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Species diversity and changes of communities of heterotrophic flagellates (protista) in response to glacial melt in King George Island, the South Shetland Islands, Antarctica

DENIS TIKHONENKOV[1,2]

[1]*Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, 152742, Russia*
[2]*Canadian Institute for Advanced Research, University of British Columbia, 6270 University Boulevard, Vancouver, BC, V6T1Z4, Canada*
tikho-denis@yandex.ru

Abstract: Glacial melt has a great influence on biological communities of the Antarctic Peninsula. Annual changes in heterotrophic flagellates from March 2008–March 2009 and effects of glacial melting on heterotrophic flagellates from December 2008–March 2009 were studied within the coastal zone of King George Island. The maximum abundance and biomass occurred in November and December (950.6–1236.2 individuals ml^{-1}; 0.02–0.035 $\mu g\,C\,ml^{-1}$), and the minimum in May and June (419.8–456.8 individuals ml^{-1}; 0.018–0.019 $\mu g\,C\,ml^{-1}$). Forty-five species were identified. The diversity of choanoflagellates, euglenids, bicosoecids, kinetoplastids and *incertae sedis* flagellates was greatest. Glacial melt between December and April resulted in the freshening of the surface water at the Collins Bay, giving rise to a vertical gradient of salinity (from 26‰ at the surface to 34‰ at the near-bottom layer). The trophic, size and species structure of the heterotrophic flagellates was simplified due to freshening of the surface waters. Eurybiontic and cosmopolitan species were significantly enriched in the freshened surface layer, with prevalence of small-sized mobile bacterio-detritovorous forms. The simplification of structure of the assemblage of heterotrophic flagellates can affect the stability of biological communities.

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Key words: abundance and biomass, climate change, glacier melting, marine plankton, species structure

Introduction

The area around the Antarctic Peninsula is experiencing the most rapid climate warming in the world (Steig *et al*. 2009). A number of studies have shown that climate change can influence the composition of biological communities (Moline *et al*. 2004). Increasing temperatures in the West Antarctic have exceeded 0.1°C per decade over the last fifty years have attributed to global warming and the increased strength of circumpolar westerlies (Steig *et al*. 2009). The most gradual air temperature increases over the past decades has been recorded from research stations (i.e. Vernadsky, Rothera, and Bellingshausen) located on the western peninsula and nearby islands (Tymofeyev 2006). The warming of the air temperatures has accelerated glacial melting. Satellite remote-sensing data of ice elevations and ice motion indicate significant glacial ice loss, up to 31–196 $Gt\,yr^{-1}$ in West Antarctica in recent years (Chen *et al*. 2009). It was shown that glacial meltwater has a great influence on hydrological characteristics of the Antarctic Peninsula, particularly on the stratification of the water column, as well as its turbidity (Dierssen *et al*. 2002). Consequently, this can impact the structure of hydrobiont communities. For example, in the nearshore coastal waters along the Antarctic Peninsula, a recurrent shift in phytoplankton from diatoms to cryptophytes has been documented (Moline *et al*. 2004, Schofield *et al*. 2010). Such change represents a shift in the physical size of the phytoplankton, making individuals smaller, which will negatively impact the zooplankton communities and the aquatic ecosystem as a whole.

Observations of marine ecosystems in this area are fragmented and do not represent shifts in the various biological communities (Usov 2007). In particular, patterns of seasonal changes of communities and populations remain unexplored. A single sampling effort often characterizes only spatial patterns of distribution, not temporal. Often, the characteristics of seasonal dynamics of plankton are reconstituted from data obtained during different seasons and years. Studies characterizing the changes in planktonic coenosis are rather sporadic, and there is no information on the climate-induced structural transformation of protozoans (heterotrophic flagellates (HFs), in particular). At the same time, HFs represent the smallest and the most mobile eukaryotes, with the fastest metabolism and reproduction. Heterotrophic flagellates are also sensitive to various external influences (e.g. salinity, Eh and pH). They are the most important consumers of bacteria in microbial assemblages, as well as important prey for other protists (ciliates, dinoflagellates) and metazoans

