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Short Communication

Phagotrophic algae in a wastewater stabilization pond

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Abstract: In the course of evolution, phagotrophic euglenoids developed before the phototrophic species. Most phagotrophic euglenoids have a robust feeding apparatus. Members of the algal genus *Peranema* are able to eat a wide variety of living organisms that have little or no mobility, such as other unicellular algae and live yeasts. Stabilization ponds are artificial environments rich in organic matter, nutrients and microorganisms, and are therefore suitable environments for growing species of Euglenophyta. This contribution records, through photographs and videos, the operation of the feeding apparatus of *Peranema* collected in a wastewater stabilization pond. The mean pheophytin content of the water was higher than the mean chlorophyll *a* content, which indicates a non-ideal environment for the growth of microalgae. Thus, *Peranema* can be used as a bioindicator of the quality of wastewater stabilization pond. The operation of the feeding apparatus of *Peranema* sp. and the strategy for phagocytizing plastids of *Lepocinclis* sp. are described.

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Introduction

The euglenoids represent an ancient and diverse eukaryotic lineage. Huber-Pestalozzi (1955) described over 800 species belonging to about 40 genera. About two-thirds of the species are non-photosynthetic. In the course of evolution, phagotrophic euglenoids developed before the phototrophic species. The phototrophic species evolved from a single secondary endosymbiotic event involving a chloroplast from a green alga, a member of Prasinophyceae (Marin, 2004).

The transition from a phagotrophic to a phototrophic lifestyle occurred several times independently in the evolution of eukaryotes. One of these events occurred within the Euglenophyta, a moderately large group of flagellates with diverse modes of nutrition. Phototrophic euglenoids constitute a clade that is nested within lineages of phagotrophic euglenoids, and originated through a secondary endosymbiosis with green algae (Yamaguchi et al., 2012). Most phagotrophic euglenoids have a robust feeding apparatus comprised of two main bundles of microtubules termed rods, and four interior vanes (Leander et al., 2007).

Members of the euglenoid genus *Peranema* are able to consume a wide variety of living organisms that lack or have low mobility, such as live yeasts and other unicellular algae. Smaller organisms are swallowed whole. Larger organisms are also swallowed, but when this is not possible *Peranema* uses its rod-organ to make a cut in the periplast of the prey and ingests the contents, including starch grains, oil droplets and protein particles (Chen, 1950).

Marin (2004) noted that algae may be colorless not only through the loss of chloroplasts, but also from having undergone a mutation that results in the formation of leucoplasts rather than chloroplasts. Leucoplasts are non-pigmented plastids that function to store reserve substances.

Stabilization ponds are artificial environments rich in organic matter and nutrients, and are therefore suitable environments for growing Euglenophyta. For instance, Soldatelli and Schwarzbold (2010) found that euglenophytes comprised 27% of the total number

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of taxa in the phytoplankton community.This contribution records, based on microphotography, the operation of the feeding apparatus of a species of *Peranema* collected in a stabilization pond.

Materials and Methods

The samples were collected in September 2011, from a facultative pond of the Trindade wastewater-stabilization pond system, Goiás, Brazil. The identifications of the organisms in living samples were based on morphological and morphometric characteristics, analyzed in an inverted microscope equipped with an Olympus CKX41 camera connected to the software Cell D®. The reference used for identification was Huber-Pestalozzi (1955).

Physical and chemical analyses of the wastewater, including temperature, electrical conductivity, orthophosphate, ammonia, biochemical oxygen demand, pH and transparency, according to APHA (1998). The transparency of the stabilization ponds was measured with a Secchi disk, and the depth of the euphotic zone was calculated as three times the transparency value (Cole, 1975).

Results and Discussion

As seen in Table 1, the dissolved-oxygen content was low (1.9 mg L^{-1}), which is common in stabilization ponds. The mean pheophytin content was higher than the mean chlorophyll content, which indicates a non-ideal environment for the growth of microalgae, i.e., they were dying. In such a situation, the phagotrophic euglenoids exploit the weakness of other algae. The pond depth was approximately 1.70 m and the euphotic zone was 0.45 m.

Figure 1 shows an individual *Peranema* sp. swallowing plastids of a weakened *Lepocinclis* sp. To access the plastids, the *Peranema* used its flagellum to make a hole in the anterior part of the pellicle of *Lepocinclis* sp. and, when it had pierced the cell, projected its feeding apparatus to phagocytize the plastids (Fig. 1A, B and C). Triemer (1997) showed that *Peranema* can ingest living cells, paramylon grains, and whole plastic beads, and has a strong preference for nonmotile or injured cells.

Table 1. Mean and standard deviation (SD) of physicochemical and biological variables analyzed in facultative pond.

Variables	mean	SD
Temperature (°C)	25.7	1.6
pH	7.7	0.1
Dissolved oxygen (mg L^{-1})	1.9	0.4
Electrical conductivity (µS cm^{-1})	1,343	7
Orthophosphate (mg L^{-1})	0.9	0.2
Ammonia (mg L^{-1})	13.2	2.0
Chemical oxygen demand (mg L^{-1})	390	39
Chlorophyll *a* (µg L^{-1})	691	475
Pheophytin(µg L^{-1})	1008	875
Transparency (m)	0.15	0.01
Euphotic zone (m)	0.45	0.03

Alternatively, *Peranema* can "drill" or tear a hole in the prey cell and then suck out the contents. The projected rod-organ seems to help to push food into the body of the cell. After phagocytizing approximately 7 plastids, *Peranema* sp. used its flagellum to exit from inside the *Lepocinclis* sp. cell (Fig. 1D, E, F). Upon exiting, *Peranema* sp. showed cell contractions for more than 1 minute (Fig. 1G, H). Figure 1G shows the hole in the anterior part of *Lepocinclis* sp. left by *Peranema* sp.

