NEW showed that ca 90% were *O. vanhoeffeni*, which is similar to the relative abundance of these 2 species in Newfoundland waters.

Next, the number of faecal pellets was recorded both within the gut of the animal and deposited in the well plate. The house rudiment (if present) was removed by dragging the upper part of the trunk against the glass surface of the slide. The tail was then cut off with a dissecting blade because of its potential confounding effect on pigment analysis due to high concentrations of beta-carotene, astaxanthin and asteraxanthin (Deibel & Head unpubl.). Next, the trunk and any faecal pellets released into the well slide were transferred to a 1.5 ml Eppendorf tube which was flash frozen in liquid nitrogen and stored in the dark at −20°C until analysis onshore. The above procedure took up to 40 min at stations where appendicularians were abundant.

After the cruise, the samples were shipped via airfreight to our home laboratory in Newfoundland in a 20 l cooler filled with dry ice. Total chlorophyll (i.e. chlorophyll [chl] $a + b + c$) was determined using a Sequoia-Turner model 450 digital fluorometer equipped with a narrow band excitation filter (NB 440) and a sharp-cut emission filter (SC 665). The instrument was calibrated using a pure chl a standard (Sigma Chemicals). This filter set is designed to exclude all a-type phaeopigments from the signal, thus avoiding the inaccuracies of phaeopigment determination using a conventional broad-band filter and the traditional acid-ratio equations in the presence of chl b and c

(Bochdansky et al. 1998). This modification of the gut pigment technique was possible because we have found that appendicularians convert a predictable proportion (mean = 79% with 95% CL of 76 to 83%) of ingested chlorophyll to phaeopigments and colorless products (Bochdansky et al. 1998). Also, the Sequoia-Turner 450 fluorometer has sufficient sensitivity to make relatively rapid determinations of GCC of single individuals with near-HPLC specificity for total chlorophyll (Bochdansky et al. 1998). This permits the analysis of large numbers of samples in a fraction of the time required for HPLC work, making large-scale mapping of ingestion rates possible, an initial strength of the gut pigment technique (Mackas & Bohrer 1976) which has been lost with the increasing reliance on time-consuming and costly HPLC analyses.

To analyze the GCC of each appendicularian, 1.25 ml of chilled acetone was added to each Eppendorf tube, which was then sonicated for 3 min in a water bath filled with crushed ice. Pigments were extracted at −20°C for 24 h. Each tube was then sonicated and centrifugued at 9500 RCF (relative centrifugal force) for 3 minutes at room temperature. After 10 more minutes in the dark at room temperature, total chlorophyll fluorescence (i.e. chl $a + b + c$) of each sample was analyzed by retrieving 1 ml of the supernatant and pipetting it into a cuvette, which was then diluted to 1.5 ml by adding 0.5 ml of 90% acetone. The fluorescence of the sample was then determined without acidification (i.e. relative fluorescence units) using a Sequoia-Turner 450 fluorometer (see above).

Appendicularians for determination of background fluorescence were collected at Logy Bay, insular Newfoundland, in Winter 1994. The same species occur at the same water temperatures in Logy Bay and in the NEW (i.e. *Oikopleura vanhoeffeni* and *O. labradoriensis*). Appendicularians were starved for 5 h, which is sufficient time for the gut to empty ca 5 times (Bochdansky et al. 1998). Following this depuration interval, the appendicularians were collected and processed for GCC as above. There was a significant allometric relationship between background GCC (GCC*, ng ind.$^{-1}$) and trunk length (TL, mm): GCC* $= 0.022TL^{2.81}$ (n = 5, $r^2 = 0.97$, $F = 109$, p < 0.01, SE of the slope = 0.27, SE of the intercept = 1.38). The size range of animals sampled for blank readings was from 2.1 to 4.4 mm, covering 53% of the size range of animals sampled in the NEW (from 0.55 to 3.88 mm, see 'Results'). We have assumed that the allometric relationship for blank readings extends linearly to small appendicularian sizes.