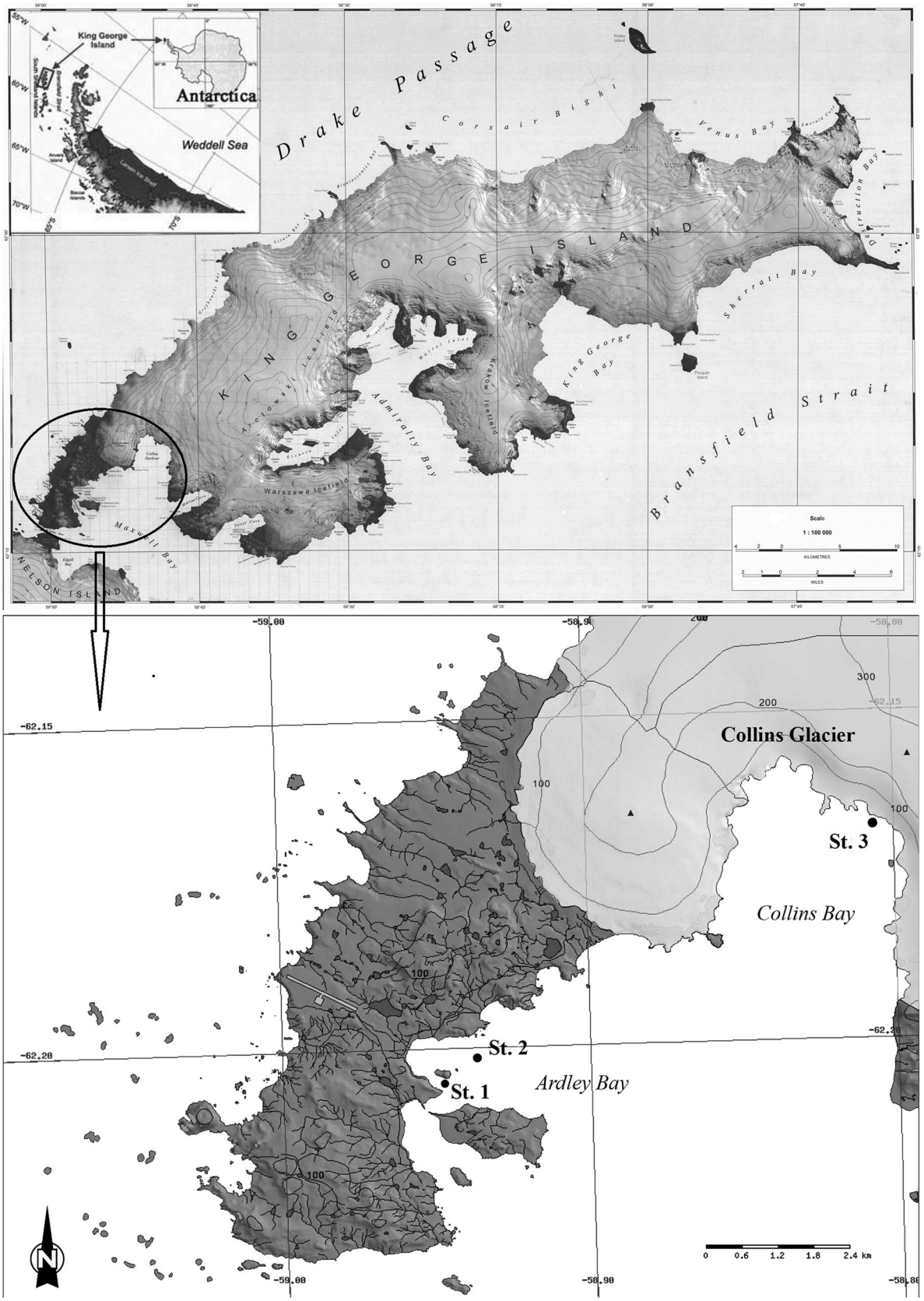

Fig. 1. Location of studied sites along the coastal zone of King George Island (the South Shetland Islands, the Antarctic) from March 2008–March 2009.

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(Sherr & Sherr 1988). Some HFs can assimilate dissolved organic matter, for which they compete with bacteria (Sanders 1991). Heterotrophic flagellates make a considerable contribution in energy flow, as well as biogeochemical cycling, and play a cardinal role in carbon and nutrient regeneration (Arndt *et al*. 2000). Thus, studying their response to environmental changes is merited.

Heterotrophic flagellates are often considered as "a black box" in ecological investigations. At that, only total abundance, biomass, and rates of food consumption are usually estimated. Characteristics of species, trophic and size structure remain unexplored. However, HFs represent a very diverse ecologic group and include species considerably different in trophic preferences (bacteriodetritophages, eukaryovorouses, omnivorouses), feeding strategies, sizes, and other ecological characteristics (Sanders 1991, Arndt *et al*. 2000, Boenigk & Arndt 2002). Cell biovolumes of different HF species vary by several orders of magnitude. Estimation of abundance of each taxonomic, trophic, and size group as well as abundance of each species is very important to understand the effects of glacial melting on HF structure under rapid climate change.

The aim of the present study is to investigate the species composition and annual quantitative and qualitative structural changes in HF along the coastal zone of King George Island, located on the South Shetland Islands in the Antarctic. Studies were carried out according to the International Polar Year project CLICOPEN (CLImate Change in COastal areas of the Antarctic PENinsula) to estimate the influence of glacial melt on sea and coastal ecosystems of the Antarctic Peninsula.

Materials and methods

Study area and sampling

We investigated species diversity, distribution, abundance, biomass and structural changes in planktonic HF at three stations along the coastal zone of King George Island (the South Shetland Islands, Antarctica) (Fig. 1). Station 1 (depth 27 m) was situated near the seashore of Ardley Bay (58°56'51"W, 62°12'20"S), station 2 (depth 60 m) was located in the open part of Ardley Bay (58°56'22"W, 62°12'09"S), and station 3 (depth 38 m) was in the north-eastern part of Collins Bay in the immediate vicinity of Collins Glacier (58°47'44.5"W, 62°10'20.5"S).

At station 1, samples were taken from the surface of the water and 10, 15 and 25 m from March 2008–March 2009. During the same period, samples were collected from the water surface and 10, 15, 25, and 50 m at station 2. At station 3, samples were taken from the surface of the water and 5, 10, 15, 25, and 35 m during the time of active glacial melting from December 2008–March 2009. Station 3 was inaccessible in winter time.

Sampling was undertaken at approximately 14-day intervals. Breaks in the sea ice allowed access to the three sites. No samples were collected on 9 July 2008 and 7 August 2008, due to adverse weather conditions. All samples were collected using reversing bathometers (BM-48). Water temperature at various depths was measured using reversing thermometers, and salinity was measured using a conductometer (YSI). The investigations were performed at the Russian Bellingshausen Station during the 53rd Russian Antarctic Expedition.

Microscopy, counting and identification

Thirty millilitres of each sample were decanted into petri dishes (15 ml in each) for viewing by light microscopy. Quantitative and qualitative analyses of HF were performed in fresh samples so that small HFs could be identified to species level. A live-counting technique was used to quantify HFs in each sample (Massana & Güde 1991, Gasol 1993). An important advantage of this method is that

