PICES-2025
W11:MEQ Workshop

QuickConc: A highly sensitive eDNA concentration method with cationic-assisted capture

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Introduction

Environmental DNA (eDNA) analysis

Environmental DNA (eDNA) analysis is a non-invasive monitoring technique that detects species by extracting and identifying genetic material shed by organisms into their environment, such as water, soil, or air.

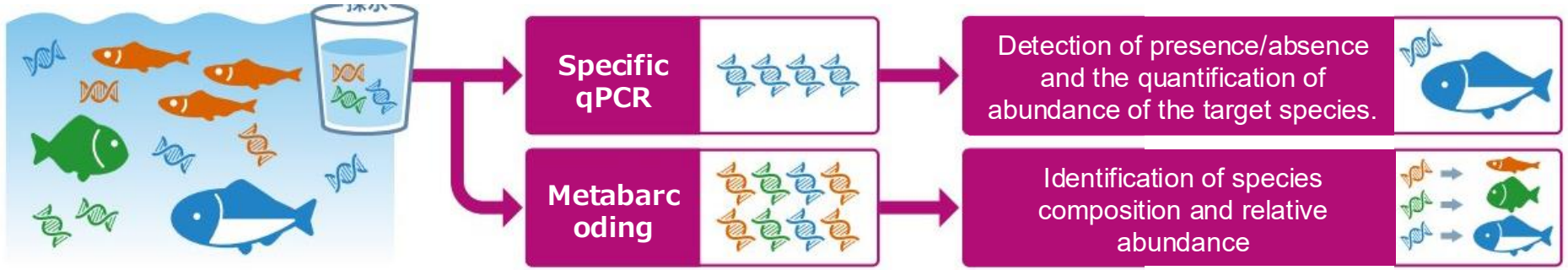
Advantages

- Non-invasive and non-destructive surveys are possible.
- Less labor-intensive and cost-effective.
- Enables detection of elusive or rare species.

Disadvantages

- Need for analysis before eDNA degradation.
- Risk of sample contamination.
- Risk of false negatives and false positives.

Workflow of eDNA analysis



Developing biomonitoring techniques is crucial for biodiversity conservation

Challenges

General eDNA concentration methods

Lab-based filtration

Glass fiber filters

On-site filtration

Sterivex cartridge

Challenges in Filtration

- ✓ Limited water volume for high-turbidity samples
- ✓ Filtration is time-consuming
- ✓ Need to improve eDNA recovery efficiency

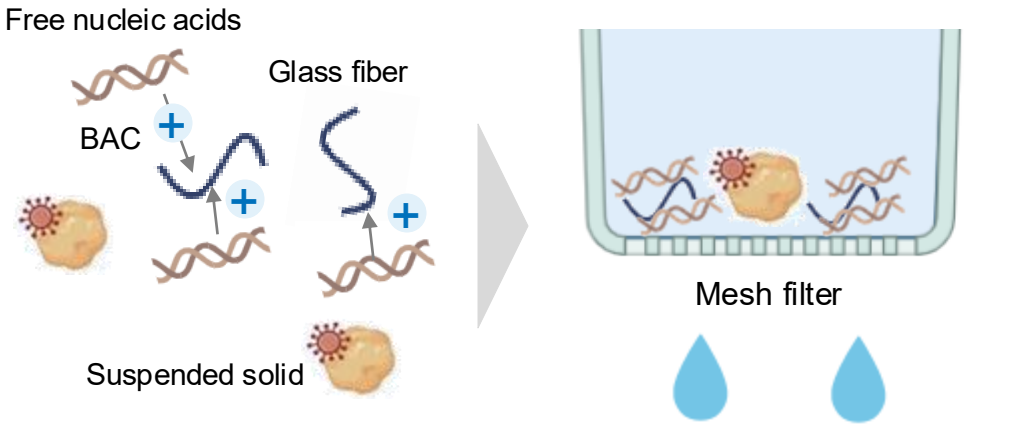
Newly developed

QuickConc™

Overview of QuickConc (QC)

