Bacterivory by phototrophic picoplankton and nanoplankton in Arctic waters

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1   **Abstract**

2   Mixotrophy, the combination of phototrophy and heterotrophy within the same individual, is

3   widespread in oceanic systems. Yet, neither the presence nor ecological impact of mixotrophs

4   has been identified in an Arctic marine environment. We quantified nano- and picoplankton

5   during early autumn in the Beaufort Sea and Canada Basin and determined relative rates of

6   bacterivory by heterotrophs and mixotrophs. Results confirmed previous reports of low microbial

7   biomass for Arctic communities in autumn. The impact of bacterivory was relatively low,

8   ranging from 0.6 x 10^3 to 42.8 x 10^3 bacteria mL^{-1} day^{-1}, but it was often dominated by pico- or

9   nano-mixotrophs. From 1-7% of the photosynthetic picoeukaryotes were bacterivorous, while

10   mixotrophic nanoplankton abundance comprised 1-22% of the heterotrophic and 2-32% of the

11   phototrophic nanoplankton abundance, respectively. The estimated daily grazing impact was

12   usually < 5% of the bacterial standing stock, but impacts as high as 25% occurred. Analysis of

13   denaturing gradient gel electrophoresis band patterns indicated that communities from different

14   depths at the same site were appreciably different, and that there was a shift in community

15   diversity at the midpoint of the cruise. Sequence information from DGGE bands reflected

16   microbes related to ones from other Arctic studies, particularly from the Beaufort Sea.

17

Introduction

Planktonic protists have traditionally been categorized based on their modes of energy and carbon acquisition as either phototrophic (algal) or heterotrophic. However, mixotrophic behavior, whereby organisms combine both of these nutritional modes within a single cell, has been increasingly recognized and documented in aquatic systems (Stoecker, *et al.*, 2009, Sanders, 2011). Phagotrophic feeding behavior occurs in a variety of algal taxa, including chrysophytes, dinoflagellates, prymnesiophytes, rhaphidophytes and cryptophytes, and these organisms have been shown to be ecologically significant as primary producers and consumers (Sanders & Porter, 1988, Unrein, *et al.*, 2007, Jeong, *et al.*, 2010, Jeong, 2011). Mixotrophic nanoplankton (MNAN) can comprise up to 50% of the total phototrophic nanoplankton (cells <20 microns) and be responsible for as much as 86% of the total bacterivory in diverse aquatic habitats (e.g.,Sanders, *et al.*, 1989, Havskum & Riemann, 1996, Havskum & Hansen, 1997, Sanders, *et al.*, 2000).

Although abundances and bacterivory by protists have been examined in the Arctic Ocean, there are no previous investigations of mixotrophy. Earlier studies in the central Arctic Ocean and Chukchi Sea found heterotrophic nanoplankton (HNAN) comprised a large portion of the microbial biomass during summer when flagellates in the $6 - 20$ µm size range tended to dominate HNAN biomass (Sherr, *et al.*, 1997); autotrophic plankton biomass during that investigation was dominated by dinoflagellates and miscellaneous flagellates at most stations in the central Arctic, with diatoms making up most of the remaining biomass (Booth & Horner, 1997). However, at some stations in the Canada and Makarov Basins, particularly under thicker ice cover, picophytoflagellates (cells <2.5 microns) tentatively identified as *Micromonas* sp. contributed up to 93% of autotrophic cell abundance and 36% of the autotrophic biomass in

41   freshly prepared samples (Booth & Horner, 1997, Sherr, *et al.*, 1997).

42   In the present study the occurrence of mixotrophy in pico- and nano-phytoplankton from

43   Arctic waters was assessed, abundances and bacterivory of heterotrophic and phototrophic

44   protists were determined, and a molecular analysis of the potentially abundant protists was

45   performed using denaturing gradient gel electrophoresis (DGGE). We hypothesized that

46   mixotrophy could be a successful strategy for these Arctic phototrophs based upon the

47   ubiquitous incidence of mixotrophic nanoplankton in subpolar and Antarctic waters (Nygaard &

48   Tobiesen, 1993, Havskum & Riemann, 1996, Bell & Laybourn-Parry, 2003, Moorthi, *et al.*,

49   2009). We expected that the grazing impacts of mixotrophic organisms could sometimes exceed

50   that of the heterotrophic plankton in the Beaufort Sea the Canada Basin region of the Arctic

51   Ocean.

52
53   **Materials and methods**

54   **Study sites and sampling**

55   Samples to examine protistan abundance and bacterivory were collected at eleven stations within

56   the Beaufort Sea and the Canada Basin of the Arctic Ocean on a cruise of opportunity aboard the

57   icebreaker USCGC Healy in September 2008 (Figure 1). Water was collected from the deep

58   chlorophyll maximum layer (DCM) and at 5 m below the surface during the upcast of a rosette

59   CTD system with 10 L Niskin bottles. These depths were considered to be representative of the

60   mixed surface layer or of a depth (DCM) with potentially increased biomass and activity of

61   microorganisms, including phytoplankton. Exact station locations and physical parameters of the

62   sampling depths from the CTD instruments are presented in Table 1.

63   Subsamples from each depth were collected immediately for microplankton counts and

64   chlorophyll *a* analysis. Microplankton (ciliates, dinoflagellates, other microflagellates, and

65 diatoms) were preserved with Lugol's solution (4.5% final concentration) and later settled and

66 enumerated using an inverted microscope at a magnification of 200X. For each chlorophyll *a*

67 determination, 100 mL of whole water were filtered onto a 47 mm GF/F filter (Whatman) and

68 frozen at -20 °C until analyzed. Filters were later extracted in 90% acetone overnight at -20 °C

69 and fluorescence was determined with a Model TD-700 fluorometer (Turner Designs,

70 Sunnyvale, CA, USA).

71

72 **Preparation of fluorescently labeled bacteria (FLB)**

73 FLB were prepared from cultured *Halomonas halodurans* (~1 µm). These were used

74 successfully in prior studies for the identification of mixotrophic nanoplankton (Sanders*, et al.*,

75 2000, Moorthi*, et al.*, 2009). *H. halodurans* was inoculated into 1 L of 0.2 µm-filtered and

76 autoclaved seawater enriched with yeast extract (0.1% final concentration). Bacterial cells were

77 grown at room temperature, harvested by centrifugation, washed using filter-sterile seawater

78 (FSW), and then stained with mixing for 3 hr at 64 ºC with 5-(4, 6-dichlorotriazin-2-yl)

79 aminofluorescein (DTAF, Sigma-Aldrich Co., St. Louis, MO, USA) at a concentration of 40 µg

80 mL^{-1}. FLB were then washed 4-5 times with FSW by repeated centrifugation, and finally filtered

81 through sterile 47 mm polycarbonate filters (Whatman, 3 µm pore size) to remove clumps.

