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Particulate and Dissolved Organic Carbon Production by the Heterotrophic Nanoflagellate *Pteridomonas danica* Patterson and Fenchel

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Abstract

We established a budget of organic carbon utilization of a starved heterotrophic nanoflagellate, *Pteridomonas danica,* incubated in batch cultures with *Escherichia coli* as model prey. The cultures were sampled periodically for biomass determinations and total organic carbon dynamics: total organic carbon, total organic carbon <1 μm, and dissolved organic carbon (DOC, <0.2 μm). During the 22 h incubation period, *P. danica* underwent biovolume variations of 3.2-fold. Gross growth efficiency was 22% and net growth efficiency 40%. *P. danica* respired 33% and egested 44% of the ingested *E. coli* carbon during lag and exponential growth phases. The form of the organic carbon egested varied. Of the total ingested carbon, 9% was egested in the form of DOC and occurred mainly during the exponential growth phase; 35% was egested in the form of particulate organic carbon (POC), ranging in size from 0.2 to 1 μm, and took place during the lag phase. *P. danica* could have reingested as much of 58% of this previously produced POC during the exponential growth phase as food scarcity increased. We concluded that POC egestion by flagellates could represent a significant source of submicrometric particles and colloidal organic matter. In addition, flagellate reingestion of egested POC could play a nonnegligible role in the microbial food web. Finally, the methodology reported in this study has proved to be a useful tool in the study of carbon metabolism in aquatic microorganisms.

Introduction

Despite their relative importance in carbon cycling, few data exist on carbon metabolism in heterotrophic nanoflagellates. Reliable data on assimilation efficiencies and egestion rates of protists are rare [5, 34, 41]. Rarer still are data on the form of egestion. Koike et al. [32] indicated that flagellate grazing could be responsible for the production of the submicrometer (0.38–1 μm) particles observed after 1 month incubation of seawater samples. Other recent studies seem to support the hypothesis that flagellates can release a large amount of submicron or colloidal organic materials [15, 40, 41]. Release of

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ingested nitrogen as dissolved free amino acids has been reported in the literature [e.g., 29, 31, 39, 40], but very little is known about dissolved organic carbon (DOC) excretion [6]. Moreover, measuring excretion or egestion rates of bacterivores is not simple because of the consumption of the egested DOC and/or small particles by the bacterioplankton [e.g., 27, 52].

The few data available on carbon metabolism in heterotrophic nanoflagellates have been obtained in the laboratory on cultures kept under balanced growth conditions. However, in their natural habitat protozoa undergo "feast and famine" situations as a result of fluctuations in the availability of food resources [17, 46]. Under starvation, cells shrink, dismantle RNA and protein synthesis, decrease the number of ribosomes, reduce the number of mitochondria, and cease to divide [9, 17, 18, 24, 26]. When fed again, cells go through a lag phase, during which they rebuild the metabolic machinery, before multiplication can proceed. Balanced growth is often not achieved again for several generations [17]. The residence time of food vacuoles in protists appears to be a constant [12, 13]. However, the efficiency of digestion likely varies with the physiological state of the grazer and perhaps is directly related to the quantity of digestive enzymes available per food vacuole [11, 54]. Thus, lag phase cells may be inefficient digesters relative to late exponential/early stationary phase cells. We may expect egestion rates to be maximal when starved cells begin to feed again.

The objective of this study was to obtain a mass balance of organic carbon utilization for a starved helioflagellate, *Pteridomonas danica* Patterson and Fenchel, by measuring carbon incorporation efficiency and carbon egestion and respiration rates. A major source of possible error when calculating incorporation efficiencies of protozoa are carbon:biovolume conversion factors. The conversion factors used in this study were measured directly for *P. danica* and its prey (*Escherichia coli*) [43] and not estimated from conversion factors obtained from other species in the literature.

