Bacterioplankton taxa compete for iron along the early spring-summer transition in the Arctic Ocean

Running title: Competition for iron in the Arctic Ocean

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Abstract

Microbial assemblages under sea ice of Dease Strait, Canadian Arctic, were sequenced for metagenomes and metatranscriptomes of the small size fraction (0.2 to 3 µm). The community from early March was typical for this season, with *Alpha*- and *Gammaproteobacteria* as the dominant taxa, followed by *Thaumarchaeota* and *Bacteroidetes*. Towards summer, *Bacteroidetes* and particularly the genus *Polaribacter*, became increasingly dominant, followed by the *Gammaproteobacteria*. Analysis of genes responsible for microbial acquisition of iron showed an abundance of ABC transporters for divalent cations and ferrous iron. The most abundant transporters, however, were the outer membrane TonB dependent transporters of iron-siderophore complexes. The abundance of iron acquisition genes suggested this element was essential for the microbial assemblage. Interestingly, *Gammaproteobacteria* were responsible for most of the siderophore synthesis genes. On the contrary, *Bacteroidetes* did not synthesize siderophores but accounted for most of the transporters, suggesting a role as cheaters in the competition for siderophores as public goods. Likely, this cheating ability of the *Bacteroidetes* contributed to their dominance in summer.

Introduction

Climate change over the recent decades is evident across the Arctic Ocean through the decline in seasonal sea ice coverage [(1), (2), (3)]. This reduction of sea ice impacts the microorganisms living in the ice and underlying water. For example, a shift towards smaller algae has been observed in phytoplankton [(4)]. The distinctive conditions for life in polar environments involve prolonged periods of ice cover and darkness in winter, generally low water temperatures, and extended irradiance in summer [(5)] The transition from winter to spring and summer involves, thus, dramatic changes in the environment and these are reflected in the composition of the microbial assemblages [(6), (7)]. Prokaryotic taxa abundant in winter such as *Thaumarchaeota* or *Deltaproteobacteria* disappear during the spring, while *Alpha* and *Gammaproteobacteria* and in particular *Bacteroidetes* increase in abundance towards the summer [(8)]. However, the traits that these different microbial taxa use to become dominant are unknown. This is partially due to the logistic difficulties of sampling during the early part of the year. Thus, we carried out a time series through the ice melt transition from early March to late July in Dease Strait of the Canadian Arctic.

Iron is an essential nutrient that is scarce in surface waters due to the insolubility of the most common form Fe(III) and to the fact that sources are of terrestrial origin. As a result, between 15 and 40% of the world's oceans are limited by iron [(9), (10)]. Even in areas where growth is limited by other nutrients (such as the Arctic Ocean) microorganisms need to develop strategies to obtain iron. A common method to get iron in aquatic media is to use siderophores [(11)]. These are small molecular weight compounds that are soluble in water and have a very high affinity for Fe(III). They are

67 synthesized by some microorganisms and usually released in the environment. Then,
68 with the appropriate receptors, the iron-siderophore complexes can be taken up.
69 Siderophores, however, require a complex set of enzymes to be synthesized and
70 excreted in the medium. And, therefore, they require considerable amount of energy
71 [(12)]. Some bacteria do not synthesize siderophores but have the receptors for them.
72 These bacteria act as cheaters, exploiting "public goods" that they have not contributed
73 to create. Due to these peculiarities, the interactions between siderophore producers and
74 non-producers have been explored with cultures of marine *Vibrio* to illustrate
75 competition for public goods [(13)] and the evolutionary implications of these
76 interactions have been reviewed [(14)]. Most experimental information, however, comes
77 from studies with pure cultures of *Proteobacteria*, particularly of different *Vibrio* and
78 *Pseudomonas* strains. But the issue has not been analyzed in nature where the large
79 diversity of coexisting microorganisms may result in complex interactions.
80
81 In the Arctic, *Bacteroidetes* become the most abundant taxon of the assemblage in
82 summer. On the other hand, *Proteobacteria*, that are the most abundant taxa in winter
83 and early spring, decrease in abundance (8). One remarkable difference between
84 *Proteobacteria* and *Bacteroidetes* is the capability to synthesize siderophores.
85 *Proteobacteria* have been shown to synthesize dozens of different siderophores (15),
86 while almost no instances of siderophore synthesis are known among *Bacteroidetes*
87 (16). However, *Bacteroidetes* are known to have receptors for iron-siderophore
88 complexes (17), (18). Therefore, we examine the time evolution of the bacterial
89 assemblage and the presence and abundance of iron-related genes through the ice
90 melting period with the aim to test whether the iron acquisition strategies may have a
91 role in the outcome of the competition among taxa.

Materials and methods

Sampling

Samples were collected in Dease Strait, lower Northwest Passage, Nunavut, Canada (69.03°N, 105.33°W; Figure 1). Water depth was 60 m and samples were collected between 7 March and 24 June 2014, as part of the 2014 Ice Covered Ecosystem-CAMbridge Bay Process Study (ICE-CAMPS). During the ICE-CAMPS campaign, Dease Strait was covered by first-year ice (1.8-2.1 m thick) with drifted snow ranging from 5 to < 25 cm in depth, until melt commenced on 8 June. Snow melt caused the ice surface to flood by 16 June (19), followed by subsequent drainage and the formation of melt ponds that persisted until ice break-up on 19 July. One week after ice break-up, the site was sampled a final time in ice-free waters on 30 July aboard the *R/V Martin Bergmann*.

Water samples were collected opportunistically thirteen times, through a 20-cm diameter hole from 7 to 18 March 2014, protected by a portable tent. For the remainder of the ice-covered season (21 April to 24 June 2014), samples were taken from a 2-m^2 sampling hole maintained within a heated tent. Finally, the 30 July sample was collected, as mentioned, form a ship. A submersible water pump mounted on an under-ice arm was used to collect ice-water interface at 2.5 m. Hydrographic profiles were also taken alongside using a conductivity, temperature and depth probe (RBR XR-620 CTD) equipped with a chlorophyll (chl) *a in vivo* fluorescence sensor. The region is in close proximity to mainland Canada, and receives freshwater inputs from the nearby

117 Coppermine, Hood, Burnside and Ellice rivers (Figure 1). Further details about the
118 study site can be found in Campbell *et al*. (20), (21), (22).
119

120 **Physico-chemical measurements**

121 Approximately at 10 m from the location of water sampling, profiles of under-ice
122 photosynthetically active radiation (PAR) were collected between 10:00 am and 12:30
123 pm local time. This was done using a spherical underwater sensor (LI-193 LI-COR)
124 lowered through an auger hole that took measurements at 1-m depth intervals from the
125 ice-water interface down to 20 m. During measurements the sampling hole was covered
126 to limit contamination by surface radiation. Disturbance of this sites for light
127 measurement was kept to a minimum, where the hole was approached along a single
128 path from the north.
129

130 Following transport to laboratory facilities, water samples that were kept in darkness
131 close to, but not below, -2˚C, and were immediately processed for chl *a* and nucleic
132 acids. Subsamples for chl *a* were filtered onto GF/F filters (Whatmann) before
133 subsequent pigment extraction in 10 ml of 90% acetone for 24 h in darkness and at 4 ˚C.
134 Fluorescence was measured before and after acidification with 5% HCl (Turner Designs
135 Trilogy Fluorometer) according to Parsons et al. (1984) and chl *a* concentration was
136 calculated using the equations of Holm-Hansen et al. (23).
137

138 In order to collect nucleic acids, approximately 10 l of water was prescreened to remove
139 visibly identifiable plankton and then filtered through a tiered stainless steel large-
140 volume filtration system (Millipore) with a succession of 20, 3 and 0.22 µm filters.

