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SHORT COMMUNICATION

Dinobryon faculiferum (Chrysophyta) in coastal Mediterranean seawater: presence and grazing impact on bacteria

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We report the presence and abundance of *Dinobryon faculiferum* (Chrysophyta) in NW Mediterranean coastal waters, as well as the first estimations of the grazing impact of this mixotrophic species on bacteria. In June 2006, *D. faculiferum* was detected at an abundance of 37 cell mL^{-1} (1.2% of total protistan abundance). By grazing with cell-specific rates of 7.8 bacteria cell h^{-1}, this single species explained 4.5% of the total bacterial grazing losses.

KEYWORDS: mixotrophy; *Dinobryon*; bacterivory

Dinobryon are planktonic pigmented chrysophytes usually forming large colonies, and very abundant in oligotrophic lakes (Kamjunke *et al.*, 2007). As most chrysophytes, several species belonging to this genus have been shown to be mixotrophic (Sanders *et al.*, 1989; McKenzie *et al.*, 1995; Kamjunke *et al.*, 2007), being capable of both photosynthesis and phagotrophy. Moreover, in some lakes and during certain periods, *Dinobryon* may reach ingestion rates as high as those of purely heterotrophic flagellates (HF) (Bird and Kalff, 1986; Domaizon *et al.*, 2003). The occurrence of this genus in lakes has typically been linked to relatively low-nutrient waters. Accordingly, this genus was recently observed reaching high density during the process of re-oligotrophication of Lake Constance (Kamjunke *et al.*, 2007). The re-appearance of *Dinobryon* has been associated to the decrease in phosphorus concentration that took place during the last decade in this lake.

Although *Dinobryon* species are more common in freshwater than in marine environments, there are at least three species inhabiting seawaters: *Dinobryon balticum* (syn. *D. pellucidum*, *Dinodrendron balticum*), *D. faculiferum* (syn. *D. petiolatum*) and *D. coalescens*. Even though the phagotrophic capability of this genus is well documented in lakes, there is no quantitative information for marine species except for *D. balticum* (McKenzie *et al.*, 1995) and *Dinobryon* spp. (Havskum and Riemann, 1996) in the low-salinity Baltic Sea.

Among the different marine species of *Dinobryon*, *D. balticum* has always been found in cold habitats like the Baltic (Hasle and Heimdal, 1992), Arctic (Kononen *et al.*, 1992) and North Atlantic (Canada) seas (Trottet

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Table I: In situ *conditions in Blanes Bay on 13 June 2006*

Maximum depth (m)	20	Heterotrophic bacteria (cell mL^{-1})	1.2×10^6
Temperature (°C)	22.4	Picocyanobacteria (cell mL^{-1})	2.6×10^4
Secchi disc (m)	12	PF total (cell mL^{-1})[a]	4021
Salinity	36.3	PF ≤ 5 μm (cell mL^{-1})	3427
Kd (extinction coefficient)	0.18	PF > 5 μm (cell mL^{-1})[a]	594
μM $PO_4^{=}$	0.14	*Dinobryon faculiferum* (cell mL^{-1})	37
μM NH_4^{+}	1.75	HF total (cell mL^{-1})	1053
μM $NO_2^{=}$	0.31	HF ≤ 5 μm (cell mL^{-1})	973
μM $NO_3^{=}$	0.51	HF > 5 μm (cell mL^{-1})	80
Chlorophyll *a* total (μg L^{-1})	0.43	Specific primary production (mgC mg Chl^{-1} h^{-1})	3.22
Chlorophyll *a* <3 μm (μg L^{-1})	0.18	Total primary production (mgC m^{-3} h^{-1})	1.38

PF, phototrophic flagellates; HF, heterotrophic flagellates.
[a]Includes *Dinobryon faculiferum*.

et al., 2007). In contrast, *D. coalescens* was reported only for the Mediterranean sea in the Gulf of Naples (D'Alcalá *et al.*, 2004; McDonald *et al.*, 2007), whereas *D. faculiferum* was found in the Arctic (Werner *et al.*, 2007), the Baltic sea (Suikkanen *et al.*, 2007) and in the Gulf of Naples (McDonald *et al.*, 2007) at abundances usually lower than 128 cell mL^{-1} (Gustavson *et al.*, 1999). Apparently, the geographic distribution of these three species seems to respond to a gradient of temperature.