In addition, we measured the form of egested carbon: quantities egested as DOC and as particulate organic carbon (POC), ranging in size from 0.2 to 1 μm and corresponding to the submicrometric particles of Koike et al. [32]. An original experimental approach was employed to avoid bacterial uptake of DOC and/or small particles egested by protozoa. The technique consists of using the enterobacterium *Escherichia coli* as food source. Added to seawater, *E. coli* does not reproduce and its metabolic activity is greatly reduced. *E. coli* reacts to this stress situation by progressing toward a viable but nonculturable dormant state [37, 42, 49]. Although of nonmarine origin, *E. coli* is a gram-negative bacterium like most marine species. In addition, the use of osmotically shocked *E. coli* rather than heat-killed bacteria avoids the denaturation of organic compounds that may affect POC and/or DOC egestion rates, or digestibility [36].

Materials and Methods

Bacterial Prey

Escherichia coli HB10B was grown on Luria–Bertani medium at 37°C in a Gyratory water bath shaker. The culture, which had reached ~1 unit optical density at 600 nm, was centrifuged at 9000 rpm for 10 min (Centrikon T-24). The supernatant was withdrawn and replaced with 0.22 μm filtered (consecutively Whatman GF/F, 0.8 μm and 0.22 μm Millipore filter units) and autoclaved seawater. Each centrifuge tube was sonicated for 1 min to detach bacteria from medium particles and centrifuged again. This procedure was repeated 5 times. This bacterial suspension, containing $2–3 \times 10^9$ cells ml^{-1}, was then diluted and immediately used in our experiments.

Heterotrophic Nanoflagellates

We used a filter-feeding protozoan, the helioflagellate *Pteridomonas danica,* isolated from Villefranche Bay (NW Mediterranean sea). Stock cultures of the flagellate are maintained in the laboratory on wheat-grain cultures of mixed marine bacteria [48]. For the experiments, *P. danica* was cultivated at 18°C with *Escherichia coli* as prey. The culture was transferred four successive times by adding 10 μl of the actual culture to 1L of 0.22 μm filtered and autoclaved seawater containing no bacterial nutrients, only osmotically shocked *E. coli.* These transfers were conducted every 48 h, when *E. coli* concentration had decreased from 30×10^6 cells ml^{-1} to $<10^6$ cells ml^{-1}. This procedure had the objective of eliminating natural bacteria from the flagellate culture. Strom et al. [53] calculated growth rates of 0.77 d^{-1} for marine bacteria grazed by protozoa, and similar growth rates were reported for marine bacteria by Zweifel et al. (0.5–1.33 d^{-1} [57]). Assuming a growth rate of 0.77 d^{-1}, no mortality, and no ingestion of these bacteria by *P. danica,* we calculated that there were virtually no marine bacteria left in our cultures after the third transfer. However, the growth rate of exponentially growing *P. danica* was 2.9 d^{-1} (data not shown), greater than for most bacteria, and the highest grazing rate measured during the experiment was of 44 *E. coli P. danica*$^{-1}$ h^{-1}. Thus, our calculations likely overestimated the presence of marine bacteria during the various transfers. In addition, microscopical observations were conducted regularly during the whole experiment (the four transfers, the starvation period, and the incubation period) to test for the presence of bacterial morphotypes other than *E. coli.* Samples were taken periodically, stained with DAPI, and observed under the epifluorescence microscope (see Analysis). One single bacterial morphotype was observed during the whole experiment,

corresponding to *E. coli*. Thus, the role of marine bacteria in our experimental setup, if present, was negligible.

Escherichia coli Experiment: Uptake and/or Release of DOC

We conducted an experiment to test for *Escherichia coli* uptake and/or release of DOC once introduced into seawater. *E. coli,* prepared as described above, was added to four 1 L bottles containing 400 ml of 0.22 μm filtered and autoclaved seawater to a final concentration of ~7×10^6 cells ml^{-1}. Sterile D-glucose was added to a final concentration of ca. 1.7 g L^{-1} to two of the bottles. Glucose was chosen because it is the most widely used bacterial carbon substrate and is also the dominant neutral monosaccharide of DOC. Glucose alone has been shown to be able to support 15–47% of marine bacterial production [47]. Addition of other nutrients was not considered necessary because NO_3^- and PO_4^{2-} concentrations at the moment of the experiment were enough to sustain bacterial growth (2.5 and 0.3 μM, respectively). All bottles were incubated at 37°C in a Gyratory water bath shaker. Dissolved organic carbon (DOC) samples were taken initially at 1-h intervals for 10 h, and then 21 h after setup. Samples for bacterial enumeration (taken initially every 3 h for 10 h, and then 21 h after setup) were fixed with 0.2 μm filtered and buffered (calcium carbonate) formaldehyde to a final concentration of 2% (vol/vol).

