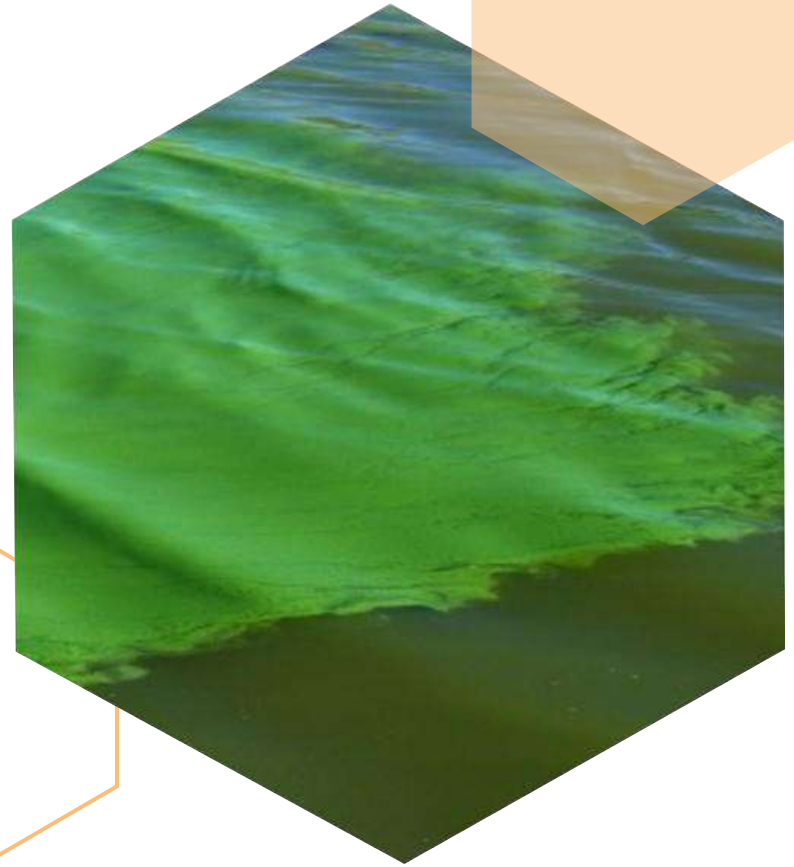
Different pasts, different responses:

How *Daphnia* react to cyanobacteria exposure?

Ivo Pinto, Luísa Azevedo, Sara C. Antunes

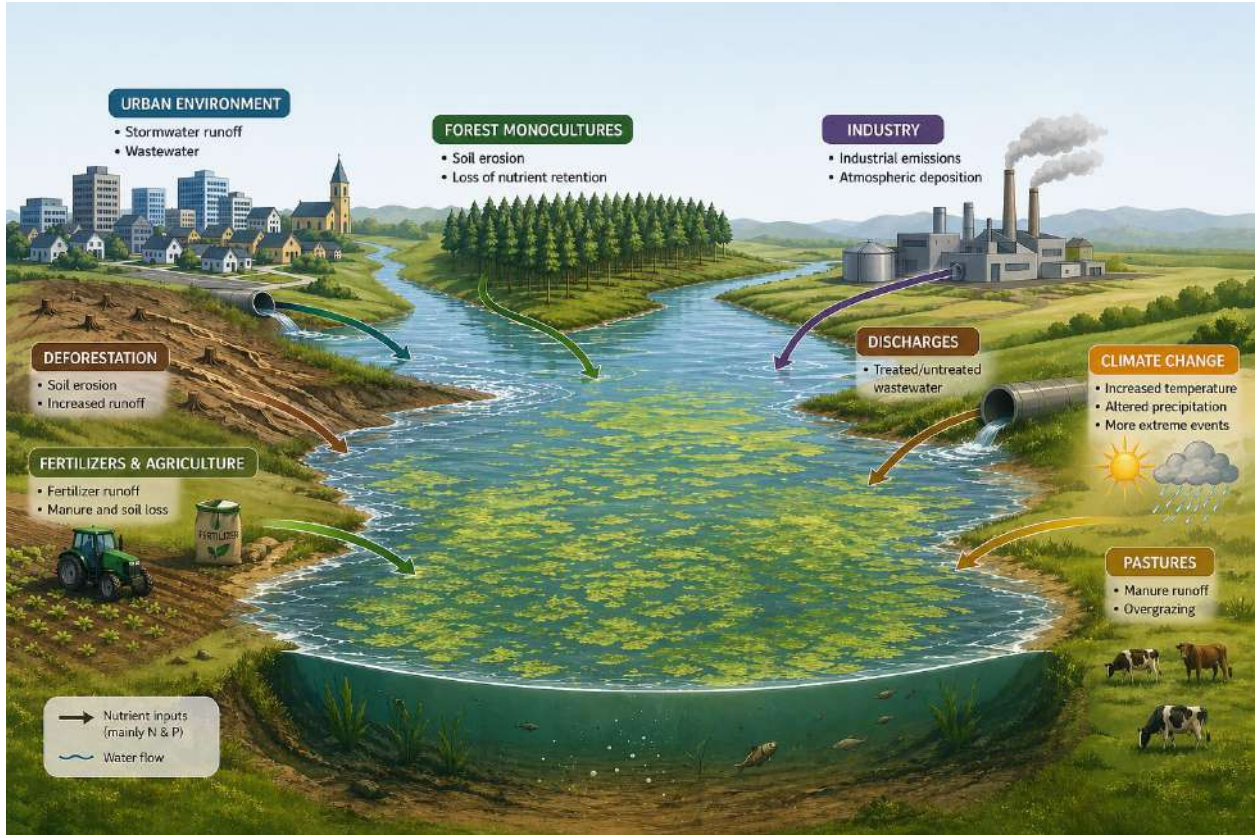
May 18, 2026

Maastricht, The Netherlands



SETAC Europe 36th Annual Meeting
Embrace the Outlier: In Science, Regulations and Networks.

