



Chasing Flamingos

Tracking synthetic DNA movement in a river

Suzanne Thompson^{1,2}, Gavan McGrath², Annette
Koenders¹, Josephine Hyde^{2,3}, Anna Hopkins¹

¹ Molecular Ecology and Evolution Group, School of Science, Edith Cowan University, Western Australia

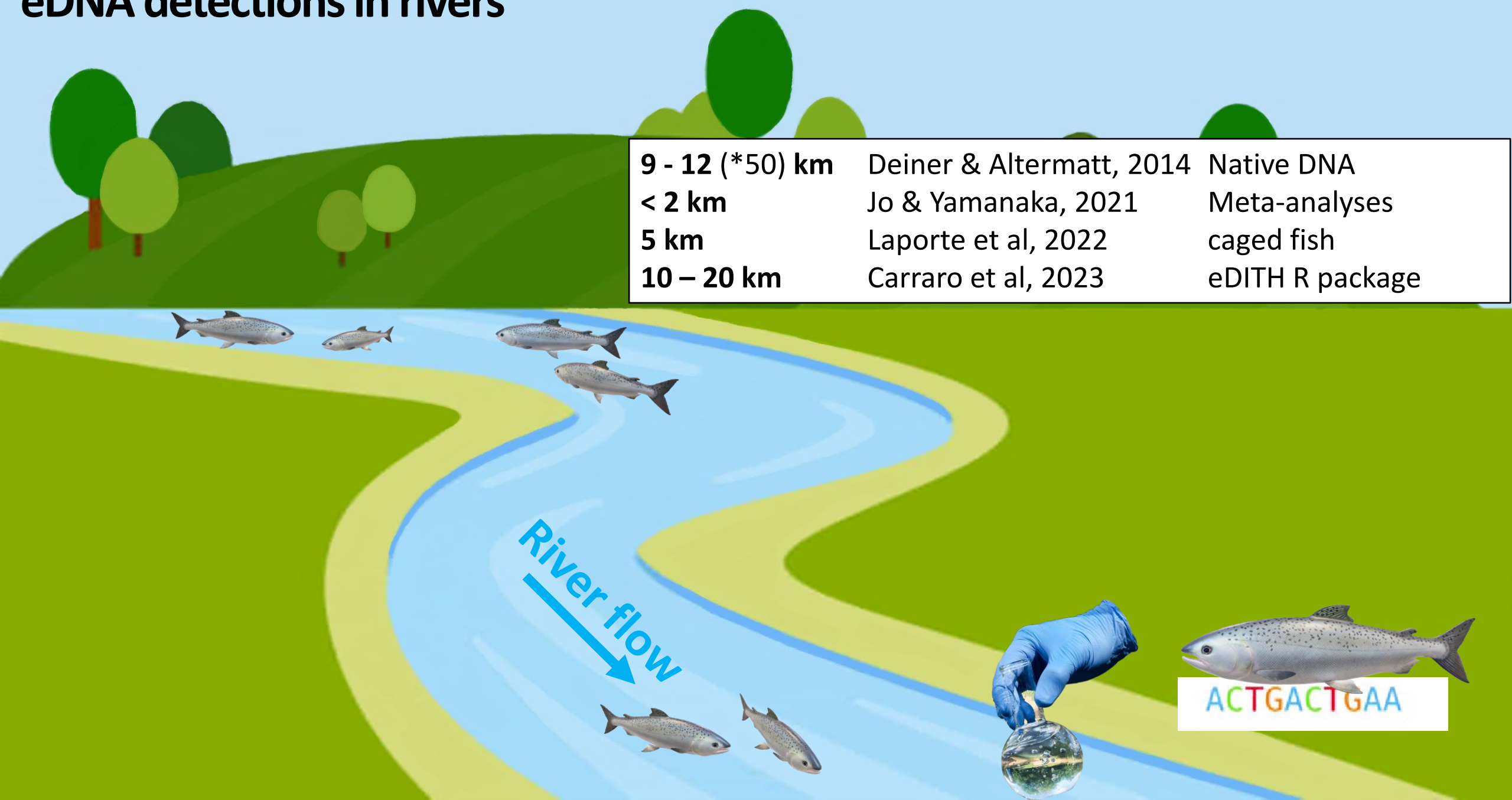
² Department of Biodiversity, Conservation and Attractions, Western Australia