Pteridomonas danica Grazing Experiment

A stationary-growth-phase culture of *Escherichia coli*-fed *Pteridomonas danica* was left to starve for a period of 5 days. Duplicate cultures of this starved *P. danica* were then started with an initial concentration of $1.4 \pm 0.1 \times 10^5$ *P. danica* and $4.7 \pm 0.3 \times 10^7$ *E. coli* ml^{-1}, and kept in an incubator at 18°C. After 0, 3, 6, 9, 12, 17, and 22 h of incubation, water samples were taken to measure flagellate and bacterial biomass (fixed with 0.2 μm filtered and buffered formaldehyde to a final concentration of 2%), and organic carbon dynamics (total organic carbon (TOC), TOC < 1 μm, and DOC). All samples were microscopically checked for the presence of bacteria other than *E. coli*.

Duplicate cultures of 0.22 μm filtered and autoclaved seawater containing only *Escherichia coli* were incubated in parallel to test for bacterial growth and DOC production and/or consumption. Initial bacterial concentration was $6.5 \pm 0.2 \times 10^7$ *E. coli* ml^{-1}. Samples were taken as indicated above, except that no samples for TOC <1 μm analysis were collected.

Analysis

Three different organic carbon fractions were measured: TOC, TOC <1 μm, and DOC. Dissolved organic carbon and TOC <1 μm samples were filtered (7 in. Hg vacuum) through acid-washed (10% HCl) 0.2 μm pore-size polyethersulfone membrane Supor filters (Gelman) and 1 μm pore-size Nuclepore polycarbonate filters, respectively. Once placed onto the filtering unit (500°C 4 h ignited Millipore glassware), the filters were washed again by filtering 20 ml MilliQ water and 10 ml sample. Twenty-ml samples were then filtered and collected in ignited Pyrex glass tubes. Samples to check Supor and Nuclepore filter blanks were also taken. Total organic carbon samples were collected directly in 20-ml ignited Pyrex glass tubes. All these samples were acidified to pH <2 with 2 N HCl and stored at 5°C pending analysis.

Samples for biomass determination (formaldehyde-fixed samples) were stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma Chemical Co.) (5 mg L^{-1} final concentration) and filtered onto black 0.2 μm pore-size Nuclepore polycarbonate filters [45]. *Pteridomonas danica* and *Escherichia coli* were then counted on a Zeiss epifluorescence microscope at a magnification of ×1250. A minimum of 400 bacteria and 100 flagellates were counted per sample. Sample replication was conducted to ensure a <10% standard deviation of the mean. Mean standard deviation of replicate measures was 1.7 ± 2 and 1.4 ± 0.5% of the mean for bacteria and flagellates, respectively. Average diameter of the spherical *P. danica* was determined microscopically on 48 h formaldehyde-fixed samples at each sampling occasion. *P. danica* diameter was measured on the epifluorescence microscope with a calibrated ocular micrometer on a minimum of 60 organisms. Particle Coulter Counter (Multisizer) average diameter of live *P. danica* was determined at two sampling occasions, at time zero (stationary growth phase cells) and 9 h (exponential growth phase cells) after the start of the incubation, with a 20 μm aperture tube calibrated with 2.09 μm latex microspheres. Carbon conversion factors of 175×10^{-15} g C μm^{-3} and 124×10^{-15} g C μm^{-3} were used for *E. coli* and *P. danica,* respectively [43]. These conversion factors had been measured for exponentially growing cells. *E. coli* had a mean volume of 0.7 ± 0.3 μm^3 and an equivalent spherical diameter (Coulter Counter) of 1.1 ± 0.2 μm.