Environmental Drivers of Cyanobacterial Blooms



Anthropogenic pressures → Water degradation → Phytoplankton proliferation → Cyanobacteria Blooms

What are cyanobacteria?

- **Prokaryotes**, blue-green color, capable of **photosynthesis**;
- *Microcystis*, *Planktothrix*, *Aphanizomenon* and others.

Why are they a concern?

- **Oxygen depletion** and **toxin production;**
- Biochemical changes, affecting **growth**, **reproduction** and **mortality** of different organisms.

Why focus on Zooplankton?

- As **primary consumers**, they connect primary producers to higher trophic levels;
- Alterations at this level can **cascade** through the entire food web.

Aim

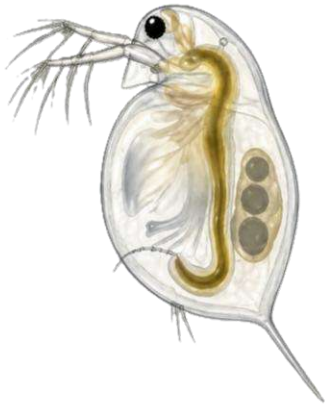
How do *Daphnia* spp. react when cyanobacteria are used as their primary food source?

HYPOTHESES

H0 No significant effects are observed.

H1 *Daphnia* spp. showed significant effects.

H2 *Daphnia* spp. from different locations exhibited different responses.



Methods

Study area characterization[1]

Rabagão reservoir

- **Location:** Northern Portugal, Cávado River basin
- **Water quality:** Oligotrophic, low nutrient concentrations, high water transparency
- **Catchment pressure:** Low, predominantly forest and natural land use
- **Main use:** Hydropower production, urban water supply and trout farming
- **WFD status:** GOOD (High ecological potential, over the three monitoring cycles)

Aguieira reservoir

- **Location:** Central Portugal, Mondego River basin
- **Water quality:** Eutrophic, high nutrient concentrations, low water transparency
- **Catchment pressure:** High agricultural activities, urban areas and wastewater inputs
- **Main use:** Hydropower production, irrigation and urban water supply
- **WFD status:** MODERATE or POOR (Low ecological potential, over the three monitoring cycles)





Rabagão reservoir



Aguieira reservoir

Two reservoirs with contrasting ecological status

(1) Pinto, I.; Azevedo, L.; Antunes, S.C. Changes in Regional Practices and Their Effects on the Water Quality of Portuguese Reservoirs. 2025. doi:10.3390/earth6020029.

Methods

1 Sampling and organism collection

Historical data[2]: 2014 – 2020
Seasonal water sampling: 2023

Target species	Identification
Cyanobacteria	Morphological + 16S gene
Daphnia	Morphological + 12S gene





2 *Daphnia* feeding rate assays[3]

T1	7.5×10^4 cells/mL	**Medium risk**
T2	1.5×10^5 cells/mL	**High risk**
T3	3.0×10^5 cells/mL	**High risk**
T4	1.2×10^6 cells/mL	**Very high risk**

Public health risk
World Health Organization [4]



3 Endpoints

- Feeding rate
- Antioxidant/detoxification defense (CAT and GSTs activities)
- Lipid peroxidation (TBARS levels)
- Cholinergic neurotransmission (AChE activity)
- Energy Reserves (ETS activity)

(2) Pinto, I.; Silva, R.; Hentschke, G. S.; Vasconcelos, V.; Morais, J.; Azevedo, L.; Antunes, S.C. Cyanobacteria and Cyanotoxins in Mediterranean Reservoirs: Ecological Variability, Risks and Implications for Water Quality Assessment. 2026. doi:10.1002/eco.70187.
(3) McWilliam, R.A.; Baird, D.J. Postexposure Feeding Depression: A New Toxicity Endpoint for Use in Laboratory Studies with *Daphnia Magna*. 2002. doi:10.1002/etc.5620210612.
(4) World Health Organization Guidelines for Safe Recreational Water Environments. Volume 1, Coastal and Fresh Waters; 2003. ISBN 9241545801.

