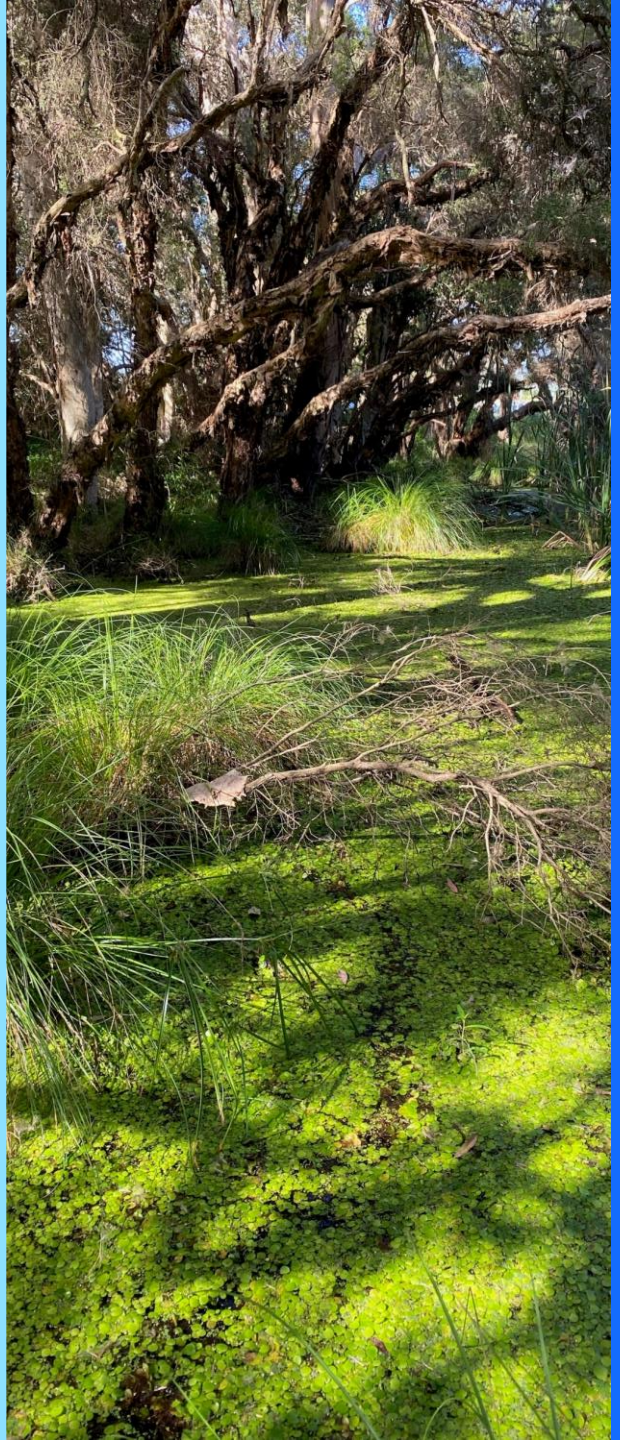
Department of Primary Industries and Regional Development



QPCR-BASED ENVIRONMENTAL RNA (ERNA) APPROACH MIGHT NOT BE SUITABLE FOR AQUATIC WEED BIOSECURITY – A case study using Amazon frogbit (*Limnobium laevigatum*)

Dr Xiaocheng Zhu
Dr Karen Bell
Dr Hanwen Wu
Dr David Gopurenko

20 February 2025

dpird.nsw.gov.au

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eDNA

“genetic material obtained directly from environmental samples without any obvious signs of biological source material”