Organic carbon samples (TOC, TOC <1 μm, and DOC) were analyzed by high-temperature catalytic oxidation (HTCO) [e.g., 50]. Samples were sparged with an artificial gas mixture (AGA, France) (containing ≤0.1 ppm CO, CO_2, or hydrocarbons) to eliminate inorganic carbon, and measured on a Shimadzu TOC-5000 instrument equipped with a high sensitivity catalyst. Organic carbon concentrations were calculated with the instrument software and a four-point standard calibration curve made with potassium biphthalate. The coefficient of variation of duplicate injections was always <2%.

Calculations

Pteridomonas danica grazing rates (*Escherichia coli* $flg.^{-1}$ h^{-1}) were determined at each sampling interval and calculated by using the equation of Frost [21] as modified by Heinbokel [28]. The highest rate observed during the incubation period was assumed to be the maximum rate under the imposed culture conditions. Clearance rates (nl $flg.^{-1}$ h^{-1}) were calculated for each sampling interval, and the highest rate of the incubation period reported. Carbon gross growth efficiency (GGE) of *P. danica* was calculated from flagellate production divided by prey ingestion during lag and exponential growth phases. Respiration was calculated from the decrease in TOC during lag and exponential growth phases.

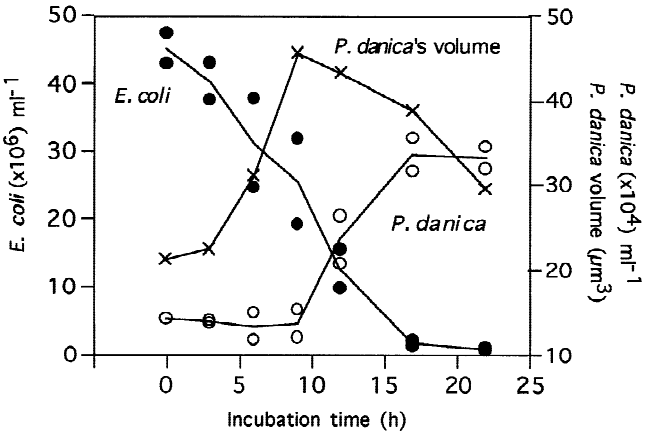

Fig. 1. Duplicate cultures of starved *Pteridomonas danica* growing on *Escherichia coli*. Changes of *E. coli* ($\times 10^6$ cells ml^{-1}) and *P. danica* ($\times 10^4$ cells ml^{-1}) concentrations, and the mean volume (μm^3) of *P. danica* during the incubation period (h).

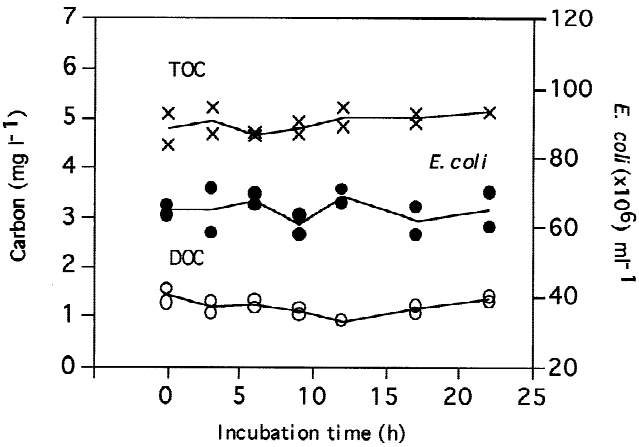

Fig. 2. Duplicate cultures of *Escherichia coli,* containing no predator and incubated in parallel with *Pteridomonas danica* cultures. Changes in *E. coli* ($\times 10^6$ cells ml^{-1}) concentration, and in total organic carbon (TOC, mg C L^{-1}) and dissolved organic carbon (DOC, mg C L^{-1}) during the incubation period (h).

