Uncoupling heterotrophic and mixotrophic predation using Rotenone, a mitochondrial inhibitor

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It cannot distinguish between heterotrophic and mixotrophic life-forms

Introduction

Microzooplankton are the main grazers of primary producers in the oceans. However, new evidence suggest that many of them are likely mixotrophs (Flynn et al., 2019)

Mixotrophs are the organisms that have the ability to photosynthesize and ingest particulate food items

Knowing the contribution of mixotrophs to the total microzooplankton–due predation will improve the assessment of Carbon fluxes within the pelagic realm

Aim & Methods

The Dilution Technique (Landry & Hassett, 1982) is the most used tool to estimate micro-zooplankton grazing rates

Mixotrophs

Rotenone inhibits the electron transport chain in the mitochondria, inhibiting the oxidative phosphorylation and ATP synthesis (Xu et al., 2016). Chloroplast-bearing organisms should be less affected

Results

Growth

Changes in cell number relative to the initial concentration (%)

24 h exposure

0 mg L⁻¹ 0.5 mg L⁻¹ 1.0 mg L⁻¹ 2.0 mg L⁻¹ DMSO

Auto Mixo Het

Figure 1: Growth of auto- mixo- and heterotrophs upon exposure to Rotenone. Different letters within the same organism highlight statistically significant differences (Tukey HSD; p-value < 0.05). Error bars = SEM.

The diatom was unaffected by Rotenone, whereas the other autotrophs displayed a progressively lower growth as the concentration increased

The lowest concentration (0.5 mg L⁻¹) did not seem to significantly affect mixotrophic growth, but higher concentrations had a negative effect

The growth of the heterotrophic dinoflagellate was affected by Rotenone, achieving maximum negative effects at the lowest concentration

Rotenone had a strong negative effect on the growth of the heterotrophic ciliate.

On the lowest concentration, the result may be explained by the DMSO toxicity

Ingestion

A Karlodinium armiger

B Micrasterias rubrum

C Gyrodinium dominans

D Strombidium sp.

The presence of Rotenone clearly affects feeding in the ciliates M. rubrum (mixotrophic) and Strombidium sp. (heterotrophic)

Part of the feeding inhibition can be explained by the sole toxicity of DMSO, which completely or partly inhibited feeding

None of the dinoflagellates was affected by the DMSO treatment

The mixotrophic K. armiger did not display any evidence of feeding in the presence of Rotenone. G. dominans required a concentration of 2.0 mg L⁻¹ to fully inhibit feeding

Conclusion

Although chloroplast-bearing organisms displayed a better resilience at low concentrations of Rotenone, their feeding was more affected than that of heterotrophs

Contrary to the expectations, the heterotrophic dinoflagellate G. dominans was the least sensitive organism when balancing growth and ingestion

Ciliates seem to have a larger growth and ingestion impairment than dinoflagellates, regardless of the trophic mode of nutrition

Rotenone cannot be used to uncouple mixo and heterotrophic predation in situ by modifying the existing Dilution Technique