Principle of QuickConc*

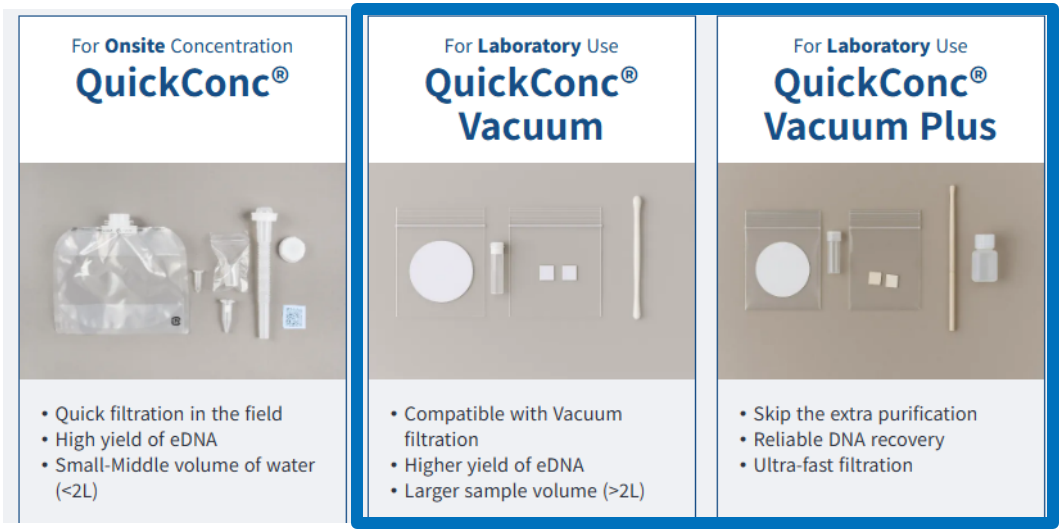
Principle of eDNA concentration



1. Positive charge of BAC promotes binding of nucleic acids to glass

2. Suspended glass fibers are filtered through a mesh filter

QuickConc® Series



Today's topic

Challenges of the manual version of QuickConc

> Difficulty filtering large-volume samples (2L or more).

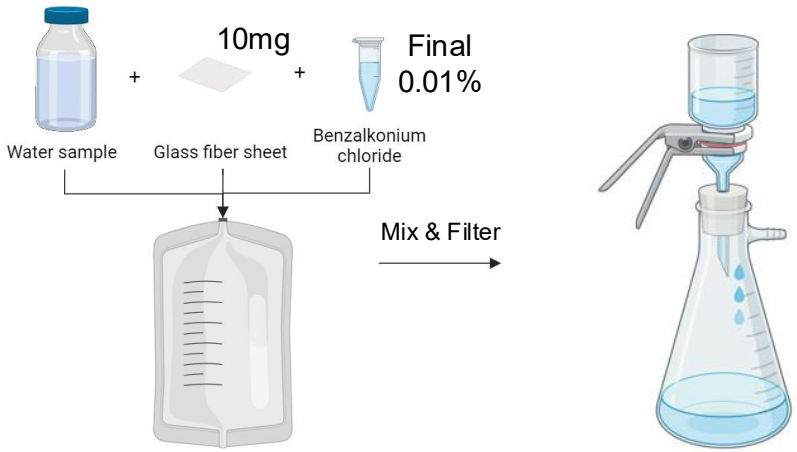
> PCR inhibition issues arise as the filtered water volume increases.

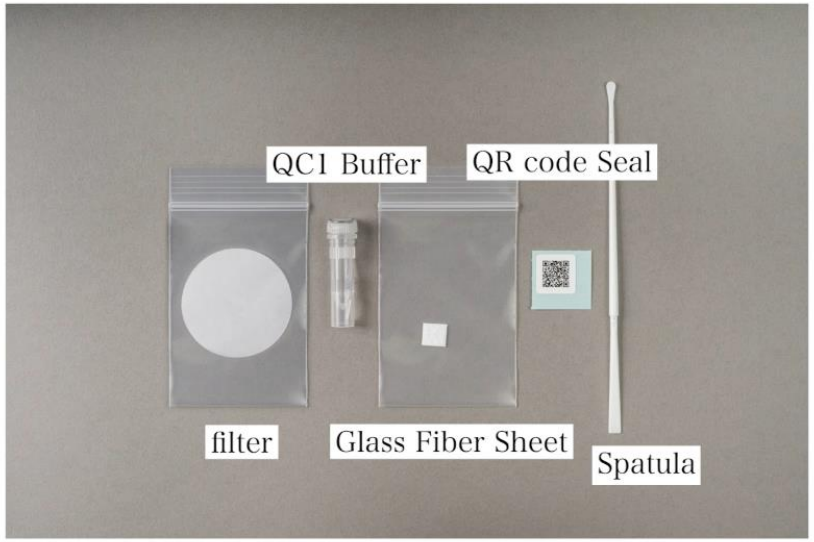
We developed a novel concentration method; QuickConc Vacuum and Vacuum Plus

*Kuroita *et al.*, ***Ecology and Evolution*** (2025)

Workflow of QuickConc Vacuum (QCV)

Workflow of QuickConc Vacuum





This kit includes all the components listed above.