Rates are shown as mean ± standard deviation of the mean. An analysis of the variance (one-way ANOVA) was carried out to test the significance of the differences among the samples.

Results

Escherichia coli Experiment: Uptake and/or Release of DOC

During the 21 h of incubation, DOC values were 1.7 ± 0.1 g L^{-1} and 1.6 ± 0.1 mg L^{-1} in the bottles containing *Escherichia coli* and incubated with and without D-glucose, respectively. No accumulation or consumption of DOC (n = 24, $p > 0.05$) occurred during the incubations. Bacterial numbers were 7.7 ± 0.5 × 10^6 *E. coli* ml^{-1} during the 21 h of incubation in bottles with and without D-glucose. No bacterial growth (n = 10, $p > 0.05$) was detected during the incubations. Similar results were obtained in *E. coli* bottles incubated in parallel with *Pteridomonas danica* bottles (see below).

Pteridomonas danica Grazing Experiment

The helioflagellate *Pteridomonas danica* exhibited a typical growth pattern, flagellate concentration increased with a decrease in bacterial prey (Fig. 1). There was a lag period of 9 h preceding the start of an exponential growth phase, but the concentration of *Escherichia coli* started to fall at time zero and continued throughout the experiment. By the time the exponential growth phase began, *E. coli* concentration had decreased to 54% of the initial concentration. The stationary growth phase started 17 h after the beginning of the incubation, when bacterial numbers had decreased by 95% and flagellate concentration had increased 2.6-fold. At the end of the incubation (22 h) there still remained 1 ± 0.2 × 10^6 *E. coli* ml^{-1} in the culture (2% of the initial concentration). Microscopical observation of all samples showed one single bacterial morphotype corresponding to *E. coli*. Maximum grazing rate was 44 *E. coli* $flg.^{-1}$ h^{-1}, and maximum clearance rate was 0.9 nl $flg.^{-1}$ h^{-1}. *E. coli* concentration did not change significantly (n = 14, $p > 0.05$) from the initial concentration during the 22 h of incubation of *E. coli*-only bottles (Fig. 2).

The diameter of exponentially growing *Pteridomonas danica* in living versus fixed cells was significantly different. After 48 h of formaldehyde fixation, cell diameter declined from 4.2 ± 0.8 μm (n = 16,430) to 3.4 ± 0.3 μm (n = 64) ($p < 0.05$). However, the diameter of stationary growth phase cells did not change significantly ($p > 0.05$) with fixation [3.0 ± 0.93 μm (n = 2,671,937) and 3.1 ± 0.4 μm (n = 64) before and after fixation, respectively]. From this information and the size of formaldehyde-fixed cells, we calculated the volume of *P. danica* prior to fixation (Fig. 1). *P. danica* volume changed during the experiment by 3.2-fold. Lag-phase *P. danica* did not divide but increased in volume immediately after prey addition from a mean initial volume of 14.1 μm^3 to a maximum volume of 44.6 μm^3 9 h later when the exponential growth phase started. *P. danica* volume decreased slightly during the exponential growth phase, and then again during the stationary growth phase (from 36.1 to 24.4 μm^3). We used *P. danica* live biovolumes and the con-

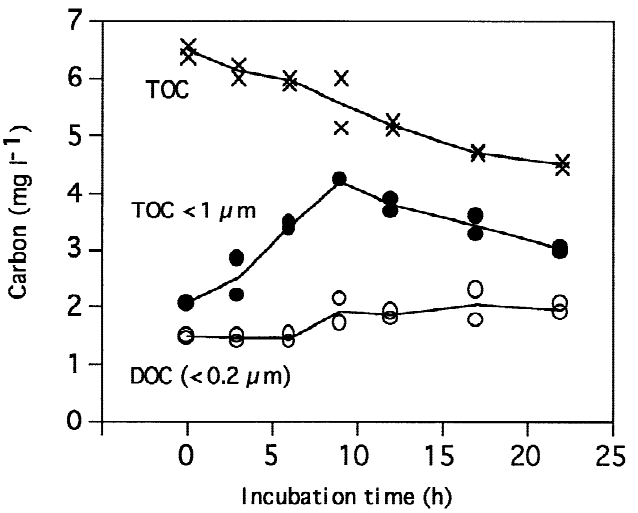

Fig. 3. Duplicate cultures of starved *Pteridomonas danica* growing on *Escherichia coli*. Changes in total organic carbon (TOC, mg C L^{-1}), total organic carbon <1 μm (TOC <1 μm, mg C L^{-1}), and dissolved organic carbon (DOC, mg C L^{-1}) during the incubation period (h).

