Some mixotrophic flagellate species selectively graze on Archaea

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ABSTRACT

Many phototrophic flagellates ingest prokaryotes. This mixotrophic trait becomes a critical aspect of the microbial loop in planktonic food webs because of the typical high abundance of these flagellates. Our knowledge of their selective feeding upon different groups of prokaryotes, particularly in field conditions, is still quite limited. In this study, we investigate the feeding behavior of three species (*Rhodomonas* sp., *Cryptomonas ovata* and *Dinobryon cylindricum*) using their food vacuole content in field populations of a high mountain lake. We used the Catalyzed Reporter Deposition - Fluorescent *in situ* Hybridization protocol (CARD-FISH) with specific probes for the domain Archaea and three groups of Eubacteria: β-Proteobacteria, Actinobacteria, and Cytophaga-Flavobacteria of Bacteroidetes. Our results provide field evidence that contrasting selective feeding exists between coexisting mixotrophic flagellates under the same environmental conditions, and that some prokaryotic groups may be preferentially impacted by phagotrophic pressure in aquatic microbial food webs. In our study, Archaea was the preferred prey, chiefly in the case of *Rhodomonas* sp., which rarely fed on any other prokaryotic group. In general, prey selection did not relate to prey size among the grazed groups. However, Actinobacteria, which were clearly avoided, mostly showed a size <0.5 µm, markedly smaller than cells from the other groups.

IMPORTANCE: That mixotrophic flagellates are not randomly feeding in the main prokaryotic groups in field conditions is a pioneer finding in species-specific behavior that paves the way for future studies according to this new paradigm. The particular case that Archaea were preferentially affected in the situation studied shows that phagotrophic pressure cannot be disregarded when considering the distribution of this group in freshwater oligotrophic systems.

INTRODUCTION

Mixotrophic behavior, the combination of phototrophic and phagotrophic nutritional modes within a single cell, has been increasingly documented in aquatic systems (1, 2). Phagotrophy occurs in a variety of phytoplankton flagellates groups including Chrysophyceae, dinoflagellates, prymnesiophytes and cryptophytes, and comprising picoeukaryotes (3-5). Currently, there is no doubt on the ubiquity of mixotrophy and its significance in the functioning of planktonic systems. In oligotrophic conditions, phototrophic flagellates can account for up to 80 % of total bacterial grazing (4, 6, 7).

Predation by protists is among the primary mortality factors of prokaryotes in planktonic communities, and thus an important selective pressure. It becomes a structuring factor of the abundance, morphology, composition and activity of bacterial assemblages (8, 9). The impact of protist predation appears to be modulated by the characteristics of the system (e.g. productivity) and predator and prey traits (10). Over the last few decades, efforts have been made to understand the selective feeding behavior of protists. General selection mechanisms have been identified (11). However, the current view is still mainly based on laboratory data, using readily growing species (12). On the other hand, the limited number of field experiments use general grazer groups rather than evaluating predation at the species level (12-14). There is a need to evaluate prey selection in natural conditions comparing flagellate species to determine more specific interactions between microbial predators and preys and assessing the relevance of selective microbial predation in the microbial loop dynamics. The scarcity of studies are in great part due to the difficulties in i) prokaryote prey identification and ii) establishing the prey and predator links at

74 the highest possible taxonomic resolution. Prey identification can be addressed
75 by techniques based on DNA fingerprinting (15, 16), which allow following
76 taxonomic changes in prey assemblages (10, 17). Linking prey and predator
77 can be addressed using techniques based on fluorescence *in situ* hybridization
78 (FISH), which allow detecting targeted preys inside of protist food vacuoles (5,
79 14, 18, 19). These techniques have been mostly performed under experimental
80 conditions (18, 20), and results suggest a high selectivity in the feeding of
81 heterotrophic flagellates and some ciliate species investigated. In contrast, the
82 few *in situ* measurements showed not clear ingestion patterns for lake
83 flagellates, even random feeding was proposed for some bacterial groups (14).
84 All in all, there is still limited information on the selective feeding of phagotrophic
85 protists in natural conditions and, more remarkably, there is a huge gap of
86 knowledge about mixotrophic flagellates species.
87 The catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-
88 FISH) protocol (21) is particularly suited to assess the phagotrophy of
89 mixotrophic protist on prokaryotes since it maintains cell and plastid integrity
90 and allows the visualization of labeled preys against plastid autofluorescence.
91 CARD-FISH can be easily applied to natural assemblages to evaluate *in situ*
92 prey preference of mixotrophic species, and other prokaryotic grazers (14, 19),
93 using the appropriate bacterial and archaeal probes. In the present study, we
94 examine phagotrophic selectivity of three mixotrophic species in natural
95 conditions. We sampled a deep high-mountain lake in which we expected
96 mixotrophic activity to be enhanced by the ultraoligotrophic conditions. Samples
97 were obtained at different times of the day to take into account potential feeding
98 variation and to assess the mean behavior better. The phytoplankton species