82 Concentrations of FLB were determined with epifluorescence microscopy, and aliquots of FLB

83 were stored at -20 ºC until just prior to use in experiments.

84

85 **Experimental setup and processing**

86 For feeding experiments, triplicate 2 L samples of seawater, prescreened through 100 µm Nitex

87 mesh (Wildlife Supply, Yulee, FL, USA) to remove zooplankton, were incubated in 2.7 L

88   polycarbonate bottles. Zooplankton were not observed in the experimental bottles or on any of

89   the slides when the samples were counted.

90   To determine the appropriate addition of tracer particles, bacterial abundance from each

91   depth was initially assessed by epifluorescence microscopy from samples filtered on 25 mm

92   black Poretics polycarbonate filters (0.2 µm pore size). Filters were stained and mounted with

93   cover slips onto glass slides using VectaShield® mounting medium containing DAPI (Vector

94   Laboratories, Inc., Burlingame, CA, USA). Replicate counts for bacteria and fluorescent particle

95   abundances used to calculate grazing impacts were determined with subsamples from each

96   incubation bottle, fixed in 1% formalin and frozen at -20 °C until analysis. Bacteria were

97   enumerated with fluorescence microscopy as described above and fluorescent particles counted

98   by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA). Our experience was in

99   agreement with Hyun and Yang (2003), who reported a minimum loss of bacterial cells kept

100   frozen in this manner and counted within 2-3 months.

101   For feeding experiments, fluorescently labeled bacteria (FLB) or 0.6 µm polycarbonate

102   microspheres (Polysciences, Inc., Warrington, PA, USA) were added at approximately 25% of

103   natural bacterial abundance. At Stations 1 and 2, FLB were the only tracers used. At Stations 3

104   and 4, FLB (1 – 1.2 µm) and microspheres (0.6 µm) were added to seawater in separate

105   incubations. From these experiments it was apparent that picoeukaryotes were not ingesting the

106   larger FLB, and the use of FLB was discontinued in subsequent incubations. In all cases,

107   fluorescent tracers were sonicated immediately prior to addition to disperse particles evenly. The

108   replicate bottles were incubated at 2 ºC under fluorescent lamps at irradiance levels between 2

109   and 7 X 10^{14} quanta sec^{-1} cm^{-2}, depending on the depth from which the original samples were

110   taken. Light level was measured with a QSL-100 Quantum-Scalar Irradiance meter (Biospherical

111   Instruments, Inc., San Diego, CA, USA). To determine rates of bacterivory, 100 mL aliquots

112   were taken from the bottles at several time points beginning immediately after particle addition

113   (T0), and fixed using the Lugol's / formaldehyde / $Na_2S_2O_3$ method to prevent egestion (Sherr &

114   Sherr, 1993). After evaluation of initial time course data indicated linear uptake of both FLB and

115   microspheres for 120 minutes, samples were taken at T0 (background correction) and 30 minutes

116   to determine ingestion.

117   Ingestion rates and abundances of phototrophic, mixotrophic and heterotrophic pico- and

118   nano-plankton were determined from examination of 100 mL of sample filtered onto 25 mm

119   Poretics polycarbonate membranes (3 µm pore size, GE Osmonics, Minnetonka MN, USA).

120   Filters were simultaneously stained and mounted with cover slips on glass slides using

121   VectaShield® as previously described for bacterial counts. To eliminate loss of chlorophyll

122   fluorescence, specimens were frozen at -20 °C until enumeration by epifluorescence microscopy

123   at 1000X magnification onboard the ship. Nanoplankton (3-20 µm) and picoeukaryotes (≤ 2.5

124   µm) were counted in at least 25 fields per filter (a minimum of 100 and 200 cells for

125   nanoplankton and picoplankton, respectively). Phototrophic and heterotrophic cells were

126   differentiated by the presence or absence of chlorophyll autofluorescence, while mixotrophic

127   cells were defined as those with chlorophyll and at least one ingested fluorescent tracer (FLB or

128   microsphere) after background correction.

129

130   **Statistical analysis of abundance and feeding data**

131   To examine the potential for environmental factors to affect rates of bacterivory and proportions

132   of mixotrophs, a Spearman correlation analysis was performed using the statistical software

133   program "R" (Hornik, 2011); the arcsine transformation was used on percentage data prior to the

134 analysis. ANOVA was used to test the effect of tracer particle (FLB, microsphere) on ingestion

135 rates by HNAN and MNAN; relative feeding rates of MNAN and HNAN were examined with a

136 paired comparisons method (Wilcoxon's signed ranks test). Statistical analysis of DGGE results

137 is described in the following section.

138

139 **Genetic analysis of samples**

140 Twenty liters of water from the surface and DCM were collected directly from the Niskin bottles

141 through 100 µm mesh prefilters onto 47mm GF/F filters (Whatman) and frozen at -20 ºC.

142 Nucleic acids were recovered following the method described in Gast *et al.* (2004). One µl of

143 each sample was amplified for DGGE analysis using the 18S rDNA primers 960FGC and 1200R

144 generating a ~250 bp fragment from the V7 region following the method described in Gast *et al.*

145 (2004). Triplicate PCR reaction products were precipitated and resuspended in a total of 6 µl of

146 sterile distilled water. Nucleic acid concentrations were estimated with a Nanodrop™ 1000

147 (Thermo Fisher Scientific, Waltham, MA, USA), and about 500 ng of each sample was loaded

148 onto the gradient gel, which was poured and run following the procedure in Gast *et al.* (2004).