version factor of 124×10^{-15} g C μm^{-3} [43] to calculate *P. danica* carbon content and biomass.

Organic carbon dynamics during the experiment are shown in Figs. 2 and 3. Total organic carbon did not change significantly ($p > 0.05$) in bottles containing only *Escherichia coli* (Fig. 2), but decreased from 6.5 ± 0.1 to 4.5 ± 0.1 mg C L^{-1} in *Pteridomonas danica* cultures ($Y = -0.09X + 6.4$, $r^2 = 0.92$, $n = 14$, $p < 0.05$) (Fig. 3). Likewise, dissolved organic carbon values did not change significantly ($p > 0.05$) in *E. coli*-only bottles (Fig. 2), but increased ($p < 0.05$) in *P. danica* cultures from an initial value of 1.5 mg C L^{-1} (0–6 h after setup) to 2 ± 0.1 mg C L^{-1} 12 h after setup and then remained constant until the end of the experiment (Fig. 3). We observed a lag phase increase in TOC < 1 μm, and a subsequent decrease during exponential and stationary growth phases (Fig. 3).

Discussion

Numbers of *Escherichia coli* ingested by *Pteridomonas danica* represented 5.5 mg C L^{-1} during the lag and exponential growth phases (17 h). We calculated that 1.8 mg C L^{-1} were respired (33%), while 1.2 mg C L^{-1} were converted into flagellate biomass (22%). Consequently, 2.5 mg C L^{-1} of the ingested food was egested. Maximum respiration rate of *P. danica* was 1 pg C $flg.^{-1}$ h^{-1} and took place at the beginning of the exponential growth phase. Assuming a respiratory quotient of 1, our rates are lower than the rates reported for nanoflagellates of volumes ranging from 50 to 200 μm^3 (1.9–5 pg C $flg.^{-1}$ h^{-1}), as expected from starved cells [16]. The percentage of carbon respired by *P. danica* during the experiment (33%) was within the rather large range of previous estimates (17–60% [6, 16, 22]).

We calculated that 9% of the ingested *Escherichia coli* carbon was egested as DOC during the exponential growth phase. Various authors have found higher protozoan releases of dissolved nitrogen and phosphorus during the exponential growth phase and an important decrease during the stationary growth phase [1, 20, 24, 39], and similar results were reported by Caron et al. [6] for DOC excretion. Caron et al. [6] reported that 10% of the POC removed by *Paraphysomonas imperforata* grazing on *Phaeodactylum tricornutum* appeared as DOC. This DOC was presumably related to flagellate feeding activity, but could also have been partly egested by *P. tricornutum*. Recent data suggest that DOC egestion could be as high as 16–37% of the ingested food carbon [53]. However, the authors considered as DOC all organic carbon passing through GF/F filters (nominal retention pore size of 0.7 μm), while in the present work we considered as DOC only the fraction passing through 0.2 μm filters.