141 Separate filters were prepared for RNA and DNA. Filters were immediately frozen at
142 -80˚C. Here we present data from the 0.22 µm filters only.
143

144 **Nucleic acid extraction**

145 DNA was extracted according to the phenol/chloroform protocol detailed by Massana et
146 al. (24). After thawing, frozen filters were cut into small pieces and placed into sterile
147 cryovials. Lysis buffer and lysozyme (final concentration: 1 mg ml^{-1}) were added to the
148 cryovials and they were incubated at 37 °C for 45 min. Sodium dodecyl sulfate (10%)
149 and Proteinase K (0.2 mg ml^{-1}) were added and the mixture was incubated at 55°C for 1
150 h. The lysate was mixed twice with an equal volume of phenol/chloroform/isoamyl
151 alcohol (25:24:1, pH 8) and centrifuged at 12 000 × rpm (10 min). A second extraction
152 with chloroform/IAA (24:1) was carried out to remove the residual phenol. The aqueous
153 phase containing the DNA was concentrated and purified by cleaning up with sterile
154 purified water using Amicon Ultra Centrifugal filters (Millipore). The DNA yield and
155 integrity were quantified in a Nanodrop spectrophotometer (NanoDrop 1000 Thermo
156 Fisher) and Qubit fluorimeter (Thermo Fisher). DNA extracts were stored at -80°C.
157

158 RNA was extracted after cutting the frozen filters into small pieces and using Qiagen's
159 RNeasy kit. After a DNase treatment with Ambion's Turbo DNA-free kit, RNA yield
160 and integrity were quantified with a Nanodrop spectrophotometer (NanoDrop 1000
161 Thermo Fisher) and a Qubit fluorometer (Thermo Fisher). The RNA extracts were
162 stored at -80°C. Prior to sequencing, ribosomal RNA depletion was performed by
163 Sequentia Biotech (http://www.sequentiabiotech.com/) using Illumina's TruSeq
164 Stranded Total RNA LT – with RiboZero.
165

166 **High-throughput sequencing (HTS)**

167 DNA samples were sequenced in two batches. The first batch included most
168 metagenomic samples and was sequenced at CNAG (https://www.cnag.crg.eu) on the
169 Illumina HiSeq2000 sequencing platform using a TruSeq paired-end cluster kit, v3. The
170 second batch included four metagenomic samples (May 10, June 1, June 15, and July
171 30) and all metatranscriptomic samples. The numbers of reads per sample are shown in
172 Supplementary Table S1. We used the SqueezeMeta pipeline (v1.3) and the SQMtools
173 R package (25), (26) for all the bioinformatics analyses. Details of the assembly are
174 shown in Supplementary Table S2. Average gene copy numbers per genome were
175 calculated as described in Puente-Sánchez *et al.*, (26) using the median coverage of 10
176 universal single-copy marker genes (27). These markers are particularly suitable for
177 normalizing metagenomic and metatranscriptomic data to provide estimates of relative
178 per-cell gene copies, because they represent constitutively expressed single-copy
179 housekeeping genes (27). Furthermore, since average copy numbers are normalized
180 against the coverage of well conserved genes, they should be unaffected by the
181 percentage of mapped and/or annotated reads, making them better to compare the
182 abundances of the same function in different samples. Thus, throughout the manuscript
183 we will use average copy numbers per genome when discussing the normalized
184 abundance of individual functions in the microbiome, and percentages of reads assigned
185 to each taxon when discussing taxonomic distributions. Sequences were deposited under
186 NCBI BioProject ID PRJNA803814.
187
188

189 **Taxonomic assignment of ORFs and contigs**

190 For each ORF DIAMOND (v0.9.22) homology searches were performed with its amino
191 acid sequence against the GenBank nr database (downloaded in September, 2019). An
192 e-value cutoff of 1e-03 was set to discard poor hits. The best hit was obtained, and then
193 we selected a range of hits (valid hits) having at least 80% of the bitscore of the best hit,
194 and with an identity to the nr database no smaller than 10% of the identity of the best
195 hit. The Last Common Ancestor (LCA) of all these hits was obtained at diverse
196 taxonomic ranks (from phylum to species). In order to allow for some flexibility, the
197 case of putative transfer events or incorrect annotations in the database, we reported a
198 taxon as the LCA as long as it was supported by 90% of the valid hits.
199
200 To ensure trustworthy annotations we also required a minimum identity to the nr
201 database in order to assign ORFs to the different taxonomic ranks. These identity
202 cutoffs were based on Luo *et al.* (28), and were 85, 60, 55, 50, 46, 42 and 40% for the
203 species, genus, family, order, class, phylum and superkingdom ranks, respectively.
204
205 Finally, for each contig, we obtained a consensus taxonomy from the annotations of all
206 the ORFs encoded in the contig, such as 50% of all the genes (regardless of whether
207 they could be annotated or not) and 70% of the annotated genes belonging to the same
208 taxon. After mapping of the reads to the contigs using Bowtie2 v2.3.4.1 (29), contig
209 taxonomies were used for calculating global taxon abundances in our samples, while
210 ORF taxonomies were used to calculate the taxonomic distribution of particular
211 functions.
212

213 **Functional annotation of open reading frames (ORFs)**

214 The ORFs were functionally annotated into Clusters of Orthologous Groups (COGs) by
215 downloading the eggNOG database (30), selecting the entries annotated as COGs,
216 creating a DIAMOND database and using it for annotation as described in Tamames
217 and Puente-Sánchez (25). COGs were used for functional annotation instead of KEGG
218 orthologs (KOs) or PFAMs, since their finer granularity allowed for a more careful
219 curation of the annotations (see next section).

220

221 **Identification of siderophore synthesis genes and pathways**

222 Pathways for siderophore synthesis usually include two types of enzymes. In the first
223 steps, fairly common enzymes are used, such as for example the protein catalyzing the
224 step chorismate to isochorismate. These enzymes are coded by genes that are common,
225 not only to several siderophore synthesis pathways, but also to more general central
226 metabolic pathways. And next, several additional steps that usually require
227 nonribosomal peptide synthetases (NRPs), polyketide synthases, and NRPs-independent
228 siderophore synthetases (NIS) that condense small and large molecules until the
229 complex structures of the siderophores are completed. In many cases, several of these
230 NRPs are common to different pathways and, thus, different gene names are classified
231 in the same COG category. For example, COG3486 has representatives in at least 13
232 siderophore synthesis pathways (see Supplementary Figure S1). Thus, detecting this
233 COG in metagenomes does not indicate which siderophore pathway is present. On the
234 contrary, some genes are assigned to more than one COG, further complicating the
235 issue. For example, gene *dhbE* in the bacillibactin synthesis pathway is assigned to
236 COGs 0318 (AMP-dependent synthetase and ligase) and COG1021 (2,3-
237 dihydroxybenzoate-AMP ligase). We checked manually all the siderophore synthesis

238 pathways in MetaCyc (31) and added a few more (Supplementary Table S3). We have
239 summarized this complexity in a network (Supplementary Figure S1) that will facilitate
240 identification of both common and unique genes in the synthesis pathways of the
241 different siderophores.
242
243 Since identifying the siderophore pathways that are present in metagenomes and
244 expressed in metatranscriptomes is complicated, we adopted the following criteria:
245 1. In order to state that the pathway for a given siderophore MAY BE present, all
246 the genes in the pathway must be present.
247 2. In order to state that the pathway IS present, besides presence of all the genes, at
248 least one of the genes must be unique to this pathway, one that is not shared
249 among different pathways.
250 3. If at least one gene of a pathway is absent, we consider the whole pathway
251 absent.
252 There was an additional difficulty. A substantial portion of the genes involved in
253 siderophore synthesis belonged to COG0318. This COG includes genes *entE* in
254 salmochelin and enterobactin pathways (three different steps in the pathways), *pvdI* and
255 *pvdL* in pyoverdin (first condensation step), *dhbE* in bacillibactin (two successive
256 steps), *asbC* in petrobactin (a ligase in two steps), and *acsA* in achromobactin (synthase,
257 four steps). It also includes several enzymes unrelated to siderophore synthesis. Thus,
258 we considered the presence of this COG necessary to fulfill criterion 1 above. However,
259 we did not count the reads assigned to this COG in the quantitation of siderophore
260 synthesis genes, because many of them might code for genes in other metabolic routes.
261 We followed a similar procedure for the rest of the COGs, using for quantitation only
262 those which could be unambiguously assigned to siderophore biosynthesis pathways

263 using the high quality – manually curated SwissProt database (32). Some of them,

264 however, should be involved in siderophore synthesis. Therefore, our counts of

265 siderophore synthesis genes are underestimates.

266

267 **RESULTS**

268

269 ## Changes in ecosystem parameters

270 Daylight increased from <12 h during early March to 24 h in 21 May. The consequent
271 increase in surface irradiance was intensified by the progressive melt of surface snow
272 and ice, with PAR transmitted to 2.5 m depth increasing steadily until 12 June,
273 thereafter, increasing nonlinearly (Figure 2a) as melt ponds formed on the surface. Chl *a*
274 increased steadily throughout the study following the increase in PAR, while salinity
275 decreased slightly at the surface due to ice melting (Figure 2b-c).