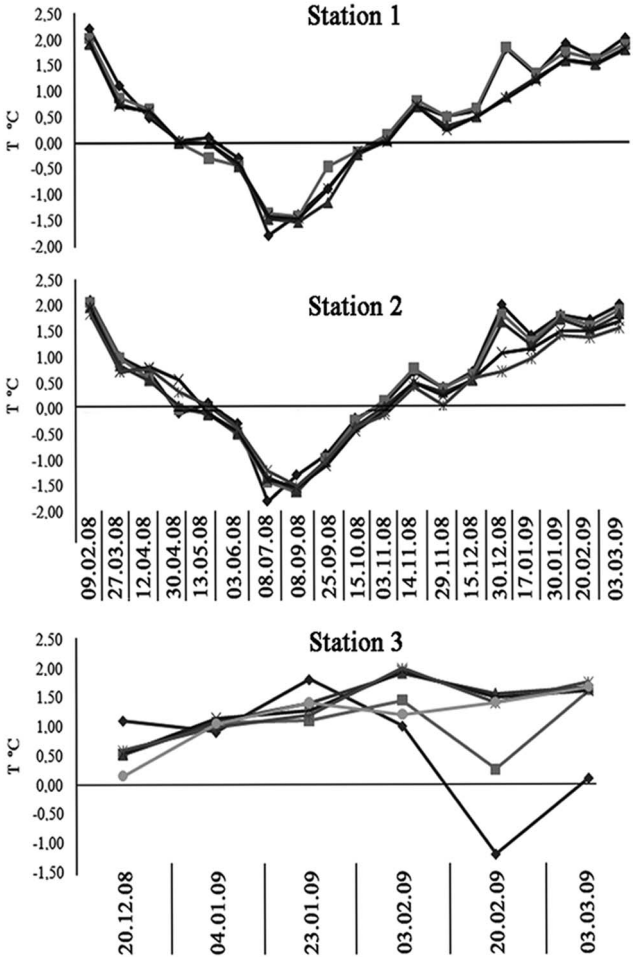

Fig. 2. Seasonal changes of average water temperature along the coastal zone of King George Island from March 2008–March 2009.

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Table I. Average values and range of temperature and salinity on different layers of water column on the stations of sampling along the coastal zone of King George Island from March 2008–March 2009.

Water layers (m)	Temperature (°C)		
	Station 1	Station 2	Station 3*
0	0.52 (2.2 to -1.8)	0.53 (2.1 to -1.8)	0.62 (1.8 to -1.2)
5			1.00 (1.61 to 0.25)
10	0.52 (2.03 to -1.43)	0.48 (2.07 to -1.63)	1.35 (1.9 to 0.51)
15	0.36 (1.91 to -1.54)	0.41 (1.98 to -1.6)	1.33 (1.94 to 0.53)
25	0.38 (1.93 to -1.47)	0.37 (1.95 to -1.54)	1.31 (1.99 to 0.59)
35			0.14 (1.66 to 0.15)
50		0.31 (1.82 to -1.52)	
Water layers (m)		Salinity (‰)	
	Station 1	Station 2	Station 3*
0	34.3 (35.1 to 34.0)	34.3 (35.1 to 34.1)	26.02 (27.0 to 25.5)
5			32.18 (32.6 to 31.8)
10	34.3 (35.1 to 33.9)	34.3 (35.1 to 34.0)	33.03 (33.2 to 32.8)
15	34.3 (35.1 to 34.1)	34.3 (35.1 to 33.9)	33.45 (33.9 to 33.2)
25	34.3 (35.1 to 34.1)	34.3 (35.1 to 34.0)	33.87 (33.7 to 34.1)
35			34.10 (34.2 to 34.0)
50		34.3 (35.2 to 34.0)	

*the measurements were carried out during the period from December 2008–March 2009 only.

morphological and behavioural features that would otherwise be destroyed in fixed samples could be used to differentiate the taxonomic, trophic, size group and species of HFs. Live cells of each species were counted in petri dishes (diameter 60 mm) in 850 fields of view (i.e. five transects were observed at a magnification of 63x; the petri dish was rotated clockwise 45° prior to viewing another transect). Direct observation provides 'real' live dimensions without shrinking or swelling effects and there is no loss of cells due to fixation or staining. Due to excystment, predation or death of cells during observation, some bias is possible. Taxonomic determination, measurement, and handling of living cells (especially for fast moving species) are difficult. Live video recordings were used to increase quality and consistency of the counts during microscopic investigations.

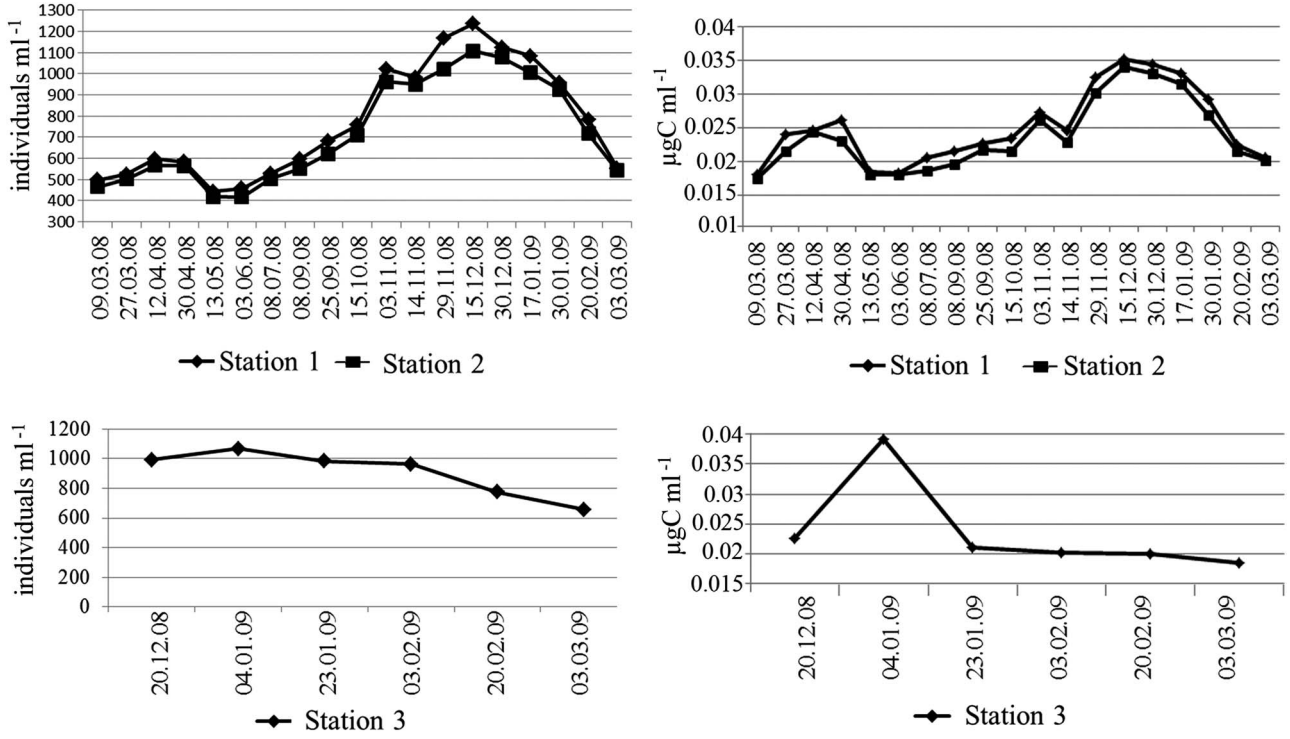

Fig. 3. Annual dynamic of heterotrophic flagellates abundance and biomass.