This study provides one of the first experimental demonstrations of the formation of Koike's submicron particles, as the increase in TOC <1 μm during the lag phase (hours 0–9, Fig. 3) appears attributable to *Pteridomonas danica* grazing activity. Since there was no growth of *Escherichia coli* during the experiment, it seems reasonable to assume that the increase in TOC <1 μm during the experiment could only originate from *P. danica* egestion. We calculated the production of POC (0.2–1 μm) at each sampling interval and compared it to bacterial ingestion during the same time period (Table 1). During the lag phase (9 h), *P. danica* egested 70% (1.9 mg C L^{-1}) of the ingested *E. coli* carbon as POC. Approximately 58% (1.1 mg C L^{-1}) of this POC produced during the lag phase, corresponding to ca. 8×10^6 *E. coli*-size particles, was presumably reingested by *P. danica* during the exponential growth phase (from 9 to 17 h after setup). Flagellates have shown positive selection for bigger food particles [2, 25, 51]; thus, larger *E. coli* were probably preferred when present. Smaller particles were ingested later in the incubation as a result of the scarcity of *E. coli*. Nagata [41] reported an increase in submicron particles in a culture of nanoflagellates grazing on bacteria, and a rapid decrease of these particles later on during flagellate stationary growth

Table 1. Lag (0–9 h) and exponential (9–17 h) growth phases of duplicate cultures of starved *Pteridomonas danica* fed *Escherichia coli*[a]

h	Ingested *E. coli* (mg C L^{-1})	Accumulated POC (mg C L^{-1})	Interval POC (mg C L^{-1})	%POC production or consumption
0	—	0.6	—	—
3	0.9	1.1	0.5	57
6	1.1	2	0.9	80
9	0.7	2.5	0.5	73
12	1.6	1.9	−0.6	−39
17	1.3	1.4	−0.5	−36
Sum	—	—	1.9 −1.1	

[a]Column 2: Mean ingested *E. coli* carbon at each sample interval. Column 3: Measured mean POC 0.2–1 μm (calculated as TOC < 1 μm minus DOC), which assuming no POC reingestion represents accumulated POC. Column 4: From data in column 2, we calculated POC produced or disappearing (negative values) at each sampling interval. Column 5: Percentage of ingested bacteria that appears, or disappears (negative values), as POC at each sampling interval. The sum of produced (3–9 h) and or reingested (12–17 h) POC is provided.

phase. He suggested coprophagy or hydrolysis by bacteria as possible mechanisms. In our study we may discount the role of the active bacteria; thus, *P. danica* coprophagy was the most plausible explanation for particle disappearance. Moreover, the formation of aggregates >1 μm retaining the 0.2–1 μm particles was not observed, and *E. coli* carbon (calculated from *E. coli* carbon content and *E. coli* concentration) was not enough to account for the TOC <1 μm measured.

POC egestion and reingestion represented 35 and 20%, respectively, of the ingested *Escherichia coli* carbon during lag and exponential growth phases (17 h). Total organic carbon egestion (DOC plus POC) was 2.4 mg C L^{-1}, close to our expected egestion rates (2.5 mg C L^{-1}). The percentage of ingested carbon that was egested by *Pteridomonas danica* was 44%, higher than previous estimates (13–43% [16, 22]). In those studies, total organic carbon egestion rates were calculated by subtracting respiration and assimilation rates from total carbon ingestion.

Table 2 shows flagellate GGE rates found in the literature, which range from 11 to 71%. Part of the variability of these rates is undoubtedly due to poorly constrained carbon:biovolume conversion factors. In our study carbon GGE was calculated by using carbon content and biovolume values measured directly for prey and predator; most studies have used conversion factors described in the literature and often measured for other organisms. Also, if particle reingestion is an important process, this could lead to underestimation of ingestion and egestion rates and to overestimation of GGE rates. In the present study, carbon GGE of *Pteridomonas danica* was 22% (18% when taking into account particle reingestion), close to the values reported by Zubkov and Sleigh (16% [56]) for hungry *P. danica* fed ^{14}C labelled *Vibrio natriegens*. Net growth efficiency [NGE, growth/(respiration + growth), 19] was 40%, lower than the data reported by other authors for balanced growth cultures of flagellates (51–70% [6, 16, 33]).