Gut passage time (GPT) and faecal pellet production rate. Appendicularians for GPT experiments were collected with a large cod end plankton net (Reeve 1981), made of 110 µm mesh, with a mouth diameter of 1 m and a 45 l acrylic cod end. The net was retrieved at ca 30 m
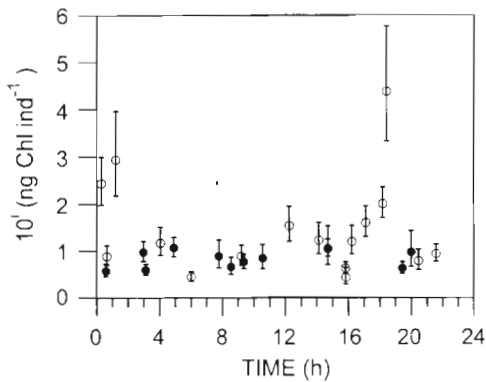
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Fig. 3. Diel patterns in body-size-normalized gut chlorophyll content (10^i) versus time of the day for all NEW stations sampled for GCC. (o, ●) Stations where chlorophyll concentration was <0.8 and >0.8 mg chl m^{-3} respectively. There were no significant differences in 10^i among six 4 h intervals for all stations pooled (Kruskal-Wallis analysis by ranks, n = 28, H = 2.95, p = 0.707), only for stations with average chlorophyll concentration <0.8 mg m^{-3} (n = 17, H = 7.29, p = 0.2), and only for stations with average chlorophyll concentration >0.8 mg m^{-3} (n = 11, H = 4.85, p = 0.3). Error bars represent 10^{i+SE} and 10^{i-SE}

accepting that the slopes were not different from one another when in fact they were different. However, an additional ANCOVA analysis of only those stations with significant within-station regressions between GCC and TL (i.e. n = 15 stations) also resulted in the conclusion that the slopes were not significantly different from one another (p = 0.33) with a common slope value of 2.22 ± 0.16 (±SE; n = 168 individuals; number of stations = 15). Furthermore, separate ANCOVA analyses made on diatom bloom stations (average chlorophyll concentration >0.8 mg m^{-3}) and non-bloom stations (average chlorophyll concentration <0.8 mg m^{-3}) did not result in rejecting the null hypothesis of equal slopes within each group (p = 0.94 and p = 0.21, respectively) and gave similar slope estimates to those above.

Although station-wise slopes of the allometric regressions of log GCC versus log TL were statistically undistinguishable, there were significant differences in intercept among stations (ANCOVA; $F_{27, 231}$ = 4.87, p < 0.001). Therefore, at each station the allometric relationship between GCC and TL was described by the equation log GCC = 2.08 log (TL) + i, where i is a station-specific intercept which can be calculated following Armitage & Berry (1987). Therefore, after reverse transformation, the allometric equation for GCC versus TL for each station is,

$$GCC = 10^i \, TL^{2.08} \qquad (1)$$

Note that, in Eq. (1), 10^i is equivalent to the GCC of a standard animal 1 mm long. In the following sections,
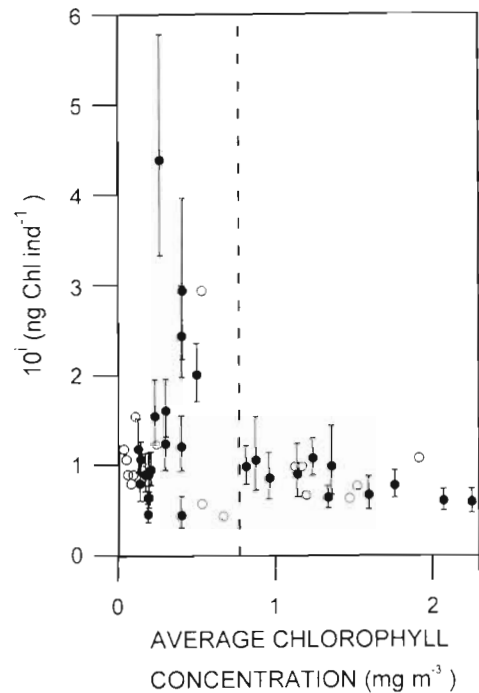

Fig. 4. Plot of the body size corrected index of gut chlorophyll content (10^i) versus the average chlorophyll concentration over the upper 50 m of the water column, determined from *in situ* fluorescence (●) and from acetone extracts of sub-samples from Niskin bottles (o). Error bars represent 10^{i+SE} and 10^{i-SE} The vertical dashed line separates stations with chlorophyll concentrations below and above 0.8 mg m^{-3}

we will use 10^i as a body-size-corrected index of overall gut fullness for each station. Over the entire 28 stations, 10^i had a mean of 1.20 ± 0.16 ng ind.$^{-1}$ (±SE), ranging between 0.44 and 4.38 ng ind.$^{-1}$. What are the most likely environmental factors accounting for this variability of 10^i among stations? We explored this question by examing the effect of time of day and food concentration on 10^i.