1. Add the glass fiber sheet and Benzalkonium chloride
2. Mix the bottle to disperse the glass fiber sheets
3. Filtered using a standard laboratory vacuum filtration system

Rapid + **Simple** + **High yield**

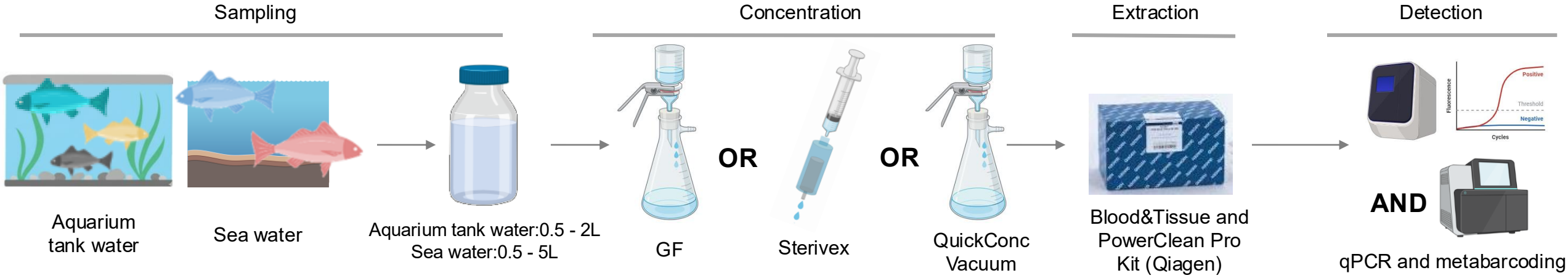
A method for achieving more efficient filtration and improved eDNA yields.

Objective and Study design

Objective

- ✓ To evaluate the utility of QCV by comparing it to conventional methods (Glass Filter and Sterivex).
- ✓ Assessing whether eDNA yield and detected number of species increase as the water sample volume increases.

Study design

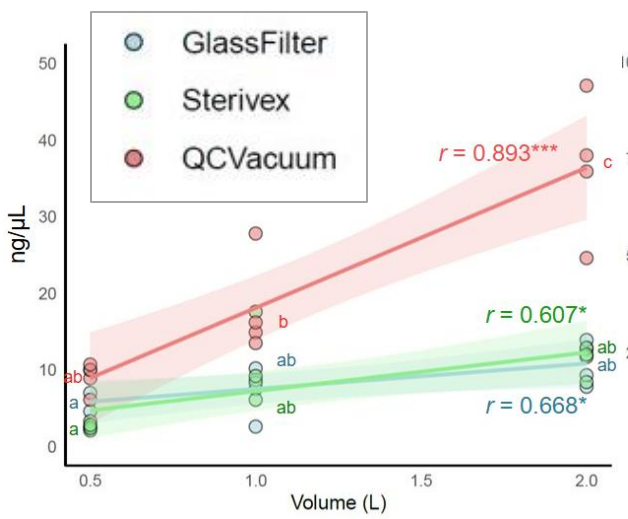


Comparison of eDNA yield, species-specific eDNA concentration, and the number of fish species

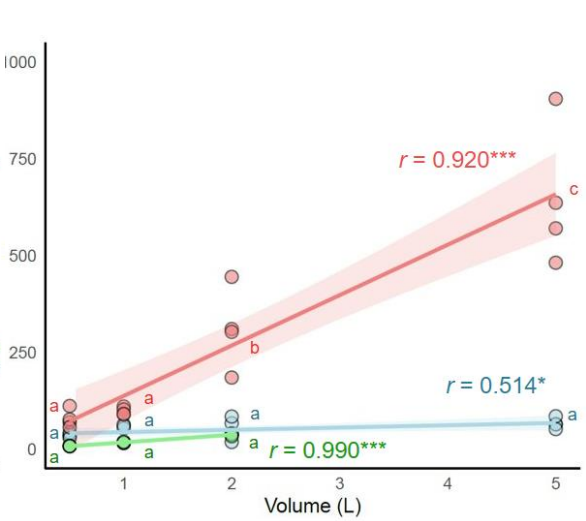
Result-1 - Comparison of total eDNA and specific eDNA yields