149 The image was analyzed using GelComparII (Applied Maths, Austin, TX, USA), and bands

150 detected manually were scored by presence/absence with a tolerance of 1%. Diversity and

151 environmental factors were analyzed using Permanova+ (Anderson*, et al.*, 2008). The DGGE

152 band data matrix was converted to a Bray-Curtis based resemblance matrix, with the

153 environmental factors of site (each station) and depth (surface, deep). Environmental variables

154 included actual depth, site, temperature, PAR, Julian date and salinity. An analysis of variance

155 was performed with Permanova+, and principal coordinates analysis (PCO) was used to visualize

156 the diversity patterns related to environmental variables.

157   Well-separated bands were picked from the gel using a sterile pipet tip to touch the

158   surface of the gel and then pipetting up and down in 5 µl of sterile distilled water. In our

159   experience, this method has reduced the recovery of multiple bands that occurs when cutting a

160   band from the gel and eluting material from the matrix. The band was reamplified from 2.5 µl of

161   the sample with the non-GC clamped primer set. Samples were precipitated overnight at -20 °C

162   with a final concentration of 0.3 M sodium acetate and 0.6 volumes of 100% isopropanol. They

163   were resuspended in 5 µl of water and 250 ng were sequenced at the Bay Paul facility (MBL)

164   using the non-GC clamped forward primer (960f). Putative band sequence identities were

165   assessed using Blast ™ (GenBank), and we did not observe any potentially chimeric sequences.

166   GenBank no longer accepts sequences shorter than 200bp, and because six of ours are below this

167   limit we have made our sequence data available in the supplemental material (Table S1).

168

169   **Results**

170   **General environmental parameters**

171   The surface salinities and temperatures observed in the Beaufort Sea and the Canada Basin

172   during the study period ranged from 23.15 to 31.49 PSU and -1.63 to 0.4 °C, respectively (Table

173   1). Photosynthetically active radiation (PAR) measured at the time of sampling ranged from 0.27

174   to 5.35 quanta cm^{-2} sec^{-1} x 10^{15} at the surface, but never exceeded 0.29 quanta cm^{-2} sec^{-1} x 10^{15} at

175   the chlorophyll maximum depth of any station (Table 1). The general oceanographic parameters

176   of salinity, temperature, and light (Table 1) are within the range of previous reports for the region

177   and the season (Cota*, et al.*, 1996, Lovejoy*, et al.*, 2007, Sherr*, et al.*, 2009, Tremblay*, et al.*,

178   2009).

179

180   **Plankton abundances**

181   Overall, abundances of microorganisms were low, as expected for the region and the autumn

182   season. Bacteria were typically present at between $1 - 2$ x 10^5 cells mL^{-1} (Table 2). The

183   maximum chlorophyll *a* concentration, observed at the deep chlorophyll maximum of Station 1,

184   was 0.87 µg L^{-1} (Table 2). The abundance of phytoplankton reflected the low chlorophyll

185   concentrations. Diatoms were conspicuously absent from most samples and had a maximum

186   abundance of <1 mL^{-1}. Dinoflagellates were present in all samples, and though the Lugol's-fixed

187   samples used for microplankton enumeration did not enable differentiation between phototrophic

188   and purely heterotrophic individuals, qualitative shipboard observations with epifluorescence

189   microscopy indicated that heterotrophic dinoflagellates tended to be more abundant than

190   phototrophic dinoflagellates by a factor of 2:1 to 3:1. The combined dinoflagellates always

191   numerically dominated the other microflagellates, with a maximum of approximately 7 mL^{-1}

192   (Table 2). Ciliate abundances frequently mirrored the "other microflagellate" category and

193   exceeded 2 mL^{-1} on only two occasions at the surface.

194   Heterotrophic (HNAN) and phototrophic nanoflagellates (PNAN), mostly in the 4 -6 µm

195   size range, were typically present at $50 - 200$ cells mL^{-1}, while mixotrophic nanoplankton

196   (MNAN) abundances were usually at $5 - 20$ cells mL^{-1} (Table 3). Photosynthetic picoeukaryotes

197   (Peuk) were the numerically dominant group, and usually exceeded 10^3 cells mL^{-1} (Table 3).

198   Mixotrophic picoeukaryotes (Mpeuk) were identified at every station after the switch was made

199   to smaller (0.6 µm) tracer particles. The abundances of Mpeuk usually exceeded that of the

200   MNAN, sometimes by an order of magnitude (Table 3). However they made up only a very

201   small proportion of the total picoeukaryotes, while MNAN comprised up to 32% of the

202   phototrophic nanoplankton (Table 3). MNAN should also be compared to HNAN since both

203   contribute to grazing impact on bacteria. MNAN were on average 10% (range 1 – 22%) of the

204   bacterivorous nanoplankton (Table 3).

205

206   **Ingestion rates and bacterivory impact**

207   Time courses run at the beginning of the cruise indicated that uptake of both FLB and

208   microspheres was linear for the first 120 minutes if incubation, after which ingestion was

209   balanced by digestion and egestion. At Stations 1 and 2, only FLB were used as tracer particles,

210   but at Stations 3 and 4 separate feeding experiments were run with the same communities using

211   either FLB or fluorescent microspheres. Grazing rates by nanoflagellates was greater on

212   microspheres than on FLB in cases where both tracer types were used (Table S2), and ingestion

213   by Mpeuks was detected only in experiments with microspheres. At Stations 3 and 4 where

214   direct comparisons were made, average calculated ingestion rates using microsphere tracers were

215   0.9 and 3.3 bacteria individual^{-1} h^{-1} for HNAN and MNAN, respectively. Using FLB, the

216   corresponding rates were 0.2 and 2.2 bacteria individual^{-1} h^{-1}. The ingestion rates determined

217   using microspheres were significantly greater than those determined with FLB for both HNAN

218   and MNAN (ANOVA, $p < 0.01$). Polar bacteria tend to be less than 1 µm in size, and the higher

219   rates observed for ingestion of microspheres may reflect size-selective feeding, although larger

220   sized particle ingestion still occurred for the nanoplankton.