276

277 ## Taxonomic composition of the prokaryotic assemblage

278 Microbial populations changed along the study (Figure 3a). In early March
279 *Proteobacteria* dominated the assemblage. Specially, *Alphaproteobacteria* accounted
280 for 36% of the reads and *Gammaproteobacteria* for 20%. The two other main groups
281 were *Taumarchaeota* (12%) and *Bacteroidetes* (9%). Other minor components were
282 *Deltaproteobacteria* (5%), *Actinobacteria* (4%), *Betaproteobacteria* (2.5%),
283 *Verrucomicrobia* (2.5%), *Euryarchaeota* (2.5%), *Planctomycetes* (2%), and
284 *Marinimicrobia* (2%). Progressive changes eventually resulted in a different
285 composition in June. For example, on 23 June the main groups were *Bacteroidetes*
286 (55%), *Alphaproteobacteria* (21%), *Gammaproteobacteria* (18%), chloroplasts (12%),
287 and *Actinobacteria* (2%).

288

289 *Alphaproteobacteria* were, as mentioned, the most abundant group in late winter. The
290 most important orders were *Pelagibacterales* and *Rhodobacterales*, followed by

291 unclassified *Alphaproteobacteria*, and small contributions from *Rhodospirillales* and
292 *Sphingomonadales* (Figure 3b). This is a fairly usual composition in the oceans.
293
294 *Gammaproteobacteria* increased up to 1 June, when they reached 37% of the reads and
295 decreased slightly afterwards (Figure 3a). The most abundant group could not be
296 classified beyond the class level. The next taxa in abundance were *Cand*. Thioglobus
297 and *Cellvibrionales* (Figure 3b). Another unusual group was the Order
298 *Pseudomonadales*. In contrast, *Gammaproteobacteria* groups commonly abundant in
299 the oceans such as SAR86 and *Alteromonadales* had only small contributions.
300
301 Throughout the study, *Flavobacteriales* were the most abundant *Bacteroidetes* order,
302 followed by unclassified *Bacteroidetes*, unclassified *Flavobacteriia*, and *Cytophagales*
303 (Figure 3b). Most of the increment in *Bacteroidetes* was due to the genus *Polaribacter*
304 (Supplementary Figure S2). In effect, at the beginning of the study, the *Bacteroidetes*
305 reads were fairly well distributed among seven taxa: N55, NS9, *Marinoscillum*, NS4,
306 *Polaribacter*, *Owenweeksia* and *Sufflavibacter* in order of abundance and several other
307 minor components. In late June, on the other hand, 60% of the reads belonged to
308 *Polaribacter* and the rest to several other taxa. The increase in *Bacteroidetes* closely
309 followed the phytoplankton summer bloom, as indicated both by chl *a* (Figure 2) and
310 chloroplasts (Figure 3a).
311
312 *Actinobacteria* were present in small amounts throughout the study. Two more bacterial
313 taxa, *Verrucomicrobia* and *Deltaproteobacteria*, were significant at the beginning of the
314 time series, but eventually disappeared. Likewise, the two archaeal phyla
315 *Thaumarchaeota* and *Euryarchaeota* were abundant in March and almost disappeared

316 with the seasonal progression. The bloom of eukaryotic algae in June was reflected as a
317 slight increase in the chloroplast reads towards the end of the study period (Figure 3a).
318

319 **Main iron transport genes**

320 We searched for almost 250 unique genes grouped in 93 COG categories related to iron
321 acquisition and siderophore synthesis (Supplementary Table S3). We pooled the genes
322 into groups with similar functions (Table 1, see scheme in Supplementary Figure S3).
323 Thus, the main transporters of iron were classified into: i) ferrous iron ABC
324 transporters, ii) ferric iron siderophore outer membrane transporters (TonB dependent),
325 and iii) ferric iron ABC transporters. These are the proteins destined to transfer ferrous
326 iron across the cell membrane and ferric iron bound to siderophores across both
327 membranes. Other categories were iv) divalent cation ABC transporters (transporting
328 iron and other cations across the cell membrane), v) transporters of iron bound to
329 dicitrate, vi) transporters of heme groups, vii) storage of iron as ferritin, and viii)
330 regulators of iron metabolism. Finally, we also examined siderophore synthesis genes
331 (see next section).
332

333 Normalized abundance of iron related genes increased from a copy number (CN) of less
334 than 5 in March to higher than 8 in late June (Figure 4a). Thus, the CN of genes devoted
335 to iron acquisition almost doubled along the time series. Three of our categories, ferric
336 iron-siderophore ABC transporters, storage, and siderophore synthesis did not change
337 significantly. Two other, heme and divalent cation ABC transporters increased
338 moderately, while ferric iron TonB dependent, dicitrate and ferrous iron ABC
339 transporters increased substantially. Iron uptake regulation genes also increased and
340 were those with highest CN throughout the study (between 2 and 4, Figure 4a).

341 Globally, regulator genes accounted for about half of the copies in the category, ferric
342 iron (TonB dependent and ABC) for a quarter and the remaining genes for another
343 quarter.
344
345 The taxonomy of these genes generally corresponded to that of the community
346 (compare Figs. 3a and 4b). In other words, the most abundant taxa were responsible for
347 most of the iron related genes. There was only one obvious difference: while
348 *Alphaproteobacteria* were always the first or the second group in abundance, they were
349 always the third one when considering only the iron-related genes, with
350 *Gammaproteobacteria* and *Bacteroidetes* alternating as those with the most genes. This
351 underrepresentation was likely due to the fact that the most abundant member of the
352 *Alphaproteobacteria* was the order *Pelagibacterales* (Figure 3b), that is known to lack
353 all TonB dependent transporters and to have a very small percent of their genome
354 devoted to iron acquisition genes (Cobo-Simón *et al*., in preparation).
355

356 **Ferric iron transport genes**

357 There were large differences in abundance and taxonomy between the outer membrane
358 and cell membrane transporters (Figure 5a and b). The TonB dependent outer
359 membrane transporters increased from a CN of 0.3 in March to reach a maximum of 1.6
360 in late June. Most of this increase was due to *Bacteroidetes* (Figure 5a). In effect, this
361 group accounted for 90% of the reads of these genes in late June. On the contrary, the
362 inner membrane ABC transporters remained almost constant around a CN of 0.9. And
363 the taxonomy was also completely different. In order of importance the main groups
364 were *Alpha*-, *Gammaproteobacteria* and *Bacteoridetes* in the third place (Figure 5b).
365

366 An alternative path to obtain ferric iron is incorporating it bound to dicitrate. The genes
367 for this process increased in parallel to the outer membrane TonB dependent
368 transporters, although at lower CNs (always below 0.6, Supplementary Figure S4a). In
369 this case, *Gammaproteobacteria* and *Bacteroidetes* were responsible for most of the
370 reads.
371
372 The last ferric iron transporter genes that we found were those devoted to transport
373 heme bound iron (Supplementary Figure S4b). These genes also increased with time,
374 mostly due to the *Gammproteobacteria*. *Bacteroidetes* did not have these genes. And
375 towards the end of the study *Actinobacteria* and *Chlorophyta* contributed significantly.
376 The CNs, however, were low, always below 0.15.
377

378 **Siderophore synthesis genes**

379 The CN of siderophore synthesis genes oscillated slightly around 0.025 (Figure 6).
380 These values are likely underestimated because we excluded all the reads of COG0318
381 (see M&M). Still, the CNs are very low compared to the genes to incorporate iron
382 bound to siderophores. Thus, the effort dedicated to siderophore synthesis was
383 moderate, and more or less constant. A portion of these genes could not be assigned to
384 particular bacterial groups. But, of those that could be assigned, most belonged to
385 *Proteobacteria*. Interestingly, *Beta*- and *Deltaproteobacteria* were significant
386 contributors in March, while *Gamma*- and to a lesser extent *Alphaproteobacteria* were
387 the main groups in June. A striking difference with previous groups of genes was that
388 *Bacteroidetes* almost did not contribute anything to siderophore synthesis.
389

390 With the criteria explained in M&M we could confidently state that pathways for eight
391 siderophores were present: alcaligin, enterobactin, mycobactin, myxochelin,
392 petrobactin, salmochelin, and staphyloferrins A and B, and that four were absent
393 (pyochelin, pyoverdin, yersiniabactin, and amphibactin). Again, considering our criteria,
394 the presence of the remaining studied siderophores could not be confirmed
395 (Supplementary Table S4). All eight siderophore synthesis pathways identified as
396 present were found in all the metagenomes except the earliest one on 9 March
397 (Supplementary Table S5).
398

399 **Ferrous iron transport genes**

400 Ferrous iron is able to diffuse through porins into the periplasmic space. There it can be
401 transported to the cytoplasm by ABC transporters, either iron specific or able to transfer
402 several divalent cations including ferrous iron. The ferrous iron specific ABC
403 transporters increased during the study from a copy number of 0.25 to almost 1 in June
404 (Figure 7a). Again, most of the increase was due to *Bacteroidetes* with
405 *Gammaproteobacteria* having a constant contribution. Several other bacterial groups
406 had significant contributions in March and disappeared along the study. These included
407 *Actinobacteria*, *Planctomycetes*, *Verrucomicrobia* and *Gemmatimonadetes*.
408

409 The divalent cation ABC transporters, on the other hand, increased very slightly from a
410 CN of 0.8 to 1.0 (Figure 7b). Interestingly, *Thaumarchaeota*, *Planctomycetes* and
411 *Verrucomicrobia* accounted for half of the reads in March and decreased through
412 spring. *Alphaproteobacteria* accounted for a constant proportion. *Gammaproteobacteria*
413 increased until 15 June and then decreased again. Finally, *Bacteroidetes* had the largest
414 increase becoming dominant in June (70% of the reads).