Comparison of total eDNA

Aquarium tank water

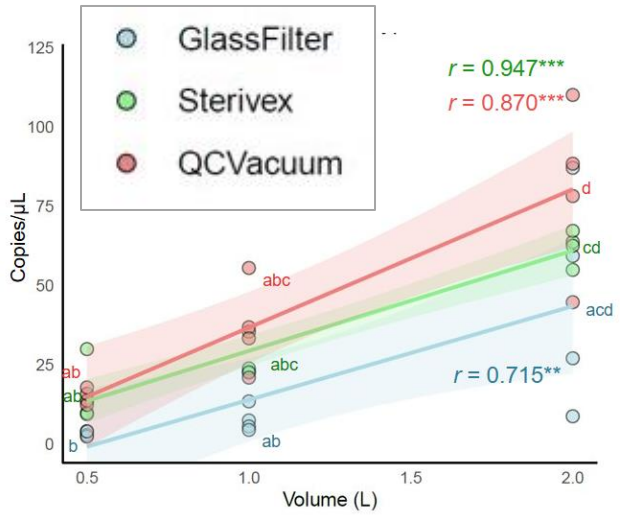


Sea water

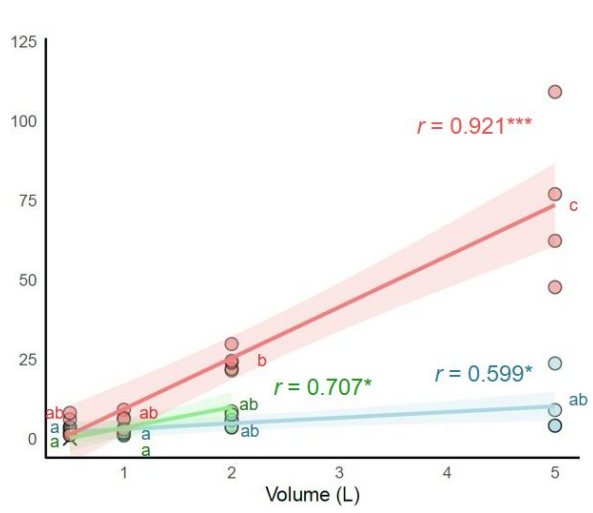


Comparison of specific eDNA yields

Aquarium tank water (*Mugil cephalus*)



Sea water (*Acanthopagrus schlegelii*)



