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Mixotrophic ciliates in an Andean lake: dependence on light and prey of an *Ophrydium naumanni* population

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SUMMARY

1. Planktonic ciliates were examined during a spring–summer period (November 1998–April 1999) in the ultraoligotrophic Lake Moreno Oeste (41°5′ S and 71°33′ W, 758 m a.s.l), which belongs to the Nahuel Huapi System (Patagonia, Argentina). The lake is deep ($Z_{max} = 90$ m) and warm monomictic.
2. Sampling was performed at a mid-lake station, where vertical profiles of temperature and light were measured *in situ*, and samples for bacteria and ciliates enumeration were taken throughout the water column.
3. The peritrich *Ophrydium naumanni*, a freshwater pelagic ciliate with endosymbiotic *Chlorella*, was the dominant ciliate in the lake.
4. *Ophrydium naumanni* and autotrophic picoplankton exhibit a clear coincidence in their vertical distribution ($P < 0.05$), preferring levels at or near the 1% of surface photosynthetically active radiation (PAR) irradiance. Both may have the same light requirements, or the coincidence may reflect a trophic relationship.
5. Dependence on light and prey by *O. naumanni* were studied using field experiments, in which we analysed ciliate grazing on bacteria, and in laboratory experiments, in which we compared particle uptake under dark and light conditions.
6. *Ophrydium naumanni* was able to ingest particles [latex microspheres and fluorescently labelled bacteria (FLB)] in field and laboratory experiment, indicating that it has the potential to affect bacteria population of Lake Moreno Oeste.
7. Ciliate particle ingestion was observed to be dependent on light availability because under dark conditions, the ingestion was lowered ($P < 0.05$).

Keywords: light availability, mixotrophy, *Ophrydium naumanni*, particle uptake, planktonic ciliates, ultraoligotrophic lake

Introduction

Ciliates are acknowledged to be an important component in pelagic food webs (Porter *et al*., 1985; Macek *et al*., 1996; Stabell, 1996). Pelagic ciliate communities are composed of many of species ranging in volume over three orders of magnitude and with different feeding strategies. They can eat bacteria, heterotrophic flagellates and a wide range of phytoplankton (Sherr & Sherr, 1987; Sanders *et al*., 1989; Weisse, 1991; Simek *et al*., 1995; Jürgens & Simek, 2000, Kisand & Zingel, 2000; Simek *et al*., 2000). In addition, they may have a mixed nutrition, termed mixotrophy, which combines autotrophic and heterotrophic nutrition in a single individual (Jones, 1994, 2000). The photosynthetic capability can be acquired by sequestration of chloroplasts from ingested prey or by possession of photosynthetic cells as endosymbionts (Reisser *et al*., 1985; Stoecker & Silver, 1987; Perris, Laybourn-Parry

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& Jones, 1994). Mixotrophy is far more common than once thought (Jones, 1997) and the evolutionary strategy seems to confer important ecological advantages, as a large number of planktonic groups have independently evolved some form of mixotrophy (Jones, 1994).

Ophrydium naumanni Pejler is a freshwater, pelagic ciliate with endosymbiotic *Chlorella*. This ciliate species is dominant in transparent oligotrophic lakes, and solitary individuals and small colonies up to 20 individuals have been reported in Andean lakes from Argentina and Chile (Modenutti, 1988; Wölfl, 1995, Modenutti, 1997; Queimaliños, Modenutti & Balseiro, 1999; Modenutti, Balseiro & Queimaliños, 2000). Recently, the vertical distribution of *O. naumanni* was studied in detail and a deep chlorophyll maximum caused by this ciliate was described (Queimaliños *et al*., 1999). Laboratory experiments revealed that *O. naumanni* has a strong dependence on light, since negative growth rates were obtained under continuously dark conditions (Queimaliños *et al*., 1999). In the present study, we explore the balance between phototrophy and phagotrophy in this species. In a field study, we tested the dependence on light and prey of *O. naumanni*, as well as grazing by the ciliate on bacteria. We also compared particle uptake of the ciliate under dark and light conditions in laboratory experiments.