were identified by the size and shape of the autofluorescent plastid based on a prior taxonomic knowledge of the assemblages, which is an advantage of investigating mixotrophic flagellates at the species level respect to the heterotrophic ones. The heterotrophic flagellates are usually grouped into operational functional groups (14, 20, 22). In our study, we used fluorescent probes for the domain Archaea and three groups of Eubacteria: β-Proteobacteria, Actinobacteria, and the Cytophaga-Flavobacteria group of Bacteroidetes. These groups dominate aquatic prokaryotes in the Pyrenean lakes and account for more of the 85% of clades present in the lake studied (23).

MATERIAL AND METHODS

Sampling site:

Lake Redon is an ultraoligotrophic high mountain lake located at 2,240 m above sea level in the Central Pyrenees. It has a surface area of 24 ha and maximum and mean depth of 73 and 32 m, respectively. The lake is dimictic, well oxygenated throughout the water column and is usually covered by ice and snow during six months a year (24). The productivity patterns and seasonal changes in the water column are typical for high mountain lakes (25, 26). This lake has been widely studied in the last 30 years, a general description of its physical, chemical and temporal changes can be found in Catalan (27, 28) and a detailed description of the microbial plankton composition in Felip et al. (29).

Sampling:

On 9 August 2004, during the summer stratification period, integrated samples (0 to 60 m) of the water column were taken at the deepest point of the lake at

124 midnight (0 a.m.), dawn (7 a.m.), morning (11 a.m.) and afternoon (3 p.m.). The
125 four sampling times aimed to an estimation of the consistency of potential
126 selective behaviors and roughly approximate the time scale of digestion, in case
127 the number of ingested preys markedly fluctuated. The goal was not to
128 investigate daily patterns with only one sampling day. The water samples were
129 screened through a 40 µm mesh net to remove large zooplankton and
130 subsequently divided into two subsamples. One of the subsamples was fixed
131 with 0.5% (vol/vol) alkaline Lugol's solution followed by 2% buffered (pH 7) 0.2-
132 µm-pore-size-filtered formaldehyde, and several drops of 3% sodium thiosulfate
133 to decolor Lugol´s fixation, following Medina-Sánchez et al. (21). After 1 h of
134 fixation at room temperature, 24 aliquots of each sample (12 of 90 ml for
135 protists and 12 of 10 ml for prokaryotes) were gently filtered (<100 mm Hg) onto
136 respective 25 mm diameter polycarbonate Millipore membrane filters (type
137 RTTP, 1 µm pore size for protists; type GTTP, 0.2 µm pore size for
138 prokaryotes). Filters were then rinsed twice with Milli-Q water, allowed to air dry,
139 and stored at -20°C until further processing. The second subsample was
140 preserved with 0.5% (vol/vol) alkaline Lugol's solution to identify algal species
141 (30).
142 *Phagotrophic activity*:
143 Three different HRP-labeled probes for the main Bacteria groups found in the
144 plankton of the Pyrenean lakes (23, 31, 32); and one for the domain Archaea
145 were used to hybridize the filters (Table 1). The probe EUB338 that targets
146 most Bacteria was used only to evaluate the hybridization yield of the other
147 probes. Two replicate filters were processed for each probe and sample.
148 Alexa488-labeled tyramide was used for signal amplification and filters were