Flagellated algae in stabilization ponds help to stabilize the organic-matter content (Madoni, 1994). In general, the protist community is directly related to the depuration state and the final effluent quality (Salvado et al., 1995). Akpor and Momba (2010) studied phosphate and nitrate removal in activated sludge, and found that using *Peranema* and acetate as a carbon source, the phosphate and nitrate concentration decreased dramatically after 96 hours.

In conclusion, *Peranema* is a good microorganism for use in sewage treatment, because it can survive in environments with high organic matter and low dissolved oxygen. Thus, *Peranema* can be used as a bioindicator of the quality of wastewater stabilization pond.

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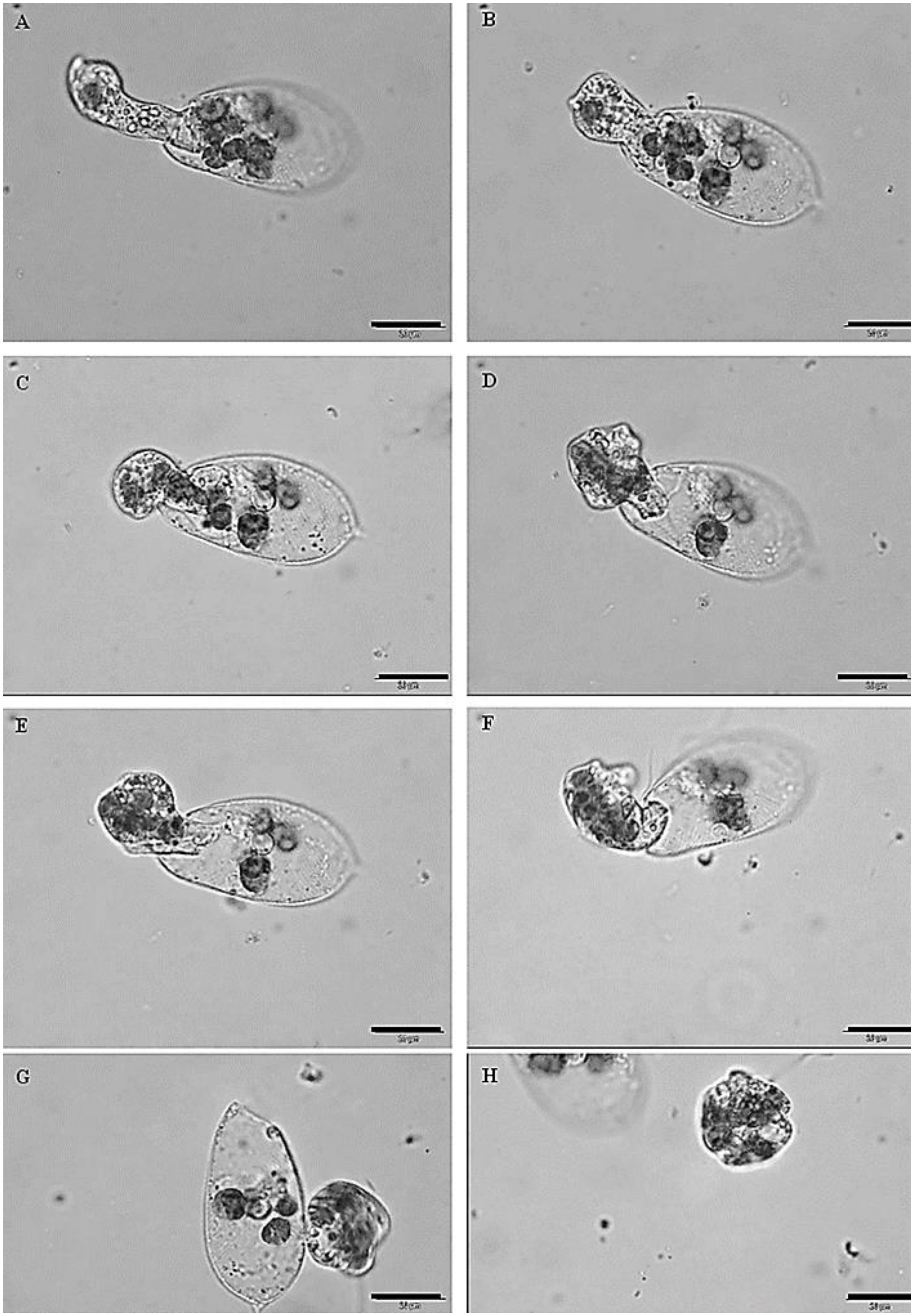

Figure 1. Sequential movements of *Peranema* sp. phagocytizing plastids of a *Lepocinclis* sp. (A B and C) opening the pellicle and phagocytizing the plastids, (D, E and F) using the flagellum to remove matter from inside a cell of *Lepocinclis* sp., and (G and H) contractions of the cell after phagocytizing the plastids (Scale = 20 μm).

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