Issue with legacy DNA

Less spatiotemporal reliable

https://www.nps.gov/articles/aps-19-1-6.htm

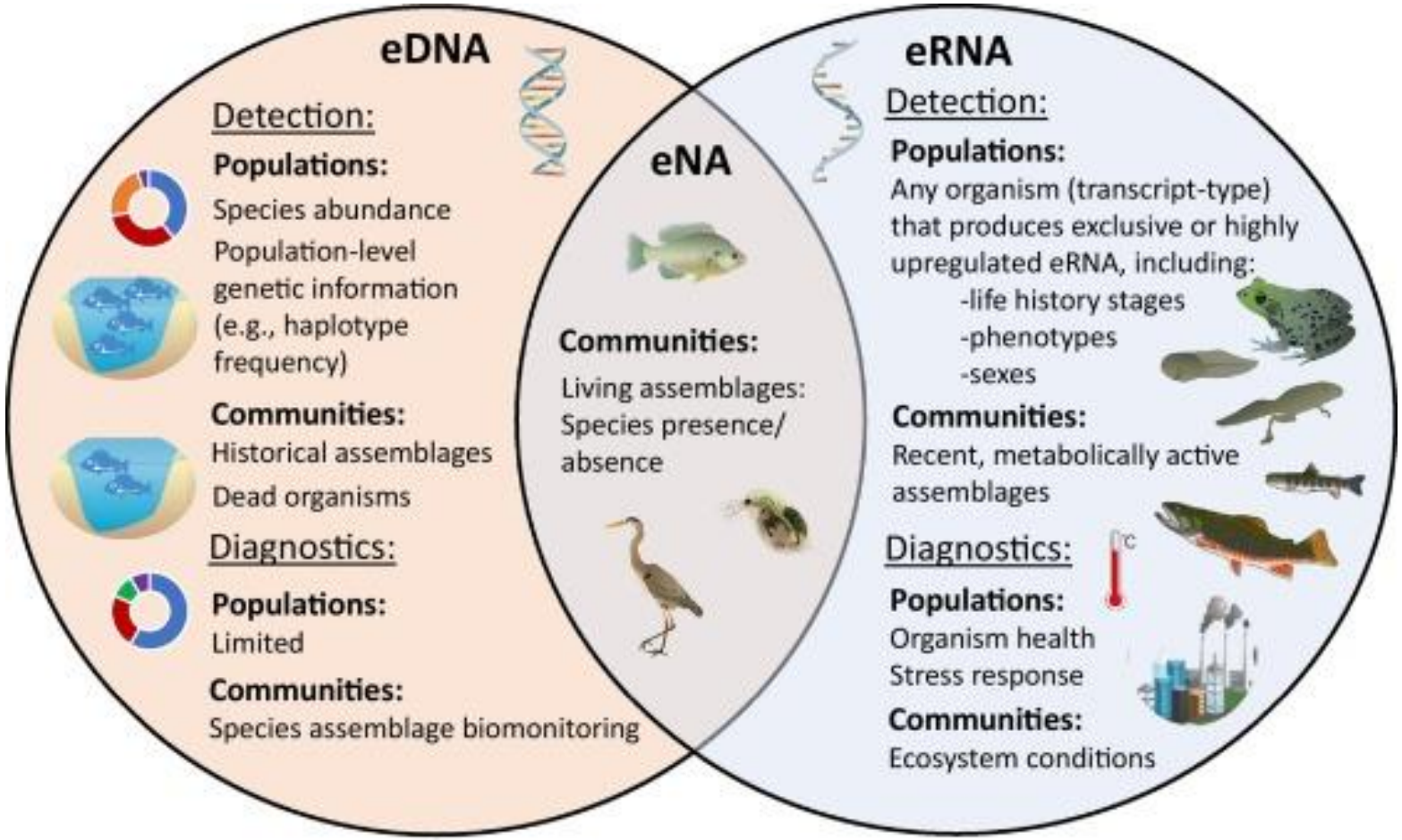
eRNA

Persist for much shorter period

Spatiotemporal precision

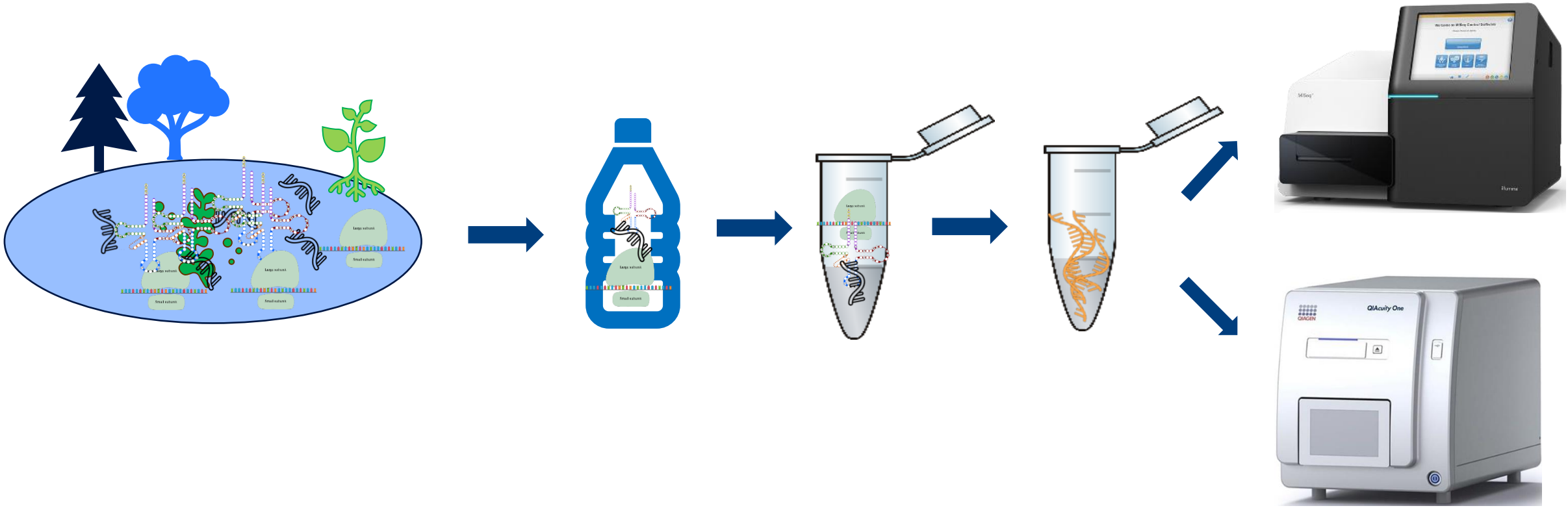
Only produced by living organisms

Life stage and health evaluation



(Yates et.al, 2021) Trends in Ecology & Evolution

eRNA workflow



Received: 17 November 2023 | Revised: 17 February 2024 | Accepted: 12 March 2024

DOI: 10.1002/edn3.536

Environmental DNA

Dedicated to the study and uses of environmental DNA for basic and applied sciences

WILEY

METHOD

Generic qPCR assays for quality control in environmental DNA research

Xiaocheng Zhu[1] | Karen L. Bell[1,2] | Meaghan L. Rourke[3] | Hanwen Wu[1] | David Gopurenko[1]

[1]NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Wagga Wagga, New South Wales, Australia

[2]School of Biological Sciences, The University of Western Australia, Perth, Western Australia, Australia