149 counterstained with DAPI (4´,6´-diamidino-2-phenylindole; 1µg ml^{-1} final
150 concentration), and mounted on glass slides by using Citifluor (Citifluor Ltd.,
151 UK). Slides were stored at -20 °C in the dark until subsequent counting.
152 Slides were examined at x1000 magnifications for bacterial groups, Archaea
153 and smaller protists (<10 µm) and at x400 magnifications for larger protists (>10
154 µm) under a Zeiss Axio Imager epifluorescence microscope. The microscope
155 was equipped with an X-Cite 120 light, appropriate filter sets for DAPI (Zeiss
156 filter set 01, BP365/12 FT396 LP397) and Alexa-Fluor488 (Zeiss filter sets 09,
157 BP450-490 FT510 LP515, o 24 DBP485/20 DFT500/600 BP515-540 + LP610),
158 a coupled camera Axio Cm Mrm and a PC-based image analysis software Axio
159 Vision 4.8. For prokaryotes, a minimum of 500 cells was counted to establish
160 the total abundance (DAPI counts) and the number of hybridized cells for each
161 specific probe to estimate their abundance and percentage of hybridization.
162 Also, cell area and perimeter were measured by image analysis software in at
163 least 100 cells of each filter. A characteristic length was calculated using the
164 square root of the cell area.
165 Protists were identified based on their plastid size and shape observed by the
166 chlorophyll auto-fluorescence under blue excitation. The identification was
167 facilitated by parallel observation of the Lugol's fixed subsample under an
168 inverted light microscope (x600 and x1000). A minimum of 100 individuals of
169 the most abundant protist species was assessed, for each, the number of
170 hybridized prokaryotic cells inside was counted.
171 *Data analysis:*
172 Protist species prey selectivity was analyzed according to Chesson's α-index
173 (33):

$$\alpha_i = \frac{\frac{r_i}{p_i}}{\sum_{i=1}^{n} \frac{r_i}{p_i}}$$

where r_i and p_i are the mean percentages of each prokaryotic group (i) inside the protist assessed and in the lake water assemblage, respectively; n is the number of prokaryotic groups distinguished (four in our case). When $\alpha_i = 1/n$ unselective feeding occurs; when $\alpha_i < 1/n$ negative selection occurs, less of the prokaryotic group *i* occurs inside the protist than expected from random feeding; when $\alpha_i > 1/n$, positive selection occurs, more individuals of the prokaryotic group *i* were ingested by the protist than expected from random feeding. One-way and two-way ANOVAs, were performed to compare prokaryotic assemblages among samplings. To test changes on protist ingestion, Kruskal-Wallis non-parametric tests were used (STATISTICA 7.1; StatSoft, Inc.).

RESULTS

Total DAPI counts ranged from 2.8 x 10^5 to 6.3 x 10^5 cell ml^{-1}, and between the 27-37 % of such total counts were hybridized with the four probes used in this study (Fig 1a). No significant differences were found in the total amount of hybridized cells between samplings (Fig 1a, ANOVA $p>0.05$). The three groups of Bacteria considered, all together, accounted for 99-104% of cells hybridized by the probe EUB338, which is generic for the domain Bacteria, indicating that not any major Bacteria group were missing in our study.

From the four tested prokaryotic groups, β-Proteobacteria was often the most abundant, ranging from 12.4 ± 3.3 to 20.2 ± 8.8 % of total DAPI counts. Only at the last sampling time the group Actinobacteria showed higher abundance

196 reaching 15.6 ± 2.7 % of total DAPI counts (Fig 1b). Cytophaga-Flavobacteria of
197 Bacteroidetes proportions were slightly variable representing between 4.0 ± 2
198 and 7.2 ± 4.5 % of total DAPI counts, whereas Archaea ranged between 2.9 ±
199 0.8 and 6 ± 0.4 %.
200 Cell length frequency distributions, evidenced that Actinobacteria were always
201 smaller and with more uniform sizes than the others prokaryotic groups (Fig 2).
202 A temporal tendency can be observed in Archaea and Cytophaga-Flavobacteria
203 distributions: cells were larger at night and progressively declined during the
204 day.
205 Three mixotrophic species, two Cryptophyta: *Rhodomonas* sp. and
206 *Cryptomonas ovata*; and the Chrysophyceae *Dinobryon cylindricum*, were
207 selected to assess the phagotrophic activity and describe their feeding
208 behavior. These species were easy to recognize by fluorescence microscopy
209 and large enough (Table 2) to accurately quantify the hybridized prokaryote
210 cells inside them (Fig 3). Their abundance and the percentage of feeding cells
211 observed for each probe were included in Table 2. In some cases, cells actively
212 feeding accounted for more than half of the population (e.g., *Rhodomonas* sp.
213 feeding on Archaea or *C.ovata* on β-Proteobacteria), whereas in others most
214 individuals showed no prey inside (e.g. *Rhodomonas* sp. feeding on
215 Actinobacteria or Cytophaga-Flavobacteria). The average food vacuole content
216 of the three flagellate species changed among the different surveys performed
217 (Fig 4). *C. ovata* and *D. cylindricum* presented a similar feeding variation.
218 *Rhodomonas* vacuole content had an elevated variation with significant
219 differences among surveys (Kruskal-Wallis $P<0.05$, Fig 4).

