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Abundance of planktonic thraustochytrids and bacteria and the concentration of particulate ATP in the Greenland and Norwegian Seas

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Abstract: Surface water samples were collected at 15 sites in the Greenland and Norwegian Seas, and total planktonic thraustochytrids and bacteria were directly counted by epifluorescence microscopy. Particulate (>0.2 μm) adenosine 5'-triphosphate (ATP) was also determined by bioluminescence photometry. Abundance of planktonic thraustochytrids varied from below the detection limit (<8.1×10^2 l^{-1}) to 2.3×10^5 l^{-1} with an overall average of 3.1×10^4 l^{-1}. Abundance of bacterioplankton ranged from 2.2×10^7 l^{-1} at the northernmost site to 6.0×10^8 l^{-1} at the southernmost site with an overall average of 2.1×10^8 l^{-1}. Particulate ATP concentration ranged from 79 pmol to 676 pmol at the mid-transect (68°N) and southernmost (62°N) sites, respectively, with an overall average of 222 pmol. The measured ATP concentrations were too low to account for the abundance of thraustochytrids and bacteria estimated from total counts and carbon-per-cell factors. However, particulate ATP was correlated with the abundance of bacterioplankton, but not that of planktonic thraustochytrids. These differing relationships suggest different physiological and biochemical strategies of starvation survival and differing substrate availability in the Arctic and sub-Arctic surface waters.

key words: adenosine 5'-triphosphate, Arctic, sub-Arctic, bacterioplankton, fungoid protists

Introduction

Thraustochytrids are marine- and estuarine-dwelling fungoid protists classified in the phylum Bigyra within the kingdom Chromista (Cavalier-Smith, 1981; Cavalier-Smith *et al*., 1994; Honda *et al*., 1999; Cavalier-Smith and Chao, 2005). Thraustochytrids have attracted little attention in marine ecology (Kimura and Naganuma, 2001; Nagao *et al*., 2002); however, they occur in detectable amounts in the water column and sediment and are associated with plant and animal tissues (reviewed by Raghukumar, 2002). The abundance of planktonic thraustochytrids was estimated to range from 10^2–10^3 l^{-1} using a culture-based method and 10^2–10^5 l^{-1} using epifluorescence microscopy direct count (Na-

ganuma *et al*., 1998; Kimura *et al*., 1999, 2001; Kimura and Naganuma, 2001; Raghukumar, 2002; Bongiorni and Dini, 2002), compared with 10^8–10^9 bacterioplankton l^{-1} according to epifluorescence microscopy in general surface water (Cole *et al*., 1988).

Planktonic thraustochytrids and bacteria play key roles in degradation and utilization of organic matter in marine ecosystems, and are differentially associated with phytoplankton blooms. Bacterioplankton abundance is generally correlated with phytoplankton abundance, increasing with phytoplankton bloom (Cole *et al*., 1988; Müller-Niklas and Herndl, 1996). In contrast, the abundance of thraustochytrids is unrelated to blooming phytoplankton abundance but is thought to be associated with post-bloom phytodetritus (Kimura *et al*., 2001; Raghukumar, 2002).

Planktonic thraustochytrids and bacteria also play a role in the initial stage of the microbial food chain, converting "sub-micron" dissolved organic matter into supra-micron particles (cells) to be ingested by larger microorganisms such as flagellates and ciliates, and it is suggested that bacteria are ingested even by thraustochytrids (Raghukumar, 1992). Bacteria might be a more important starter of the food chain than thraustochytrids with regard to their number and specific growth rate; however, a thraustochytrid cell of 4–20 μm wide is 85–21400 times larger than a typical 0.5×1–2 μm bacterial cell (0.72–0.91 μm equivalent spherical diameter) in natural waters, and thus, serves as a larger starter in microbial food chains with fewer trophic links and less trophic-level losses (Naganuma *et al*., 1998; Kimura *et al*., 1999; Raghukumar, 2002).

In the Arctic pelagic food web, bacterioplankton are known to be an important secondary producer (Müller-Niklas and Herndl, 1996; Aagaard *et al*., 1999). Heterotrophic protists such as ciliates and dinoflagellates mainly prey on bacteria, and, in turn, >10 μm-protists are grazed by copepods (Levinsen *et al*., 2000), suggesting that >10 μm-thraustochytrids might also contribute to zooplankton production. For example, the calanoid copepod *Calanus finmarchicus* in the sub-Arctic Norwegian Sea is known to actively feed on phytoplankton (Irigoien *et al*., 1998; Meyer-Harms *et al*., 1999); however, its reproduction is unrelated to the spring phytoplankton bloom (Niehoff *et al*., 1999), and thus, is likely supported by >10 μm-protists including thraustochytrids.