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Table II. Species list, relative abundance*, % and trophic type of heterotrophic flagellates along the coastal zone of King George Island.

Morphospecies	Station 1	Station 2	Station 3	Trophic group
OPISTHOKONTA				
Choanomonada				
Acanthocorbis sp.	1.25	1.16	0	BF
Acanthoeca sp.	1.7	0.56	0	BF
Bicosta sp.	0	0.48	0	BF
Cosmoeca sp.	0.8	1.36	0	BF
Diaphanoeca grandis Ellis	1.4	3.64	0	BF
Diaphanoeca sp.	0.9	1.12	0	BF
Kakoeca antarctica Buck & Marchant	3.55	3.28	1.2	O
Salpingoeca infusionum Kent	0	0	1.67	BF
S. tuba Kent	0	0	1.43	BF
Salpingoeca sp.	1.6	0.64	0	BF
Stephanoeca diplocostata Ellis	7	10.6	6.37	BF
RHIZARIA				
Cercozoa				
Cryothecomonas armigera Thomsen, Buck, Bolt & Garrison	4.3	3.56	1.03	O
Metromonas simplex (Griessmann) Larsen & Patterson	0.95	0.48	0	C
Protaspa simplex Vørs	0	0	2.57	BC
P. verrucosa Larsen & Patterson	1.2	1.24	0	BC
Protaspa sp.	0.95	1.88	0	BC
CHROMALVEOLATA				
Cryptophyceae				
Goniomonas amphinema Larsen & Patterson	0.9	0	0	O
*G. pacifica*** Larsen & Patterson	2.55	1.56	5.87	O
Stramenopiles				
Caecitellus parvulus (Griessmann) Patterson, Nygaard, Steinberg & Turley	1.1	0	0	BC
Cafeteria roenbergensis Fenchel & Patterson	8.85	9.2	8.9	BF
Cafeteria sp.	1.75	2.8	0	BF
Ciliophrys infusionum Cienkowsky	1.35	2.28	0	BF
Discoselis saleuta Vørs	3.65	0.76	1.13	BC
Pseudobodo tremulans Griessmann	1.15	2.12	2.13	BF
Paraphysomonas sp.	8.2	8.08	9.3	O
Pteridomonas danica Patterson & Fenchel	2.85	0	0	BF
Alveolata				
Amphydinium sp.	0.8	0.56	0	C
EXCAVATA				
Heterolobosea				
*Percolomonas cosmopolitus*** (Ruinen) Fenchel & Patterson	1.95	1.32	3.47	BF
Stephanopogon colpoda Entz	0	0.88	0	C
Euglenida				
Notosolenus sp.	1.15	1.16	0	O
Petalomonas minor Larsen & Patterson	0	0	2.77	BC
P. minuta Hollande	1.1	0	4.37	BC
P. pusilla Skuja	0.85	0.64	5.2	BC
Petalomonas sp.	0	0.48	0	BC
Ploeotia sp.	1.1	1.76	0	O
Kinetoplastea				
Bodo saltans Ehrenberg	0.55	0.56	1.63	BC
Neobodo curvifilus (Griessmann) Moreira, Lopez-Garcia & Vickerman	0	0	0.9	BC
Neobodo designis (Skuja) Moreira, Lopez-Garcia & Vickerman	10.5	11.4	10.3	BC
Neobodo saliens (Larsen & Patterson) Moreira, Lopez-Garcia & Vickerman	3.25	2.44	0	BC
Rhynchomonas nasuta (Stokes) Klebs	0	0	6	BC
*Procryptobia sorokini*** (Zhukov) Frolov, Karpov & Mylnikov	10.5	11.2	6.77	BC
APUSOZOA				
Ancyromonas sigmoides Kent	9.2	10.5	11.4	BC
Thecamonas mutabilis (Griessmann) Larsen & Patterson	0	0	1.97	BC
Eukaryotes *incertae sedis*				
Kiitoksia kaloista Tong, Vørs & Patterson	0	0.28	0	BC
Kiitoksia ystava Vørs	1.1	0	3.63	BC

*Relative abundance of each species was calculated as a portion (%) of total abundance of species in total abundance of HFs on the station during the study period. Data on all water depths and sampling dates summarized.

**Species were identified from clonal cultures.

BC = bacterio-detritophage collector feeders, BF = bacterio-detritophage filter feeders, O = omnivorous, C = carnivorous.

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Size and biovolume were estimated from video-micrographs. Species were distinguished in accordance with cell shape and size, flagella movement and arrangement, and behaviour and locomotion of the cell (Arndt *et al*. 2000). The observed species were identified using the following as taxonomic guides: Larsen & Patterson (1990), Leatbeater (1991), Thomsen & Buck (1991), Vørs (1992, 1993), Ikävalko & Gradinger (1997), Tong *et al*. (1997), Lee & Patterson (2000). All HFs were identified to species or genus. Only colourless (presumably heterotrophic) species of dinoflagellates were recorded. Light microscopy observations were made on a MBI-3 microscope equipped with phase contrast and water immersion objectives at a total magnification of 700x. Video recordings were carried out for more precise identification and measurement. Clonal cultures were used for morphological investigation of some forms. Depending on the concentration of organisms in the samples, 10–100 µl aliquots were serially diluted in 250 µl well-plates with sterile marine water enriched by *Pseudomonas fluorescence* bacteria in a 96-well cell culture plate. The plate was then sealed with parafilm and incubated at room temperature for a few days. Wells containing a single morphospecies were transferred into a 70 mm petri dish with sterile marine water enriched by *Pseudomonas fluorescence* bacteria. Other wells required repeated serial dilution to obtain pure cultures. We established three cultures (*Procryptobia sorokini*, *Percolomonas cosmopolitus* and *Goniomonas pacifica*). Fixatives and scanning or transmission electron microscopes were not available for use during this study. Therefore, organisms for which these methodologies are necessary for confident identification to the level of species (e.g. some chrysomonads, cercozoans and choanoflagellates) were identified to the genus level.