415

416 ## Other iron related genes

417 We also looked for iron storage genes, mostly related to ferritin (Supplementary Figure
418 S5a). There was only a moderate increase in CN, from 0.2 to 0.3. *Thaumarchaeota* were
419 abundant in March, while *Gammaproteobacteria* and *Bacteroidetes* were abundant in
420 June. Finally, genes involved in the regulation of iron metabolisms were always the
421 most abundant ones (Supplementary Figure S5b), with CNs increasing slightly from 2.1
422 to 3.8 The three most abundant groups of bacteria were also responsible for most of the
423 reads belonging to these genes.

424

425 ## Gene expression

426 Taxonomic assignments of the metatranscriptomic reads are shown in Figs. S6 and S7.
427 On several dates more than half of the reads were either unmapped or unclassified. As a
428 result, the information they provide is limited. Thus, we have only used the
429 metatrancriptomes to show that, considering the mapped and classified reads,
430 expression was proportional to abundance of the taxa. Therefore, there were no
431 differences among the different taxa in the level of expression.

432

433

434 # Discussion

435

436 ## Community composition

437 The observed change in microbial composition from early spring to summer has
438 previously been reported for the Amundsen Gulf (6), which is approximately 600 km
439 from our study site. The winter community is generally dominated by *Alpha*- and
440 *Gammaproteobacteria*. *Archaea* are important in winter and decrease towards spring
441 and summer (33). Other minor but significant groups in winter are *Betaproteobacteria*,
442 *Verrucomicrobia* and *Actinobacteria*. All of these groups typically decrease and even
443 disappear in summer (8). In contrast, *Bacteroidetes* increase along the spring and
444 become one of the dominant taxa. Predominance of the genus *Polaribacter* is known to
445 be common in polar waters (34). This general structure of groups *Alpha*- and
446 *Gammaproteobacteria* and *Bacteroidetes* dominating is fairly typical of the Arctic
447 Ocean (8), and is supported by this study. This is in contrast to the usual community
448 composition in temperate waters, where *Alphaproteobacteria* are generally dominant
449 accounting for around 50% of the community, and are followed by
450 *Gammaproteobacteria* and *Cyanobacteria*, while *Bacteroidetes* usually account for only
451 4% and *Betaproteobacteria* are barely detectable (8).

452

453 In general, both abundance and expression of genes involved in iron uptake was
454 proportional to abundance of the organisms. *Gammaproteobacteria*,
455 *Alphaproteobacteria* and *Bacteroidetes* were the most important. Debeljak et al. (35)
456 studying heterotrophic bacterial assemblages around Kerguelen Islands, found that
457 *Gammaproteobacteria* and *Bacteroidetes* were the main taxa in the uptake of iron-
458 siderophore complexes, while *Alphaproteobacteria* had a more significant role in Fe(II)

459 and Fe(III) uptake, just like we found. Likewise, Fourquez et al. (36), using
460 microautoradiography, found that *Gammaproteobacteria* and *Bacteroidetes* were the
461 main bacterial taxa active in iron uptake.
462

463 **Iron in the Arctic Ocean**

464 There have been several studies determining iron concentrations in the Arctic Ocean.
465 Most of these have been either in the Central Arctic close to the Eurasian coast ((37),
466 (38), concentrations between 0.4 and 0.6 nM) or in the Chuckhi Sea and the Canadian
467 Basin (39), (40), (41), (42). In the Bering Sea, Aguilar-Islas et al. (43) found iron to be
468 limiting in the outer shelf not influenced by ice, but it was sufficient in the outer shelf
469 influenced by ice. This was due to release of dissolved iron from the melting ice. To our
470 knowledge, Colombo *et al*. (44) presented the only study in the Canadian Archipelago.
471 Here, a higher concentration of iron was found than in the deep-water areas to the East
472 and West, with surface concentrations between 0.40 and 1.91 nM above 40 m and
473 between 1.10 and 1.15 between 70 and 100 m. They also noted the well-known inverse
474 correlation between iron concentration and salinity, due to the known influence that
475 freshwater river discharge and ice melt have on enriching sea water in iron. Therefore,
476 iron concentrations in the area are much higher than in most of the oceans. Moreover,
477 N:P ratios in the ice-water interface at our study site suggested nitrogen limitation (21).
478 Thus, iron was not expected to be limiting in these waters. Despite this, iron is an
479 essential nutrient for all microorganisms and it requires special proteins to acquire it.
480 While iron in ocean water could not be directly sampled in this study, our metagenomic
481 data showed presence of iron acquisition genes in all three dominant bacterial taxa, with
482 copy numbers larger than 1 in some cases.
483

484 Unlike the Arctic Ocean, the Southern Ocean is one of the main HNLC areas in the
485 world, where phytoplankton growth is limited by iron. However, there is considerable
486 variability. Thus, in studies of the waters around Kerguelen Islands, Obernosterer *et al*.
487 (45) could compare HNLC waters on the windward side of the islands with naturally
488 iron enriched waters on the leeward side. These authors found that heterotrophic
489 bacteria were limited by iron in both regions. Debeljak *et al*. (35) analyzed the
490 expression of several iron acquisition genes in the same area and found that transcripts
491 for Fe(II) and Fe(III) uptake genes were found in higher proportions at the HNLC
492 stations as compared to the Fe-fertilized sites. In contrast, no such pattern was observed
493 for siderophore-uptake (35). However, when they analyzed the taxa specific expression,
494 siderophore uptake transcripts were higher in the HNLC station than in the iron
495 enriched zones. In principle, we should expect in the Arctic Ocean a situation similar to
496 that in the iron enriched zone. That is, an abundance of iron uptake genes with those for
497 siderophores outnumbering those for Fe(II) and Fe(III) and, in effect, this is what we
498 found. Therefore, it seems a general feature of marine bacterial assemblages that iron
499 acquisition is a major need of bacteria even under non limiting conditions.
500

501 **Siderophores**

502 In the aerobic ocean, iron is predominantly in the Fe(III) oxidation state, which has a
503 very low solubility and is therefore hard to obtain. One of the most common ways for
504 microorganisms to thus acquire ferric iron is to synthesize siderophores, that can be
505 carried out by many marine microorganisms (11). The chemistry of siderophores in
506 marine microorganisms has been reviewed (46), (47). These studies involve pure culture
507 isolates of marine bacteria in which the siderophores are produced in large enough
508 quantities to be characterized chemically. Most of these belong to

509 *Gammaproteobacteria* genera *Halomonas*, *Vibrio*, *Marinobacter*, *Ochrobacter*,
510 *Pseudoalteromonas*, *Shewanella*, as well as cyanobacteria such as *Synechoccocus* (15).
511
512 Concentrations of siderophores have been measured in a vertical profile at ALOHA
513 station off Hawaii (48), transects from the coast offshore in the southeast Pacific (49),
514 the California Current System (50), and along a latitudinal transect in the oligotrophic
515 Atlantic Ocean (51). In all these studies, ferrioxamines were found in surface waters,
516 while amphibactins were typically more abundant in deep waters and in high nutrient
517 low chlorophyll zones. Extrapolating this distribution to the costal Arctic, we would
518 expect genes for ferrioxamines but not those of amphibactins in our 60 m deep study
519 region. Actually, we found the whole set of genes for ferrioxamines B and E, which
520 would be in agreement with the previous studies. However, none of these genes was
521 unique to these pathways and, therefore, we cannot assert whether these siderophores
522 were present in our samples or not.
523
524 The pathways for synthesis of amphibactins have not been as well characterized.
525 However, the pathway in *Vibrio neptunius*, a pathogen of bivalve mollusks has been
526 determined (52). In our metagenomes we found all the genes for this pathway but one.
527 Moreover, all the genes found were common to other siderophore synthesis pathways.
528 According to our conservative criteria, amphibactin synthesis pathways were not
529 present in our samples. This would agree with the absence of amphibactins from surface
530 ocean waters. Amphibactins have a polar head with the function of binding iron linked
531 to a fatty acid tail that may be anchored in the outer cell membranes, thus avoiding the
532 dispersion of the siderophore beyond the reach of the producing organism (53) (15).
533 This is a way to avoid utilization of siderophores by alien microorganisms and would