Methods

Study area and sampling

We conducted studies in Lake Moreno Oeste (41°5′ S and 71°33′ W, 758 m a.s.l), which belongs to the Nahuel Huapi System (Patagonia, Argentina). Lake surface area is 6 km^2 and maximum depth is 90 m. The thermal regime is warm monomictic, remaining stratified from late November until April (spring–summer months) (Queimaliños *et al*., 1999; Modenutti *et al*., 2000). The lake is ultraoligotrophic, with epilimnetic Chlorophyll-*a* concentrations less than 1 μg L^{-1}, and total phosphorus between 2 and 4 μg L^{-1}, and with high transparency (Secchi depth: 20 m) (Queimaliños *et al*., 1999; Modenutti *et al*., 2000).

Water samples were obtained from a central sampling point located at the deepest part of Lake Moreno Oeste; samples were collected from 0 to 52 m at 4 m intervals. Samples were obtained with a 12-L Schindler-Patalas trap, and subdivided in different sampling bottles for different purposes. All samplings were carried out at mid-day, 1 h before astronomic noon. Temperature and light [photosynthetically active radiation (PAR), 400–700 nm] profiles were measured with a PUV 500B submersible radiometer (Biospherical Instruments Inc., San Diego, CA, USA). We collected water and *O. naumanni* for experimentation on several occasions during southern spring and early summer at the same sampling site.

Bacterial abundance and fluorescently labelled bacteria (FLB) preparation

Samples for total bacteria enumeration were fixed with formaldehyde (final concentration 2% v/v) and stained with fluorochrome 4′, 6-diamidino-2-phenylindole (DAPI; final concentration 0.2% w/v) according to Porter & Feig (1980). Samples for autotrophic picoplankton enumeration were fixed with formaldehyde-cacodylate. Samples were stored in darkness and refrigerated, and quantified within 2 weeks of sampling. Counting was performed on black polycarbonate filters (Poretics, 0.2 μm pore size) at 1000× magnification in an Olympus B×50 epifluorescence microscope using UV light (U-MWU filter) for total bacteria, and blue light (U-MWB filter) for autotrophic picoplankton. A minimum of 1000 bacteria per sample was counted and processed with an image analysis system (Image ProPlus; Media Cybernetics, Silver Spring, MD, USA).

Fluorescently labelled bacteria were prepared following Simek & Straskrabova's (1992) procedures. Bacterioplankton from Lake Moreno Oeste was concentrated on 0.22 μm pore-size filters after prefiltration through 2 μm pore-size filters (Osmonics Inc., MN, USA). Bacterial cells were detached from the filter surface by sonication, and were heat-killed and stained with 5-[(4,6-dichlorotriazin-2-yl)amino] fluorescein according to Sherr, Sherr & Falon (1987). FLB were stored frozen in 1 mL aliquots. The resultant FLB were coccoid-shaped of 0.85 μm in diameter.

Ophrydium naumanni grazing and abundance

Ophrydium naumanni cells were quantified from 250 mL samples fixed with Lugol solution. *Ophydrium* cells were enumerated with an inverted microscope using 50 mL Utermöhl chambers and quantification

was carried out by scanning the entire surface of the chamber at 200× magnification.

Two series of *in situ* grazing experiments were carried out on 28 November and 18 December with FLB and unflavoured fluorescent latex microspheres of 0.5 μm diameter (Polyscience, Inc., Worrington, PA, USA). Water samples (2 L) were collected at 25 m depth where *O. naumanni* showed higher abundance. Grazing experiments were performed in two replicates using 300 mL ground-stoppered flasks, in which 250 mL of the collected water were dispensed. After 20 min of acclimation, in two flasks FLB were added at a final concentration of 1.02×10^5 mL^{-1} (equivalent to 6% of total bacteria abundance). In another set of flasks, microspheres were added at a final concentration of 1.8×10^5 mL^{-1} (equivalent to 10% of total bacteria abundance). The flasks were incubated in the lake at 5 m depth. Subsamples of 40 mL were taken at 5, 10, 15, 20 and 40 min after FLB or microsphere addition. These samples were fixed with 0.5% of Lugol solution, followed by 2% formaldehyde and several drops of 3% sodium thiosulphate to clear the colour (Sherr & Sherr, 1993). Within 3 days after fixation, 40 mL were stained with DAPI and filtered through 1 μm black polycarbonate filters (Poretics Products, Livermore, CA, USA). The number of ingested prey (FLB or microspheres) was quantified with an epifluorescence microscope (Olympus B×50) and the same image analysis system.