This communication is the first report to document the abundance of planktonic thraustochytrids and the concentration of particulate adenosine 5'-triphosphate as a microbial biomass indicator (Karl, 1980; Karl and Dobbs, 1998; Karl and Björkman, 2001), as well as bacterioplankton abundance, in the surface waters of the Greenland and Norwegian Seas.

Materials and methods

Fifteen surface (0–1 m deep) seawater samples were collected along a cruise track from 76°04.5'N, 7°15.1'E to 62°14.9'N, 4°31.3'E (Fig. 1, Table 1; Tamaki *et al*., 2001; Okino *et al*., 2002). A plastic bucket sampler was pre-washed and pre-rinsed with autoclaved distilled water then re-washed with seawater from the same site immediately before sample collection. A total of four filters (see below) were prepared from a single water sample: two for duplicated counting (n=2) of planktonic thraustochytrids and bacteria, and the remaining two for duplicated measurement (n=2) of particulate ATP concentration. Cell count variances within a filter and between two filters were at the same

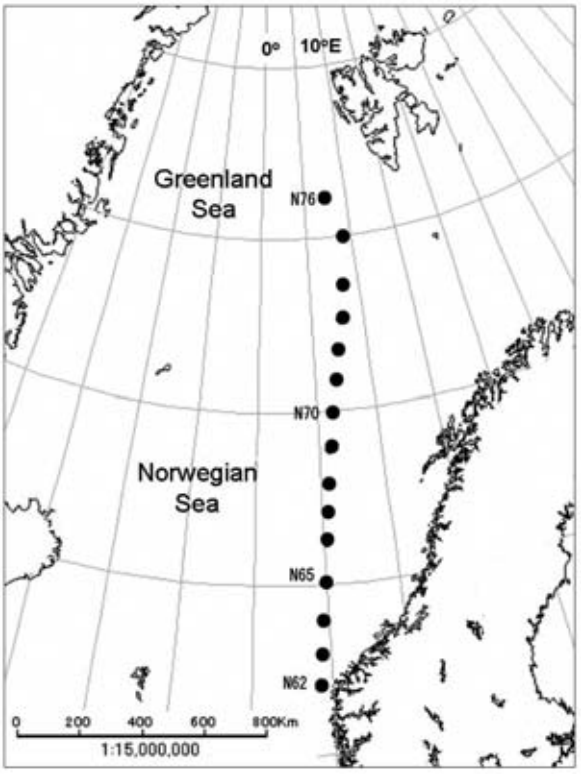

Fig. 1. Locations of sample collection sites in the Greenland Sea and Norwegian Sea during the Knipovich-2000 Cruise, September 2000.

level, and thus, the raw counts from both filters were used to calculate an average and standard deviation.

Planktonic thraustochytrids and bacteria were collected on pre-blackened polycarbonate filters (Isopore, Millipore; pore size, 0.2 μm; diameter, 25 mm) immediately after water sampling. For bacterial cell counts, 0.5–2-m*l* samples of water were filtered, and for thraustochytrid cell counts 10–100-m*l* samples. The remaining water samples were fixed with 0.2 μm-filtered formalin at a final concentration of 5% v/v and refrigerated until use. Thraustochytrid and bacterial cells were counted separately with acriflavin and acridine orange, respectively, using epifluorescence microscopy (Raghukumar and Schaumann, 1993; Kimura *et al*., 2001). Thraustochytrid (mostly 5–20 μm in diameter) and bacterial cells were counted in at least 100 and 20 microscopic view fields, respectively. To avoid uncertainty, thraustochytrid-like cells smaller than >5 μm were excluded from counting.

The concentration of particulate ATP was determined on board by luciferin-luciferase bioluminescence assay using an ATP photometer (TOA Electric Ltd., Tokyo, Japan). Particles in a water sample of 10–100 m*l* were collected on a 0.2-μm Isopore filter, which was then directly immersed in the reaction solution provided by the reagent kit

Table 1. Abundance of planktonic thraustochytrids and bacteria and the concentration of particulate ATP in the surface water of the Greenland and Norwegian Seas.