Here, we report, to the best of our knowledge, the first estimations of the grazing impact of *D. faculiferum* on bacteria and the first record of *Dinobryon* in Blanes Bay, NW Mediterranean coastal waters. We place these values in the context of the rates of bacterivory that have been estimated for this genus in different environments.

Short-term grazing experiments were performed on 13 June 2006 with fluorescently labelled bacteria (FLBs) prepared with a culture of *Brevundimonas diminuta*. We followed the same methodology applied previously for an annual survey (Unrein *et al.*, 2007). Briefly, water samples were collected from the Microbial Observatory of Blanes Bay (NW Mediterranean 41°40′N, 2°48′E), 800 m offshore, transported to the laboratory in the dark and gently filtered through a 100-μm net mesh. Grazing experiments were performed in a culture chamber, in triplicate bottles, at 20°C and 200 μE m^{-2} s^{-1} of light intensity. FLBs were added to each bottle to reach about 25% of the natural bacterial concentration. Samples for FLB ingestion were taken at the beginning of the experiment and after 40 min of incubation (Unrein *et al.*, 2007). *In situ* conditions are presented in Table I.

The nanoplankton community on the date of sampling (13 June 2006) was mainly composed of heterotrophic (aplastidic) flagellates and phytoflagellates. The later group was dominated by haptophytes, dinoflagellates and cryptophytes, as far as could be inferred by epifluoresence microscopy. All of them were

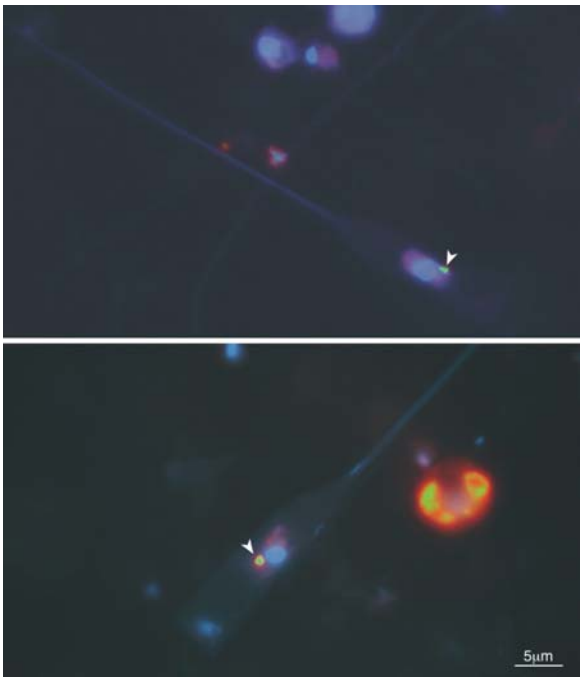

Fig. 1. Epifluorescence micrographs of *D. faculiferum* ingesting fluorescently labelled bacteria, FLB. The images are overlays of the same cell observed (i) under UV radiation showing the nucleus in blue after DAPI staining and (ii) the chloroplast in red and the FLBs in yellow–green (indicated with the arrows) under blue light excitation.

observed with ingested FLBs. Unexpectedly, the chrysophyte *D. faculiferum* was also observed in the samples, feeding on bacteria (Fig. 1). In spite of the fact that the phytoplankton from Blanes Bay has been intensively studied for decades (cf. Margalef, 1945), *Dinobryon* was never observed at this site before. Nevertheless, this species had been previously reported in the Mediterranean Sea in the Gulf of Naples (McDonald *et al.*, 2007) and seen sporadically off the coast of Barcelona, 70 km South from Blanes it where never reached more than 12 cell mL^{-1} (L. Arin *et al.*, personal communication).