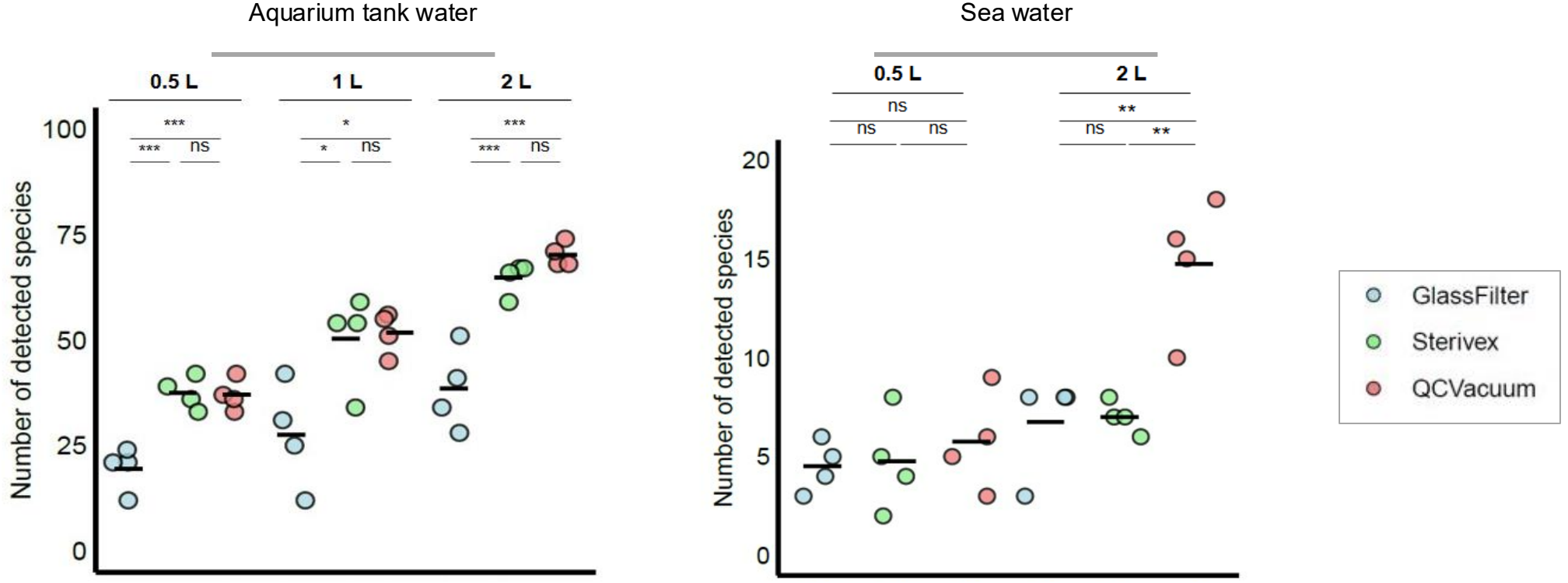
- ✓ **For all methods, both the total eDNA and the fish-specific eDNA amounts increased in correlation with the sample volume.**
- ✓ **The QCV method showed a significantly higher yield than the other two methods. As the sample volume increased, the difference in yield became much wider.**

QCV collected much more eDNA, especially from large volumes of sea water.

The different letters (abcd) are significantly different factor levels ($p < 0.05$) according to Tukey's HSD.
Statistically significant Pearson's correlation coefficients (r) are listed (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Result-2 - Metabarcoding

Comparison of the number of detected species



- ✓ **For all methods, the number of detected species increased as the sample volume increased.**
- ✓ **In Aquarium tank water, QCVacuum and Sterivex detected a significantly higher number of species.**
  In Sea water, QCVacuum detected significantly more species, particularly at the 2L sample volume.

QCV not only provided a high eDNA yield, but also detected a greater number of species in metabarcoding.

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Statistical analyses of the differences among groups were performed using Tukey's HSD ($^{ns}p \geqq 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

New Product: QuickConc® Vacuum Plus

Challenges in QuickConc

✓ To minimize the effects of PCR inhibitors, such as humic acid.

New!!

QuickConc® Vacuum Plus

Original QuickConc® Vacuum

- Higher sensitivity: more effective than conventional methods
- Large-volume sample compatibility
- Reduced operational workload

+

New Functions

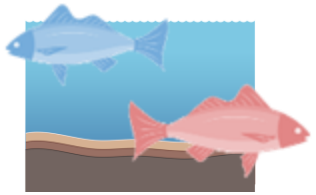
NEW
Removal of PCR inhibitors

NEW
Concentration of turbid water samples

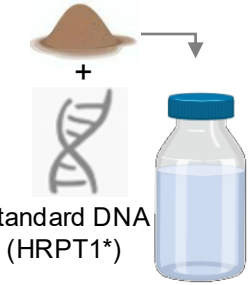
QCV Plus can simultaneously concentrate eDNA and remove PCR inhibitors by integrating an adsorbent

Result-3 - Evaluation of QuickConc Vacuum Plus (QCVP)

Sampling | Concentration & Extraction | Detection



River water



Standard DNA (HRPT1*)

Soil and Standard DNA addition to water sample

Exp-(A): Evaluation of PCR inhibition

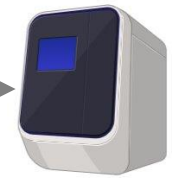
- QuickConc Vacuum
- **QuickConc Vacuum Plus**
- QuickConc Vacuum + Competitor kit(X)

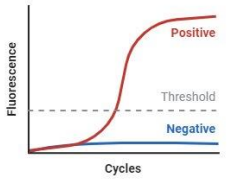
Exp-(B): Comparison of methods

GF **OR** Sterivex **OR** **QuickConc Vacuum Plus**

Internal positive control DNA (IPC**)

Blood&Tissue Kit (Qiagen)