221   Grazing rates were not consistently affected by depth (surface versus DCM). For all three

222   grazing groups, ingestion was greater in surface waters than at the DCM about half the time.

223   Rates were greater in the DCM only twice for HNAN and MNAN and only once for Mpeuk. For

224   the remainder of the incubations, there was no significant difference between depths. Over all

225   experiments, the calculated individual grazing rates were greater for MNAN than for HNAN ($p <$

226   0.001, Table S2). Using microsphere tracers, the average ingestion rates for HNAN and MNAN

227   were 1.3 and 5.1 bacteria individual^{-1} h^{-1}, respectively. For Mpeuk the average ingestion rate

228   (from microsphere incubations) was 2.9 bacteria individual^{-1} h^{-1}. The reported ingestion rates per

229   cell for MNAN and Mpeuk are inflated relative to those of HNAN due to the method of

230   calculation. HNAN that do not ingest tracers during an experiment can be counted, while

231   potential mixotrophs that are "inactive grazers" usually cannot be distinguished from pure

232   autotrophs. Therefore, the total number of mixotrophs is based only on those ingesting tracers.

233   This does not, however, affect the relative grazing impacts (see discussion).

234   The potential grazing impact of protists on bacteria ranged from <1 to 25.2 percent of

235   bacterial standing stock per day; the impact was <5% of standing stock d^{-1} in 15 of 20

236   incubations (Table S3). HNAN, frequently identified as the major planktonic bacterivores,

237   dominated the grazing impact in about half of the experiments, while mixotrophs were more

238   important in the rest (Fig. 2). Mpeuks tended to dominate bacterivory in experiments where the

239   highest total impacts were determined (Table S3). Regarding correlations between ingestion rates

240   and environmental parameters, HNAN rates were positively correlated to light, but there were no

241   other significant relationships (Table S4). MNAN as a proportion of total nanoplankton

242   bacterivores (MNAN/[MNAN+HNAN]) was negatively correlated to salinity and positively

243   correlated to light. Mpeuk as a proportion of Peuk was positively correlated to chlorophyll a,

244   dinoflagellates and total Peuk abundance,

245

246   **Community structure and comparison**

247   The DGGE results are shown in Figure 3, with the bands successfully recovered for sequencing

248   numbered in panels A and B, and the PCO results in panel C. Generally there were fewer than 5

249   predominant bands in each sample that were possible to recover for sequencing. Many more

250   were identifiable for community analysis using GelComparII (Applied Maths). Taxonomic

251   affiliation of DGGE band sequences were determined using Blast ™. Sequences recovered

252   include dinoflagellates, diatoms, copepods, dinoflagellate parasites (Syndiniales), and

253   *Micromonas* (a mixotrophic picoeukaryote), with bands from the same position in different

254   samples giving the same sequence results (Table S5). Permanova+ indicated that both depth

255   (p=0.0086) and location (p=0.0001) were significant in describing the diversity between

256   samples, but that there was no synergistic interaction between the factors (p=0.7085). Site and

257   date variables were co-linear, so date was removed from further analyses. The PCO analysis

258   illustrated the effect of site/date and depth on the grouping of samples (Fig. 3C). Separation of

259   two groups along the first axis corresponded to the midpoint of the cruise and described 28.8%

260   of the total variation. The second axis described slightly less of the overall variation (19.3%),

261   and appeared to correspond to depth.

262

Discussion

264   Protists play important roles as both primary producers and consumers in southern and northern

265   polar waters (Sherr, *et al.*, 2003, Riedel, *et al.*, 2007, Pearce, *et al.*, 2010), However, previous to

266   the current investigation, nothing was known about mixotrophic protists in the Arctic. Our study

267   confirms the presence of both nano- and pico-planktonic mixotrophs in the Arctic Ocean, and

268   their potential for substantial impact on bacterial communities in the ice-covered Arctic region of

269   the Beaufort Sea in early autumn.

270

271

272 **Protistan abundance**

273   Our microscopic investigation of bacterial and protist distribution indicated that densities

274 were low, but within the range of previous reports for the Canada Basin and Beaufort Sea during

275 the late summer to early fall (Table 4). Picophytoplankton were the most abundant protists,

276 typically outnumbering hetero- and auto-trophic nanoplankton by an order of magnitude (Table

277 3). A small percentage of the picoeukaryotes, from <1 – 7%, were identified as mixotrophic by

278 ingestion of fluorescent tracers, but at most stations they were still more abundant than

279 mixotrophic nanoplankton (MNAN) identified in the same manner (Table 3). At least one

280 cultured strain of the picoprasinophyte *Micromonas* was previously found to be phagotrophic

281 (González*, et al.*, 1993), and most of the mixotrophic picoeukaryotes enumerated with

282 epifluorescence microscopy in our study resembled the "typical *Micromonas*-like cell stained

283 with DAPI" as presented in a color photomicrograph in Lovejoy *et al.* (2007). Coupled with the

284 frequent occurrence of bands in the DGGE gels that were linked to *Micromonas* (Fig. 3, Table

285 S5), our data indicate potential for relatively large impacts by this picoprasinophyte as a

286 bacterivore in the Arctic ecosystem at this time. Furthermore, the wide-spread occurrence of

287 *Micromonas* (Sherr*, et al.*, 2003, Not*, et al.*, 2005, Lovejoy*, et al.*, 2007, Tremblay*, et al.*, 2009)

288 suggests that mixotrophy could be common in the Arctic throughout the year.

289   After picoplankton, HNAN were the next most numerous protists, though HNAN

290 abundance was usually within a factor of two of combined PNAN and MNAN assemblages.

291 Sherr et al. (1997) also found that the <5 µm HNAN were numerically dominant heterotrophs,

292 but noted that the 6 – 20 µm size class tended to dominate heterotrophic biomass in integrated

293 samples (0 to 50 m) along a cruise track from the Chukchi Sea to the Nansen Basin from July

294 through August (Sherr*, et al.*, 1997, Wheeler*, et al.*, 1997). The microplankton size-fraction in

295   our samples was always dominated by dinoflagellates and ciliates, though abundances were on

296   the low end of ranges reported previously for arctic and sub-arctic waters (Sherr, *et al.*, 1997,

297   Levinsen *et al.*, 2000, Strom, *et al.*, 2007, Vaqué, *et al.*, 2008). Diatoms were observed at very

298   low abundances, if at all, in our microscope counts (Table 2). Likewise, Sherr et al. (2003)

299   reported Arctic Ocean winter diatom abundances ≤ 1 cell mL^{-1} and Terrado et al. (2009) reported

300   diatoms represented only 4% of autumn clone library sequences in Franklin Bay.