Is *Escherichia coli* representative of natural prey? The bacterium *E. coli* has been used successfully as a food source for flagellates in a number of studies [e.g., 2, 44, 55]. In addition, the generation time of *Ochromonas* sp. feeding on *E. coli* was similar to that found when feeding on natural bacteria [55]. However, there is the possibility that pure cultures of bacteria do not contain all nutrients required by protozoa. Apparently, cultured *E. coli* may contain less carbon when compared to natural assemblages [30, 35]. Goldman et al. [23] found that GGE of bacteria increased as the C:N ratio of the substrate decreased relative to the C:N ratio of the bacterial biomass. Thus, GGE efficiencies of nanoflagellates could also be affected by the elemental composition of their prey. However, the few studies conducted on protozoa did not show any significant difference in carbon metabolism or in nitrogen regeneration within nanoflagellate cultures fed prey items containing different amounts of nitrogen or phosphorus [7, 14]. In addition, there are no significant differences in GGE among the nanoflagellate cultures fed different prey items reported in Table 2.

In conclusion, starved *Pteridomonas danica* had low GGE and NGE, indicating inefficient food processing. We show a simplified carbon budget for *P. danica* grazing activity in Fig. 4. Approximately 9% of the ingested food was available again for bacteria as DOC, but 33% was lost as carbon dioxide. POC (0.2–1 μm) production by bacterivorous nanoflagellates could be a nonnegligible contributor to oceanic submicron particles and colloidal organic materials, comprising 35% of the ingested food in this study. POC reingestion in the present study could represent as much as 20% of the ingested food. Thus, POC reingestion, reported only once prior to the present study [41], could be a significant process in the microbial food web. The traditional methodology involving bacterial disappearance may underestimate actual grazing rates. Finally, the methodology reported in this study has shown to be a useful tool in the study of carbon metabolism in aquatic microorganisms.

Table 2. Comparison of gross growth efficiencies (GGE, in terms of %C:C) of heterotrophic and mixotrophic nanoflagellates

Predator species	Prey species	GGE	Reference
Cafeteria sp.	Marine bacteria	21.5–38.5	26
Monas sp.	Marine bacteria	23	4
Paraphysomonas imperforata	Bacteria or diatoms	58	6
Paraphysomonas imperforata	Phytoplankton	53–71	7
Paraphysomonas imperforata	Marine bacteria	20	9
Paraphysomonas imperforata	*Vibrio natriegens*	21	56
Parabodo attenuatus	Marine bacteria	18–20	33
Pleuromonas jaculans	*Pseudomonas* sp.	43	16
Poterioochromonas malhamensis	*Pasteurella* sp.	43	8
Pteridomonas danica	*Vibrio natriegens*	16	56
Pteridomonas danica	*Escherichia coli*	22	This study
Ochromonas sp.	*Pseudomonas* sp.	34	16
Ochromonas sp.	*Escherichia coli*	43	55
Stephanoeca diplocostata	Marine pseudomonad	40	22
Heterotrophic flagellates	Marine bacteria	33, 35	3, 10
Heterotrophic flagellates	Picoplankton	11–53	38

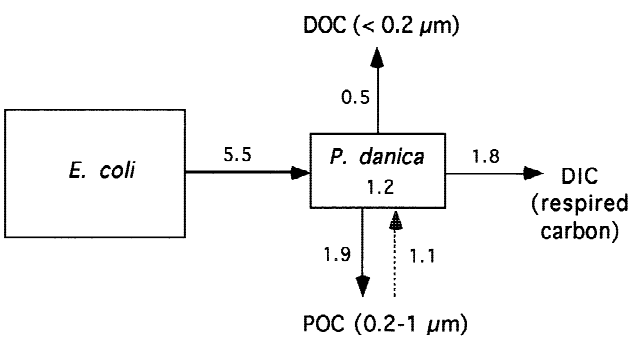

Fig. 4. Starved *Pteridomonas danica* growing on *Escherichia coli*. Simplified carbon budget of *P. danica* grazing activity during 17 h incubation: dissolved organic carbon (DOC) egestion, particulate organic carbon (POC) 0.2–1 μm egestion, and dissolved inorganic carbon (DIC) respiration. Part of the egested POC could have been reingested by *E. coli* (dotted arrow). Organic carbon units are in mg C L^{-1}.

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