534 seem an adaptive strategy in the oceans. However, as discussed, they do not seem to be
535 abundant in surface marine waters and we did not find the complete pathway in our
536 study.
537

538 **Siderophores in Proteobacteria and Bacteroidetes**

539 *Proteobacteria* is the bacterial phylum in which siderophore synthesis and uptake has
540 been most studied, particularly concerning pathogenic *Gammproteobacteria* (54), (55).
541 In contrast, experimental evidence of siderophore synthesis in *Bacteroidetes* is scarce.
542 Some pathogenic *Flavobacteria* can synthesize siderophores with genes in a plasmid
543 (56). Delmont *et al*. (57) found siderophore synthesis genes in three *Bacteroidetes*
544 metagenomic assembled genomes (MAGs), including two *Polaribacter* and one
545 *Cryomorphaceae,* in a bloom in Antarctica. The *Proteobacteria* MAGs they found did
546 not have these genes. Guan *et al*. (16) found a ferrichrome (*fhuA*) receptor in the fish
547 pathogen *Flavobacterium columnare* that was shown to also synthesize siderophores
548 with the chrome azurol assay. Chen *et al*. (47) reviewed siderophores from marine
549 bacteria and found over 80 of all the known siderophore families. Most siderophores
550 were synthesized by *Gammaproteobacteria* and a few by *Alpha*- and
551 *Betaproteobacteria* and *Actinomycetes*. Only one siderophore was synthesized by a
552 *Bacteroidetes*. This was bisucaberin B made by *Tenacibaculum mesophilum* isolated
553 from a tropical sponge. Fujita *et al*. (58) claimed this was the first siderophore made by
554 a *Bacteroidetes*. In *Allivibrio salmonicida* (*Gammaproteobacteria*) the gene cluster for
555 synthesis and uptake of this siderophore is flanked by transposases. This suggests that
556 this cluster may have been acquired by lateral gene transfer. The same might have
557 occurred in *Tenacibaculum*. Bisucaberin was one of the siderophores that might be
558 synthesized in the Arctic, but the reads did not belong to *Bacteroidetes*.

559

560 On the other hand, there is evidence of *Bacteroidetes* being able to use siderophores
561 made by other bacteria. D'Onofrio *et al*. (17) found that a *Maribacter polysiphoniae* (a
562 member of the family *Flavobacteriaceae*) could grow on solid agar when supplemented
563 with a siderophore from a producer bacterium such as *Microccocus luteus* isolated from
564 the same sediment environment. Zhu *et al*. (18) showed that *Bacteroides*
565 *thetaiotaomicron* could use enterobactin and salmochellin from *E. coli* and
566 *Pseudomonas* in the intestine with *xusABC* genes. Our results are consistent with this
567 model of *Bacteroidetes* contributing very little to synthesis but considerably to uptake
568 of siderophores.

569

570 **Iron-siderophore complexes as common goods**

571 In our Arctic study, the time series followed the development of the seasonal
572 phytoplankton bloom (5), which is triggered by the increasing intensity and duration of
573 solar radiation available (59). In addition to the increased growth of primary producers
574 as ice melts, large amounts of polysaccharides made by ice-algae may be released in the
575 water column (60). For this reason, *Bacteroidetes* are known to grow better when
576 accompanying algal blooms and have a large array of outer cell membrane proteins to
577 take up polysaccharides from them (61), (62). This is likely the main factor in the
578 dominance by *Polaribacter* within this study. However, given the substantial amounts
579 of genes for siderophore uptake and their active expression, it seems that iron was a
580 desirable commodity. Our data on the siderophore synthesis and uptake genes are
581 consistent with competition for siderophores between *Gammaproteobacteria* and
582 *Bacteroidetes*. Both taxa dedicated most iron acquisition genes to the uptake of iron-
583 siderophores. *Bacteroidetes* seemed to act as cheaters while *Gammaproteobacteria* were

synthesizing them. It seems likely that this characteristic, among others, may have helped *Bacteroidetes* to become the most abundant group at the end of the study. Our results confirm the idea of siderophores as public goods, highlight the fact that iron-siderophores are competitively sought after even in environments not limited by iron, and open a fascinating avenue of research for future Arctic cruises.

Author contributions (not necessary in ISME journal)

Conceived and designed the work: C.J. Mundy, CPA.

Acquired and processed data: C.J. Mundy, K. Campbell, Marta Royo-Llonch, Vanessa Balagué, P. Sánchez, L. Macías, F, Puente-Sánchez, J. Tamames.

Drafted the manuscript: L. Macías, F. Puente-Sánchez, CPA.

All authors contributed to discussion of the results, finalization and approval of the manuscript.

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Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflicts of interest.

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805 **FIGURE LEGENDS**

806 **Figure 1**. Map of study location near Cambridge Bay, Nunavut. Inset includes
807 approximate locations of the main rivers in the area.

808

809 **Figure 2.** (a) Integrated chlorophyll *a* (mg m^{-2}) and PAR (µEinst m^{-2} s^{-1}) along day of
810 year. (b) Time-depth diagram of chlorophyll *a* (µg L^{-1}). (c) Time-depth diagram of
811 salinity (‰).

812

813 **Figure 3.** Percent of reads in metagenomes at two different taxonomic levels: phyla and
814 Proteobacteria classes (a) and orders (b).

815

816 **Figure 4**. (a) Copy numbers of iron related genes through the study period in
817 metagenomes. (b) Percent of the reads assigned to different taxonomic groups (bars, left
818 hand scale) and copy numbers (line and right hand scale). **(b).** Taxonomy of iron related
819 genes.

820

821 **Figure 5.** Taxonomic assignments and copy numbers of ferric iron transporter genes
822 along the study: outer membrane ferric siderophore TonBD (above) and cell membrane
823 ABC (below).

824

825 **Figure 6.** Taxonomic assignments (bars) and copy numbers (line and right scale) of
826 siderophore synthesis genes.

827

828 **Figure 7**. Taxonomic assignments and copy numbers of (a) ferrous iron ABC
829 transporter genes and (b) divalent cation ABC transporter genes along the study.

769

770

771 **Table 1**. Categories of iron transport and siderophore synthesis genes used in the present

772 paper.

Category	Function	Transporter type	Location	Examples
Fe(II)	ferrous iron transport	ABC	Cell membrane	*efeUOB*
Fe(III)-OM	ferric iron +sideorphore transport	TonBD	Outer membrane	*fhuA*
Fe(III)-CM	ferric iron +sideorphore transport	ABC	cell	*fhuBCD*
Divalent cation	divalent cations transport including iron	ABC	cell	*sitABC*
Dicitrate	iron + dicitrate transport	TonBD, ABC	out, cell	*fecABCD*
Heme	iron + heme transport	TonBD, ABC	out, cell	*hemR, hemUV*
Storage	ferritin	-	cytoplasm	*bfr*
Regulators	regulation of iron related genes	-	cytoplasm	*fur, irr*
Siderophore synthesis	Synthesis of siderophores	-	cytoplasm	*entABCDF*

773

774

FIGURES

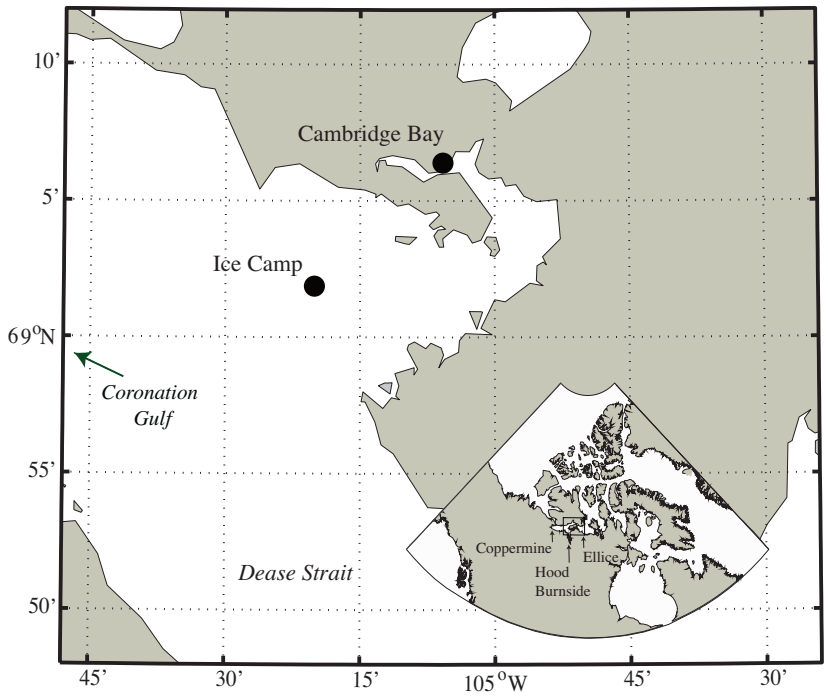


Figure 1. Map of study location near Cambridge Bay, Nunavut. Inset includes approximate locations of the main rivers in the area.