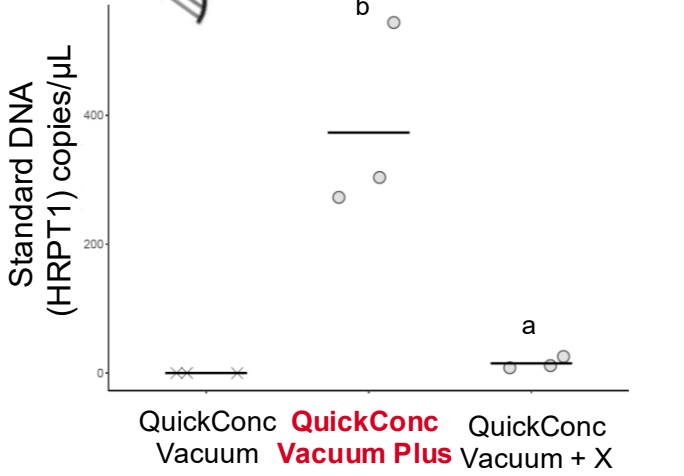


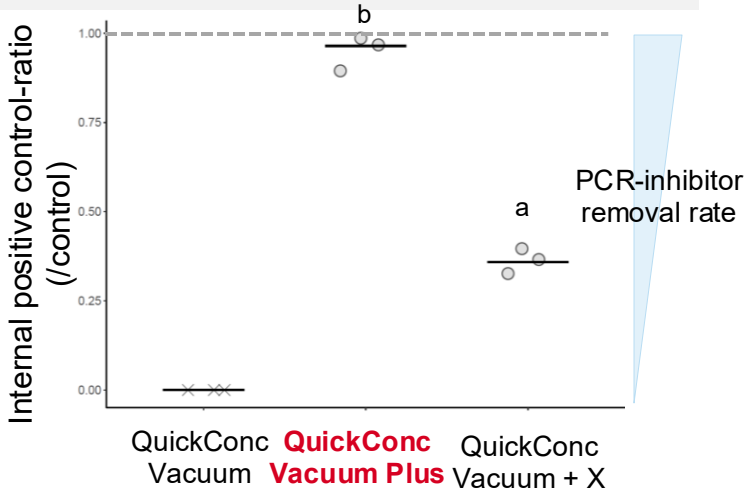


qPCR
- Exp-(A): HRPT1 and IPC
- Exp-(B): Cyprinus carpio

*HPRT1: Hypoxanthine Phosphoribosyl Transferase 1
**IPC: Internal positive control

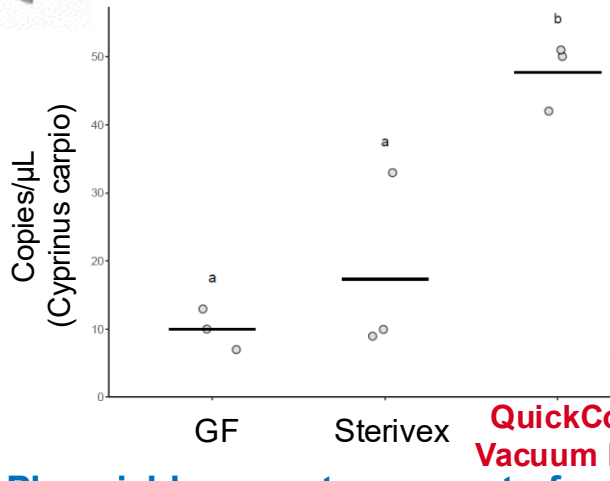
Exp-(A): Evaluation of PCR inhibition





QCV Plus more effectively removes PCR inhibitors than competitor kits.

Exp-(B): Comparison of methods



QCV Plus yields a greater amount of species-specific eDNA compared to conventional methods.

QCVPlus can not only concentrate eDNA at a high yield, but also simultaneously remove PCR inhibitors.

Summary

- The QuickConc Vacuum (QCV) method successfully overcomes the volume limitations of the original manual method.
- QCV demonstrated higher eDNA yield and a greater number of detected fish species compared to existing methods in environmental samples.
- QCV Plus shows promise as an environmental water concentration method that can simultaneously concentrate eDNA and remove PCR inhibitors.
- QCV/QCV Plus is expected to be a valuable new tool for biodiversity monitoring.

Thank you for listening