Results

Historical phytoplankton patterns in the two reservoirs

Rabagão reservoir

Low phytoplankton dominance

Low cyanobacteria occurrence

Low bloom risk

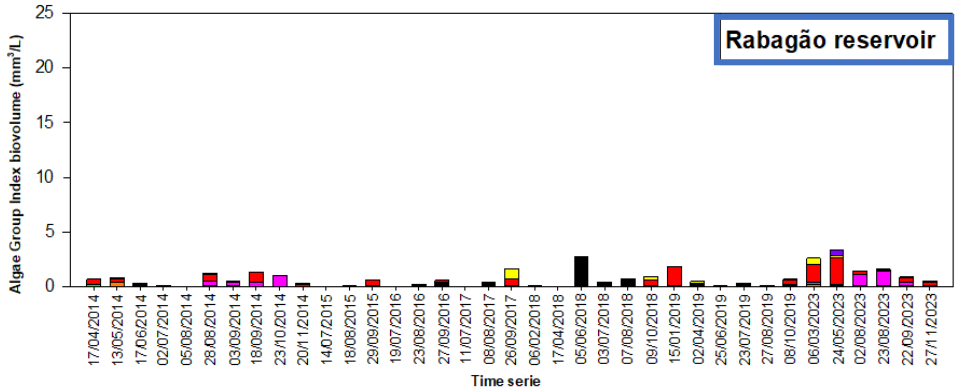
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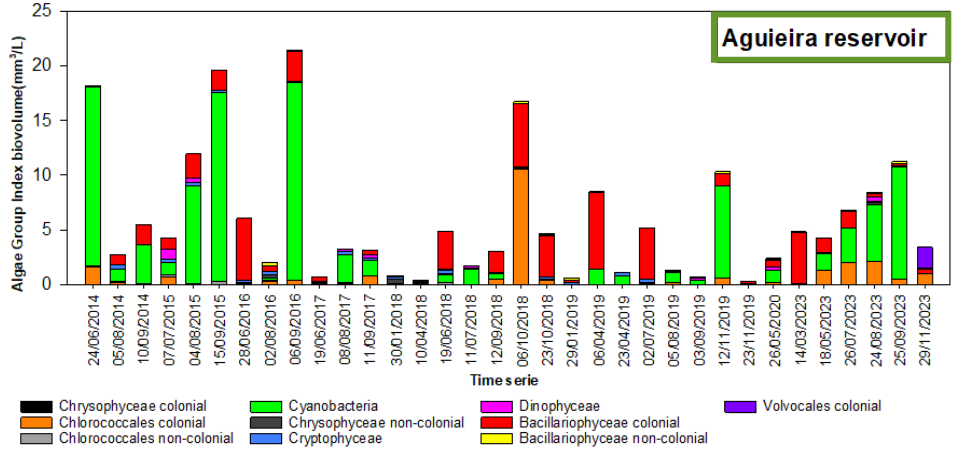
Aguieira reservoir

High phytoplankton dominance

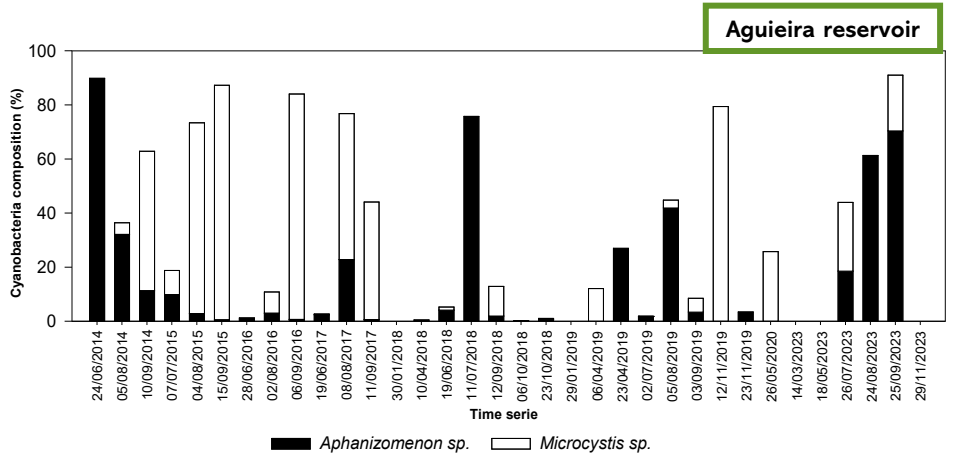
High cyanobacteria occurrence

High bloom risk





Historical cyanobacteria records in **Aguieira** were mainly dominated by *Microcystis* spp. and *Aphanizomenon* spp.

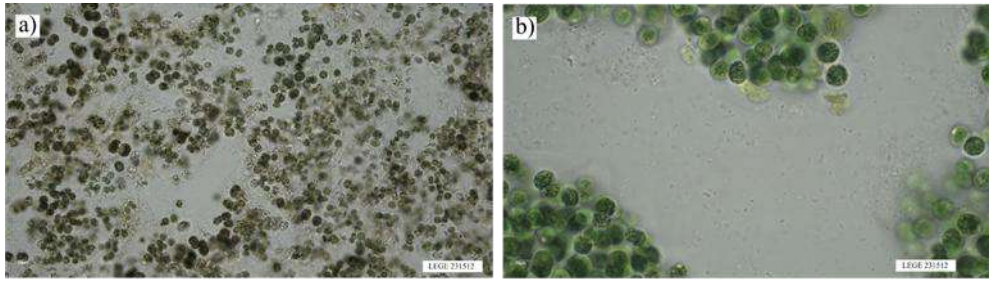


Results

Cyanobacteria characterization

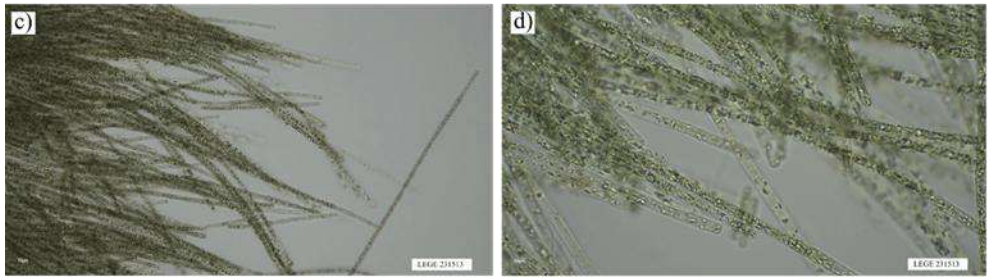
Microcystis aeruginosa LEGE 231512

- Spherical or irregular colonies
- Numerous aerotopes
- Mucilage diffluent and colourless



Aphanizomenon flos-aquae LEGE 231513

- Parallel-arranged and cylindrical
- Isodiametric or longer than wide
- Straight trichomes
- Rounded ends and numerous aerotopes



