Trophic switching rewires mitochondrial–chloroplast energetics, redox balance, and ROS physiology in *Euglena gracilis*

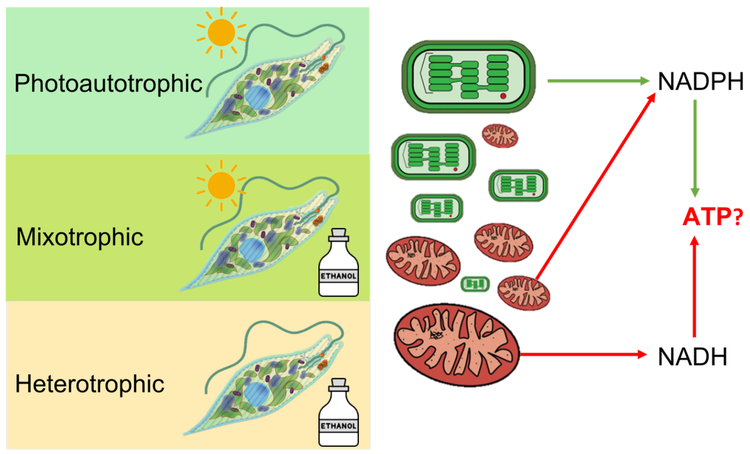
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Abstract

Euglena gracilis is a metabolically versatile alga capable of adapting to diverse trophic conditions. This study investigated mitochondrial physiology of *E. gracilis* cultured in Hutner's medium under three trophic conditions: photoautotrophic (light without external carbon), mixotrophic (light with 1% v/v ethanol), and heterotrophic (dark with 1% v/v ethanol). Physiological parameters including growth, mitochondrial mass (MitoTracker Green™, MTG), chlorophyll content, reactive oxygen species (ROS), energy cofactors, and redox cofactors were analyzed. Mixotrophic cells showed the highest growth rate (0.71 day^{-1}), followed by heterotrophic (0.68 day^{-1}), while photoautotrophic cells grew the slowest (0.28 day^{-1}). MTG signals were strongest in heterotrophic and mixotrophic cells, whereas chlorophyll was maintained only in photoautotrophic and mixotrophic conditions. Photoautotrophic cells exhibited the highest ROS and H_2O_2 levels. Redox analysis showed higher NADP(H) pools under photoautotrophy and higher NAD(H) pools under mixotrophy and heterotrophy. Although total adenylate levels were highest in heterotrophic cells, the ATP/ADP ratio and energy charge were highest in photoautotrophic cells. These results indicate that *E. gracilis* exhibits strong metabolic plasticity, reallocating energy and redox pathways to adapt to different trophic environments.