We expected 10^i to have no diel cycle and to increase with increasing ambient chlorophyll concentration. There was no apparent relationship between 10^i and time of day when considering all stations or when the stations where sorted by their average chlorophyll concentration (Fig. 3). Thus, our first hypothesis was supported. However, a scatter plot of 10^i versus vertically averaged chlorophyll concentration did not reveal a monotonic increase with increasing food concentration, but a complex relationship with 2 domains of points (Fig. 4). At chlorophyll concentrations below 0.8 mg m^{-3}, 10^i was variable, with values ranging from 0.44 to 4.5 ng ind.$^{-1}$ and 6 stations having 10^i > 1.5 ng ind.$^{-1}$. At chlorophyll concentrations above 0.8 mg m^{-3}, 10^i was significantly less variable, with much lower values and no stations having 10^i > 1 ng chl ind.$^{-1}$ (Levene's test for

homogeneity of variances p < 0.01; Kolmogorof-Smirnov non parametric test, $Z = 1.37$, n = 28, p = 0.047) (Fig. 4). Comparative data from extracted pigment samples also indicate higher variability of 10^i below a pigment concentration of 0.8 mg m^{-3} (open circles in Fig. 4).

Next, we asked the question of whether the relationship between GCC and ambient chlorophyll concentration was fundamentally different for appendicularians of differing body sizes. To do this, we plotted observed GCC versus ambient chlorophyll concentration for all appendicularians subdivided into 9 body size classes, from <0.88 mm to >2.63 mm (Fig. 5). All body size classes except the largest one had a pattern of GCC versus chlorophyll concentration essentially the same as that of 10^i versus chlorophyll concentration, indicating maximum GCC at low or intermediate food concentrations and decreasing GCC at higher food concentrations (compare Fig. 4 with Fig. 5A to I). The largest individuals >2.63 mm long were the only ones having maximum GCCs at food concentrations above 0.8 mg m^{-3} (Fig. 5I), indicating some ability to ingest the larger cells responsible for the higher chlorophyll concentrations. Thus, our hypothesis of monotonically increasing GCC with increasing chlorophyll concentration was rejected for all body size classes except the largest.

The simplest explanation for the inverse relationship between 10^i and chlorophyll concentration (Fig. 4) is that the inlet filters of the house are clogged by the large diatom cells responsible for the high chlorophyll concentrations (see 'Discussion'). From this hypothesis we would predict that when the relative abundance of large phytoplankton is high, GCC should be low, regardless of the concentration of smaller, ingestible particles. Next, we tested this prediction.

10^i was significantly correlated with 2 environmental descriptors, average temperature and the ratio of the abundance of large (>5 µm) to total phytoplankton (i.e. the ratio BL:BT) (Table 2). The relationship between 10^i and BL:BT was the stronger of the 2 correlations. Furthermore, as BL:BT increased, the value of 10^i decreased, in accordance with our prediction of an inverse relationship of 10^i with the relative abundance of large particles.