Results

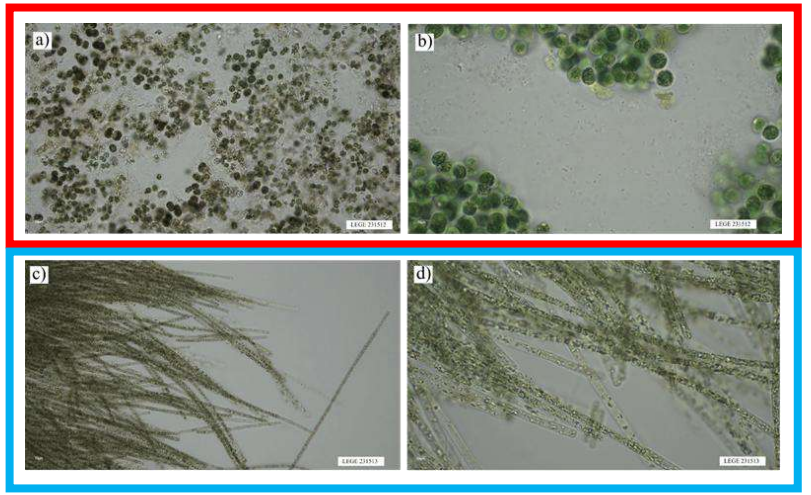
Cyanobacteria characterization

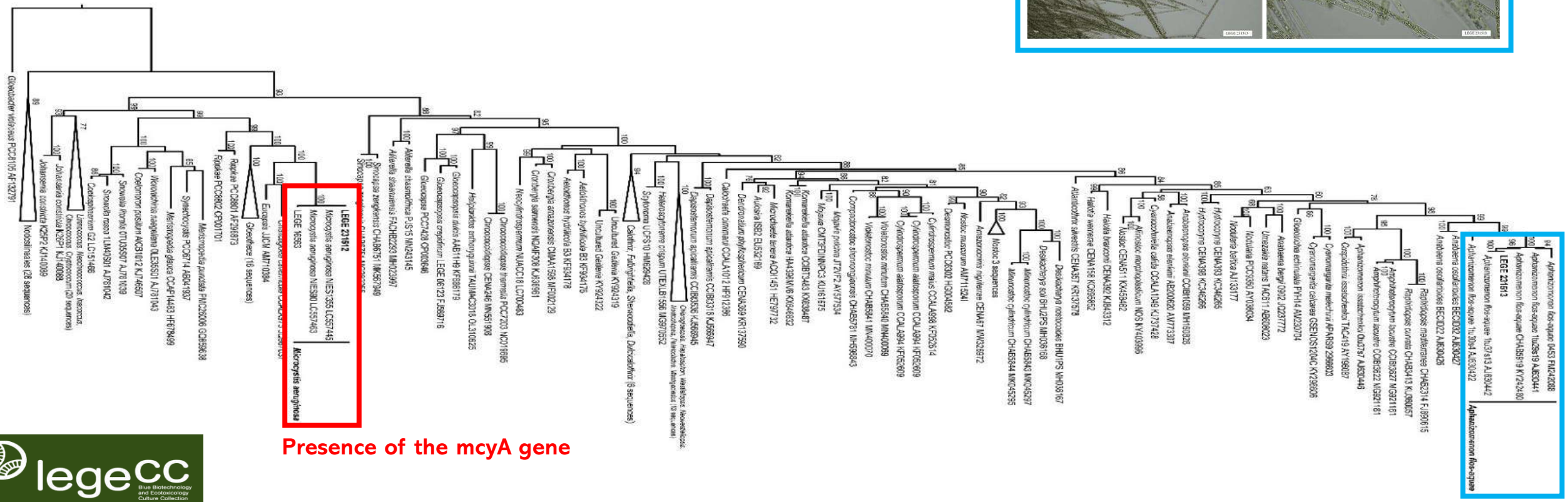
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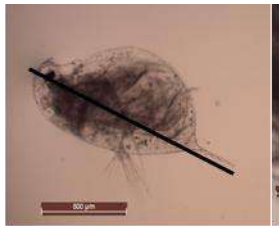
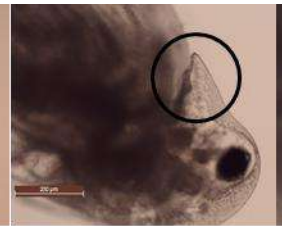
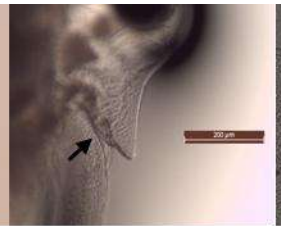
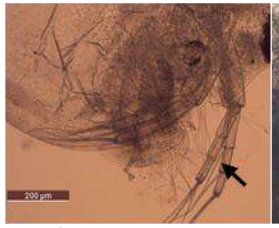
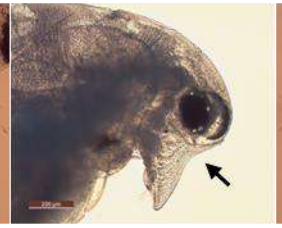
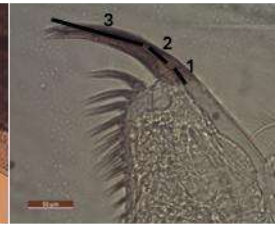
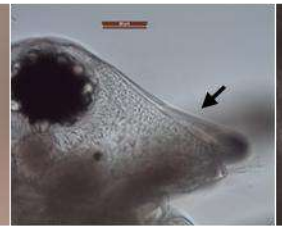
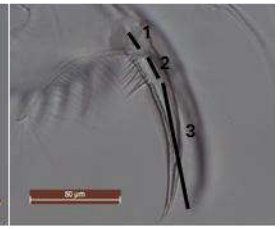