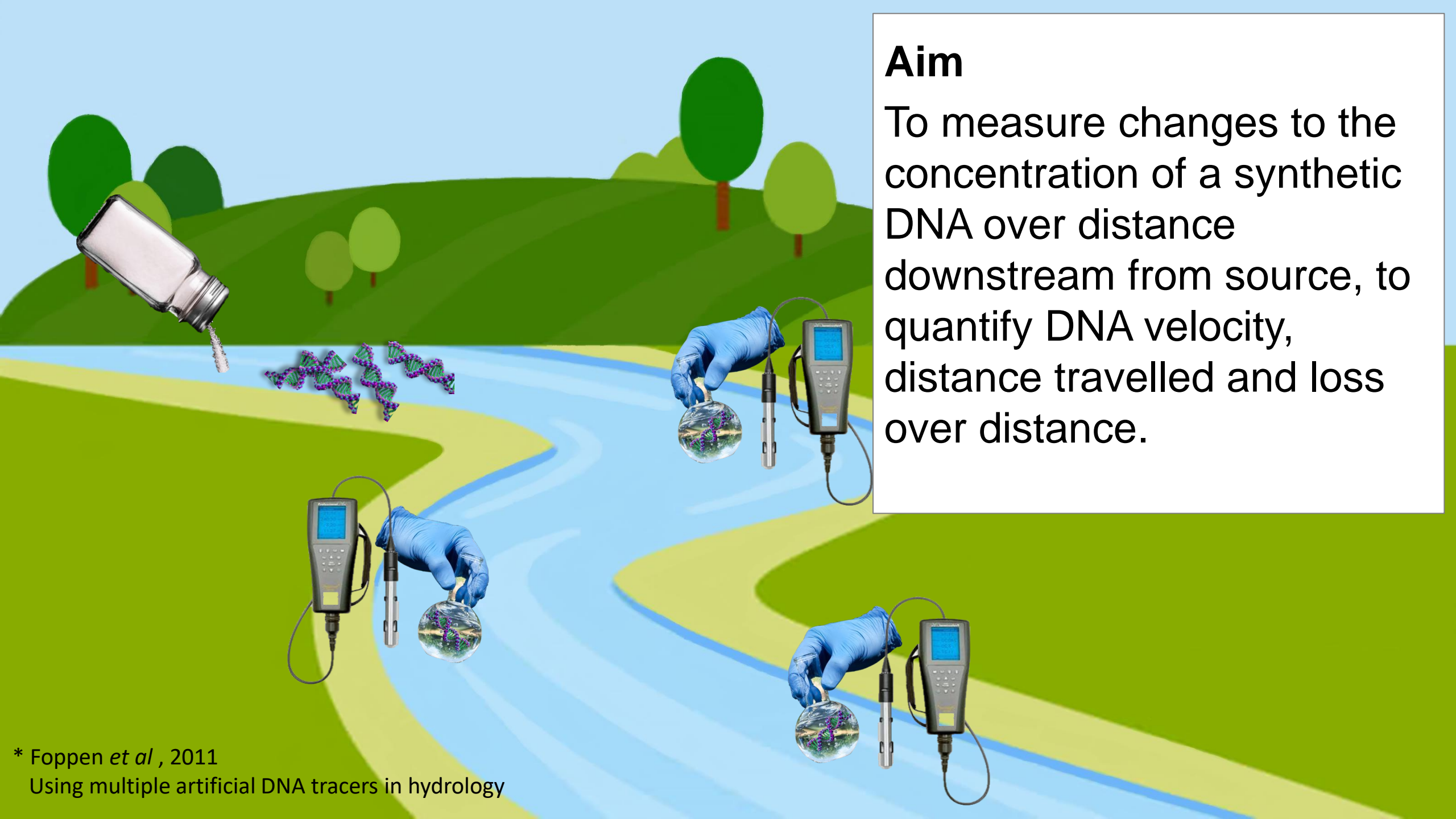
³ Biologic Environmental



eDNA detections in rivers

9 - 12 (*50) km	Deiner & Altermatt, 2014	Native DNA
< 2 km	Jo & Yamanaka, 2021	Meta-analyses
5 km	Laporte et al, 2022	caged fish
10 – 20 km	Carraro et al, 2023	eDITH R package





Aim

To measure changes to the concentration of a synthetic DNA over distance downstream from source, to quantify DNA velocity, distance travelled and loss over distance.