An appealing characteristic of the index BL:BT is that it has been used by Pesant et al. (1996) as a metric
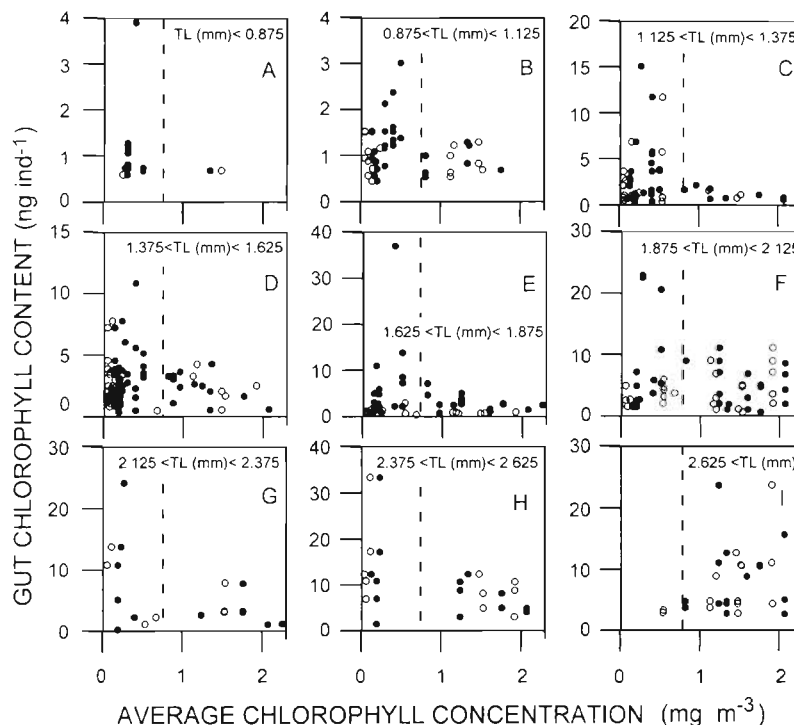

Fig. 5. (A–I). Each panel represents a plot of gut chlorophyll content versus vertically averaged chlorophyll concentration for appendicularians belonging to 9 different trunk length (TL) classes. Except for the lowest and highest TL classes, each class is 0.25 mm wide. Symbols as in Fig. 4

to divide the NEW into several biogeographic provinces, including Zones 1 and 2, dominated by blooms of large diatoms, and Zone 5, dominated by cells <5 µm in diameter. Thus, we used BL:BT to build a regression equation to predict GCC of oikopleurid appendicularians in the NEW Our multiple linear regression model included log TL and log (BL:BT) as independent variables and log (GCC) as the depen-

Table 2. Correlation of the body-size-corrected gut chlorophyll content (GCC, 10^i) with environmental co-variables. Because 10^i was not normally distributed, we have used the non-parametric correlation statistic, Kendall's Tau. BT: total chlorophyll in bottle extracts, BS: <5 µm and BL: >5 µm chlorophyll in bottle extracts. By far the strongest correlation was with the index of phytoplankton size composition BL:BT (inverse correlation). ns: not significant

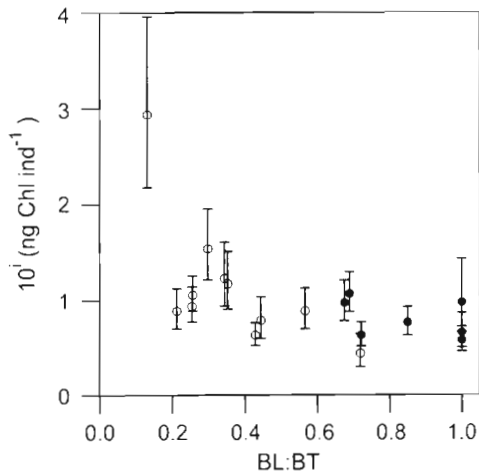| Co-variable	Tau	p	n
Mean temperature	−0.26	<0.05	28
Mean salinity	0.22	NS	28
Ice cover	0.08	NS	23
Mean *in situ* chlorophyll	−0.17	NS	28
BT	−0.18	NS	18
BS	0.10	NS	18
BL	−0.18	NS	18
BL:BT	−0.73	<0.05	18