301

302   **Protistan diversity**

303   Low biomass as indicated by the microscopic and pigment observations would not necessarily

304   indicate low diversity. However, microscopy suggested that only a limited number of taxa were

305   present, with diatoms absent from most samples. DGGE also yielded only a few predominant

306   bands for each sample, although many faint bands were also present.

307   When the recovered Arctic DGGE bands were compared with those recovered from the

308   Antarctic (Gast, *et al.*, 2004) at a level of 97% similarity, the only overlap in sequence

309   information was for the copepod *Oithona*. Prior genetic studies of microbial eukaryotes in the

310   Arctic have used both DGGE and clone libraries to examine the community structure (Lovejoy,

311   *et al.*, 2006, Hamilton, *et al.*, 2008, Terrado, *et al.*, 2009, Tremblay, *et al.*, 2009, Bachy, *et al.*,

312   2011, Lovejoy & Potvin, 2011). The DGGE fragment used in this study targeted a different

313   region of the ribosomal gene than some of those projects, and most of those studies have been

314   directed towards the <5 micrometer size group, yet there are similarities. The groups commonly

315   identified by molecular diversity surveys of Arctic waters included alveolates (ciliates,

316   dinoflagellates, group I & II alveolates), unidentified marine stramenopile genotypes (MAST),

317   dictyophytes, prasinophytes, haptophytes, diatoms, bolidophytes, cryptophytes and the newly

318   identified picobiliphytes. In common with this work, other studies have also reported the

319   abundance and wide distribution of *Micromonas* (Sherr*, et al.*, 2003, Lovejoy*, et al.*, 2007,

320   Terrado*, et al.*, 2008), as well as the diatom *Chaetoceros*, novel alveolate group II Syndiniales,

321   and other dinoflagellates.

322

323   **Mixotrophy, bacterivory, and grazing impacts**

324   The ubiquitous occurrence of mixotrophic plankton found in the current study and in a recent

325   reports from Antarctic waters (Moorthi*, et al.*, 2009) suggest that mixotrophy may be a

326   successful strategy for some phytoplankton in polar marine environments. The potential benefits

327   of particle ingestion by phytoplankton include the acquisition of organic carbon, energy, major

328   nutrients, and/or micronutrients including vitamins and trace metals (e.g.Caron*, et al.*, 1993,

329   Nygaard & Tobiesen, 1993, Maranger*, et al.*, 1998). If, as has been suggested by Tremblay and

330   Gagnon (2009), primary production in seasonally ice-free waters of the Arctic Ocean is

331   controlled by nitrogen supply, then mixotrophy there may act as a competitive mechanism for

332   nitrogen uptake. Increased mixotrophy under nutrient limitation has been noted for nanoplankton

333   and suggested for picoeukaryotes in other marine systems (Nygaard & Tobiesen, 1993, Zubkov

334   & Tarran, 2008), although the environmental drivers of mixotrophic behavior are likely to vary

335   with species.

336   In the Ross Sea Antarctica, photosynthetic and heterotrophic nanoplankton range over

337   three orders of magnitude from about 2 mL^{-1} to 7 x 10^3 mL^{-1} and peak in austral summer

338   (Dennett*, et al.*, 2001, Moorthi*, et al.*, 2009). MNAN were typically <200 mL^{-1} in plankton

339   assemblages south of the Polar Front of the Southern Ocean, but still comprised 8% - 42% of

340   bacterivorous nanoplankton in the water column, and 5-10% of phototrophic and 3-15% of

341   phagotrophic nanoflagellates present in ice cores (Moorthi*, et al.*, 2009). The abundance of

342   MNAN during the Arctic autumn ranged from 2 to 300 mL^{-1} and comprised the same relative

343   abundances when compared to total heterotrophic (1-22%) and total phototrophic (2-32%)

344   nanoplankton (Table 3) as was observed during austral summer. A major difference in our

345   studies of mixotrophs in the Arctic and Southern Oceans was the abundance and impact of the

346   picophytoplankton. While phototrophic picoeukaryotes were not noted in our Antarctic samples,

347   they numerically dominated many of the Arctic samples and were important as bacterivores.

348   Food size appeared to be of consequence for the mixotrophic picoeukaryotes; ingestion

349   was observed when the 0.6 µm microspheres were used, but never when the 1 – 1.2 µm FLB

350   were offered. Grazing by nanoflagellates on microspheres also was significantly greater than on

351   FLB (p< 0.001, ANOVA), though absolute differences were not large. Overall, the mixotrophic

352   community (MNAN and Mpeuks) ingested 2X as many bacteria-sized particles as the

353   heterotrophs, indicating that they had an equivalent or greater grazing impact on bacteria as that

354   of the more traditional (heterotrophic) consumer population.

355   As a community, the pico- and nano-plankton removed from 0.06 – 2.6 x 10^4 bacteria

356   mL^{-1} d^{-1}, dependent to a large degree on the abundance of picoeukaryotes. This compares to a

357   grazing impact, estimated using FLB, of 0.1 – 4.6 x 10^4 bacteria mL^{-1} d^{-1} by heterotrophic

358   plankton during the Arctic summer (Sherr*, et al.*, 1997). Anderson & Rivkin (2001) used the

359   dilution technique to examine bacterivory, and also noted significant grazing impact during early

360   summer blooms and during winter in Resolute Bay, Northwest Territories, Canada.