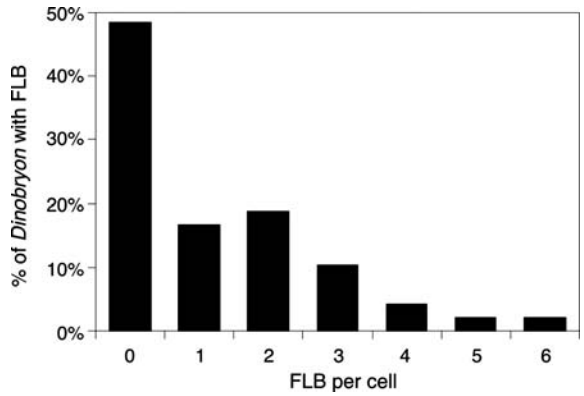

Fig. 2. Data distribution of uptake rates (FLB cell^{-1}) after 40 min of incubation. A total of 48 *Dinobryon* cells were inspected in three replicates.

In contrast to the rest of *Dinobryon* species that form large colonies, *D. faculiferum* is one of the few solitary planktonic species. At the time of sampling, the lorica ranged between 4.3 and 5.4 μm in width, and 60–80 μm in length, with extremely long basal spines of about 2–3 times the length of the receptacle. The mean cellular width (± SD) was 2.8 ± 0.4 μm and average length was 8 ± 1.6 μm.

Even though *D. faculiferum* abundance was not very high in our samples (37 cell mL^{-1}), this alga showed very high grazing activity. At the end of the incubation (40 min), 54% of the *Dinobryon* cells contained FLBs, most of them with more than one FLB per cell (Fig. 2). Mean (SD) ingestion rate was 1.82 (0.67) FLB cell^{-1} h^{-1}. Our measured clearance rates (CRs) (5.4 nL cell^{-1} h^{-1}) reached higher values than those calculated for strictly HF in the same sample: 2.1 nL cell^{-1} h^{-1} for HF $<$ 5 μm and 3.6 nL cell^{-1} h^{-1} for HF $>$ 5 μm. At this site, we had previously calculated an annual average CR of 2.2 and 6.1 nL cell^{-1} h^{-1} for each of the above-mentioned HF size fractions, respectively (Unrein *et al.*, 2007).

CRs have previously been estimated only twice in marine *Dinobryon* species (Table II). Both estimates are substantially lower than the CR here reported; nevertheless, neither of them are for *D. faculiferum*. Our estimates of CR are in the middle range of the reported values when including the many estimates from freshwater environments (Table II). The highest CRs were recorded for *D. cylindricum* (up to 51 nL cell^{-1} h^{-1}) and *D. bavaricum* (up to 15 nL cell^{-1} h^{-1}), whereas *D. sociale* and *D. divergens* showed lower values. Unfortunately, many rates have been calculated for the genus *Dinobryon* spp. as a group, without discrimination by species (Bird and Kalff, 1987). CRs estimated on cultures usually gave lower figures than those assessed with natural samples, probably because prey abundance and nutrient concentrations in culture are higher that those found in natural environments. In these conditions, grazing rates are more meaningful and better compare with *in situ* estimates. In addition, most estimations were performed using latex beads as surrogate food particles, whereas here we used heat-killed bacteria (FLB). Bird and Kalff (Bird and Kalff, 1986) reported that *Dinobryon* spp. preferentially ingested radioactive-labelled bacteria over beads by a factor of 1.32. However, the preference of *Dinobryon* for different types of particles is contradictory, because Jones and Rees (Jones and Rees, 1994a) asserted that *D. divergens* did not discriminate between latex beads and FLBs.

A positive relationship between CR and light intensity was observed in *D. cylindricum* (Caron *et al.*, 1993), *D. divergens* and *D. sertularia* (Jones and Rees, 1994b). Moreover, the three species were unable to survive under complete darkness, suggesting that even having a high phagotrophic capability, these algae are obligate phototrophs that use their bacterivorous behaviour to acquire essential nutrients, especially phosphorus (Kamjunke *et al.*, 2007). A positive relationship with temperature (Bird and Kalff, 1987) was also reported for *D. sociale*, whereas it was demonstrated that under culture conditions *D. sertularia* had its maximum CR close to 20°C (Jones and Rees, 1994b). The preference for particles of intermediate size (ca. 0.5–0.9 μm, 0.065–0.110 μm^3) was also confirmed for many species of *Dinobryon* (Bird and Kalff, 1987; Jones and Rees, 1994a, b; Kamjunke *et al.*, 2007). The optimal conditions occurring in our experiment: high temperature, sufficient illumination, low nutrient concentration, intermediate prey size and use of FLBs instead of latex beads, may explain the high CR calculated here.