Introduction

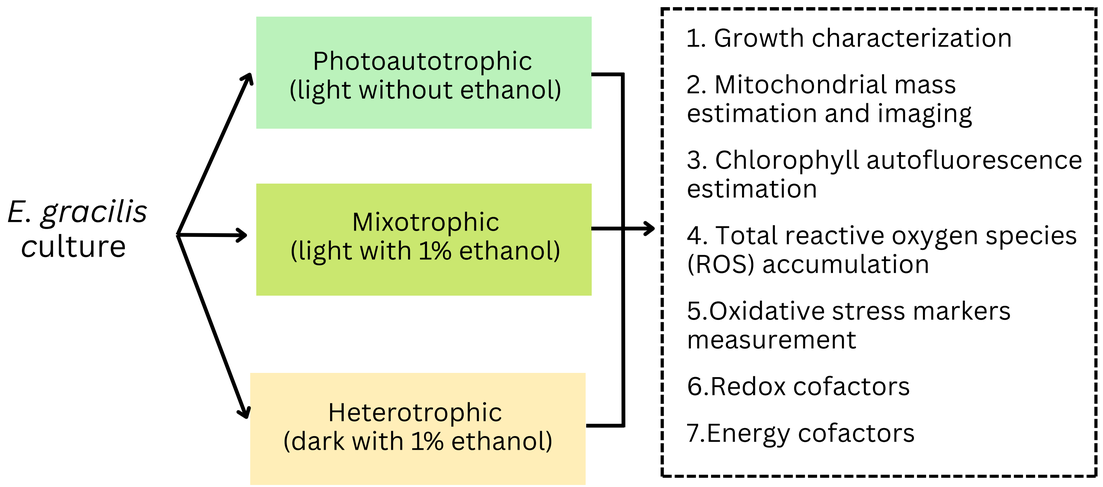


- *Euglena gracilis* exhibits diverse metabolism under photoautotrophic, mixotrophic, and heterotrophic conditions.
- The mechanisms regulating energy production in these modes remain unclear.

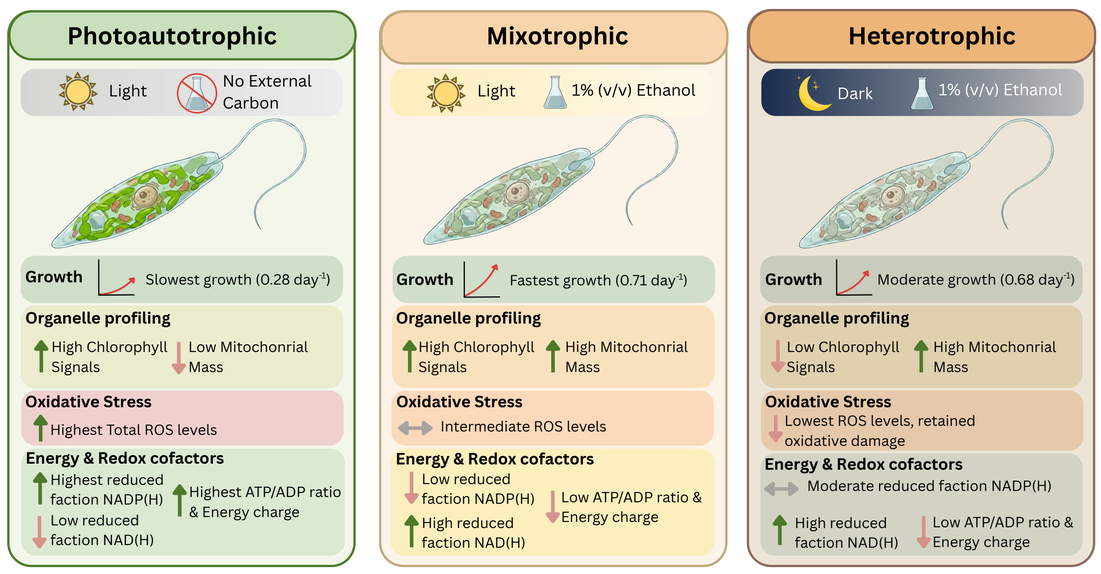
Objectives

This study aims to understand the metabolic adaptation of *E. gracilis* under changing conditions, to investigate the mechanisms of energy production under the three trophic conditions, and to examine how trophic conditions affect ROS levels.