361   This is the first study to demonstrate mixotrophy by phytoflagellates in Arctic waters, and

362   the data suggest that a *Micromonas*-like picoprasinophyte was an important bacterivore in the

363   Canada Basin during autumn. The role of picoeukaryotes as quantitatively important grazers has

364   been demonstrated only once previously – in the North Atlantic Ocean (Zubkov & Tarran, 2008),

365   though it was conjectured to occur in the Arctic (Sherr, *et al.*, 2003). Since these

366   picoprasinphytes are known to persist through winter darkness and grow exponentially from late

367   winter to early spring (Lovejoy, *et al.*, 2007), phagotrophy may contribute importantly to

368   survival during winter darkness and give the organisms a relatively large seed population at the

369   beginning of the spring growth period. If global climate change freshens the Arctic Ocean as

370   proposed by Li *et al.* (2009), the impact of picoeukaryotes as bacterivores may become

371   especially important.

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373   RWS and RJG contributed equally to this paper

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Table 1. Sampling stations, date (in 2008), locations (lat/long), collection depth (m), water column depth Z_{max} (m), salinity (PSU), temperature (°C), and light (quanta cm^{-2} sec^{-1} x 10^{15}) measurements from the CTD sensor.

Station	Date	Location	Depth	Z_{max}	Salinity	Temp	PAR
1	7 Sept	77 24.53 N 151 18.84 W	5	3840	27.43	-1.12	2.33
			60		30.99	-1.08	0.08
2	8 Sept	79 34.20 N 147 13.75 W	5	3820	27.97	-1.39	4.63
			50		31.24	-1.45	0.21
3	11/12 Sept	80 36.97 N 130 21.72 W	5	3500	29.24	-1.56	0.02
			50		30.71	-1.44	0.01
4	13 Sept	80 03.84 N 132 04.51 W	5	3620	27.69	-1.48	5.10
			50		30.72	-1.54	0.28
5	15 Sept	79 36.21 N 146 50.63 W	5	3800	27.11	-1.44	2.72
			37		30.68	-1.28	0.29
6	19 Sept	78 32.16 N 124 57.08 W	5	2450	30.14	-1.63	0.55
			60		30.49	-1.59	0.02
8	21 Sept	78 24.5 N 134 35.2 W	5	3650	21.68	-1.42	5.35
			30		24.40	-1.06	0.85
			70		25.05	-1.34	0.09
9	24 Sept	78 17.69 N 140 56.22 W	5	3800	26.98	-1.45	1.27
			45		30.79	-1.18	0.05
10	27 Sept	76 24.32 N 131 7.58 W	5	2770	27.52	-1.48	0.27
			55		31.04	-1.29	0.15
11	29 Sept	72 14.196 N 140 55.53 W	5	2990	23.15	0.40	1.31
			75		30.55	-0.65	0.03

Table 2. Station microbial characteristics. Chlorophyll a concentration (µg l^{-1}), heterotrophic and autotrophic microplankton abundance (ml^{-1}), and bacterial abundance (x 10^5 ml^{-1}) in the Beaufort Sea. * = not observed.

Station	Depth (m)	Chl a	Ciliates	Dino-flagellates	Other Flagellates	Centric Diatoms	Pennate Diatoms	Bacteria
1	5	0.16	0.51	2.54	*	*	0.20	5.50
	60	0.87	0.91	3.86	*	0.30	0.20	–
2	5	0.20	0.25	2.85	2.85	*	*	2.20
	50	0.51	0.44	4.50	0.19	0.06	*	1.81
3	5	0.29	2.03	7.25	0.25	*	0.10	1.80
	50	0.42	0.36	2.18	0.30	*	*	2.20
4	5	0.28	2.22	5.71	1.84	*	0.25	2.49
	60	0.30	0.41	3.25	0.86	*	0.05	1.93
5	5	0.44	1.78	7.00	1.42	*	0.10	1.70
	37	0.54	1.37	5.68	1.83	*	0.05	1.98
6	5	0.20	0.36	1.37	0.41	*	*	1.84
	60	0.35	0.91	2.43	0.71	*	*	2.18
8	5	0.26	1.22	5.17	0.30	0.05	*	1.33
	30	0.29	0.36	2.23	*	*	*	1.17
	70	0.34	0.10	2.33	0.20	*	*	1.60
9	5	0.24	1.07	5.88	1.62	0.05	*	1.65
	45	0.40	0.51	3.25	0.81	*	*	1.37
10	5	0.23	0.91	2.94	1.12	*	*	1.31
	55	0.30	0.20	2.33	0.61	*	*	2.51
11	5	0.22	1.32	4.26	0.71	*	*	1.65
	55	0.28	0.41	1.62	0.30	0.10	*	1.24

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Table 3. Abundances (no. ml^{-1} ± S.E.) of heterotrophic nanoflagellates (HNAN), phototrophic nanoflagellates (PNAN), mixotrophic nanoflagellates (MNAN), photosynthetic picoeukaryotes (Peuk) and mixotrophic picoeukaryotes (Mpeuk), and mixotrophs as a percentage of all similarly-sized phototrophs and heterotrophs. Picoeukaryotes did not ingest FLB, and microspheres were not used until Station 3. Note that the percentage calculations include mixotrophs as part of the total nano- and pico-plankton abundance.

STA	Depth (m)	HNAN	PNAN	MNAN	Peuk	Mpeuk	% of PNAN	% of HNAN	% of Peuk
1	5	93 ± 5	25 ± 4	7 ± 1	–	–	21	7	–
2	5	45 ± 6	15 ± 5	2 ± 1	–	–	13	5	–
	50	23 ± 4	82 ± 8	3 ± 0	–	–	4	12	–
3	5	66 ± 15	71 ± 6	11 ± 3	1 217 ± 164	30 ± 5	14	15	2
	50	52 ± 10	48 ± 17	5 ± 1	872 ± 283	18 ± 4	9	9	2
4	5	90 ± 3	54 ± 5	3 ± 1	1 901 ± 108	56 ± 22	5	3	3
	60	57 ± 15	34 ± 3	16 ± 1	880 ± 45	6 ± 6	32	22	1
5	5	121 ± 30	46 ± 11	19 ± 2	2 757 ± 242	220 ± 16	30	14	7
	37	143 ± 20	77 ± 22	16 ± 6	2 807 ± 251	155 ± 17	17	10	5