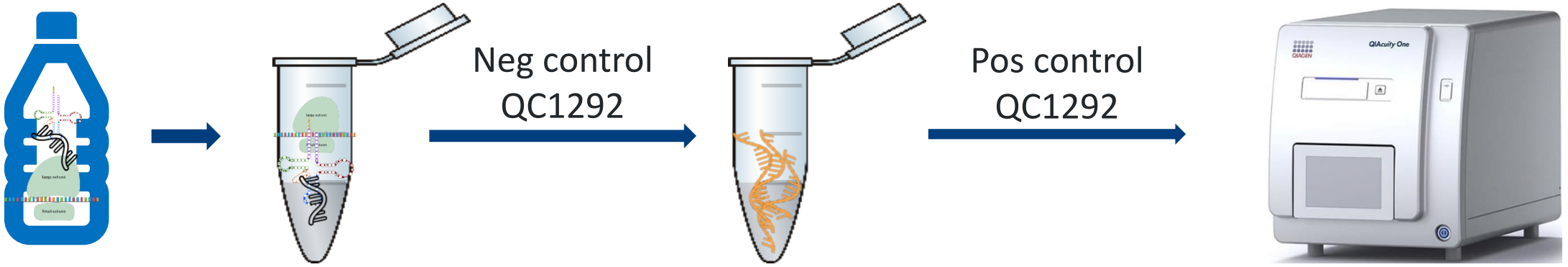
[3]NSW Department of Primary Industries, Narrandera Fisheries Centre, Narrandera, New South Wales, Australia

Correspondence

Abstract

Environmental DNA (eDNA) has been widely used for species surveillance. However, the lack of adequate quality control in many eDNA research projects and applications can lead to false-negative results, greatly affecting biosecurity surveillance and conservation efforts. Exogenous DNA is routinely added to eDNA samples and used as a positive control, typically after DNA extraction. However, this type of positive control is only able to identify false negatives due to errors at the amplification stage. Therefore, errors in upstream processes, such as sample collection will not be