Presence of the mcyA gene

legeCC
Blue Biotechnology and Ecotoxicology Culture Collection

Results

Daphnia characterization

Species	Morphological characteristics				Rabagão reservoir	Aguieira reservoir
 ***Daphnia longispina***	 Shell spine along the body axis	 Rostrum pointed, sensory setae originate far from the tip	 Antennular mound low	 1=2=3 combs	✓	
 ***Daphnia galeata***	 Proximal segment of the 3-segmented branch with well developed seta	 Head large, often convex near the eye	 Mound high, antennules reduced	 1=2=3 combs		✓
 ***Daphnia middendorffiana***	 Optic vesicle touches margin of head	 Rostrum long and sharp	 Rostrum with long and narrow polygons	 2>1>3 combs	✓	✓

Results

Daphnia characterization

Sequences clustered into two clades:

Daphnia and ***Hyalodaphnia***

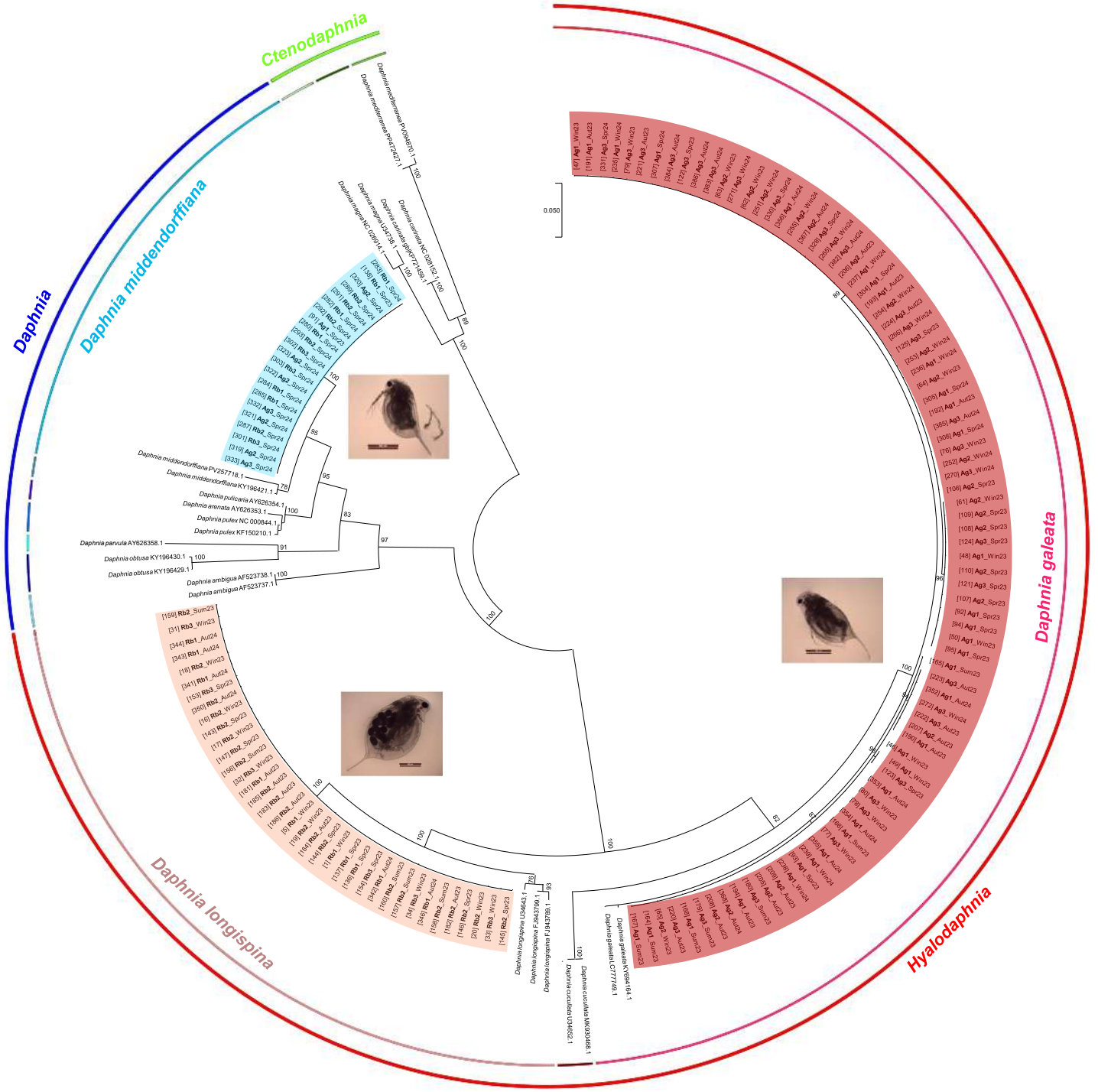
Morphological identification were **confirmed by molecular data**

Specimens with **ambiguous morphology** were **confidently assigned** to species-level clades

---

Genetic variability:

- *D. galeata* showed 4 polymorphic sites (2 A/G and 2 A/T variations)



Results

Daphnia feeding rate responses

Cyanobacterial diets **reduced feeding rate** in *Daphnia* spp., with interspecific differences.