Specific grazing rates estimated for *D. faculiferum* (7.8 bacteria cell^{-1} h^{-1}) are also in the middle range of the reported values for *Dinobryon* spp. (Table II). In our samples, *D. faculiferum* represented only 1% of the total protistan abundance, but they accounted for 4.5% of the total bacterivory and 11% of the bacterivory exerted by the pigmented nanoflagellates. Thus, the relative contribution of this species to total bacterivory in this system was certainly very high, with specific values higher than those of the strictly HF (3.1 and 5.2 bacteria cell^{-1} h^{-1} for HF $<$ 5 μm and HF $>$ 5 μm, respectively). Given the great diversity of bacterivorous protists inhabiting seawater (Jürgens and Massana, 2008), it is remarkable that a single species explains a substantial fraction of the total bacterivory. The same trend is observed, in fact, when we include estimations performed on other *Dinobryon* species (Fig. 3). Therefore, thanks to their very high specific grazing rates, small increments in *Dinobryon* abundance

Table II: CRs of Dinobryon species in several environments

Environment	Trophy conditions	Taxon	Tracer (size)	Specific grazing rate (bacteria cell^{-1} h^{-1})	Clearence rate (nL cell^{-1} h^{-1})	Source
Marine						
Bay of Aarhus, Denmark	Mesotrophic	*Dinobryon* spp. (*D. balticum* and *D. faculiferum*)	FLBn	0.4	0.4	Havskum and Riemann (1996)
Conception Bay, Newfoundland	?	*Dinobryon balticum*	Beads (0.46 μm)	—	0.1–0.5	McKenzie *et al.* (1995)
Blanes Bay, Mediterranean Sea	Oligotrophic	*Dinobryon faculiferum*	FLBc (0.47 × 0.94 μm)	7.8	5.3	This study
Freshwater						
Sep Reservoir	Oligo-mesotrophic	*Dinobryon cylindricum*	Beads (0.5 μm)	62.3–137.6	28.4–51.6	Thouvenot *et al.* (1999)
Lake Pavin	Oligo-mesotrophic	*Dinobryon cylindricum*	Beads (0.5 μm)	2.4–35.3	0.7–26.9	Carrias *et al.* (1996)
Lake Oglethorpe	Eutrophic	*Dinobryon cylindricum*	Beads (0.57 μm)	6–12	1.9–6.2	Sanders *et al.* (1989); Bennett *et al.* (1990)
		Dinobryon bavaricum		8–38	2.8–15.2	
Lake Memphremagog	Mesotrophic	*Dinobryon* spp. (mainly *D. bavaricum*)	Beads (0.6 μm)	29.2	5.8	Bird and Kalff (1986)
Lake Memphremagog	Mesotrophic	*Dinobryon* spp. (mainly *D. bavaricum*)	Beads (0.6 μm)	—	8.6–9	Bird and Kalff (1987)
Lake Cromwell	Mesotrophic	*Dinobryon* spp.		36	13.6	
Lake Magog	Mesotrophic	*Dinobryon* spp.		—	13.1–13.7	
Lake Orford	Oligotrophic	*Dinobryon* spp.		—	7.6–7.7	
Lake Bowker	Oligotrophic	*Dinobryon* spp.		—	4.7–9.5	
Lake Croche	Oligotrophic	*Dinobryon* spp.		—	3.8–3.9	
Lake Gilbert	Oligo-Mesotrophic	*Dinobryon* spp. (mainly *D. sociale*)		6–59	0.6–9.5	
Lake Gilbert	Oligo-Mesotrophic	*Dinobryon* spp. (mainly *D. sertularia*)	Beads (0.6 μm)	63.2	2.9	Bird and Kalff (1989)
Lake Annecy	Oligotrophic	*Dinobryon* spp.	Beads (0.5 μm)	6–43.2	3.5–12	Domaizon *et al.* (2003)
Lake Ossian Sarsfjella	Oligotrophic	*Dinobryon* spp.	FLBn	0.3–4.9	0.8–4.2	Laybourn-Parry and Marshall (2003)
Lake Tvillingvatna	Oligotrophic	*Dinobryon* spp.	FLBn	0.8–1	1.8–4.4	
Artificial pond	Eutrophic	*Dinobryon* spp.	FLBc[a]	0–1.3	0–0.54	Hitchman and Jones (2000)
Lake Constance	Mesotrophic	*Dinobryon sociale*	Beads (0.49 μm)	0.5–5.8	0.2–1.4	Kamjunke *et al.* (2007)
		Dinobryon divergens		2–13	0.7–3.2	
Lake Skärlen	Oligrotrophic	*Dinobryon* spp. (*D. crenulatum* and *D. divergens*)	Beads (1 μm)	0.04–0.4	0.1–0.3	Pålsson and Granéli (2003)
Lago Paione Superiore	Oligotrophic	*Dinobryon sertularia*	FLBn (0.047 μm^3)	1.2–11.2	1.3–15.9	Callieri *et al.* (2006)
Cultures						
Culture (freshwater)	—	*Dinobryon cylindricum*	FLBn	0.6–11.2	0.02–0.7	Caron *et al.* (1993)
Culture (freshwater)	—	*Dinobryon sertularia*	Beads (0.74 μm)	—	0.7–2.7	Jones and Rees (1994a)
		Dinobryon divergens	Beads (0.49 μm)	—	1.9–2.7	
Culture (freshwater)	—	*Dinobryon divergens*	Beads (0.49–0.92 μm)	—	0–10.9	Jones and Rees (1994b)