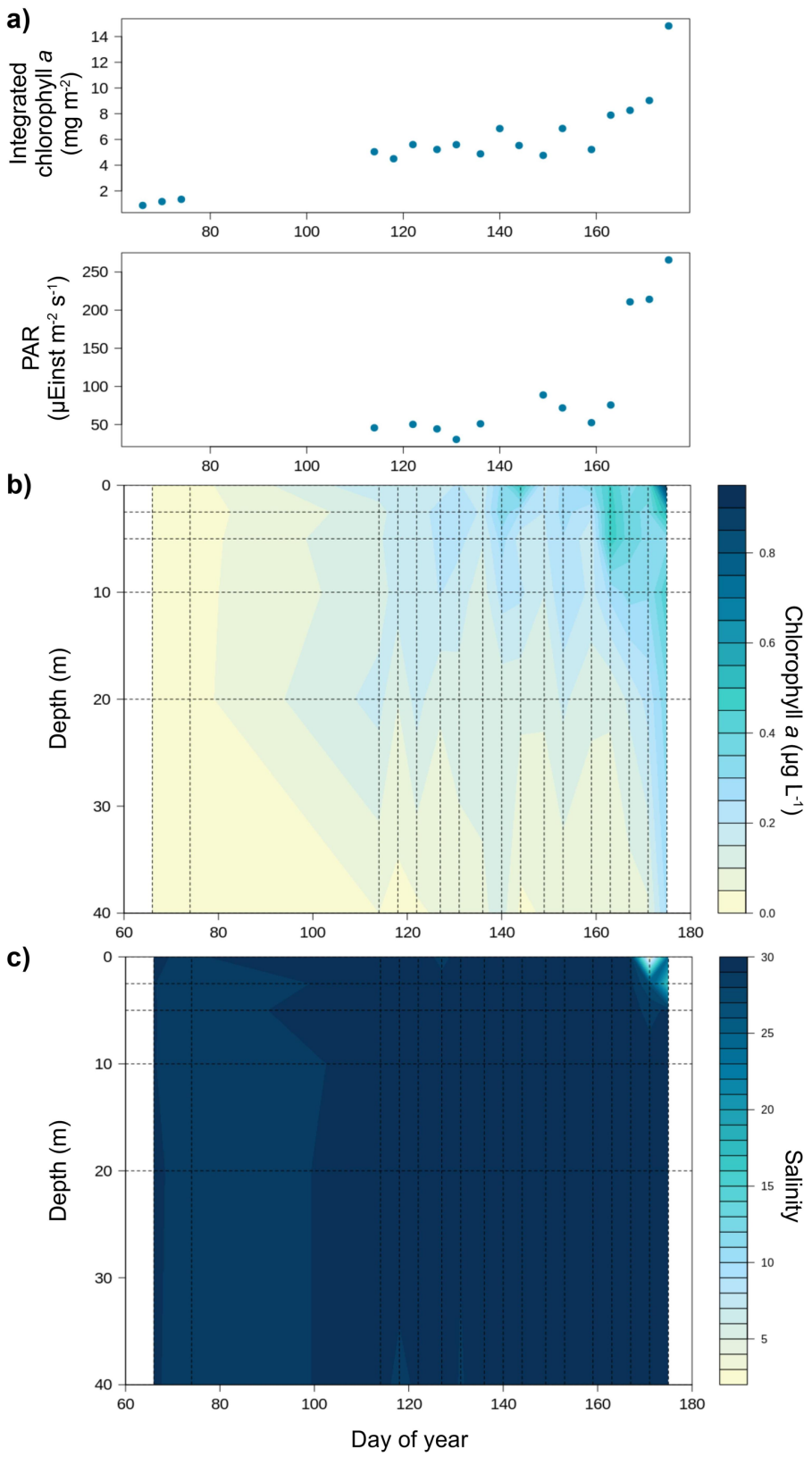
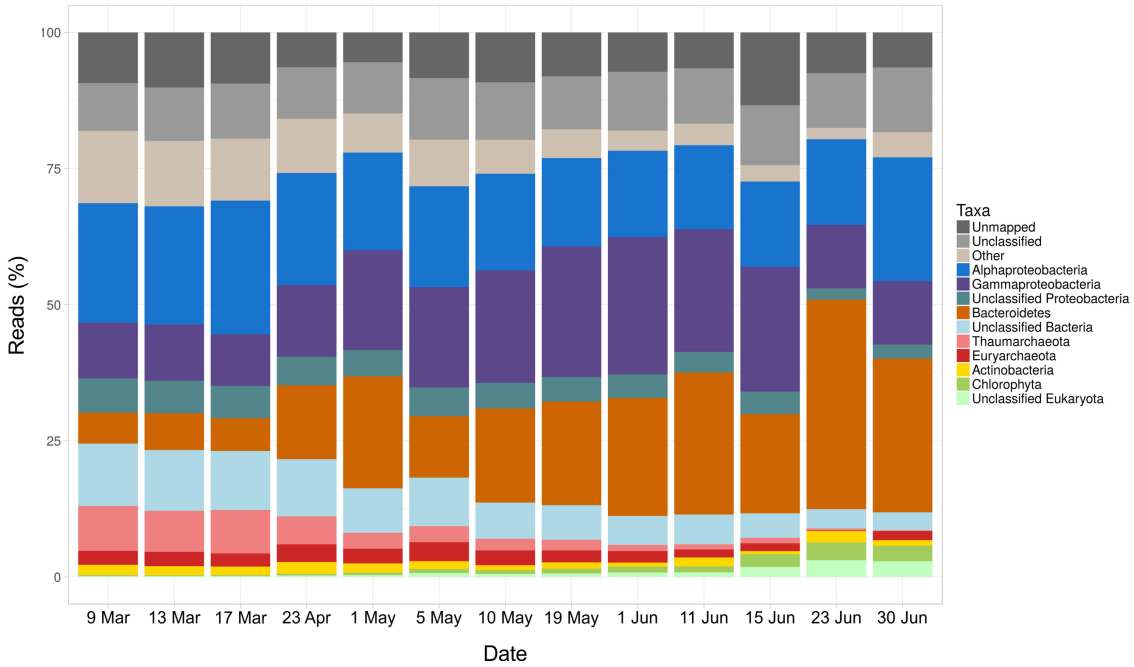


Figure 2. (a) Integrated chlorophyll *a* (mg m^{-2}) and PAR (µEinst m^{-2} s^{-1}) along day of year. (b) Time-depth diagram of chlorophyll *a* (µg L^{-1}). (c) Time-depth diagram of salinity (‰).