Control validation

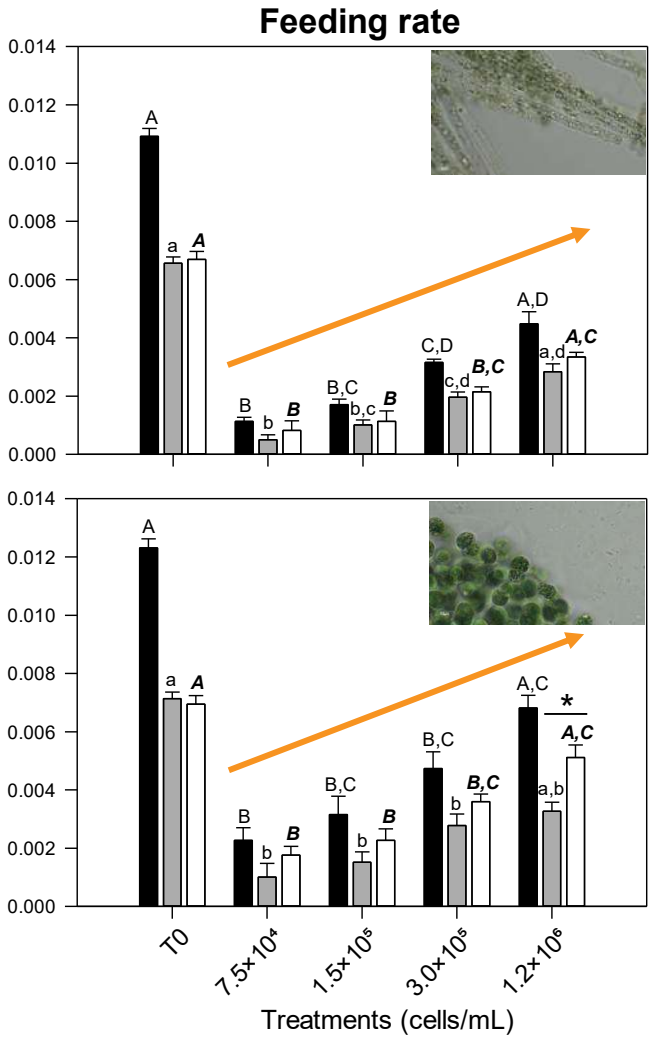
Control group (T0) with normal feeding rates, validating the assay.

Dose response

Feeding rates show a recovery trend with increasing cyanobacteria concentration

Species-specific differences

Significant differences between the two field species under the highest *M. aeruginosa* concentration