Uptake rates (clearance rates) were calculated by linear regression of average number of FLB or microspheres per ciliate versus time (Sherr & Sherr, 1993). To estimate total *Ophrydium* grazing rate, average grazing rates were multiplied by total *in situ* abundances.

Laboratory experiments

A series of uptake experiments were carried out during the spring–summer season to assess particle uptake of the ciliate under dark and light conditions. Ciliates were sampled from the lake at 25 m depth and separated in the laboratory under a stereomicroscope (Wild M5APO, Wild Heerbrugg, Germany) with a high magnification objective (100×). Twenty solitary individuals were transferred to 10 mL test tubes with lake water filtered through GF/C filter. The water used in the experiments was freshly collected the same day of experimentation at 25 m depth.

Before starting the experiments, all vessels and test tubes were carefully cleaned and sterilised (121 °C, 1 atm, 20 min) and the ciliates were transferred to experimental vessels and counted with a sterilised pipette. The experiments consisted of incubation of the ciliates (20 ciliates per 10 mL) in Moreno Oeste filtered lake water with the addition of fluorescent latex microspheres of 0.5 μm diameter at a final concentration of 2.4×10^5 mL^{-1}. We used incubation times of 10, 20 and 30 min to allow ingested beads in the food vacuoles to be counted precisely. All incubations were run with four replicates. Experiments were conducted in a growth chamber at 12 °C and light intensity of 39.0 μE m^{-2} s^{-1}. These conditions resembled the summer epilimnion of the lake at 20 m depth (Queimaliños *et al*., 1999).

To test the dependence on light of *O. naumanni* grazing we ran two different treatments with four replicates each. The two treatments differed in the light condition 15 h prior to the experiments and during the experimentation. In one case, ciliates were maintained under full light, while the other was conducted under dark conditions (test tubes were wrapped with aluminium foil). The two treatments were run for 20 min. Time of addition of microspheres to the test tubes was taken as initial time. At the end of the feeding trial, the tubes were fixed as per the '*in situ*' experiments. The entire sample was filtered on black 1 μm polycarbonate filters and the number of ingested microspheres was quantified using an epifluorescence microscope (Olympus B×50).

Correlation analyses between vertical distribution of *O. naumanni* and picoplankton abundances were performed for each sampling date. Simple linear regression analyses between ingested particles and time were carried out. In order to compare particle uptake under dark and light conditions, *t*-tests were applied. In all the statistical analysis, normality and homoscedasticity were tested when required.

Results

Field abundance and vertical distribution

The lake was sampled during the period of direct stratification when it develops a marked thermocline around 30 m depth, and temperature ranges from 11 to 18 °C in the epilimnion and 7 °C in the hypolimnion. Dissolved oxygen concentration remained at

saturation levels throughout the water column and was represented by an orthograde curve, typical of an unproductive lake. The lake has high transparency and the diffuse extinction coefficient (Kd PAR) was low and fairly constant (Fig. 1a), varying between 0.121 and 0.154 m^{-1}.

During the sampling period, *O. naumanni* was recorded all through the water column, with densities between 1 and 6 cell mL^{-1} (Fig. 1a). *Ophrydium naumanni* was the dominant ciliate species over almost the whole period (Modenutti *et al.*, 2000), with an increase in cell abundance at or below 30 m depth in November, December and January (Fig. 1a). A higher abundance of *O. naumanni* was observed near the limit of the euphotic zone (Fig. 1a) (receiving 3.65–10 μE m^{-2} s^{-1} of PAR) and just below the upper limit of the metalimnion (temperatures from 11 to 7 °C).

During the study period, total bacteria abundances were fairly constant through the water column and values were below 2×10^6 cell mL^{-1}. Nevertheless, autotrophic picoplankton showed higher abundances around the 30 m depth, especially during the January–February samplings (Fig. 1b). The position in the water column of this fraction closely resembles that of *O. naumanni*. Moreover, correlation analyses showed positive significant results (Table 1) in all sampling dates except in autumn (April), when both populations decreased their abundances (Figs 1a, b).