Data analysis

Cell volumes of HF were calculated by approximation to simple geometrical figures using our own measurements. The results of the calculations were further used for estimation of biomass (the density was assumed to equal 1 g cm^{-3}). The wet biomass of HFs was converted to carbon according to Børsheim & Bratbak (1987). The dimensional spectral construction was carried out according to standard technique (Sheldon *et al*. 1972). For this purpose organisms were subdivided into dimensional classes (Mazei *et al*. 2005). The values of a relative biomass of organisms were plotted against them.

Observations of feeding in samples and cultures (live culture collection of protists of the Institute for Biology of Inland Waters (IBIW), Russian Academy of Sciences (RAS), Borok) and the literature (Larsen & Patterson 1990, Sanders 1991) were used for the analysis of trophic structure. Shannon's diversity index was applied in order to obtain the integral characteristic of community structure.

The classification of communities (R-analysis) by their species structure (quantitative data) was conducted with the aid of a cluster analysis (complete linkage), based on the matrix of Simpson similarity indices. To reveal common trends in the distribution of heterotrophic flagellates (Q analysis), species ordination was carried out by means of principle components. Relative abundance of each species (%) in total abundance of HFs on the different depths of the stations for all the sampling periods was used for analysis. Spearman's correlation coefficients between the values of principal components (factor loadings) and salinity were calculated. These coefficients were significant at $P < 0.01$. All computations were performed with the program package PAST 1.89.

Results

Temperature and salinity

The water stations in this study were characterized by a pronounced seasonality (Fig. 2) with a maximum temperature at the beginning of March (2.0–2.2°C) and

Table III. Average abundance A (individuals ml^{-1}) and biomass B (µgC ml^{-1}) of dominant taxa and species diversity, with standard error of the arithmetic mean given below.

Taxa	Station 1		Station 2		Station 3	
	A	B	A	B	A	B
Choanoflagellida	320.84	0.0103	260.68	0.0075	256.18	0.0073
	± 43.71	± 0.0013	± 35.95	± 0.0007	± 34.73	± 0.0007
Euglenida	292.12	0.0070	252.36	0.0068	180.82	0.0039
	± 41.71	± 0.0011	± 34.28	± 0.0011	± 25.36	± 0.0004
Bicosoecida	186.24	0.0064	144.98	0.0046	138.24	0.0035
	± 25.68	± 0.0011	± 23.06	± 0.0004	± 19.71	± 0.0004
Kinetoplastea	168.44	0.0053	138.62	0.0042	124.46	0.0040
	± 22.40	± 0.0007	± 20.05	± 0.0004	± 19.18	± 0.0004
Incertae sedis	94.78	0.0024	84.26	0.0020	66.84	0.0013
	± 12.84	± 0.0002	± 11.76	± 0.0002	± 9.42	± 0.0002
Total species-richness	31 (30*)		29 (29*)		20	
Shannon diversity index	2.64		2.42		1.31	

*values calculated only for the period from December 2008–March 2009.

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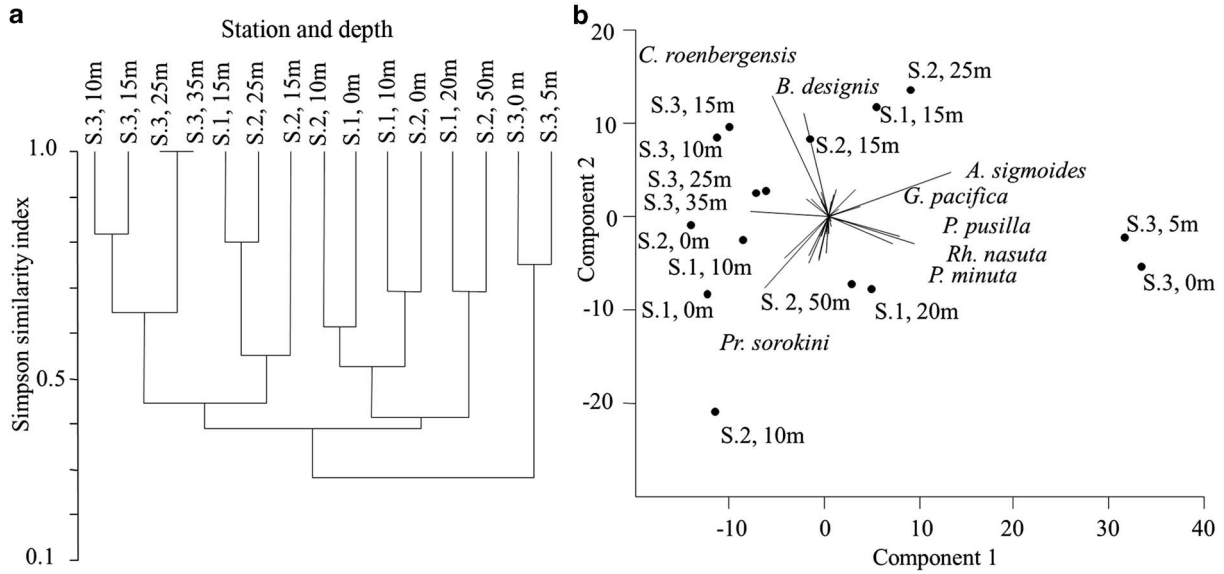

Fig. 4. Results of **a.** classification, and **b.** ordination of heterotrophic flagellates: species (marked by lines) *Ancyromonas sigmoides*, *Petalomonas minuta*, *P. pusilla*, *Rhynchomonas nasuta*, *Goniomonas pacifica* and stations (marked by dots) S.3, 0 m and S.3, 5 m plotted alongside and against other species and stations along the first principal component on b.

minimum temperature at the beginning of July (-1.8°C). The waters of Ardley Bay were characterized by negative temperatures from the middle of May to the middle of October. The ice cover during the winter period was not uniform, due to considerable wind and the choppy water surface. The temperature stratification of the water column (direct during the spring–summer period and reverse in the autumn–winter period) was revealed at stations 1 and 2 (Table I). The values of water temperature at station 3 were non-uniform (Fig. 2, Table I). Perhaps the water temperature at station 3, particularly the upper layer, was defined by intensity of glacial meltwater flow, which was highly variable on different sampling and measurement dates.