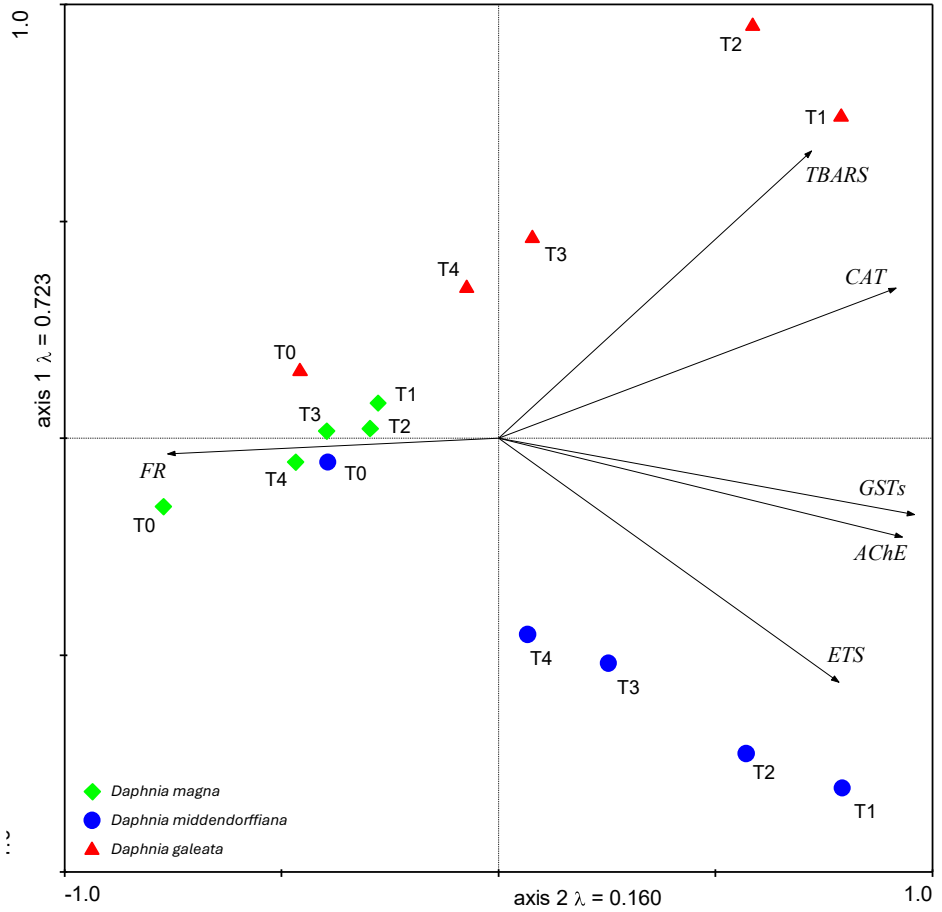


Results

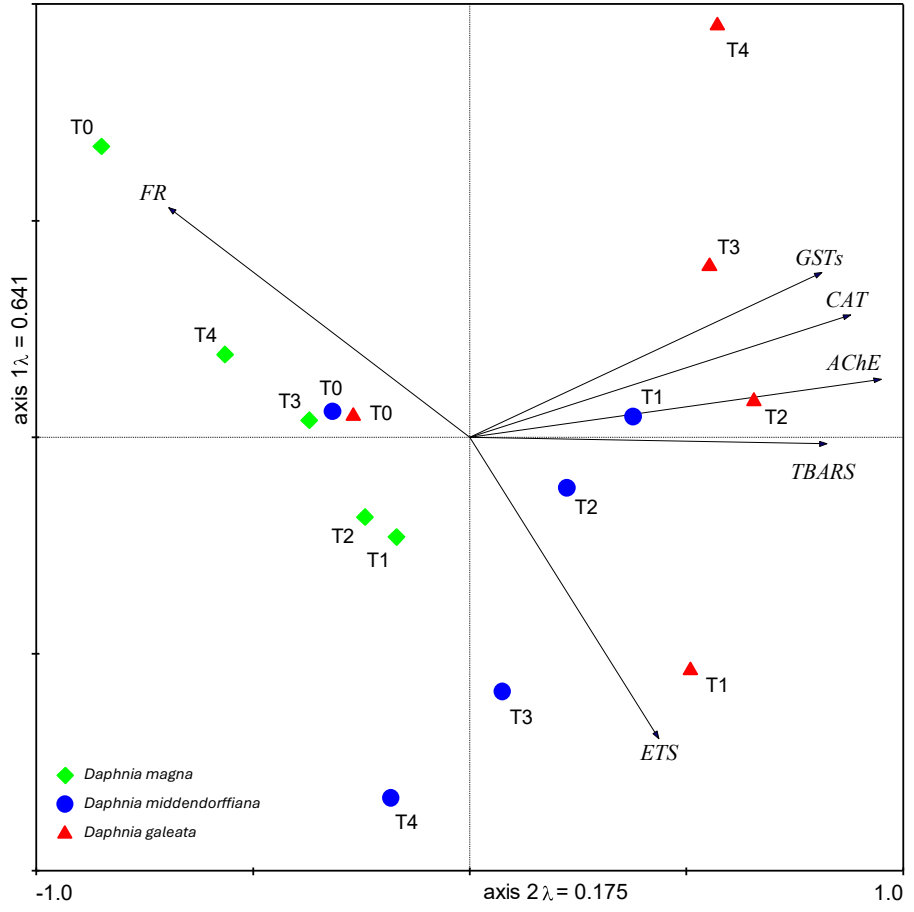
Daphnia spp. integrated assessment

In both cyanobacterial diets, the three *Daphnia* species showed **distinct response patterns**

Aphanizomenon flos-aquae



Microcystis aeruginosa

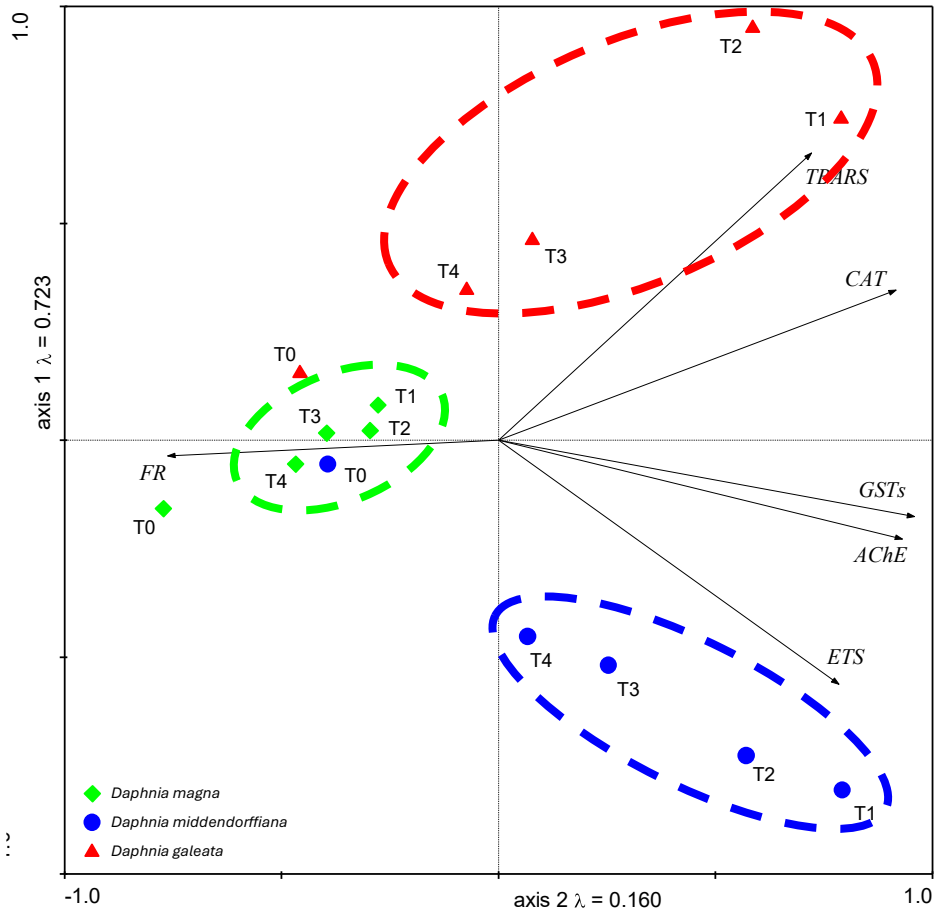


Results

Daphnia spp. integrated assessment

In both cyanobacterial diets, the three *Daphnia* species showed **distinct response patterns**

Aphanizomenon flos-aquae



D. magna affected regardless of the concentration

Field species (*D. galeata* and *D. middendorffiana*) exhibited concentration-dependent responses.

As *A. flos-aquae* concentration increases, the *Daphnia* spp. response improves, but remains lower than the control.

T4 - 1.2×10^6

(Very high risk)

Results

Daphnia spp. integrated assessment

In both cyanobacterial diets, the three *Daphnia* species showed **distinct response patterns**