Site code	Latitude (N)	Longitude (E)	Thrausto. ($\times 10^2$ l^{-1})	±SD*	Bacteria ($\times 10^8$ l^{-1})	±SD	ATP (pmol)	±SD
N76	76°04.5'	7°15.1'	ND**	—	0.22	0.02	117.0	3.5
N75	74°59.2'	8°16.7'	ND	—	2.48	1.71	94.8	7.2
N74	73°57.2'	7°51.2'	33	565	0.32	0.01	190.0	29.2
N73	73°01.1'	7°25.1'	ND	—	1.89	1.00	109.2	9.7
N72	72°00.5'	6°58.5'	67	798	1.26	1.22	80.4	14.1
N71	70°54.5'	6°36.5'	100	975	1.37	1.26	128.2	15.9
N70	69°56.9'	6°17.6'	ND	—	3.25	4.06	270.9	44.5
N69	68°58.8'	5°59.9'	83	997	0.40	0.02	170.1	31.6
N68	67°58.7'	5°43.5'	50	847	2.33	2.12	79.4	1.4
N67	67°02.5'	5°29.5'	417	2198	4.43	1.96	443.8	68.9
N66	66°06.2'	5°16.4'	333	2212	0.83	0.93	286.8	8.5
N65	65°07.1'	5°03.6'	ND	—	1.77	1.14	267.0	53.5
N64	64°01.8'	4°50.6'	1146	3620	2.12	1.20	277.2	5.1
N63	63°06.5'	4°40.3'	2293	4843	2.11	1.12	143.8	33.3
N62	62°14.9'	4°31.3'	125	1219	6.01	2.98	675.6	52.6
Average			310		2.05		222.3	

*SD, Standard deviation. ATP measurements were duplicated (n=2).

**ND, not detected. No thraustochytrids were observed in 100 microscopic view fields (0.016 mm^2) of 100 ml-passed filter (effective filtering area of 130 mm^2), yielding a detection limit of 8.1×10^2 l^{-1}.

(AF-3L1) of the ATP photometer.

The cellular ATP content of thraustochytrids was determined with the ATCC-derived thraustochytrid, *Schizochytrium limacinum* SR21 (ATCC MYA-1381). Cell number and cellular ATP content at 0 h (lag phase), 12 h (early log phase), 18 h (mid log phase), 24 h (mid-to-late log phase), 48 h (late log phase), and 72 h (early stationary phase) of liquid cultivation were determined in triplicate (n=3). Thraustochytrid cells were counted in 100 microscopic view fields (Fig. 2). *S. limacinum* SR21 was cultured in autoclaved ATCC 790 By+ medium containing 0.1% (w/v) yeast extract, 0.1% peptone, and 0.5% D+-glucose in artificial seawater at room temperature. *S. limacinum* SR21 is a representative heterotrophic producer of polyunsaturated fatty acids in ecological as well as biotechnological relevance (Chin *et al*., 2006), and was chosen to determine the ATP per cell because of its availability in public culture collections and fast proliferation, allowing it to yield a sufficient cell mass. However, *S. limacinum* SR21 is a tropical species (Honda *et al*., 1998), and thus, produces more ATP in culture conditions than in the *in situ* conditions of the studied cold oligotrophic waters. Therefore, the measured range of 1.11±0.29 pmol at the lag phase to 39.26±3.43 pmol at the log phase (Fig. 2) should be regarded as an overestimate. On the other hand, *S. limacinum* SR21 remained at 5–10 μm in diameter during culture, against the usual 10–40 μmm diameter of other thraustochytrids (Raghukumar, 2002). The cell volume of *S. limacinum* SR21 is not thought to be too far from that observed in natural waters, and thus, was not believed to have significantly affected the ATP per cell measurements, which depend on cell volumes (Raghuku-

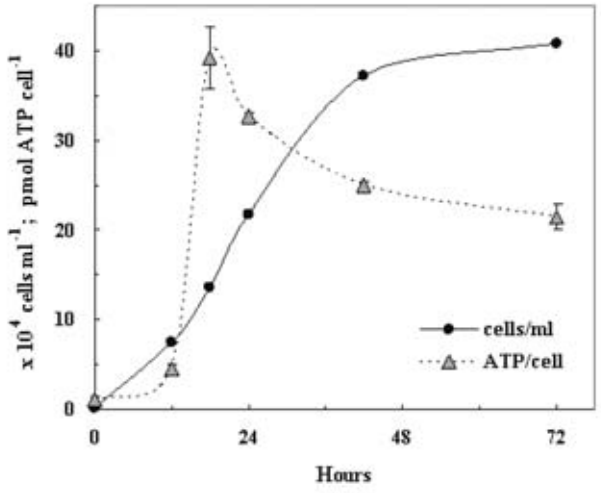

Fig. 2. Cellular ATP content of the cultured thraustochytrid *Schizochytrium limacinum* SR21 (ATCC MYA-1381).