Water salinity at coastal stations 1 and 2 in the open part of Ardley Bay changed from 33.9‰–35.1‰ throughout the year. No vertical salinity gradient was observed at these stations (Table I). Generally, salinity at different depths at stations 1 and 2 did not differ considerably (by 0.1‰). The greatest difference between maximum and minimum salinity was 0.7‰. However, glacial melt from December–April resulted in the freshening of surface water at the station close to Collins Glacier (station 3), giving rise to a clear vertical salinity gradient from 26‰ at the surface layer to 34‰ at the near-bottom layer 35 m deep (Table I).

Seasonal changes in HF

The maximum abundance and biomass occurred in November and December (950.6–1236.2 ind. ml^{-1}; 0.02–0.035 μg C ml^{-1}). As a whole, abundance and biomass of HF increased from winter to summer (Fig. 3). The lowest abundance and biomass were in May and June (419–456 ind. ml^{-1}; 0.018–0.019 μg C ml^{-1}). It was not possible to clearly characterize the seasonal changes of quantitative characteristics of the community at station 3 due to the period of investigations being too short.

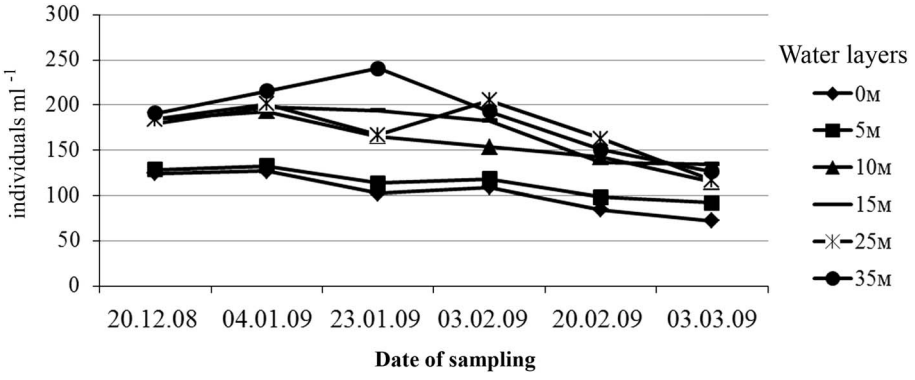

Fig. 5. Changes of average abundance of heterotrophic flagellates per depth on station 3 during the summer.

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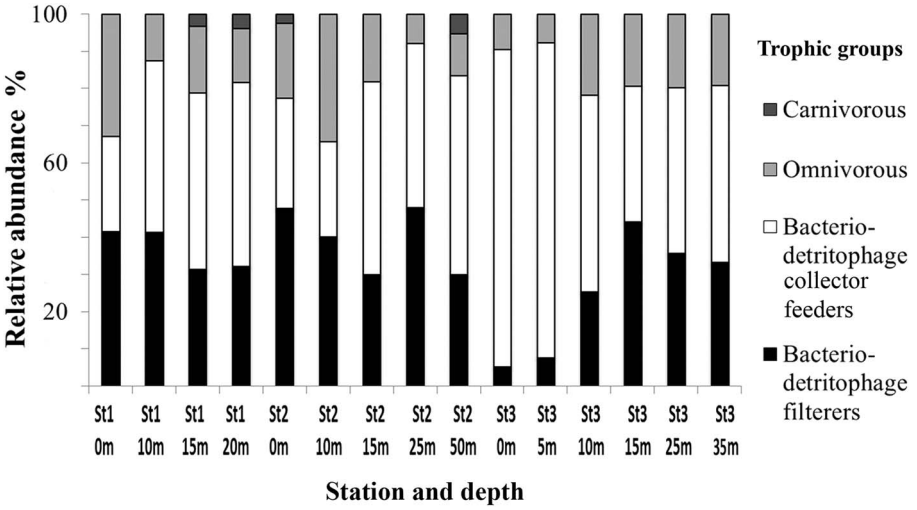

Fig. 6. Trophic structure of heterotrophic flagellates communities: percentage of abundance of different trophic groups for all the sampling periods.

The vertical distribution and abundance of organisms at stations 1 and 2 was irregular. The average species abundance was non-uniform throughout the water column during the year. This can potentially be explained by the absence of pronounced vertical gradients for temperature and salinity.

Forty-five species of HFs were identified (Table II). The diversity, abundance and biomass of choanoflagellates, euglenids, bicosoecids, kinetoplastids and *incertae sedis* flagellates were highest (Table III). The coastal stations 1 and 2 in the open part of the Ardley Bay showed similar levels of species diversity and richness (31 and 29 species, respectively). The obligate marine species *Stephanoeca diplocostata*, *Cafeteria roenbergensis*, and *Procryptobia sorokini* occurred most frequently.

Effects of glacial melting on HF

Station 3, which was subjected to glacial melt, was characterized by low species diversity (Shannon's index = 1.31) and richness (20 species) (Table III). Obligate marine species were replaced by freshwater-inhabiting eurybiontic and euryhaline species, particularly in the surface water layers of station 3.

The assemblages in the freshened surface water layers of station 3 (S.3, 0 m and S.3, 5 m) were characterized by a different structure compared to stations 1 and 2 (Fig. 4a). Similarly, the deeper water layers of station 3 (e.g. 10, 15, 25, and 35 m) exhibited low species number and contained assemblages that varied from those taken at depth (e.g. 10, 15, 25, and 50 m) at the other stations.

Principal component analysis was performed to identify species associated with a particular biotope (Fig. 4b). The first four principal components (PC) explained 79% of the total dispersion of species composition. The communities of the freshwater layers at station 3 could be subdivided along the first PC; the characteristic species were *Ancyromonas sigmoides*, *Petalomonas minuta*, *P. pusilla*, *Rhynchomonas nasuta*, and *Goniomonas pacifica*. The first PC showed a strong inverse correlation to salinity ($R = -0.7$, $P = 0.003$).