D. magna showed concentration-dependent effects

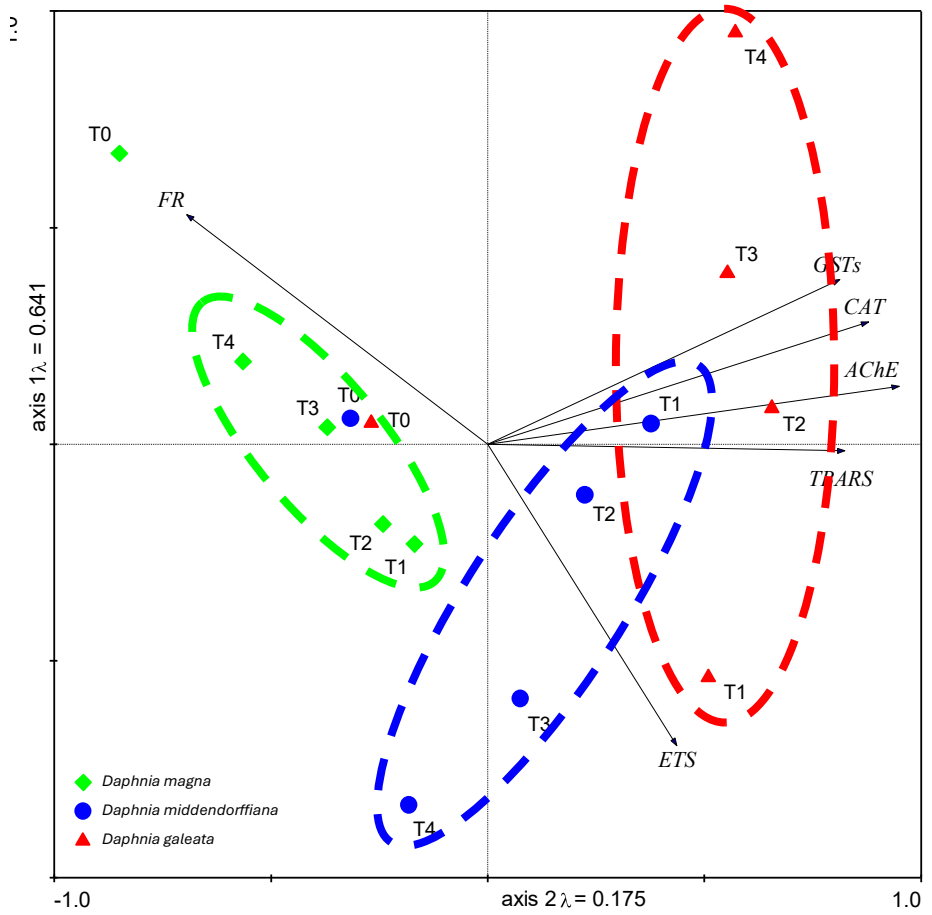
Field species (*D. galeata* and *D. middendorffiana*) showed concentration-dependent responses:

- *D. galeata* - increased antioxidant and detoxification responses
- *D. middendorffiana* - increased energetic demand

M. aeruginosa concentration increases induced persistent physiological alterations in field *Daphnia* populations

Possible production of microcystins

Microcystis aeruginosa



Conclusion



Aguieira reservoir



Rabagão reservoir

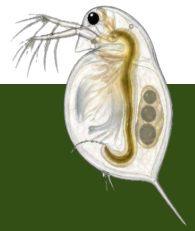
H1 ***Daphnia*** **spp. showed significant effects.**

Responses differed among species and diets

H2 ***Daphnia*** **spp. from different locations exhibited different responses.**

D. galeata (Aguieira reservoir) – strong biochemical alteration

D. middendorffiana (Rabagão reservoir) – energetic cost



Different pasts generated different responses to cyanobacteria

Prior exposure to recurrent blooms may enhance resilience through adaptation

Team







Ivo Pinto[1,2,3,4]

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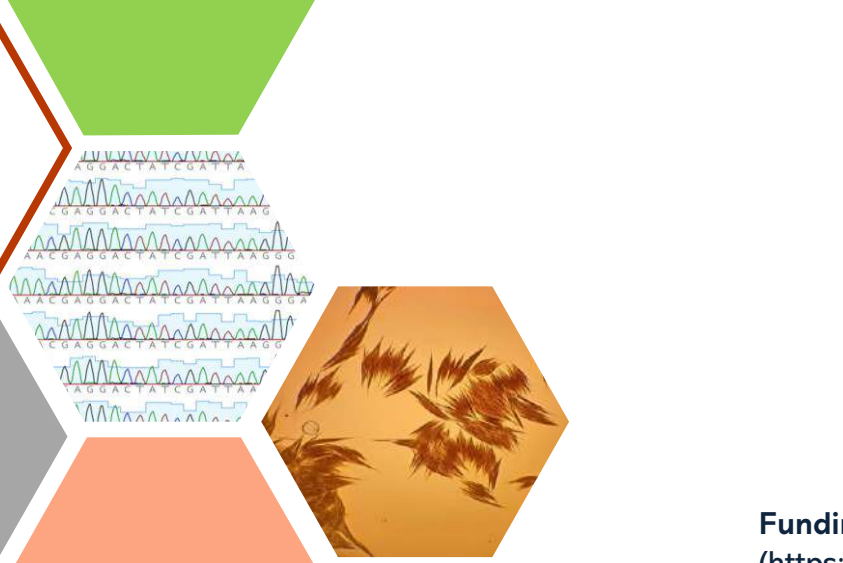
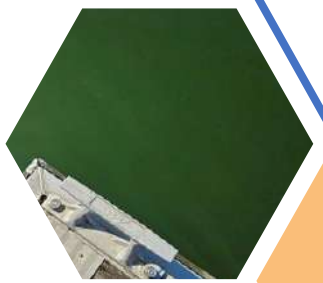
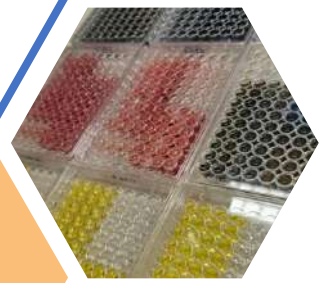
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1 School of Medicine and Biomedical Sciences, University of Porto
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Thank you

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By the project "2Qua - Water Quality and Quantity, the challenge for the present and future" (ref. COMPETE2030-FEDER-0691700 - https://doi.org/10.54499/2023.17517.ICDT).

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