Fig. 6. Plot of the body-size-corrected index of gut chlorophyll content (GCC, 10^i) versus the ratio of chlorophyll concentration in particles >5 μm in diameter to the concentration of total chlorophyll (BL:BT). (o) Non-bloom stations (chlorophyll concentration <0.8 mg m^{-3}) and (●) bloom stations (chlorophyll concentration >0.8 mg m^{-3}). Error bars represent 10^{i+SE} and 10^{i-SE}. The point with the lowest BL:BT and the highest 10^i corresponds to a group of stations showing high 10^i (up to 4.38 ng chl ind.$^{-1}$) and located at the northernmost part of the Belgica Bank (see Fig. 1)
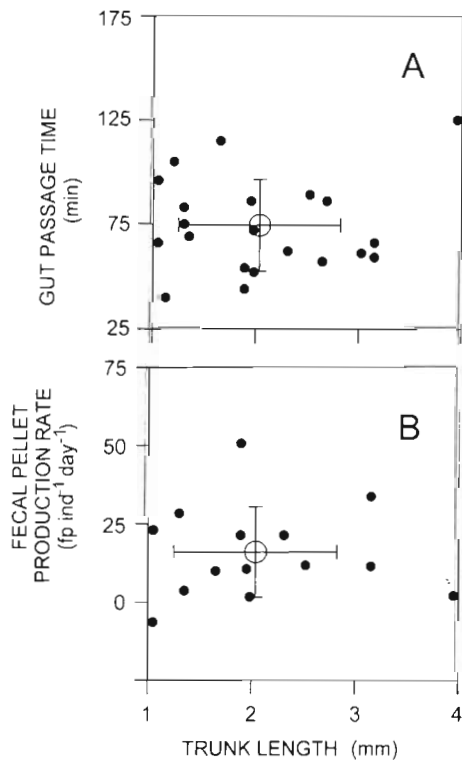

Fig. 7. (A) Plot of gut passage time versus trunk length. (O) Means and (——) standard deviations. (B) Plot of fecal pellet production rate versus trunk length. Symbols as in (A)

dent variable (TL in mm; BL:BT has no units). Log transformation was used to account for the allometric relationship between GCC and TL (Fig. 2) and the non-linear relationship between GCC and BL:BT (Fig. 6). The resulting equation is

$$\log(\text{GCC}) = (-0.19 \pm 0.07) + (-0.41 \pm 0.11)\log(\text{BL:BT})$$
$$+ (2.14 \pm 0.19)\log(\text{TL}) \qquad (2)$$

All 3 regression coefficients were significantly different from 0 (t-statistic for testing H_0: coefficient = 0 was $t = -2.8$, p = 0.006 for the intercept, $t = -3.7$, p < 0.001 for the slope of log (BL:BT) and $t = 11.3$, p < 0.001 for the slope of log (TL)). The percentage of explained variance was 43%, with n = 173 and F = 64. When back-transformed, equation 2 becomes

$$\text{GCC} = 0.65 \, \text{BL:BT}^{-0.41} \text{TL}^{2.14} \qquad (3)$$

Note that this equation is consistent with the variance structure as depicted by ANCOVA analysis (Eq. 1). GCC is related to TL by an allometric function with an exponent of 2.14, very close to our estimate of a common slope of 2.08. The term 10^i in Eq. (1), or GCC of a standard appendicularian measuring 1 mm, is given in Eq. (3) by 0.65 BL:BT$^{-0.41}$. In fact, when Eq. (3) is solved for TL = 1, it nicely describes the trend of 10^i versus BL:BT in Fig. 6 (graph not shown). Eq. (3) is robust, spanning stations with almost no diatoms (i.e. BL:BT < 0.2, Pesant's Zone 5), to stations with almost pure diatom blooms (i.e. BL:BT 1.0, Pesant's Zone 1). It is also a simple equation with biologically meaningful parameters which can be used to estimate the GCC of appendicularians in the NEW based on body size and the relative abundance of large phytoplankton. It can be applied to the goal of estimating the ingestion rate of appendicularians at any station in the NEW (see below).

Gut passage time (GPT) and ingestion rates

At a constant temperature of 0°C, GPT of appendicularians in the NEW averaged 74 ± 23 min (±SD, n = 21), with a range of 40 to 125 min. GPT was statistically independent of body size over the wide spatial and temporal scales of the NEW (Fig. 7A). Faecal pellet production rate (FPPR) had a mean of 41 ± 15 faecal pellets d^{-1} (±SD, n = 21), ranging from 19 to 76 faecal pellets d^{-1}. FPPR also was statistically independent of body size (Fig. 7B). Since GPT in *Oikopleura vanhoeffeni* is essentially constant over a wide range of food concentrations (Bochdansky et al. 1998) and TL (Fig. 7a), ingestion rate can be directly calculated from GCC by,