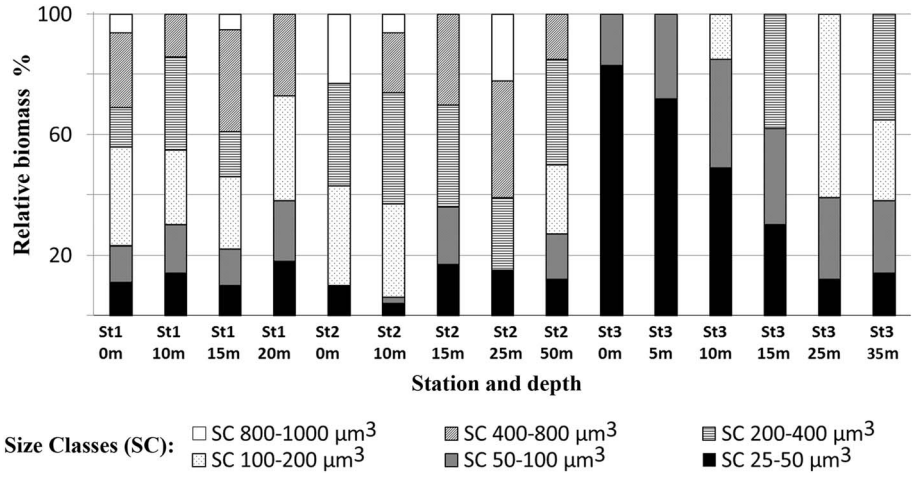

Fig. 7. Size classes (SC) of heterotrophic flagellates: percentage of biomass of different size groups for all the sampling periods.

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At station 3, which did possess a vertical salinity gradient, the assemblage characteristics increased with depth (Fig. 5). The largest difference in flagellate abundance was observed between the freshened layers of water (0 and 5 m) and the layers below.

At the same time species composition of HFs changed with depth at station 3. The eurybiontic and cosmopolitan species, such as *Ancyromonas sigmoides*, *Goniomonas pacifica*, *Petalomonas minuta*, *P. pusilla*, and *Rhynchomonas nasuta* (see videos S1–S5, which will be found at http://dx.doi.org/) were abundant in the freshened surface layer, whereas total species number here was low. All these organisms are among the twenty most commonly reported species of HF (Patterson & Lee 2000). The obligate polar and sub-polar species (e.g. *Kakoeca antarctica* and *Cryothecomonas armigera*) which were observed in the habitats with higher marine salinity, were not observed in the lower salinity samples taken from the freshened surface water layers.

The trophic, size and species structure of the assemblages were simplified owing to the freshening of the surface water layer. No carnivorous (eukaryovorous) HFs were observed at station 3 (Fig. 6). Small mobile bacterio-detrivorous feeders constituted a considerable part of the flagellate communities populations in the freshened surface layers, whereas bacterio-detritovorous filter feeders (accounting for 38% of the species at other stations) were scarce. In the non-freshened layers, trophic structure changed insignificantly: bacteriovorous HFs were dominant, while filter feeders and collector feeders were present in equal proportions. Carnivorous HFs (e.g. *Amphydinium* sp., *Metromonas simplex*, and *Stephanopogon colpoda*) were the trophic group encountered least often.

Only two size classes of flagellates (25–50 μm^3 and 50–100 μm^3) were observed in the freshened water layers (Fig. 7). The smallest flagellates (30 μm^3) were the most abundant species assemblages. Heterotrophic flagellates of sizes ranging from 25–1000 μm^3 were observed in other biotopes. Populations in normally saline layers were characterized by more complex dimensional structure, and larger HFs (size classes 100–200 μm^3, 200–400 μm^3, and 400–800 μm^3) dominated.

Discussion

We used light-microscopy to observe living HFs with the goal of obtaining an exhaustive species list, and to explore factors influencing the composition of flagellates in the Antarctic. We quantified living cells representing ten taxonomic groups of HFs, and estimated the change in their abundance and biomass over a one-year period. We used live cell counting and live videos in order to reliably identify individual cells and accurately determine the species and group affiliation. Although this approach is sometimes considered less suitable for cell quantification than fixation and epifluorescence microscopy, it enabled us to accurately describe the species, taxonomic and trophic structures. Obtaining these data is essential to understand the effects of glacial melting on HF populations and the microbial food web. In contrast, epifluorescence provides information only on total cell abundance, and does not allow a definite assignment of eukaryotic cells to 'heterotrophic flagellates' since flagella are often invisible (Boenigk & Arndt 2002). As such, epifluorescence nanofaunal counts may include small naked amoebae, yeasts, and zoospores belonging to a range of organisms, disrupted cells from unrelated eukaryotes and possibly even large bacteria with a large nucleomorph (Arndt *et al*. 2000). In addition, a non-uniform shrinkage of flagellates during fixation makes reliable estimation of biovolume difficult (e.g. Sonntag *et al*. 2000).

It has been shown that despite the considerable variability in abundance, the taxonomic composition of dominant HFs is surprisingly uniform (Hewes *et al*. 1990, Arndt *et al*. 2000, Auer & Arndt 2001). On average, the annual pelagic HF biomass comprises about 20–50% of heterokont taxa (mainly chrysomonads and bicosoecids), 5–40% choanoflagellates, and 1–8% kinetoplastids. Five other HF groups (small dinoflagellates, thaumatomastigids, apusomonads, colourless cryptomonads and euglenids) are also usually present assemblages, but comprise a minor fraction of the total biomass (Arndt *et al*. 2000). Choanoflagellates and bicosoecids were the dominant pelagic groups, while the diversity, abundance and biomass of chrysomonads were not significant. Small bacterivorous and osmotrophic euglenids and *incertae sedis* flagellates (such as *Kiitoksia ystava*, *K. kaloista*, and *Metromonas simplex*).

The seasonal population dynamics of marine choanoflagellates have been previously studied in the coastal zone of eastern Antarctica at Davis Station (Marchant & Perrin 1990). The species structure of choanoflagellates was similar to the species composition we observed. It has also been shown that flagellate abundance is positively correlated with bacterial biomass and productivity and increases several times in the summer (10–1000 individuals ml^{-1}) compared to the winter minimum (0.1 individuals ml^{-1}) (Buck & Garrison 1988). Similar dynamics of abundance was demonstrated for this dominant taxa. However, the differences in absolute values were not as great: from 72 individuals ml^{-1} in the winter to 391 individuals ml^{-1} in summer.