220 The main ingested prokaryotic group differed between the three protists (Fig 4)
221 and the Chessons' selectivity index (α_i) clearly indicates that the three species
222 were not randomly feeding (Fig. 5). Archaea were usually positively selected by
223 the three protists. The preference was extreme in the case of *Rhodomonas sp.*,
224 which hardly grazed on any other prokaryotic group. In contrast, Actinobacteria
225 were always apparently avoided, only once *D. cylindricum* selected this group
226 positively. Finally, β-Proteobacteria and Cytophaga-Flavobacteria were only
227 strongly avoided by *Rhodomonas* and randomly grazed or slightly avoided by *C.*
228 *ovata* and *D. cylindricum*.
229
230 **DISCUSSION**
231 *CARD-FISH performance*
232 Technical capacity for distinguishing the food vacuole content of protists is
233 crucial to study their selective feeding. The CARD-FISH protocol (21) offers this
234 possibility. Critical steps in the procedure are the probe selection and the
235 percentage of hybridized cells achieved. The probes used in the present study
236 included the most abundant prokaryotic groups described in the plankton of the
237 Pyrenean high-mountain lakes (31). Previous studies in Lake Redon and on 17
238 lakes more in the same area of the Pyrenees have shown that β-Proteobacteria,
239 Bacteroidetes, and Actinobacteria always account for more than 75% of the
240 total Bacteria clades, more than 85% in the Lake Redon (see Fig S2 from (23)).
241 The remaining clades proportion divided into 7 secondary groups and other
242 minority ones. Our results show that no primary Bacteria group was missing in
243 our assessment. The three groups of Bacteria considered accounted for 99-
244 104% of cells hybridized by the probe EUB338. There is no reason to assume

245 that we were missing any large part of the prokaryotic community with our
246 probes, as long as we considered the three most important groups of Bacteria
247 in addition to Archaea. The same probes were used to characterize Bacteria
248 composition in alpine lakes plankton of other ranges (34, 35).
249 The relative low hybridization compared with DAPI counts (27-37%) is due to
250 the efficiency of the procedure. Low efficiency may be due to detection technical
251 issues and to the cell physiological states (36). Cells with low rRNA content are
252 common in samples from oligotrophic environments due to cell low metabolic
253 activity or dormant state. Different assessments provide a small percentage of
254 active cells in the plankton of the Pyrenean lakes, respiring prokaryotes ranged
255 between 2–7 %, whereas viable cells were seldom higher than 50 % in deep
256 Pyrenean lakes (37). Even though CARD-FISH appears suitable for the
257 detection of prokaryotes with small quantities of rRNA molecules (38), if cells
258 are scarcely active or small, the percentage of hybridized cells compared to
259 DAPI counts decline (36, 39). Thereby, the ratio between CARD-FISH, and
260 DAPI counts is often low and highly variable in lake plankton samples (19, 35,
261 40) and are not unexpected to be particularly low in an ultraoligotrophic lake
262 such as Redon. In a recently phosphorous enrichment experiment performed in
263 this lake, the % hybridized cells (using the same four probes we applied here)
264 raised from 36% of DAPI counts in low P and lake conditions to near 100% in
265 the most productive mesocosm (Supplemental material). This is clear evidence
266 that the apparent low hybridizing efficiency we obtained is not a probe problem
267 but a constraint resulting from the little activity of many cells. In our study, the
268 percentage of hybridization did not significantly change over time (Fig 1) and

thus did not affect our objective to compare the food vacuole content against the plankton assemblage composition.

The ARCH915 probe has been widely used to detect archaeal cells. Occasionally, some biases of the probe hybridizing members of the phylum Bacteroidetes have been reported (41-43). This doubling hybridization can inflate the Archaea counts. It does not seem to occur in our case, for several reasons. No correlation was found between ARCH915 and CF319a counts neither in water samples nor in vacuoles. Indeed, contrasting extreme values between the two groups were found in some protist food vacuoles (Fig 4). Also, the Archaea proportions in the water samples of our study (3-6%) were similar to those in previous studies in Lake Redon and other Pyrenean lakes using 16S rRNA gene tag sequencing: 0-8 %,(44); 0-6 %, (45).