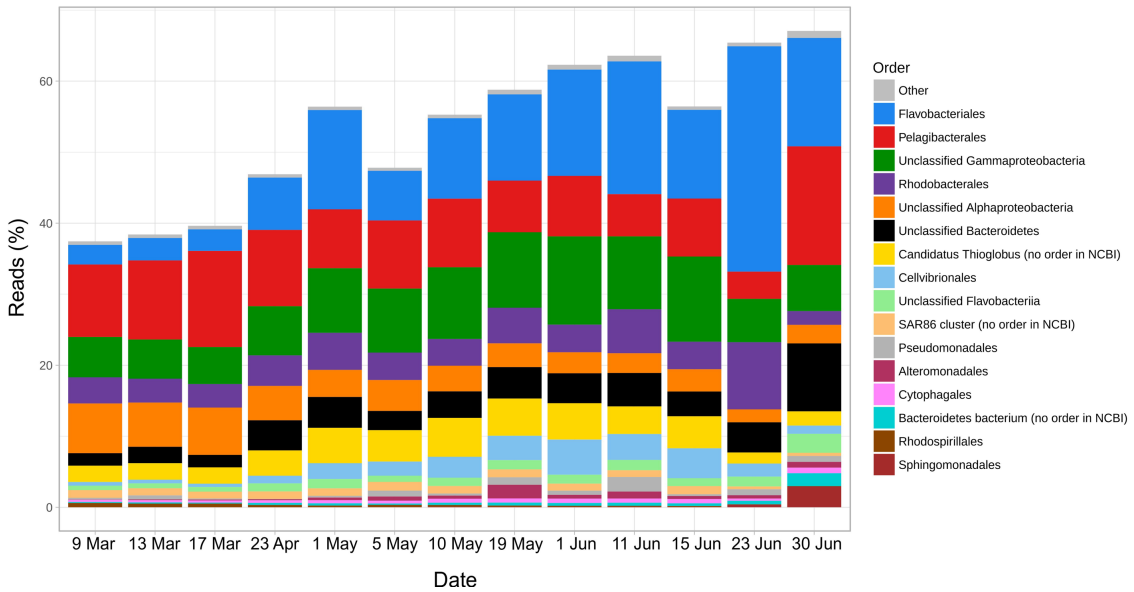
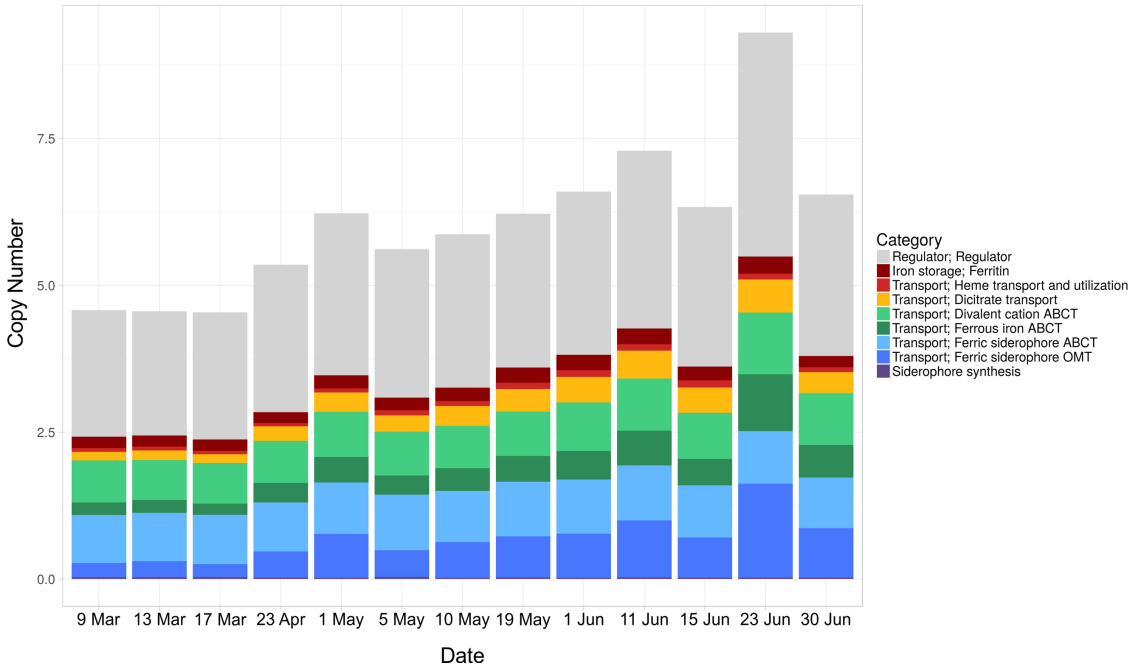


Figure 3. Percent of reads in metagenomes at two different taxonomic levels: phyla and Proteobacteria classes (a) and orders (b).



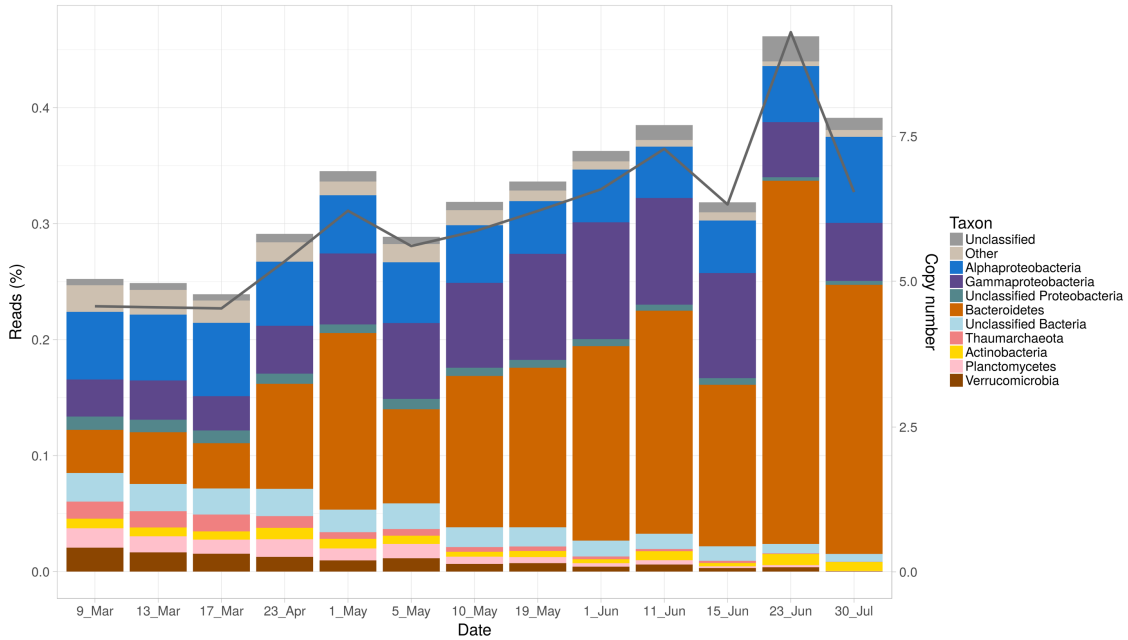
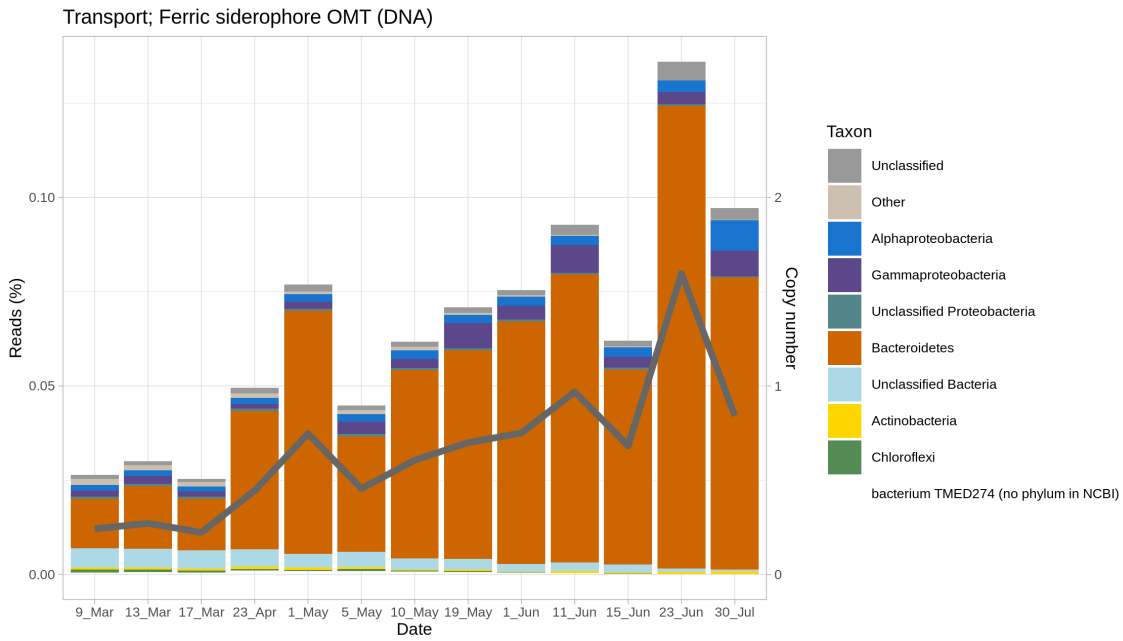


Figure 4. (a) Copy numbers of iron related genes through the study period in metagenomes. (b) Percent of the reads assigned to different taxonomic groups (bars, left hand scale) and copy numbers (line and right hand scale). **(b).** Taxonomy of iron related genes.