eRNA workflow



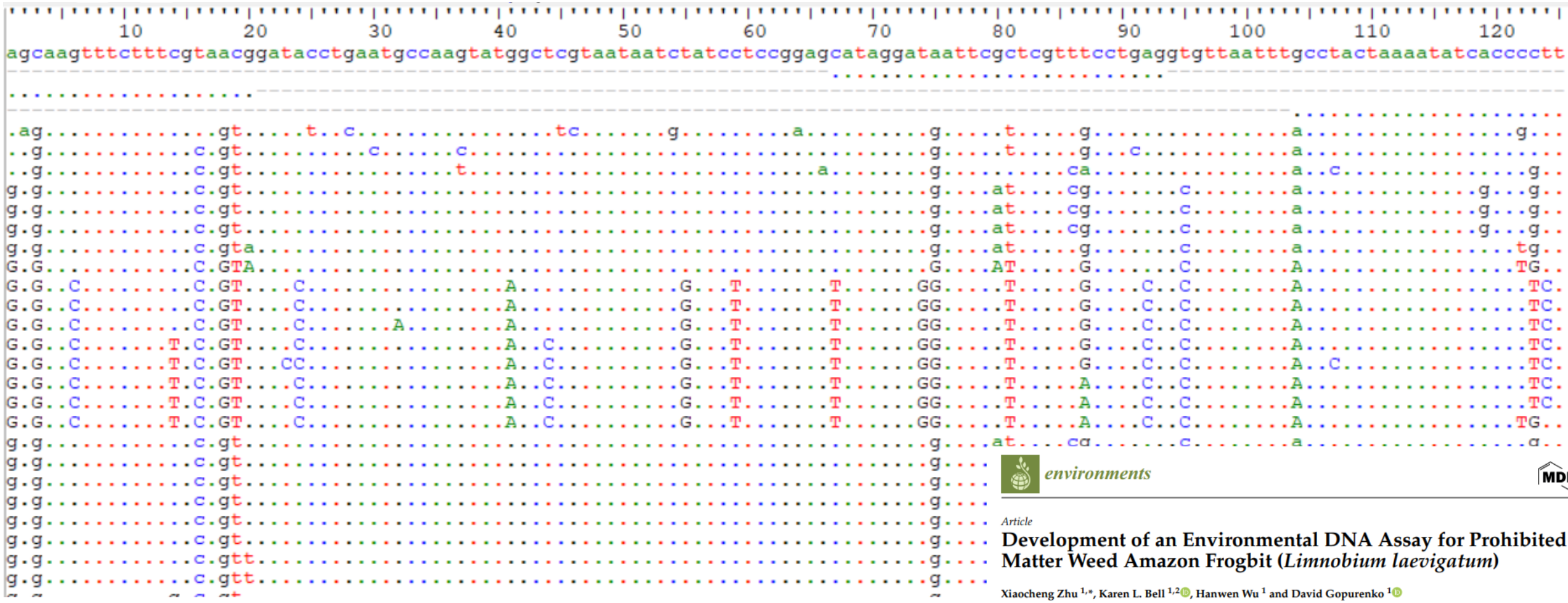
Verify gDNA removal

Verify RNA extraction and cDNA synthesis

Target species: Amazon frogbit (*Limnobium laevigatum*)

- Prohibited Matter Weeds
- Found in QLD, NSW, WA, NT and VIC
- Perennial, fast-growing, freshwater species
- Free-floating or with the roots anchored in the underwater substrates
- Dispersed by water
- Most part of the plant is submerged in the water

Amazon frogbit assay



environments

MDPI

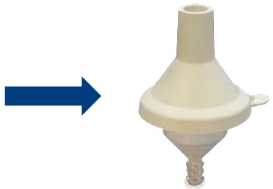
Article