Abundance, biomass, and trophic structure of HFs have been investigated in benthic microbial communities in streams saturated mainly by glacial meltwater near Jubany Station located 10–15 km away from our research site (Dietrich & Arndt 2004). It was shown that the HF populations of meltwater are composed predominantly of small (5–10 μm) cosmopolitan species, with chrysomonads, kinetoplastids and euglenids being the most dominant. Kinetoplastids *Rhynchomonas nasuta*, *Bodo saltans*, *Neobodo curvifilus*, and *N. designis* formed

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10–40% of the total abundance; euglenids *Petalomonas pusilla*, *P. minuta*, *Dylakosoma* sp., *Peranema* spp., *Anisonema* sp. formed 6–40%. The results of the present study are consistent with these observations; the same eurybiontic and cosmopolitan species of kinetoplastids and euglenids were dominant in the surface meltwaters at station 3. Furthermore, *Bodo saltans*, *Neobodo designis* and *Rhynchomonas nasuta* are the most halotolerant species among HFs (Arndt *et al*. 2000).

The majority of flagellates observed were bacteriovorous and therefore likely to play an important role in the control of abundance, production and structure of the bacteriocenoses. The observed species have different feeding strategies (raptorial-feeding, interception feeding, filter feeding, and diffusion feeding). Among these, active searching and capturing of food (raptorial feeding) prevailed, which is attributed to fast-moving species with special structures for food capturing and absorption. However, classification of HFs into trophic types was challenging, because protist feeding behaviour can be very complex and requires a combination of different techniques (observations of feeding in the samples and clonal cultures, live-counts, study of bacterial numbers, environmental measurements of DOM etc.). In addition, nutritional versatility occurs in extreme environments. Therefore, it is not clear whether previous reports on HF nutrition are relevant to Antarctic waters. To understand whether the trophic structure of HF populations changes with decreasing salinity, we classified heterotrophic flagellates into four distinguishable trophic groups based on the predominant type of feeding. This classification is based mainly on contrast between bacteriodetritophage collector feeders to filter feeders, which represent two distinct morpho-physiological and behavioural types. All choanoflagellates were classified as filter feeders (although it is known that some of them can take up dissolved organic carbon (DOC)). The feeding in most species was observed during counting. For other species our previous data on feeding in clonal cultures (live culture collection of protists of IBIW RAS, Borok) and available literature data were used. Despite the high uncertainty of such classification, we believe that this approach is suitable for the purpose of the present investigation.

Overall, few publications have been devoted to marine planktonic protozoan populations in the Antarctic. Clearly, these organisms act as consumers of bacterio- and phytoplankton (Kivi & Kuosa 1994, Becquevort 1997, Vaque *et al*. 2004) and are an essential food source for zooplankton and Antarctic krill (*Euphausia superba* Dana) (Schmidt *et al*. 2006). As a result, studying the dynamics of protists (HFs in particular) and the structural variation of their coenoses is important for a precise understanding of their role in the functional organization of the Antarctic ecosystems. The function of the microbial food web depends to a considerable extent on the structure of the protists (mainly HFs and ciliates) being the intermediate link in the transformation of matter and energy from phytoplankton to zooplankton. Omnivorous and eukaryovorous species of HFs can feed on phytoplankton and picophytoplankton. Bacterivorous flagellates feed mainly on heterotrophic bacteria, which exploit the pool of DOC largely derived from the phytoplankton. Additionally, some omnivorouses are able to exploit the DOC pool directly.

Important data on meltwater inducing structural changes in the plankton have been obtained: in the coastal waters along the Antarctic Peninsula, a recurrent shift in phytoplankton community structure from diatoms to cryptophytes has been documented (Moline *et al*. 2004, Schofield *et al*. 2010). This shift was connected with the glacial meltwater, lowering salinity and increasing turbidity. A change from diatoms to cryptophytes represents a shift in the size structure of the phytoplankton, which would, in turn, impact the zooplankton community. The problem is that the small cryptophytes are not grazed efficiently by Antarctic krill, a keystone species in the Antarctic food web. This scenario can lead to an increased occurrence and abundance of large swarms of salps (Desmomyaria) within the region.

A reduction in salinity and meltwater-induced alteration of HF can affect organisms at other trophic levels of the entire ecosystem. Fluctuations in HF should therefore be taken into account in estimates of future changes in the Antarctic. Studies of the seasonal dynamics of zooplankton at Bellingshausen Station in 2006–07, which used the same sampling scheme as the present study (stations 1 and 2), demonstrated that small planktonic forms increased in abundance during the winter (Povazhny & Neelov 2007/2008), with maximum abundance in June–July and March (Usov 2007). A negative correlation of abundance is evident from the comparison of HF and zooplankton for present data and 2006–07 respectively. This can indicate importance of top-down control from grazers in seasonal dynamics of HFs. Marine zooplankton of the studied area is dominated by small-sized Cyclopoida *Oithona similis* Claus (Usov 2007). *Oithona* can be a protozooplankton predator. Nakamura & Turner (1997) reported that *O. similis* had a diet based on autotrophic/heterotrophic (dino)flagellates, ciliates and nauplii.

Glacial meltwater influence on marine biota is also connected with the nutrient dynamics (Dierssen *et al*. 2002). Glacial flow carries macro- and micronutrients, including phosphates, nitrates, and silicates important for bacterial and phytoplankton production. Additionally, concentrations of micronutrients, such as iron, have been shown to limit the maximum production in pelagic regions of the Southern Ocean (Martin *et al*. 1990). All these can impact HFs and require further analysis of nutrient concentrations in meltwater flows and the water column.

Overall, glacial meltwater has a great influence on hydrological characteristics and biological communities of the Antarctic Peninsula. We have shown that the trophic,

size and species structure of HF was simplified due to the glacial melting and freshening of the surface waters. The eurybiontic and cosmopolitan species were abundant in the freshened surface layer; however, the total species diversity was low. These results can be applied to the development of environmental monitoring for observing changes in ecological conditions in the Antarctic Peninsula.

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Supplemental material

Five supplemental video clips of *Ancyromonas sigmoides*, *Goniomonas pacifica*, *Petalomonas minuta*, *Petalomonas pusilla* and *Rhynchomonas nasuta* can be found at http://dx.doi.org/10.1017/S0954102013000448.

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