Ophrydium naumanni clearance rates

In our *in situ* grazing experiments, *O. naumanni* was able to ingest the two types of prey offered (FLB and fluorescent microspheres in a concentration <10% of total bacteria abundance); *O. naumanni* was able to ingest prey <1 μm in diameter (0.5 μm microspheres and approximately 0.85 μm FLB). Ciliate ingestion of both prey types increased linearly with time up to 40 min incubation period (Figs 2a–c). However, FLB were cleared at a higher rate than microspheres (Fig. 2).

The uptake rate for FLB was 0.24 μL $ciliate^{-1}$ h^{-1} (November) and 0.60 μL $ciliate^{-1}$ h^{-1} (December). For microspheres, only a single rate of 0.19 μL $ciliate^{-1}$ h^{-1} was obtained because the experiment conducted during December failed. Thus, at natural *O. naumanni* densities (4 ciliate mL^{-1}), the population clearance rates were around 23–57 μL mL^{-1} day^{-1}, based on FLB consumption.

We also tested the consumption in 10, 20 and 30 min trials under laboratory conditions and observed that *Ophrydium* ingestion also increased linearly with time (Fig. 2d). There was always of

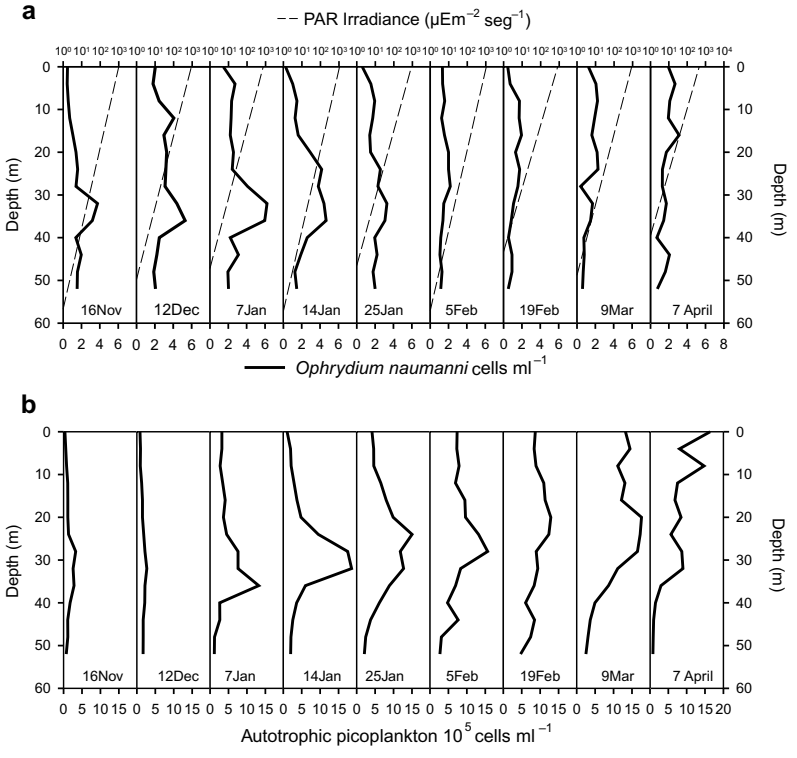

Fig. 1 Vertical distribution of (a) *Ophrydium naumanni* (solid line) and log-transformed light irradiance photosynthetically active radiation (PAR) (dashed line) and (b) autotrophic picoplankton during the study period in Lake Moreno Oeste.

Table 1 Results of the correlation analysis between *Ophrydium naumanni* and picocyanobacteria abundances throughout the water column for each sampling date

Sampling date	Correlation coefficient (r)	Probability level (P)	Significance
17 November	0.681	0.00724	$P < 0.05$
12 December	0.545	0.04360	$P < 0.05$
6 January	0.863	0.00006	$P < 0.05$
14 January	0.773	0.00117	$P < 0.05$
25 January	0.533	0.04930	$P < 0.05$
5 February	0.858	0.00008	$P < 0.05$
19 February	0.733	0.00282	$P < 0.05$
9 March	0.633	0.01510	$P < 0.05$
7 April	0.406	0.14900	NS

NS = non-significant.

fraction of ciliates that did not ingest particles during the experimental period (Fig. 3), a situation that was also observed in the field incubation experiments.