mar *et al*., 1987). Raghukumar *et al*. (1987) reported an ATP per cell of 0.04×10^{-3} to 2.68×10^{-3} pmol (0.02–1.32 pg ATP) for the thraustochytrium *Corallochytrium limacisporum* (phylogenetically a non-thraustochytrid; Cavalier-Smith and Allsopp, 1996), which is even smaller than that of bacterioplankton (0.05 pmol; see below).

The thraustochytrid carbon pool was estimated based on 1.65×10^{-10} g C for a cultured thraustochytrid cell (Kimura *et al*., 1999). Bacterial carbon measurements of carbon-to-ATP (250; Karl, 1980) and carbon-to-cell (0.025 pg C cell^{-1} in central Arctic Ocean; Sherr *et al*., 1997) were used to calculate the cell-to-ATP rate of 1×10^4 cells per 1 pg ATP. This value corresponds to an ATP-to-cell rate of about 20 cells per 1 pmol ATP, or 0.05 pmol per bacterioplankton cell, based on an ATP molecular weight of 507. The carbon-to-cell value of 0.025 pg C cell^{-1} coincides with the geometric average of 0.015–0.042 pg C cell^{-1} in Norwegian coastal water (Tuomi *et al*., 1995).

Results and discussion

Cell counts and carbon pool estimates

The abundance of planktonic thraustochytrids varied from values below the detection limit of 8.1×10^2 l^{-1} (sites N76, N75, N70 and N65) to 2.3×10^5 l^{-1} (site N63) with an overall average of 3.1×10^4 l^{-1}, showing patchiness with great variability (Table 1) but generally within the previously reported ranges of 10^2–10^5 l^{-1} (Naganuma *et al*., 1998; Kimura *et al*., 1999, 2001; Kimura and Naganuma, 2001; Raghukumar *et al*., 2001; Bongiorni and Dini, 2002). Larger standard deviations were due to the patchiness of cell counts from the 100 microscopic view fields, probably reflecting the clump-forming nature of thraustochytrids (Raghukumar, 2002). The thraustochytrid abundance remarkably increased to a maximum level of $>10^3$ l^{-1} at N64 and N63, the latter site being located

within 200 km from the coast of Möre og Romsdal and Sogn og Fjordane, Norway. Terrestrial influence on thraustochytrid distribution may therefore be considered (Kimura and Naganuma, 2001; Kimura *et al*., 2001); however, abundance did not simply increase with adjacency to land, and actually decreased to a 10^2 l^{-1} level at site N62, which was closest to land. The estimated thraustochytrid carbon pool was 0–37.8 μg C l^{-1}; however, this culture-based carbon-per-cell rate might be an overestimation for ecological application.

The abundance of bacterioplankton varied within a narrow range from 2.2×10^7 l^{-1} at the northernmost site (N76) to 6.0×10^8 l^{-1} at the southernmost site (N62) with an overall average of 2.1×10^8 l^{-1} (Table 1). The low abundance of 10^7–10^8 bacterioplankton l^{-1} reflects the oligotrophic conditions of the offshore Arctic and sub-Arctic waters, compared with 10^8–10^{10} l^{-1} in eutrophic coastal waters (Naganuma *et al*., 1998; Kimura *et al*., 1999, 2001; Kimura and Naganuma, 2001). The estimated bacterial carbon pool wasss 0.5–15.0 μg C l^{-1} based on the cell-to-carbon rate of 2.5×10^{-14} g C for Arctic bacterioplankton (Karl, 1980; Sherr *et al*., 1997), and was occasionally smaller than that of the thraustochytrids, ~37.8 μg C l^{-1}. Thraustochytrid carbon was likely overestimated, and therefore, determination of carbon-per-thraustochytrid cell as well as ATP-per-thraustochytrid cell in nature is needed.