Development of an Environmental DNA Assay for Prohibited Matter Weed Amazon Frogbit (*Limnobium laevigatum*)

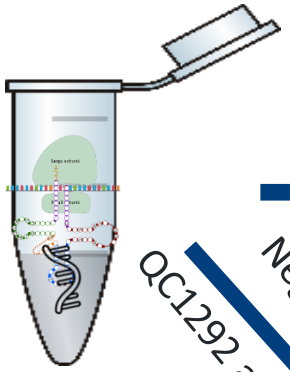
Xiaocheng Zhu [1,*], Karen L. Bell [1,2], Hanwen Wu [1] and David Gopurenko [1]

Experimental design

Sampling points: 0h, 2h, 6h, 24h, 48h, 72h, 96h, 7D

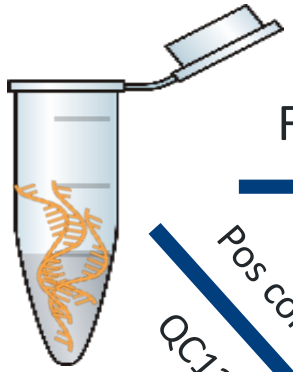


500ml
0.45µm



Neg control
QC1292 and Frogbit assay

Verify gDNA removal



Pos control
QC1292

Frogbit assay

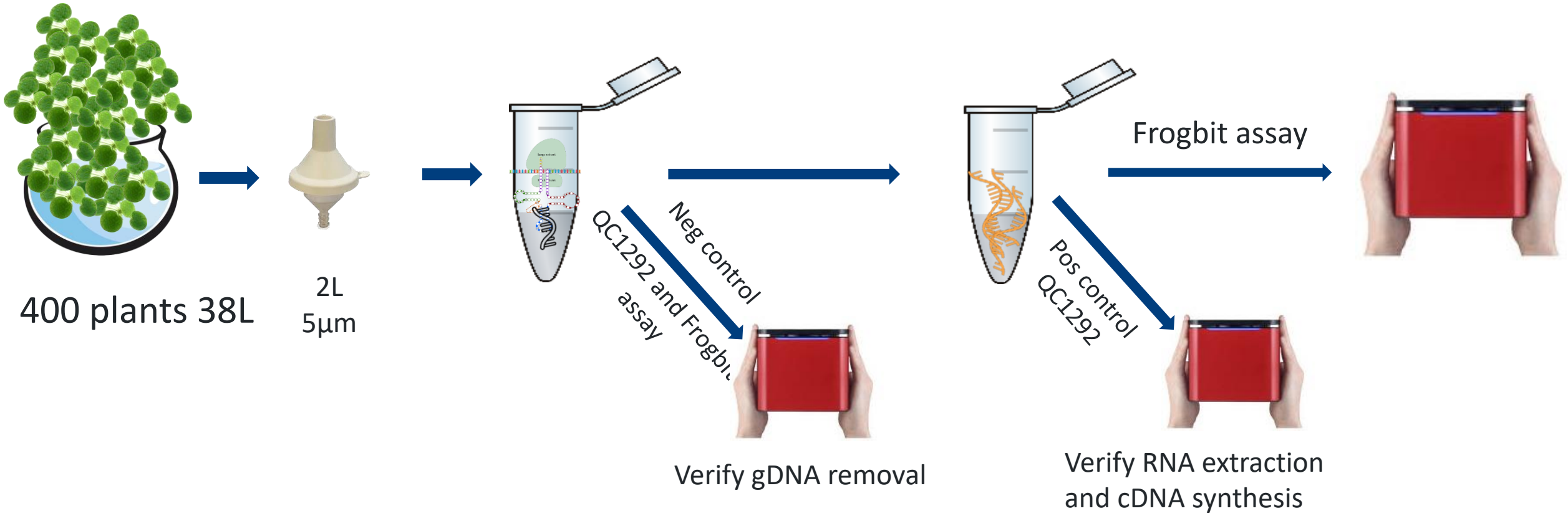
Verify RNA extraction and cDNA synthesis