In the other laboratory experiments, *O. naumanni* cells were acclimatised to the presence or absence of light for 15 h prior to the feeding experiments. Under light conditions, the ciliate ingested significantly more particles than in darkness (t-test, $P < 0.05$). In the former, *O. naumanni* consumed between 23 and 27 microspheres per ciliate in 20 min, while this number decrease to three and five in the dark treatment (Fig. 4a). Similar results were obtained in the second experimental trial (December) (Fig. 4b). In both experiments the observed differences between treatments were significant (t-test, $P < 0.05$).

Discussion

Both ciliates and autotrophic picoplankton exhibit a clear coincidence in their vertical distributions, preferring levels at or near 1% of surface PAR irradiance. It seems possible that both fractions have the same light requirements. In other oligotrophic lakes, picocyanobacteria have been observed to be preadapted to 1% PAR irradiance (in the range 500–600 nm) by the presence of phycoerythrin (Gervais, Padisak & Koschel, 1997). In Lakes Riñihue and Pirehueico in Chile, Wölfl (1995) found a significant positive correlation between Secchi depth and *O. naumanni* abundance. In Andean lakes of Argentina, Modenutti (1997) indicated that *O. naumanni* was only present in deep, transparent lakes with low dissolved organic carbon concentration, and Queimaliños *et al*. (1999) showed that high ciliate densities caused a deep chlorophyll maximum situated at levels near the lower limit of the euphotic zone.

In the present study, on eight sampling occasions in spring and summer, we found a significant positive correlation between ciliate and autotrophic picoplankton vertical abundances. During examination of the symbiotic *Chlorella* of *O. naumanni* cells with epifluorescence microscopy we were able to distinguish autotrophic picoplankton inside food vacuoles (personal observation). Picoplankton has been reported as an important food item for ciliates both in marine and freshwater environments (Simek *et al*., 1995; Christaki *et al*., 1998). Therefore, the deep location of *O. naumanni* might be because of a trophic relationship. The results of our uptake experiments showed that *Ophrydium* effectively ingest particles <1 μm in diameter, within the size range of Lake Moreno autotrophic picoplankton.

As previously demonstrated (Queimaliños *et al*., 1999), *O. naumanni* needs light to survive; therefore, it is to be expected that the ciliate would increase its phagotrophy under dark conditions. The laboratory experiments carried out with the cells acclimatised under dark and light conditions showed that in darkness the cells ingest fewer food particles,

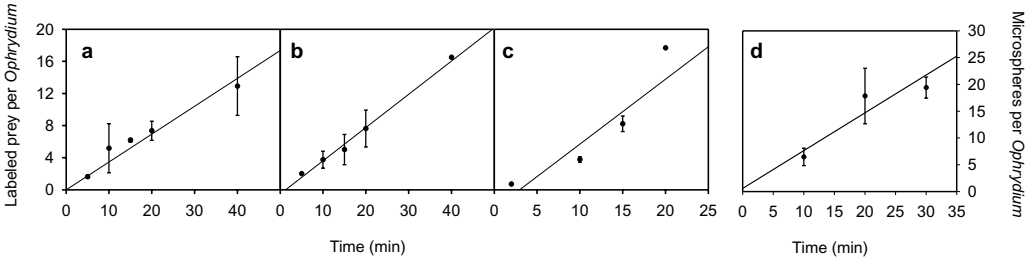

Fig. 2 Number of ingested prey over time in the grazing experiments (spring and early summer) (a) November *in situ* experiment (spring) with fluorescent latex microspheres of 0.5 μm diameter, (b) November *in situ* experiment (spring) with fluorescently labelled bacteria (FLB), (c) December *in situ* experiment (early summer) with FLB and (d) in the laboratory uptake experiment with fluorescent latex microspheres of 0.5 μm diameter. Error bars represent standard errors.