The abundance of planktonic thraustochytrids and bacteria showed no significant correlation (Table 1), possibly because thraustochytrids overexploit bacterioplankton by active bacterivory (Raghukumar, 1992) and because thraustochytrids and bacterioplankton utilize organic materials of different sources (Kimura *et al*., 2001); forms of organic materials utilized by thraustochytrids and bacterioplankton were not characterized in this study. Phytoplankton bloom and post-bloom conditions should therefore be included in a future observation strategy to elucidate whether the forms of organic materials, *e.g*., labile exudates and refractory phytodetritus, control the abundance of planktonic thraustochytrids and bacteria.

ATP pool, cell count estimates and correlations

The concentration of particulate ATP ranged from 79.4 pmol at site N68 to 675.6 pmol at the southernmost site N62 (Table 1) with an overall average of 222.3 pmol; this was within the known range of 50–900 pmol for the upper euphoric zone (0–100 m deep) on a global ocean average (Karl and Dobbs, 1998). The findings of this study do not indicate that planktonic thraustochytrids and bacteria contribute greatly to the *in situ* ATP pool, as phytoplankton and heterotrophic flagellates and ciliates likely account for most of the ATP pool in the Greenland and Norwegian Seas. However, the *in situ* ATP concentration was converted to the cell abundance of thraustochytrids or bacterioplankton conceptually to evaluate the applicability of the thraustochytrid and bacterial ATP-per-cell rates in sub-Arctic seas.

Applying these values to the *in situ* ATP concentration, a thraustochytrid abundance of 2 to 609 cells l^{-1} was estimated [the estimated range was calculated from the lowest-highest particulate ATP concentration (79.4–657.6 pmol l^{-1}; Table 1) divided by the highest-lowest ATP-per-cell rate (39.26–1.11 pmol cell^{-1}; Fig. 2), respectively]. This ATP-based abundance is in contrast with the actual abundance of ~2.3×10^5 l^{-1} (Table 1). Similarly, the *in situ* ATP concentration and bacterial ATP-per-cell rate (0.05 pmol) yielded a value of 1600–13500 cells l^{-1} corresponding to 0.001–0.012% of the actual abundance,

compared with 0.09–0.18% in the oligotrophic North Pacific Ocean (calculated from Table 2.8 of Karl and Dobbs, 1998). These estimates are much lower than the generally expected proportion of metabolical activity; for example, <10% cells of total bacterioplankton (*e.g.*, Sherr *et al.*, 2001). Cellular ATP contents of thraustochytrids in nature may be <1/100 of that in culture, and the conventional ATP content of bacterial cells in nature (0.05 pmol per cell) may vary to as low as <1/10 depending on the oligotrophic waters examined. Possible variation in ATP-per-cell rates due to culture conditions, *e.g.*, temperature, salinity, and nutrient availability, should be further studied with cultured strains from *in situ* waters. Without these considerations, particulate ATP concentrations can not be considered as representative of the *in situ* abundance of planktonic thraustochytrids and bacteria.

No significant correlation was found between the thraustochytrid abundance and particulate ATP concentration (Table 1), implying that planktonic thraustochytrids are associated with non-ATP-related rather than ATP-related organic materials; post-bloom phytodetritus and terrestrial lignocellulosic matter may account for non-ATP-related organic materials. In contrast, the bacterioplankton abundance (y, 10^8 l^{-1}) and particulate ATP concentration (x, pmol) were significantly correlated (y=0.0086x, r^2=0.56, p<0.01), implying that the bacterioplankton abundance is associated with ATP-related organic materials such as phytoplankton-derived dissolved organic matter, particularly exudates, in the Greenland and Norwegian Seas, as often observed in diverse waters. It is also suggested that thraustochytrids and bacteria may have different physiological responses, with variable ATP-per-cell rates, against starvation in oligotrophic waters.

Conclusions

The abundance of planktonic thraustochytrid and bacterioplankton and concentration of particulate ATP in the Greenland and Norwegian Seas varied within the known oligotrophic ranges. The particulate ATP pool accounted for only fractions of the planktonic thraustochytrids and bacteria populations, suggesting that they are less active in Arctic and sub-Arctic surface waters. The particulate ATP concentration was correlated with the abundance of bacterioplankton with statistical significance, but not to the abundance of planktonic thraustochytrids. These differing relationships suggest different preferences to organic sources, despite the lack of supportive data at present. The influence of the quality and quantity of dissolved organic matter on growth of planktonic bacteria and thraustochytrids should be analyzed in future studies, and similarly, efforts to isolate autochthonous thraustochytrid strains should be made to investigate their ATP-per-cell variation wit regard to culture conditions and starvation.

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