Results

	0h		2h		6h		24h		48h		72h		96h		7D	
	Cq	Conc	Cq	Conc	Cq	Conc	Cq	Conc	Cq	Conc	Cq	Conc	Cq	Conc	Cq	Conc
Frogbit assay																
cDNA	36.66	2.21	36.23	0.99	37.04	0.58	-	0	36.28	0.95	36.28	1.91	36.16	1.08	-	0
RNA	32.89	18.58	34.16	3.91	-	0	-	0	-	0	-	0	-	0	-	0
QC assay																
cDNA	16.10	2.19E+06	16.23	1.88E+06	16.55	1.49E+06	17.69	8.82E+05	16.30	2.10E+06	15.93	2.37E+06	15.58	2.98E+06	14.86	7.89E+06
RNA	32.54	15.19	32.56	5.17	34.17	5.87	33.73	3.95	32.17	13.64	31.05	30.58	31.29	58.17	30.38	120.88

Conc: Concentration (copies/µL)

Experimental design



Results

Amazon frogbit eRNA detection

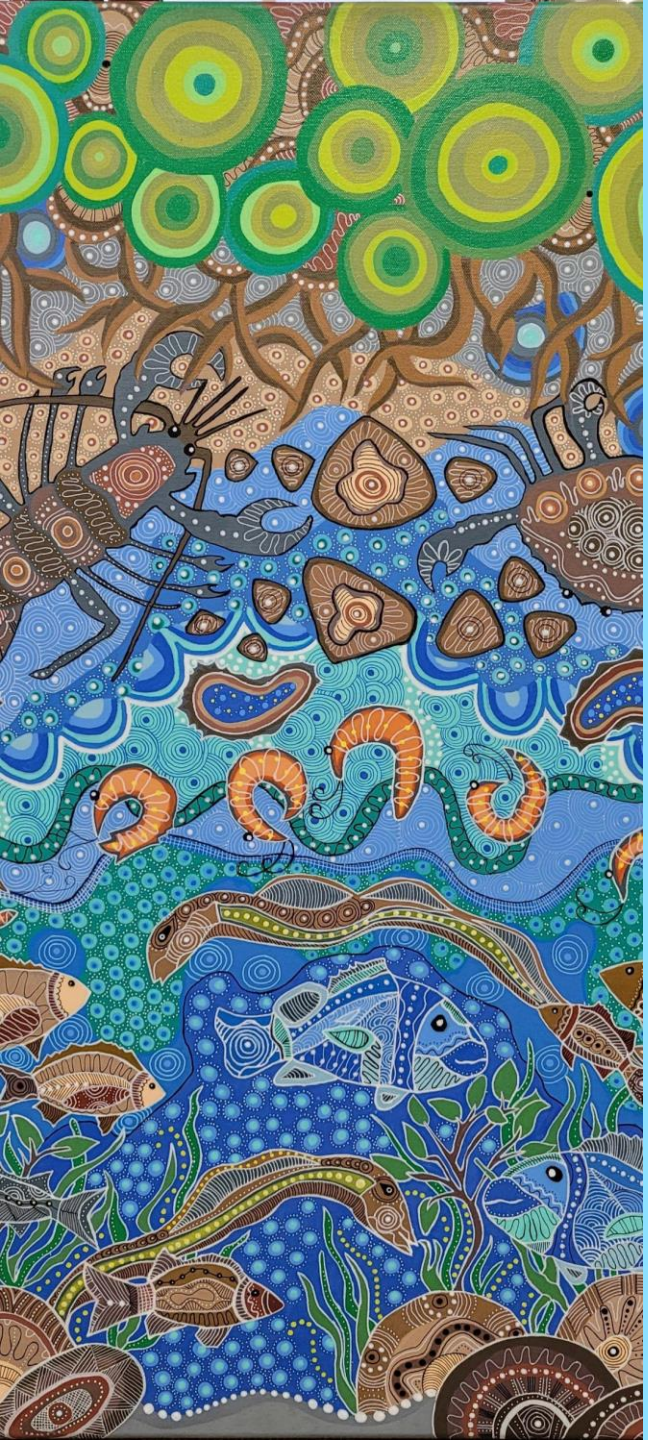
gDNA removal method	cDNA synthesis method	Cq	Copies per µL (Mean ± SE)
Column and Enzyme	Random primers	33.2	102.95 ± 13.08^{ab}
Enzyme only	Random primers	33.5	75.23 ± 5.77^{a}
Column and Enzyme	Specific primer	32.7	135.8 ± 7.91^{bc}
Enzyme only	Specific primer	33.1	103.26 ± 6.28^{ab}
Column and Enzyme	Specific and random primers	32.5	151.12 ± 6.99^{c}
Enzyme only	Specific and random primers	32.8	119.47 ± 8.47^{bc}
RNA negative		-	0

Quality control eRNA detection

gDNA removal method	cDNA synthesis method	Cq	Copies per µL (Mean ± SE)
Column and Enzyme	Random primers only	10.7	2.30E+08 ± 6.36E+07
Enzyme only	Random primers only	12.1	7.83E+07 ± 3.90E+06
Column and Enzyme	Specific primer	21.3	2.48E+05 ± 6.55E+04
Enzyme only	Specific primer	18.9	6.11E+05 ± 8.57E+04
Column and Enzyme	Specific and random primers	11.6	1.08E+08 ± 2.41E+07
Enzyme only	Specific and random primers	13.1	3.91E+07 ± 2.82E+06
RNA negative		36.7	1.33 ± 0.47

Take home messages

- eRNA of Amazon frogbit is detectable only when the plant is highly abundant.
- rRNA sequence might be more abundant but is difficult for qPCR assay design.
- Metabarcoding approach targeting rRNA sequence might work better in case of plant biosecurity.
- For biosecurity purpose, detection of highly abundant target species might not be efficient.
- eRNA will still be a useful tool for conservation studies.



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Acknowledgement

- NSW DPIRD Agriculture & Biosecurity for funding support
- Department of Environment Parks & Water Security, NT
- QLD Department of Agriculture and Fisheries
- Many weed officers, individuals and organizations

Thank you

Dr Xiaocheng Zhu
Research Scientist, Weeds and Plant biosecurity
NSW Department of Primary Industries and Regional Development
Wagga Wagga Agricultural Institute
Xiaocheng.Zhu@dpi.nsw.gov.au

23S rRNA alignment

16S rRNA alignment