Selective predation

The significance of predation on bacterial activity and community structure in natural aquatic systems has been demonstrated (10, 46-49). Consequently, the effort is currently placed on understanding the details and dynamics of the grazing by protists (11). From lab experiments, evidence exists that certain taxa feed selectively (50, 51). In natural conditions little is known about flagellate grazing preferences, and whether they tend to be specialists or generalists predators. In a recent microcosm study, Glücksman et al. (17) showed that closely related and morphologically similar flagellated species can have different impacts on natural bacterial communities. Beyond phylogeny, some general protist traits, namely, cell size and morphological plasticity, explained variation in prey composition. Accordingly, our results show that two highly

294 related species (i.e., the two cryptophytes), inhabiting the same system and
295 feeding in the same prokaryotic assemblage, markedly differ in their selectivity
296 (Fig 4 and 5).
297 The species studied are closer to specialist predators than generalist (Fig 5).
298 *Rhodomonas* sp., the smallest species analyzed, showed a more differentiated
299 feeding pattern (Fig 4). Unfortunately, the comparison of only three species
300 does not have the statistical power to evaluate the relationship between traits
301 and selectivity. In any case, we show that selectivity may be extreme and thus
302 with substantial implications for the dynamics of the prokaryotic assemblages.
303 The three protist species analyzed were the most abundant mixotrophs in the
304 phytoplankton community at the time of the study (Table 2). The selectivity, at
305 least, on high taxonomic levels shown by our results pave the way to studies
306 focusing on finer prokaryotic taxonomy, using more specific probes, and
307 evaluating whether grazing by protist constitutes a differential selective pressure
308 within each of the large prokaryotic groups with ecological and evolutionary
309 implications.
310
311 *Cell size and grazing pressure*
312 Protist prey selection may occur at various feeding steps, namely capture, prey
313 processing, ingestion and digestion (11, 52). Prey size is a trait easy to
314 measure. There is evidence indicating that flagellates tend to graze on a limited
315 size range of prokaryotic cells, thus removing medium-sized cells and shifting
316 the size distribution of the preys towards larger and smaller cells (53-55). In our
317 case, the positively selected Archaea showed similar average cell size than β-
318 Proteobacteria and Cytophaga-Flavobacteria, although large cells (i.e., quartile

75% in Fig 2) were slightly smaller in Archaea than in the other two groups. Very likely, other factors than cell size must explain the strong preference for Archaea. During the sampling day, the cell length declined with time in both Archaea and Cytophaga-Flavobacteria, which may reflect the dynamic effect of grazing throughout the day (Fig 2), yet other factors may influence such as cell division. In all the samples, Actinobacteria were markedly smaller than the other prokaryotic groups (Fig 2) and were less affected by protist grazing (Fig 5). This observation agrees with previous studies that indicate lower grazing pressure on Actinobacteria by heterotrophic nanoflagellates (18, 20) and by the mixotrophic Chrysophyceae *Ochromonas* sp. (56). Indeed, if small size (i.e., <0.5 μm) constitutes a refuge against grazing, Actinobacteria may be negatively selected only in appearance. Either because the limiting size might be an evolved defense mechanism and thus cells tend to be always smaller than in other groups or, on the contrary, because preference by grazers is so high that the group is permanently confined to the small size refuge. It has been suggested that cell miniaturization alone might not be sufficient to explain grazing avoidance (57) and that other resistance mechanisms, such as wall structures present in Gram-positive Actinobacteria, could be involved in determining a limited edibility (58). Research on prokaryotic grazing is still in its infancy, but our results indicate that random grazing cannot be the paradigm.

Preference for Archaea

In the last decade, several studies have shown the broad distribution and abundance of Archaea in aquatic ecosystems (59-61), and their potential relevance in the sulfur, nitrogen and carbon cycles (62-65). However, their

specific ecological interactions within the microbial communities remain largely unknown. There was no evidence that Archaea behaved fundamentally different than Bacteria on predatory interactions (66). Our results indicate that this may not always be the case. The three mixotrophic flagellate species studied here selected Archaea positively. The amount of such prokaryotic group inside the protist food vacuoles was always higher than the expected by random feeding (Fig 5). In *Rhodomonas* sp., the preference for Archaea was extreme. It could be thought that the Archaea dominance in the vacuoles could respond to a stable symbiosis. However, this appears unlikely. The average Archaea densities within the vacuoles and the number of *Rhodomonas* cells without any Archaea inside markedly changed between the four sampling times (Fig 4, table 2). This pattern favors the view of selective predation and relatively quick digestion rather than a stable symbiosis.

The differences among the three protist species suggest that the strength of grazing on Archaea can highly depend on the protist present. Nevertheless, the three species show a preference for Archaea, which opens the question about how important is grazing in determining the low proportion of this group in many planktonic microbial assemblages.