STA	Depth (m)	HNAN	PNAN	MNAN	Peuk	Mpeuk	% of PNAN	% of HNAN	% of Peuk
6	5	129 ± 13	97 ± 17	36 ± 3	444 ± 77	9 ± 5	27	22	2
	60	126 ± 23	143 ± 35	22 ± 8	324 ± 59	18 ± 6	13	15	5
8	5	130 ± 12	45 ± 5	8 ± 4	3 021 ± 310	87 ± 3	15	6	3
	30	102 ± 10	126 ± 18	10 ± 5	1 598 ± 54	61 ± 2	8	9	4
	70	108 ± 21	91 ± 10	5 ± 1	952 ± 101	4 ± 3	5	4	< 1
9	5	206 ± 36	106 ± 9	2 ± 1	3 047 ± 695	139 ± 7	2	1	4
	47	178 ± 13	100 ± 7	19 ± 3	1 523 ± 123	77 ± 4	16	10	5
10	5	97 ± 26	51 ± 18	8 ± 3	1 862 ± 290	12 ± 6	13	8	1
	55	114 ± 9	65 ± 4	2 ± 2	914 ± 278	16 ± 6	3	2	2
11	5	193 ± 7	45 ± 11	13 ± 1	2 011 ± 208	0	22	6	< 1
	75	66 ± 6	25 ± 12	7 ± 3	1 061 ± 161	25 ± 18	23	10	2

Table 4. Abundances of microorganisms reported from Arctic waters.

Location	Dates	Bacteria (# ml^{-1})	Synecho (# ml^{-1})	HNAN (# ml^{-1})	Peuk (# ml^{-1})	PNAN (# ml^{-1})	Ciliates (# ml^{-1})	Dinoflag (# ml^{-1})
S. Canada Basin[1]	Nov 1997-May 98	1-3 x 10^5		90 - 490	"hundreds"	3 – 1500	0.1 - 2	1 - 12
Beaufort Sea/ NW Passage[2]	Feb-May 2004				10 – 9500			
Canadian Arctic/ Franklin Bay[3]	Dec 2003-May 04	1-5 x 10^5		200 - 600		30 -450	0.1 - 1.3	
Central Arctic[4]	July-Sept 1994				1000 – 10000	42 - 910		9 - 105
Central Arctic[5]	July-Sept 1994	4-12 x 10^5	Not observed	250 - 1900	1000 – 10000	Not reported	0.1 - 17	0.1 - 39
Chukchi Plateau/ Mendeleyev Basin[1]	June-Sept 1998	2-7 x 10^5		210 – 2300	1000 - 28000	100 – 28000	0.1 - 2	1 - 12
North Baffin Bay[6]	Aug-Sept 2005		0 - 17		660 – 10365			
NW Passage[6]	Aug-Sept 2005		1 - 70		860 – 18360			
Beaufort Sea/ Amundsen Gulf[6]	Aug-Sept 2005		1 - 120		150 – 16990			
North Baffin Bay[7]	Sept-Oct 1999				18 – 4070	130 – 3080		
Mackenzie Shelf/ Amundsen Gulf[8]	Sept-Oct 2002		470 – 2425		215 -2110			
Beaufort Sea/ Canada Basin[9]	Sept 2008	1-5 x 10^5	Not observed	20 - 200	320 – 3050	15 - 140	0.2 - 2.2	1 - 7

Abbreviations: Synecho, *Synechococcus* spp.; HNAN, heterotrophic nanoflagellates; Peuk, picoeukaryotes; PNAN, phototrophic nanoflagellates; Dinoflag, dinoflagellates. [1] Sherr et al. 2003, [2] Lovejoy et al. 2007, [3] Vaqué et al. 2008, [4] Booth & Horner 1997, [5] Sherr et al. 1997 (pooled from upper 50 m, same cruise as Booth & Horner 1997), [6] Tremblay et al. 2009, [7] Mostajir et al. 2001, [8] Waleron et al. 2007, [9] This study.

Figure Legends

Figure 1. Location of sampling sites within the Beaufort Sea and Canada Basin of the Arctic

Ocean. North pole is at the upper right corner of the chart.

Figure 2. Relative impact of mixotrophic nanoflagellates (MNAN), mixotrophic picoeukaryotes

(Mpeuk) and heterotrophic nanoflagellates (HNAN) as grazers of bacterioplankton.

Figure 3. Denaturing gradient gel electrophoresis results from the Beaufort Sea and the Canada

Basin. A) Samples from stations 1-6 and B) Samples from stations 6-11 (numbers indicate

bands successfully recovered and sequenced). C) Principal coordinates analysis plot with

environmental variable vectors at the right; deep = deep chlorophyll maximum, surface =

5m, numbers indicate each station. Axis 1 represents 28.8% of the total variation, while

axis 2 represents 19.3% of the total. These appear to represent the variables of site/location

and depth respectively.