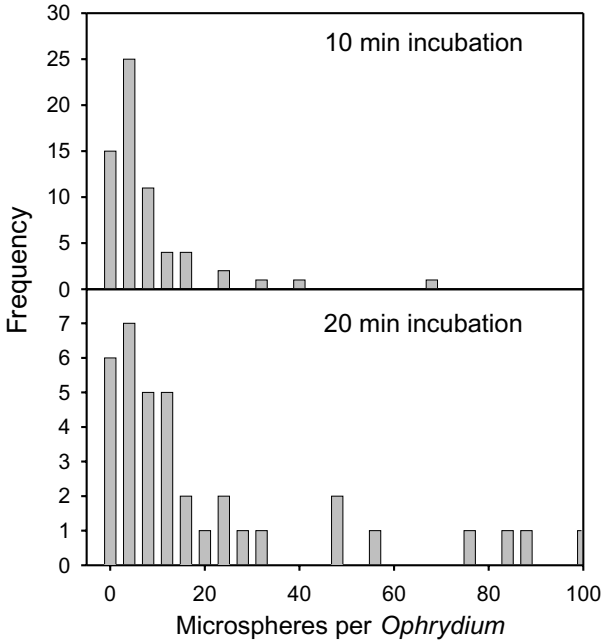

Fig. 3 Frequency distribution of microspheres uptake data of *O. naumanni* at 10 and 20 min of exposure in laboratory experiments, representing the highest number of ciliates inspected.

indicating that the primary energy source for the cells are endosymbiotic algae. Sand-Jensen, Pedersen & Geertzhansen (1997) indicated that another *Ophrydium* species, *O. versatile*, in many ways resembles unicellular algae more than symbiotic organisms with thick tissue, as the thin cell ensures a high light exposure for the symbiotic algae. *O. versatile* is a sessile ciliate that forms gelatinous colonies up to several centimetres in diameter, and up to 600 indiv cm^{-2} of lake bottom (Sand-Jensen *et al*., 1994). Self-shading among ciliates can be expected, although this has not been evaluated precisely (Sand-Jensen *et al*., 1997). On the other hand, *Ophrydium naumanni* lives free in the water column as

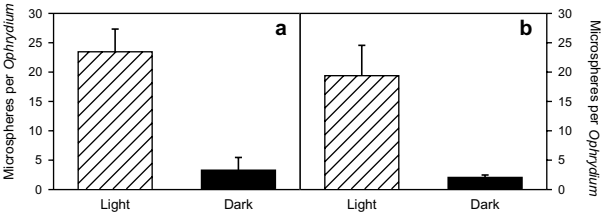

Fig. 4 Number of ingested prey of the uptake laboratory experiments with fluorescent latex microspheres under light and dark condition (*O. naumanni* cells acclimatised 15 h previous the experiment). (a) October experiment and (b) December experiment. Error bars represent standard errors.

solitary individuals or as colonies with low individual numbers. Thus, light exposure may be optimised in a situation where self-shading is absent and the cells can adjust their positions.

Nevertheless, *O. naumanni* was able to ingest particles in field and laboratory experiments indicating that it would be responsible to some extent for bacterivory in Andean lakes. The combined results of our grazing experiments and the growth experiments of Queimaliños *et al*. (1999) show that the primary energy intake of *O. naumanni* is light. However, phagotrophy is probably necessary to fulfil elemental requirements in such poor environments as Andean lakes. But the particle ingestion is dependent itself on light availability, because under dark conditions ingestion rate was lowered and opposite to what might have been expected had the two feeding modes been used as alternatives. Given that phototrophy appears in *O. naumanni* as a primary nutrition mode and phagotrophy may provide some elemental requirements, it is possible to assign this species to Group C in Jones (1997) classification. In this group, Jones (1997) states that ingestion rate is proportional to light intensity as demonstrated for the flagellates *Uroglena* and *Dinobryon* (Kimura & Ishida, 1985; Jones & Rees, 1994).

In lake Moreno Oeste, *O. naumanni* prefers a deep position in the water column. It can be hypothesised that this ciliate regulates light intensity for endosymbiotic photosynthesis to the availability of limiting elements obtained through phagotrophy. In this sense, an increase in light intensity would probably require more phagotrophy for internal maintenance of the symbiotic consortium. Moreover, Modenutti *et al*. (2000) showed that the addition of inorganic nutrient did not increase the ciliate growth rates in short-term experiments. This suggests that *O. naumanni* is unable to acquire inorganic nutrients unless they are obtained through phagotrophy. It seems that this symbiotic association results in effective exploitation of the water column in nutrient-poor, high light ecosystems like those of Andean lakes.

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