The reason for high positive selectivity on Archaea remains highly speculative. It could be related to a poor development of resistance mechanisms in this domain. Or, it could be related to unknown stoichiometric features of the Archaea, it has been shown that bacteria with low C:P ratio may be ingested at higher rates by flagellates (67, 68). Or, it could be the chemical composition of some particular group of the Archaea within them, as they differ in the cell wall structure. Or even, it could be some chemical cues released to water than could

enhance encounter rates between prey and predator (69). Archaea studies in Lake Redon indicated that most of the 16S rRNA gene sequences matched Thaumarchaeota (close to 90%) and only a few Euryarchaeota were detected. MG 1.1 dominated most of the water column in summer. This clone has a >97% identity with *Nitrosoarchaeum limnia*, an ammonium oxidizing Archaea (44). However, as new primers are applied new groups of Euryarchaeota are found in the lake (70). In any case, the high positive selectivity on Archaea observed in the mixotrophs of Lake Redon pave the way towards new ecological and physiological studies on Archaea. Protist grazing is a factor that cannot be ignored for understanding the Archaea's distribution and abundance in planktonic microbial assemblages.

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593 **FIGURE LEGENDS**

594 **Figure 1.** (a) Prokaryotic cell abundance (average ± SD of total
595 DAPI counts) and the total amount of hybridized cells by the four
596 probes used in this study (average ± SD, n=8), at the four sampling
597 times. (b) Prokaryotic assemblage composition as hybridized cell
598 abundance (average ± SD, n=2) of the four targeted groups at the
599 four sampling times.

600 **Figure 2.** Cell length cumulative distributions for the four targeted
601 prokaryotic groups at the four sampling times.

602 **Figure 3.** Epifluorescence microscope photographs of the three
603 mixotrophic flagellates species studied (a) *Rhodomonas* sp., (b)
604 *Cryptomonas ovata* and (c) *Dinobryon cylindricum*. CARD-FISH
605 staining with ARCH915 probe (a and c) and BET42a probe (b),
606 showing chloroplast autofluorescence (red), DAPI stained nucleus
607 (blue) and targeted prokaryotes (green). Dashed white lines draw
608 cell outlines and green line surround food vacuoles. Scale bar = 12
609 µm (a) and scale bar = 20 µm (b and c).

610 **Figure 4.** Food vacuole content, average ± SD (n=2) of hybridized
611 cells by the four probes, for the three mixotrophic flagellates at the
612 four sampling times.

613 **Figure 5.** Chesson's selectivity index for the three mixotrophic
614 species (plots distributed vertically) and for each of the targeted
615 prokaryotic group (plots distributed horizontally) at each sampling
616 times. The horizontal dashed lines indicate the value above which

positive selection on a particular prokaryotic group is assumed (0.25 = 1/n, being n the number of prokaryotic groups).

Table 1. Oligonucleotide probes used in this study

Probe name	Specificity	Sequence (5´- 3´) of probe	% FA*	References
EUB338	*Bacteria*	GCTGCCTCCCGTAGGAGT	55	**(71)**
ARCH915	*Archaea*	GTGCTCCCCCGCCAATTCCT	40	**(21)**
CF319a	*Cytophaga-Flavobacteria of Bacteroidetes*	TGGTCCGTGTCTCAGTAC	55	**(72)**
HGC69a	*Actinobacteria*	TATAGTTACCACCGCCGT	30	**(73)**
BET42a	*ß-Proteobacteria*	GCCTTCCCACTTCGTTT	55	**(73)**

* Formamide (FA) concentration in the hybridization buffer.

633 **Table 2.** Protist average data: mean size, mean abundance and percentage of active feeding cells for each probe analyzed

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SPECIES	SIZE	ABUNDANCE	PERCENTAGE OF ACTIVE FEEDING CELLS			
	(μm)	(cells ml $^{-1}$)	β-Proteobacteria	Actinobacteria	Cytophaga-Flavobacteria	Archaea
***Rhodomonas* sp.**	11 x 6	28 ± 8	9.4 ± 12.3	3.3 ± 2.6	2.9 ± 3.2	52.5 ± 47.7
Cryptomonas ovata	30 x 14	16 ± 6	50 ± 19.4	19.2 ± 14.7	44.1 ± 9.9	36.6 ± 16
Dinobryon cylindricum	14 x 6	139 ± 47	28.5 ± 9	7.8 ± 7.3	11.5 ± 5.4	13.3 ± 10.2

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