$$\text{IR} = (\text{GCC/GPT}) \, k \qquad (4)$$

where IR = ingestion rate (ng total chlorophyll animal^{-1} d^{-1}), GCC = gut chlorophyll content (ng animal^{-1}), GPT = gut passage time (d) and k = a correction factor to account for that portion of ingested chlorophyll which is converted either to phaeopigments or to non-fluorescent products, both of which are lost to our analytical technique (see 'Methods'). k could not be determined during the present study. However, experiments conducted in Newfoundland, with the same species and at the same water temperatures as in the NEW, suggest that chlorophyll conversion efficiency is 79% (95% CL of 76 to 83%), indicating that 21% of ingested chlorophyll remains intact and undegraded during gut passage (Bochdansky et al. 1998). Thus, a value for k of 4.76 (1 ÷ 0.21) is a reasonable approximation over a wide range of food concentrations. Therefore, in the present calculations, we used a GPT of 1.23 h (i.e., 0.051 d) and a k of 4.76. We assumed that the value of k does not vary with food concentration or food type (see 'Discussion').

Oikopleurid appendicularians in the NEW had a mean herbivorous ingestion rate of 379 ng chl ind.$^{-1}$ d^{-1}, with quartiles of 112 to 441 ng chl ind.$^{-1}$ d^{-1}. Assuming a C:chl a ratio of 60 (Booth & Smith 1997), the mean daily ingestion rate of phytoplankton carbon was 23 µg C ind.$^{-1}$ d^{-1}. Dividing this mean daily ingestion rate by the carbon mass of an appendicularian of mean size, calculated according to the equation of Deibel (1986), gives a mean, herbivorous daily ration of appendicularians in the NEW of 117% body carbon d^{-1}.

A generalized equation for predicting individual ingestion rates

Because the ingestion rates in the previous section have been calculated from the original, raw GCC data, their allometric structure is the same as that shown in Fig. 2. In fact, we can combine Eq. (3) with Eq. (4) to derive an allometric equation describing the relationship between the ingestion rate of chlorophyll (IR, ng chl ind.$^{-1}$ d^{-1}) and the body size of appendicularians (TL, mm) in the NEW,

$$IR = 2.5 \, BL{:}BT^{-0.41} TL^{2.14} \tag{5}$$

This ingestion rate can be converted to units of ng C ind.$^{-1}$ d^{-1} by multiplying by a C:chl ratio of 60.

DISCUSSION

Comparison of new method to published values

In this paper, we report the first shipboard feeding measurements using net-collected appendicularians of which we know. Achieving these experiments has allowed us to examine ingestion rates in relation to several abiotic and biotic environmental conditions. The gut pigment technique is especially suited for the study of arctic oikopleurid appendicularians, because of the absence of diel feeding rhythms (Fig. 3; see also Redden 1994), the wide size range of ingested particles (Urban et al. 1992) and the lack of particle selection by the mucous filters within this range (Deibel & Lee 1992, Bochdansky et al. 1998). Furthermore, *Oikopleura vanhoeffeni* is a relatively large appendicularian, allowing for the pigment analysis of individual guts.

Since the ingestion rates we report are based on the amount of phytoplankton pigment present in the guts of the appendicularians, they are true measures of the quantity of autotrophic particles removed from suspension that were actually consumed by the appendicularians. Therefore, no correction for the quantity of particles removed from suspension but remaining stuck to the mucous filters of the house is necessary (Gorsky 1980). This 'correction factor' has needed to be applied throughout the appendicularian feeding literature, as almost all publications have been based on determination of the disappearance of cells or particles from suspension in the water surrounding feeding appendicularians.

Although there are no previously published, herbivorous ingestion rate studies with which we can compare the present data, Bochdansky et al. (1998) and Bochdansky & Deibel (1999a) have recently completed a systematic study of the clearance rates of *Oikopleura vanhoeffeni* using a suite of techniques, including particle removal determined using a Coulter Counter, video estimates of water flow rate and the quantity of gut pigment and GPT. These rates were then compared to 2 published studies of *O. vanhoeffeni* conducted *in situ*, one using radio-labeled algae (Knoechel & Steel-Flynn 1989) and one using the uptake of fluorescent plastic beads (Deibel 1988). When corrected for loss using the same chlorophyll conversion efficiency we have used (i.e. 79% over all food concentrations), the clearance rates based on gut pigments were within a factor of 2 of those determined by Knoechel & Steel-Flynn (1989) and Deibel (1988). Most importantly, the rates from all 3 studies were not significantly different from one another (Bochdansky & Deibel 1999a). Although the chlorophyll conversion efficiency of *O. vanhoeffeni* did increase with decreasing gut fullness (Bochdansky et al. 1998), application of a variable conversion efficiency to the determination of clearance rates did not result in a statistically significant change in comparison with the constant conversion factor of 79% (Bochdansky et al. 1998). Therefore, we elected to use the simplest approach here, with a constant conversion efficiency of 79%. We have assumed also that this conversion efficiency deter-

