

Free-living alga *Chlorella vulgaris* as a freshwater ecosystem inhibitor?

Tramontana, P.¹, Kovačević, G.², Petrinec, D.³, Peharec - Štefanić, P.² and Špoljar, M.³

¹ University of Zagreb, Faculty of Science, Department of Biology, Croatia, ² University of Zagreb, Faculty of Science, Department of Biology, Croatia, ³ University of Zagreb, Faculty of Science, Department of Biology, Croatia

Free-living unicellular algal species *Chlorella vulgaris* Beij. [K&H, 1992] strain SAG 211 - 11b is often found in freshwater habitats and overall shows high adaptability. Turbellaria are one of the traditional sub-divisions of the phylum Platyhelminthes (flatworms). As predators they inhabit freshwater or moist terrestrial environments. They move by cilia on the ventral dermis, allowing them to glide along on a film of mucus. In this experiment were used free-living unicellular photoautotrophic alga *C. vulgaris* and turbellarians *Dugesia gonocephala* (Duges, 1830) and *Polycelis felina* (Dalyell, 1814). The influence of algae on the behavior of turbellarians was assessed.

Algae were cultured on a sterile deep stock agar and were growing in test tubes of 16 cm in length and 15 mm in diameter in air chamber under sterile conditions at 24 °C and constant light. By standardizing the method of maintaining algal culture, a constant amount of clonal cultures suitable for performing the experiment was obtained. Turbellarians were isolated from their natural habitat, cultivated at 13.5 °C and fed with aquatic crustaceans *Artemia salina*. The experiment was conducted under different temperature and light conditions: at 25 °C and exposed to daylight (photoperiod 8 hs light, 16 hs dark) and at 13.5 °C in the dark. Different number of turbellarians was added in experimental dishes (60 ml): 5 dishes with one specimen in each (first setup with *P. felina* and second with *D. gonocephala*) and 5 dishes with 5 specimens in each (first setup with *P. felina* and second with *D. gonocephala*) at both temperatures, including both fed and starved animals separately, and in both sets with constant concentrations of *C. vulgaris* added to each experimental dish. The results were recorded immediately after the experiment setup and every 1, 8 and 24 hs after the beginning of the experiment. There were 5 replicas for each experiment, including controls. Altogether 170 experimental dishes, 240 *P. felina* and 240 *D. gonocephala* individuals were used. For analysis of algae, light and cTEM microscopy were used. After one hour noticed was the sediment of algae formed at the bottom of each experimental dish as well as the inhibition of turbellarians *D. gonocephala* by *Chlorella*, mitigating their movement and coordination. In the suspension of algae, *D. gonocephala* exhibited extremely numb behaviour. Individuals that were sporadically moving had extremely slow and uncoordinated behaviour and this was specifically expressed only on the walls of the glass dish where no algal sediment was present. *D. gonocephala* that rested, mostly rested in the algal sediment at the bottom of the dish. This phenomenon of inhibition of motion and coordination was also present in *P. felina*, but it was extremely expressed in *D. gonocephala*.