Figure 1. Location of sampling sites within the Beaufort Sea and Canada Basin. North pole is at
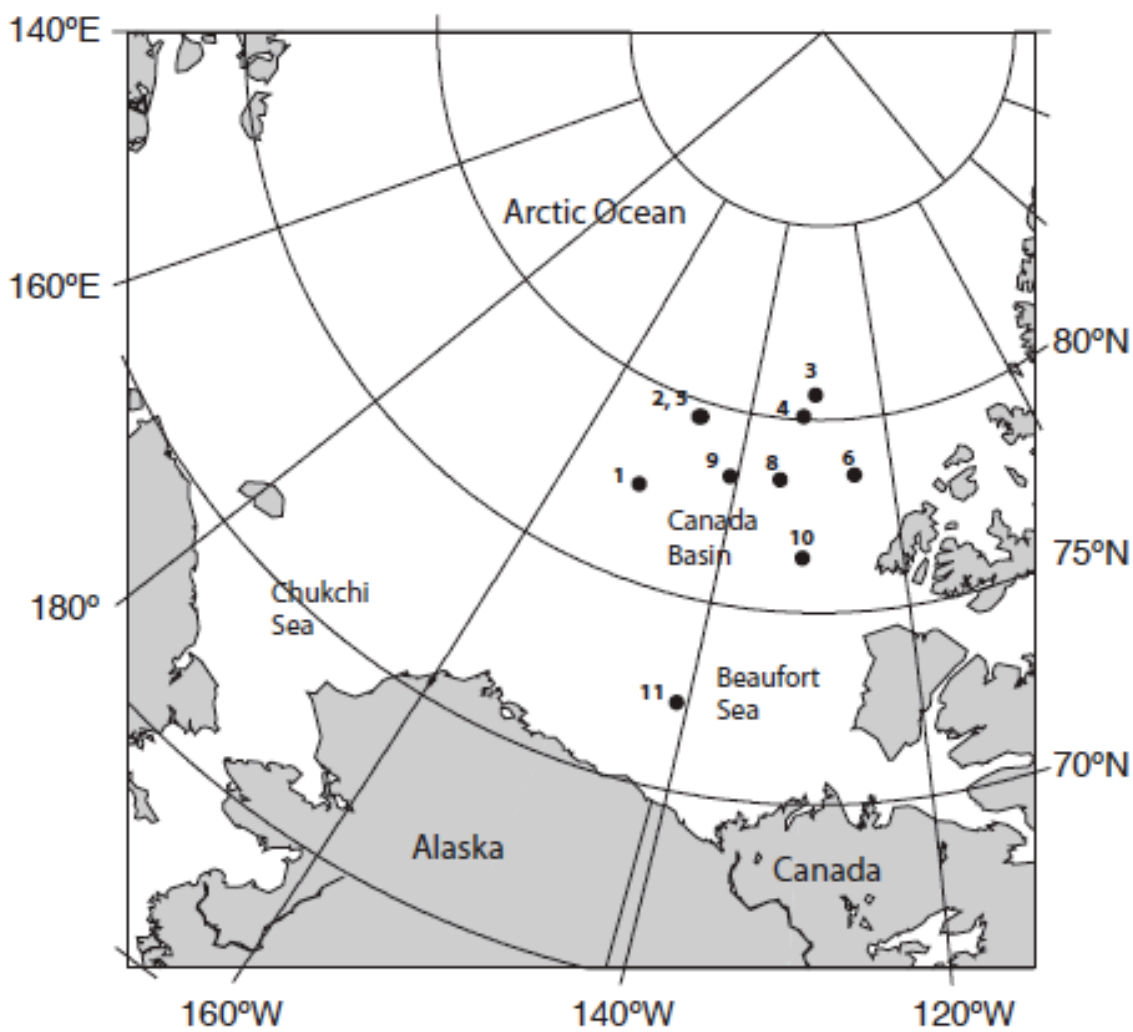
the upper right corner of the chart.

Figure 2. Relative impact of mixotrophic nanoflagellates (MNAN), mixotrophic

picoeukaryotes (Mpeuk) and heterotrophic nanoflagellates (HNAN) as
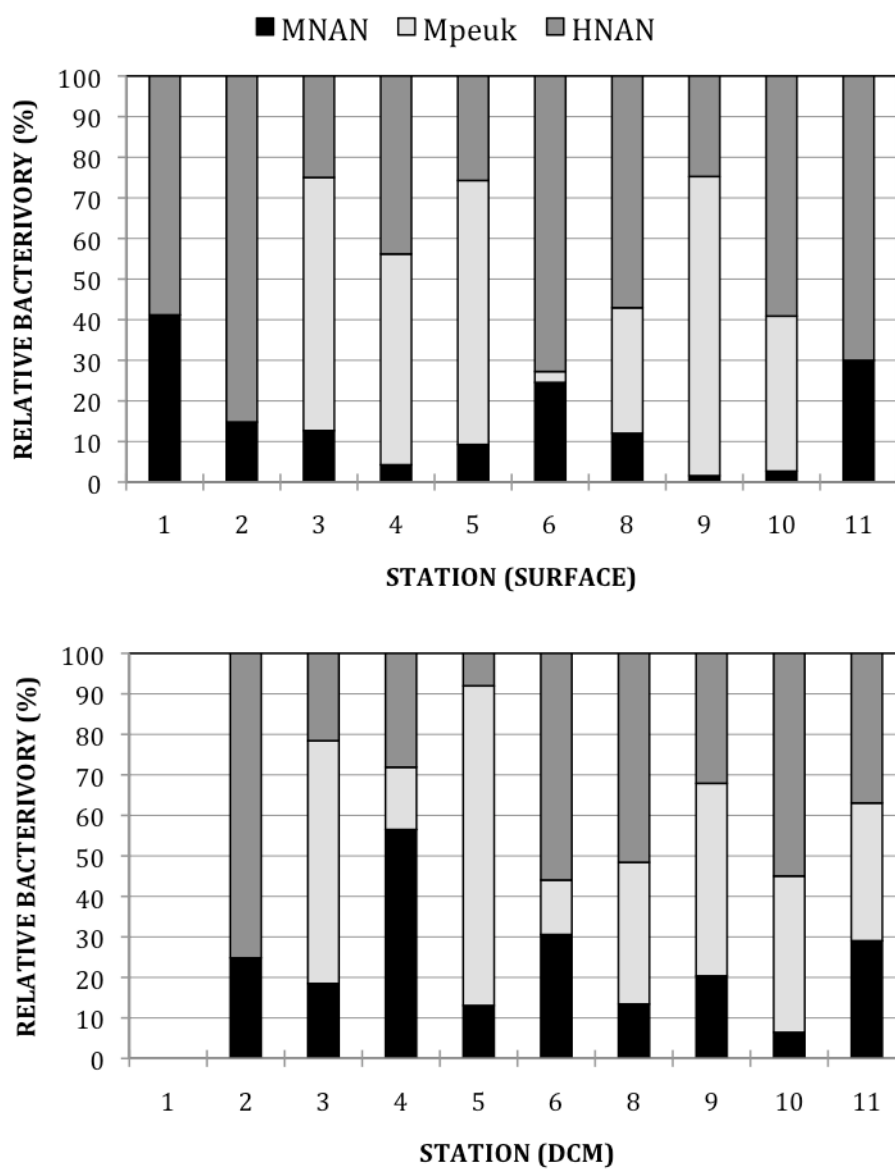
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