FLBn, fluorescently labelled bacteria prepared with natural bacteria; FLBc, FLB prepared with a bacterial culture.
[a]Strain isolated from the lake.

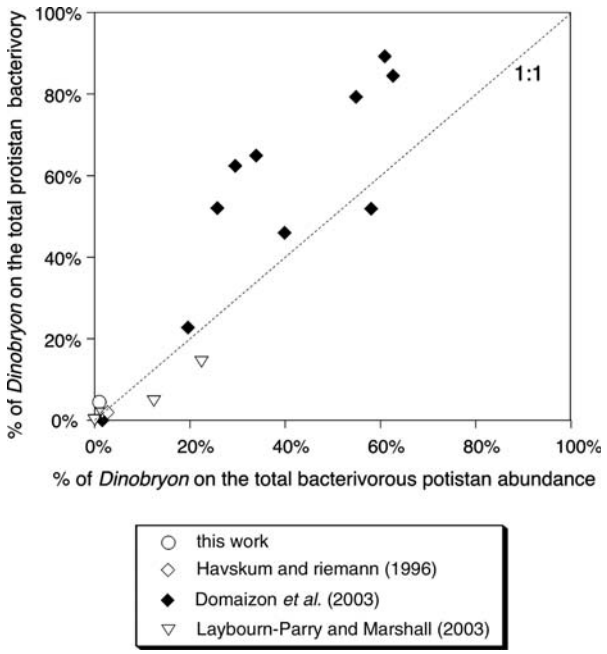

Fig. 3. Relative contribution of *Dinobryon* spp. to total bacterivory exerted by protists (heterotrophic and mixotrophic flagellates, and ciliates), related to the total bacterivorous protistan abundance in the database of Table II.

may result in a significant impact on the bacterial community, as it has already been observed in some lakes (Bird and Kalff, 1986; Thouvenot *et al.*, 1999).

The presence of *Dinobryon* has been associated in freshwaters to the decrease in phosphorus concentration (Kamjunke *et al.*, 2007). Even though *Dinobryon* is not exclusive from freshwater systems, its presence in marine samples is not very frequent. The fact that this species had not been detected before in the Blanes Bay might be related to the putative current oligotrofication of this area (Sarmento *et al.*, submitted for publication). Even though, this is yet very speculative, if this process involves species substitution in the plankton, and the newly arriving species have large grazing rates, fluxes of C between the components of the microbial food web might be altered in ways we cannot easily forecast.

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