

# Molecular quantification of potentially toxin-producing cyanobacteria via qPCR targeting gene markers

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## Aim of the STSM

- Utilize qPCR to identify and quantify cyanobacteria capable of producing four primary cyanotoxin groups: microcystins, anatoxins, cylindrospermopsins, and saxitoxins, in 60 biofilm samples collected from large rivers and tributaries in Croatia's Pannonian ecoregion.
- Contribute to CA23160 Working Group 1, focusing on "Early warning methods for detection of blooms, risk assessment, and citizen science."
- Lay the groundwork for analyzing benthic cyanotoxins in Croatian freshwater ecosystems.
- Foster international collaboration between Croatia and Slovenia.

## Methodology

**Targeted gens**

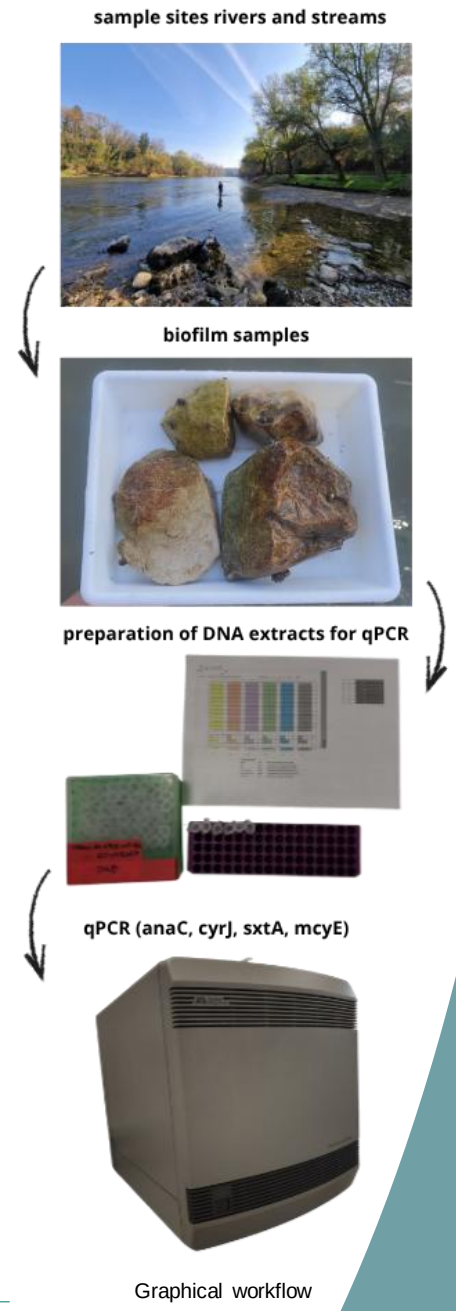
- **anaC**-anatoxin synthesis (Jablonska & Eleršek, 2025)
- **cyrJ**-cylindrospermopsin synthesis (Campo et al., 2013)
- **sxtA**-saxitoxin synthesis (Al-Tebrineh et al., 2010)
- **mcyE**-microcystin synthesis (Al-Tebrineh et al., 2011)
- **16S-cyano rRNA**-DNA extraction and qPCR inhibition control (Zupančič et al., 2021)

**qPCR workflow**

- Samples were prepared in triplicates, each experiment included positive controls (specific synthetic DNA fragments) and two negative controls (nuclease-free water; Sigma-Aldrich). Sample preparation and amplification conditions adhered to Zupančič et al. (2021).
- Analysis were performed on an Applied Biosystems 7900HT qPCR cycler (Thermo Fisher Scientific) using SYBR Green chemistry (Thermo Fisher Scientific).

**Data analysis**

- Results were analyzed using SDS software (version 2.4.1, Thermo Fisher Scientific) following the methods described by Zupančič et al. (2021).
- Samples were considered positive if (i) all three technical replicates were positive and (ii) the melting temperature (Tm) of the amplicon matched the Tm (±0.9 ◦C for 16S rRNA, ±0.5 ◦C for all other assays) of cyanobacterial cultures. Samples with two positive replicates were considered potentially positive. For all positive and potentially positive samples, average Cq values were calculated from the Cq values of all positive replicates.




Graphical workflow

## Main Outcomes & Follow-up

**Results:**

- **All 60 samples were analysed** according to sugessted workplan and time frame
- Out of 60 samples: **1 sample** tested **negative for the 16S-cyano rRNA gene**
- **10 samples** tested **anatoxin positive; 3 samples** were **potentially positive** using the **anaC-1039 assay; 4 samples** were **microcystin positive** using the **mcyE assay**, while **1** additional **sample** was **potentially positive** for microcystin
- Only 1 sample was positive for both - anatoxin and microcystin genes
- All samples were negative for cylindrospermopsins (cyrJ) and saxitoxins (sxtA)

**Follow-up**

- Data analysis in collaboration with host institution regarding relative quantification of positive samples using the calibration curve approach for autotrophs based on the 16S rRNA gene, potential anatoxin and microcystin producers based on anaC and mycE genes and data collected from overall PhD research.
- Data obtained from this STMS will be disseminated through scientific publications
- Collaboration will also continue through joint scientific publications and participation in international conferences

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