mined for *O. vanhoeffeni* feeding on diatoms applies to the mixed prey community encountered in the field. This assumption is necessary because of the absence of any published or unpublished information on chlorophyll conversion during gut passage of non-diatom prey, due in part to the lack of a comparative technique to the sensitive [68]Ge ratio approach for diatoms. At least some of the gut contents of *O. vanhoeffeni* are diatoms (Urban et al. 1992, Acuña et al. 1996, J. L. Acuña, D. Deibel, P. Saunders, B. Booth & E. Hatfield unpubl.) justifying our assumption for this first-order attempt at estimating *in situ* ingestion rates of *O. vanhoeffeni*. These estimates will be improved once chlorophyll conversion efficiencies for mixed populations of naturally occurring prey are determined.

Bloom versus non-bloom feeding dynamics

Appendicularian GCC and chlorophyll ingestion rates were highly variable over the space and time scale of our study. We recognized 2 separate domains of available food particles in the NEW, which we have called non-bloom and bloom stations. Below 0.8 mg chl *a* m^{-3} (i.e. the vertically averaged concentration over the upper 50 m at non-bloom stations) GCC was variable, and reached its highest values, while above 0.8 mg chl *a* m^{-3} (i.e. at bloom stations) GCC was low and relatively constant (Fig. 4). All of our non-bloom stations fall within regime 5 of Pesant et al. (1996), which is a biogeographically defined group of stations in which the production and biomass of phytoplankton is dominated by small cells <5 µm in diameter. Although this size of particle is below the threshold for effective retention of most copepod species, it is well within the capability of *Oikopleura vanhoeffeni* to retain this particle size with 100% efficiency (Acuña et al. 1996). Thus, it is not surprising that appendicularians in the NEW have maximum gut fullness at the non-diatom-bloom stations (Fig. 4). This conclusion is consistent with predictions from a theoretical, fluid mechanical model of particle selection by *O. vanhoeffeni*, which postulated that 71% of the total ration of carbon in the NEW should come from particles <20 µm in diameter (Acuña et al. 1996).

What are likely mechanisms for the inhibition of ingestion at diatom bloom stations? *Oikopleura vanhoeffeni* and *O. labradoriensis* have mucous pre-filters on the outside of their houses that screen the inflowing water by trapping large and spiny particles (Deibel 1986). The pore width of these filters ranges from 40 µm for juvenile *O. vanhoeffeni* to 140 µm for adults (Deibel 1986). Thus, these filters could become clogged by large centric diatoms or by tabular chains of pennate diatoms, leading to decreasing ingestion

rates and gut fullness. We have found observational support for the hypothesis that the herbivorous ingestion rate of appendicularians is controlled in part by the relative abundance of large particles (Fig. 6). This confirms over a wide spatial and temporal scale the *in situ* observations of Knoechel & Steel-Flynn (1989), who found an inverse relationship between the clearance rate of *O. vanhoeffeni* and the concentration of large particles in coastal Newfoundland waters.