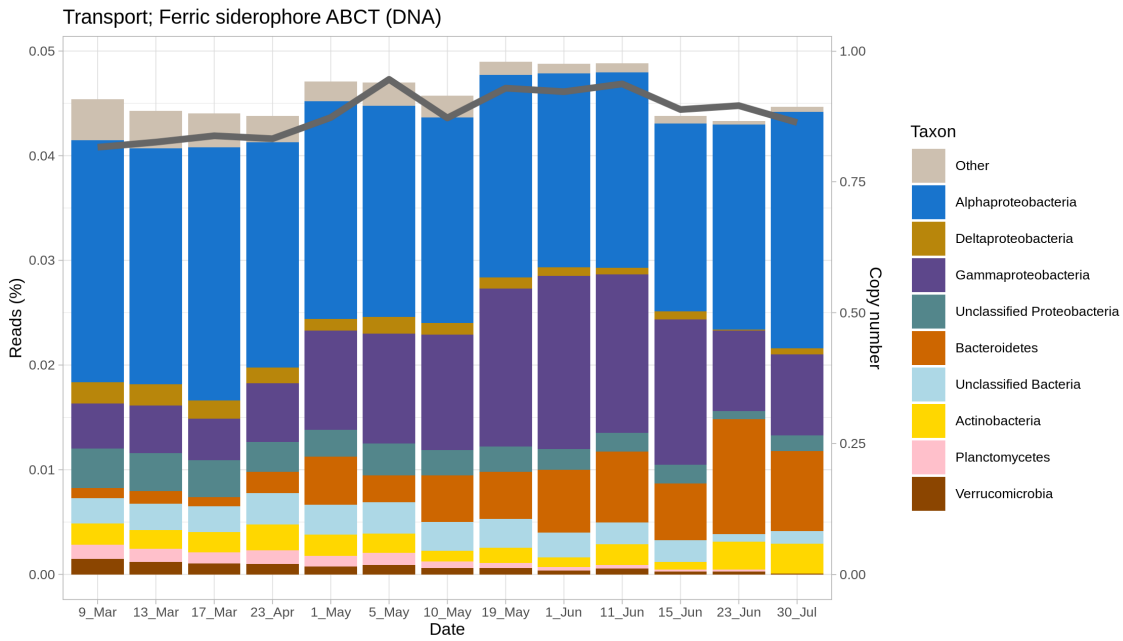


Figure 5. Taxonomic assignments and copy numbers of ferric iron transporter genes along the study: outer membrane ferric siderophore TonBD (above) and cell membrane ABC (below).

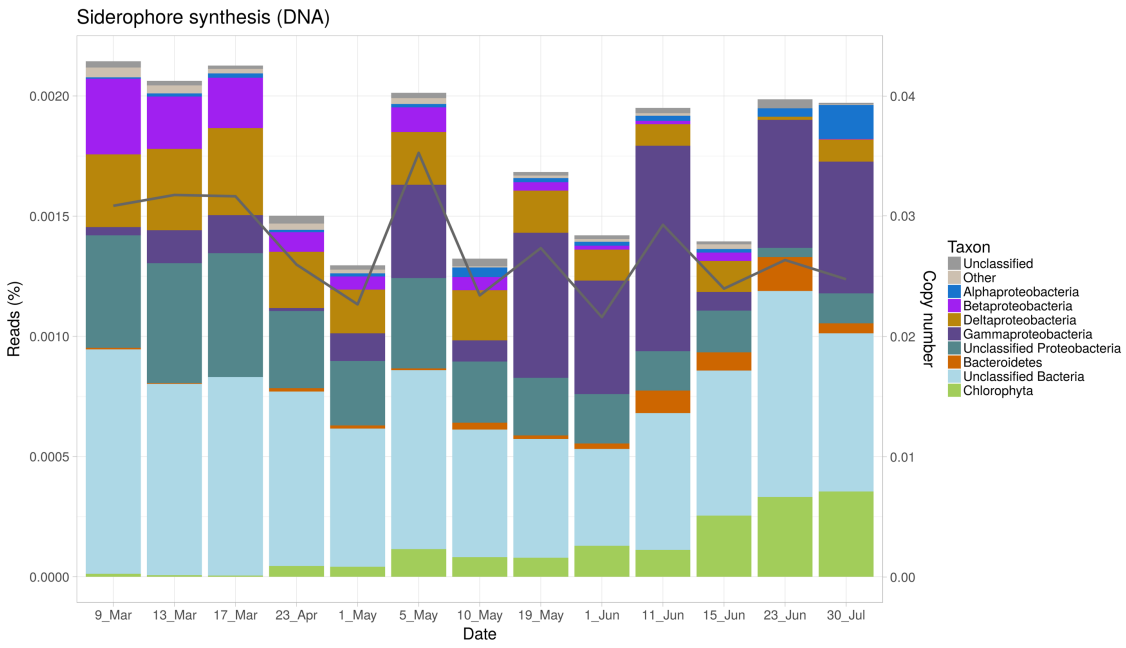


Figure 6. Taxonomic assignments (bars) and copy numbers (line and right scale) of siderophore synthesis genes.

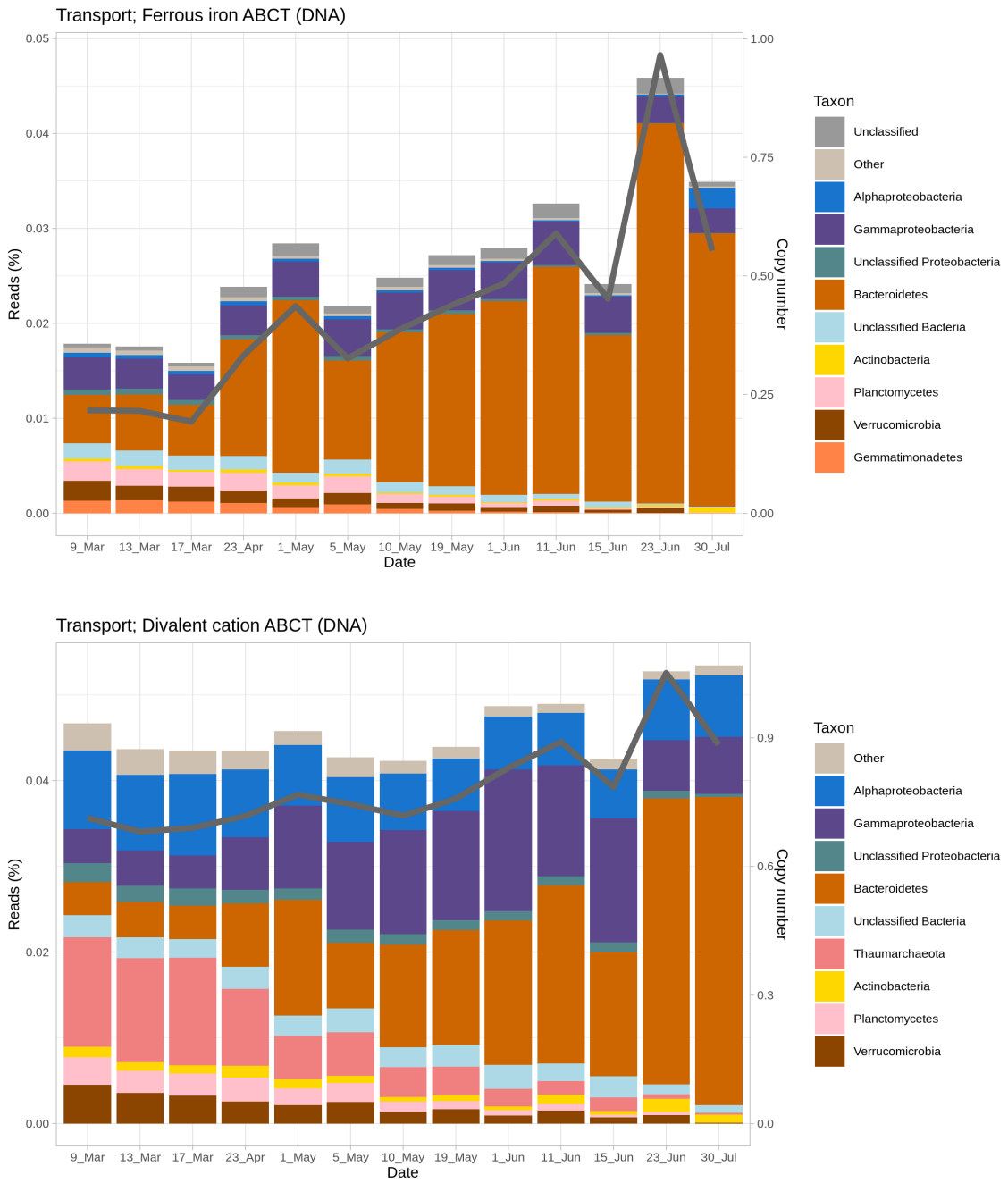


Figure 7. Taxonomic assignments and copy numbers of (a) ferrous iron ABC transporter genes and (b) divalent cation ABC transporter genes along the study.