Methods

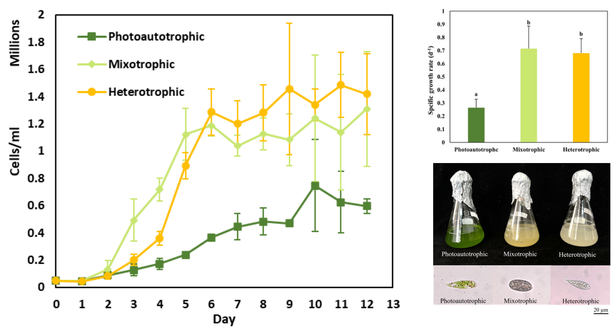


Conclusion



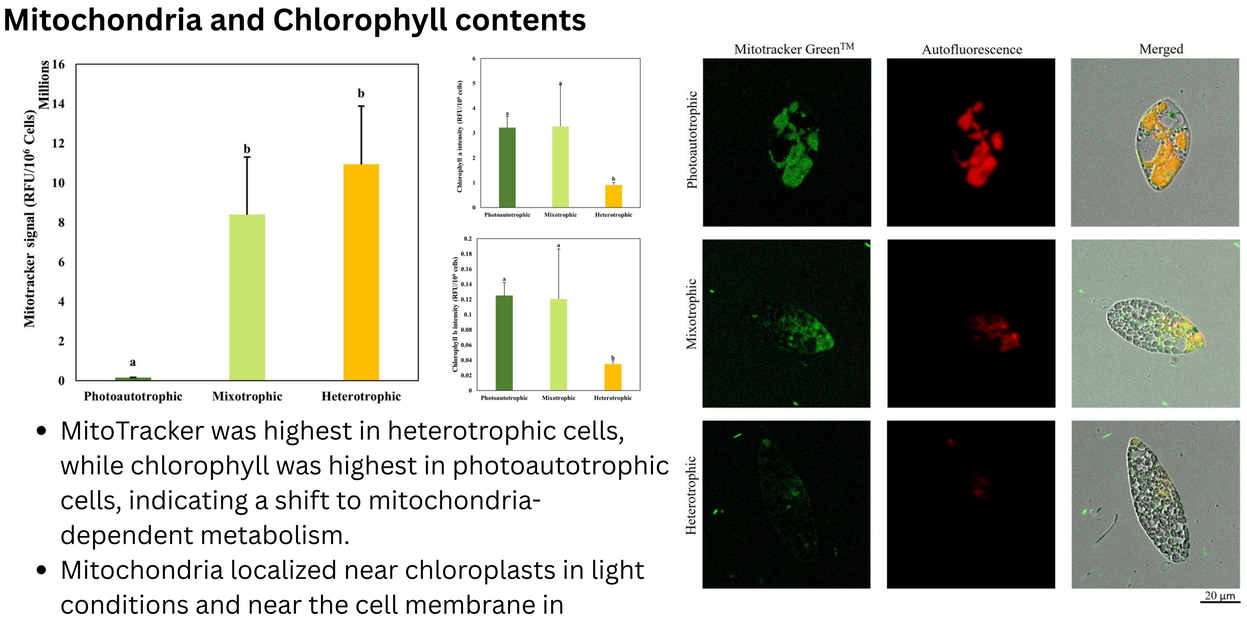
Results and discussion

Growth characteristics of *E. gracilis*



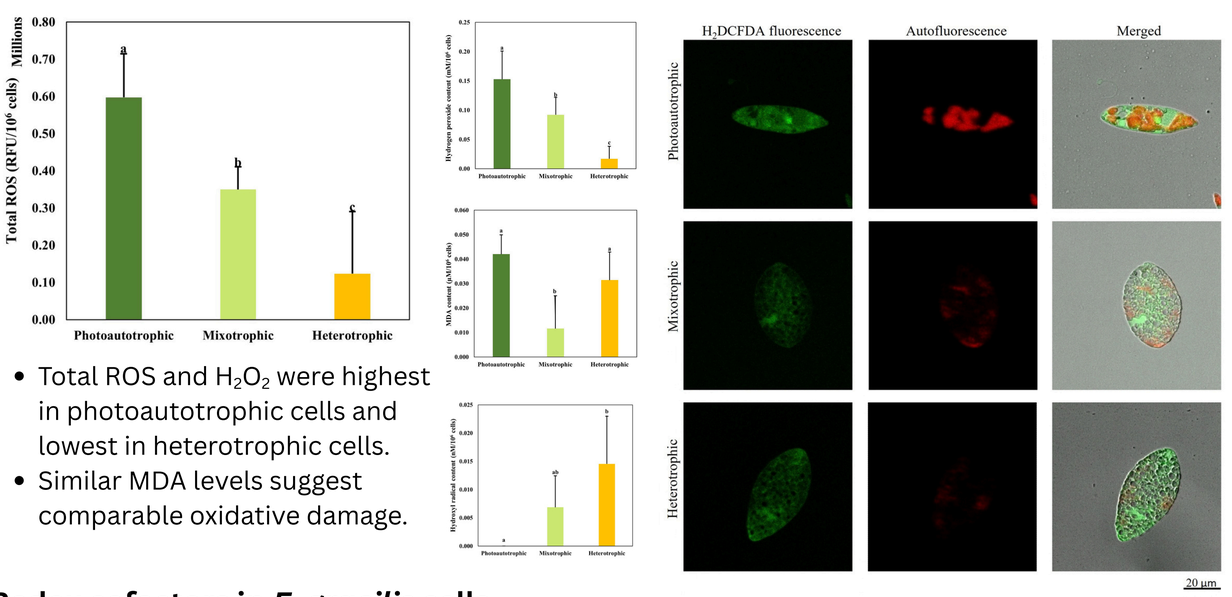
- Growth was highest under mixotrophic conditions, followed by heterotrophic and photoautotrophic conditions.
- Ethanol promotes a phenotypic shift by converting to acetyl-CoA, which enters the TCA cycle to support energy and biomass production.