Since Cushing (1989) first proposed copepods as the dominant mesozooplankton grazers in classical, nitrate-driven, pelagic food chains, there has been a growing consensus that appendicularians dominate mesozooplankton grazing in regenerative, microbial loop systems (Deibel 1998). This paradigm has been based on the perception that appendicularians have evolved towards the concentration and capture of very small particles (Flood et al. 1992). This concept was challenged by the discovery of relatively large diatoms in faecal pellets of *Oikopleura vanhoeffeni* (Urban et al. 1992, 1993), perhaps due to the large body size of *O. vanhoeffeni* and the large pore size of the inlet filters. However, the qualitative study by Urban et al. (1992, 1993) is not directly comparable to the present quantitative results. Urban et al. (1992, 1993) report simply on the presence of undigested phytoplankton remains in the faecal pellets as determined using electron microscopy, not on the absolute abundance of phytoplankton mass in the pellets nor on the relative abundance of diatoms versus smaller phytoplankton. Thus, Urban et al. (1992, 1993) did not collect data from which it is possible to determine the ingestion rate of diatoms. Although we cannot determine the relative ingestion rate of diatoms versus smaller cells from our gut chlorophyll measurements, the inverse relationship between ingestion rate and the relative abundance of phytoplankton larger than 5 µm (Fig. 6) suggests that large particles not only form a small fraction of the biomass of consumed phytoplankton (see also Acuña et al. 1996) but also have a deleterious effect on ingestion rates.

Estimation of ingestion rates

Another important goal of this work was to derive a simple predictive model for the individual ingestion rates of appendicularians in the NEW. This task has been simplified by the absence of diel feeding rythms in arctic oikopleurid appendicularians (Fig. 3), which would have complicated the sampling design and data analysis (Head 1986) and would require including time of day as an additional independent variable. The task was also simplified by the low variability of GPT, and the stability of GPT over a wide range of body sizes

(Fig. 7), food quantity and food quality. This result has been confirmed in laboratory studies of *Oikopleura vanhoeffeni* by Bochdansky et al. (1998). Thus, we can use a single value for GPT to estimate ingestion rates of appendicularians in the NEW.

In building the predictive equations, we have tried to achieve simplicity, and to give our equations not only an empirical, but also a functional basis. We have used body size and the relative concentration of large particles as predictor variables in an equation which can be used to estimate individual ingestion rate at any of the stations in the NEW (Eq. 5).

There is a considerable amount of variance unexplained by the model (ca. 50%). One possibility is that this residual variance is due to our lack of information on the small-scale concordance of appendicularians and their prey. We believe this is unlikely however, since a similar magnitude of unexplained variance is shared by field studies of appendicularians using tracer particles (Alldredge 1981, Deibel 1988, Knoechel & Steel-Flynn 1989), and even by experimental studies under highly controlled conditions and a unialgal diet in the laboratory ($r^2 = 24\%$ for log clearance rate versus log body size, Bochdansky & Deibel 1999a, J. L. Acuña & M. Kiefer unpubl.). This is very close to the variation of GCC explained by body size in nature, of 29% (see legend for Fig. 2). Thus, even when there is no small scale variation in the available chlorophyll concentration in laboratory experiments, there is high residual variation in feeding rates, similar to the residual variation in the field. This suggests that food patchiness is not responsible for the residual gut chlorophyll variation in the field. In addition, the clearance rate of a single individual can vary markedly over time due to the age and degree of clogging of the filters of the house, with a 70% reduction in clearance rate of an old versus a new house (Bochdansky & Deibel 1999b). This 3-fold range in feeding rate within a single individual depending upon the age of its filtration apparatus is greater than the 2-fold range among various techniques for determining feeding rate (see above) and suggests that much of the residual variance observed in the field is real and due to recent feeding history.

Thus, we are confident that Eq. (5) gives a biologically meaningful, average prediction of feeding by appendicularians which can be extrapolated over the surface of the polynya. Moreover, there is a clear functional basis for the application of this equation, which is that the ingestion rate of appendicularians in the NEW clearly depends upon both body size (Fig. 2) and the relative abundance of large particles (Table 2, Fig. 6) which are known to clog the inlet filters and retard ingestion rates (Knoechel & Steel-Flynn 1989). There is also a clear ecological basis for application of this equation, because all of the stations in the NEW have been divided into 3 biogeographic provinces (2 bloom and 1 non-bloom) based on the relative concentration of large phytoplankton cells (Pesant et al. 1996). According to Pesant et al. (1996) there are fundamental differences in trophic structure and ecosystem dynamics between diatom bloom stations, which correspond to export systems, and non-bloom stations, which correspond to regenerative systems (Pesant et al. 1996). Here we have shown that these contrasting ecosystem structures have profound implications on the trophic ecophysiology of appendicularian consumers.

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