Mitochondria and Chlorophyll contents



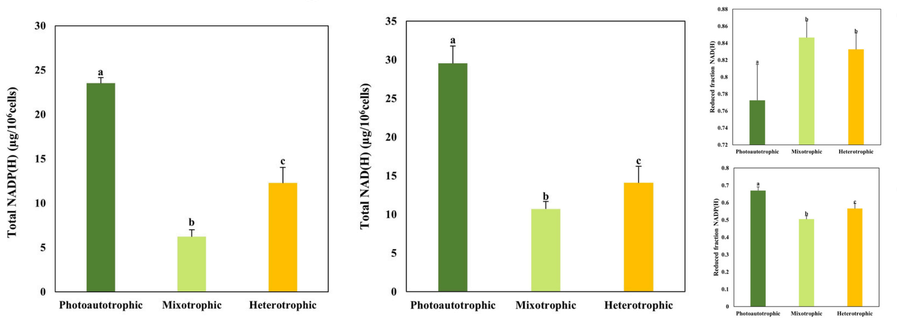
- MitoTracker was highest in heterotrophic cells, while chlorophyll was highest in photoautotrophic cells, indicating a shift to mitochondria-dependent metabolism.
- Mitochondria localized near chloroplasts in light conditions and near the cell membrane in heterotrophic cells.

Reactive oxygen species (ROS) accumulation in *E. gracilis* cells



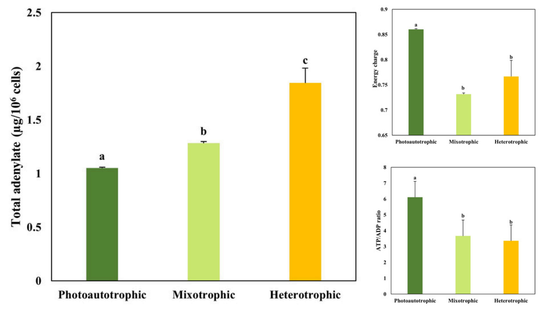
- Total ROS and H_2O_2 were highest in photoautotrophic cells and lowest in heterotrophic cells.
- Similar MDA levels suggest comparable oxidative damage.

Redox cofactors in *E. gracilis* cells



- Photoautotrophic cells showed higher NADP(H) reduction, indicating NADPH production exceeds consumption.
- Mixotrophic and heterotrophic cells showed higher NAD(H) reduction from ethanol metabolism.

Energy cofactors in *E. gracilis* cells



- Total adenylate was highest in heterotrophic cells.
- ATP/ADP ratio and energy charge were highest in photoautotrophic cells, indicating ATP production exceeds consumption.
- Lower energy charge in mixotrophic and heterotrophic cells likely reflects continuous ATP use for growth and biosynthesis.

Acknowledgements

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