Experiment zone

- Southern River, Perth
- 1.4 km
 - water level gauges
 - Discharge measurements upstream and downstream
- Optimal flow rates for experiment
- Volume of experiment reach



DNA design

gBlock gene fragment

- Integrated DNA Technology
- Double strand
- < 1,000 bp



Template DNA



American flamingo
(*Phoenicopterus ruber*)
Cytochrome B

+

Random DNA sequence =

```

GCGCACCTTCTTAATCTAC
CACTGGTTCTGCATGTAAC
ATGCGCCAGCTTCTAACTAC
GGACAACCGCAGTTACTAC
CCACGCTGCACTGTAATAC
TAGATTAAGGGTCTGGATC

```



Design New Primers

synthetic DNA

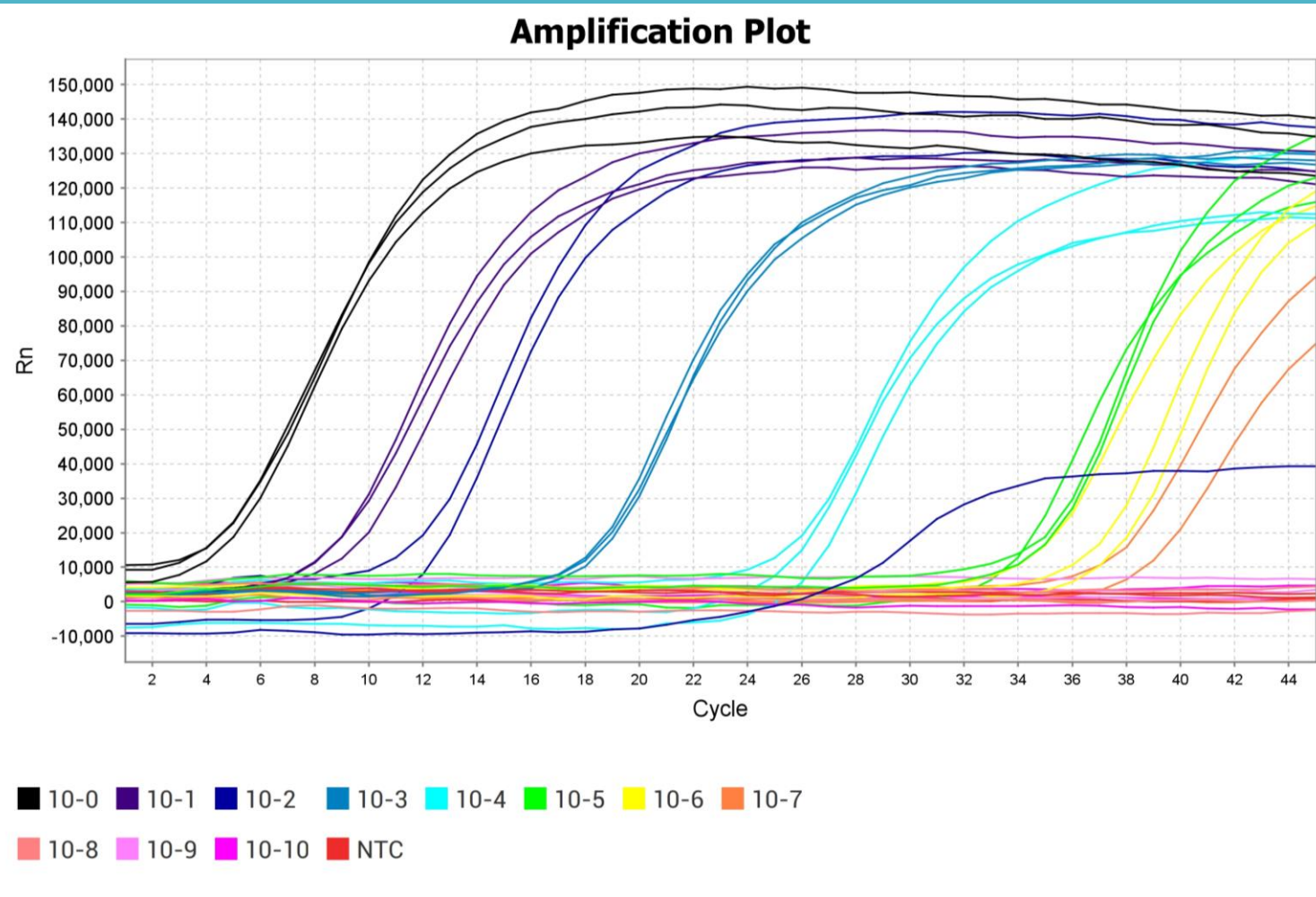


Fake flamingo
(*Phoenicopterus plastica*)

Test DNA



- 1) In silico testing
 - Uniqueness
 - Check for complex structure
- 2) In vitro specificity testing:
 - Select best primers
 - Optimise assay
 - Assay sensitivity



97,000 copies /L river = 581 trillion

Salt

100 kg in 450 L



Fake Flamingo DNA

2.8 quadrillion copies

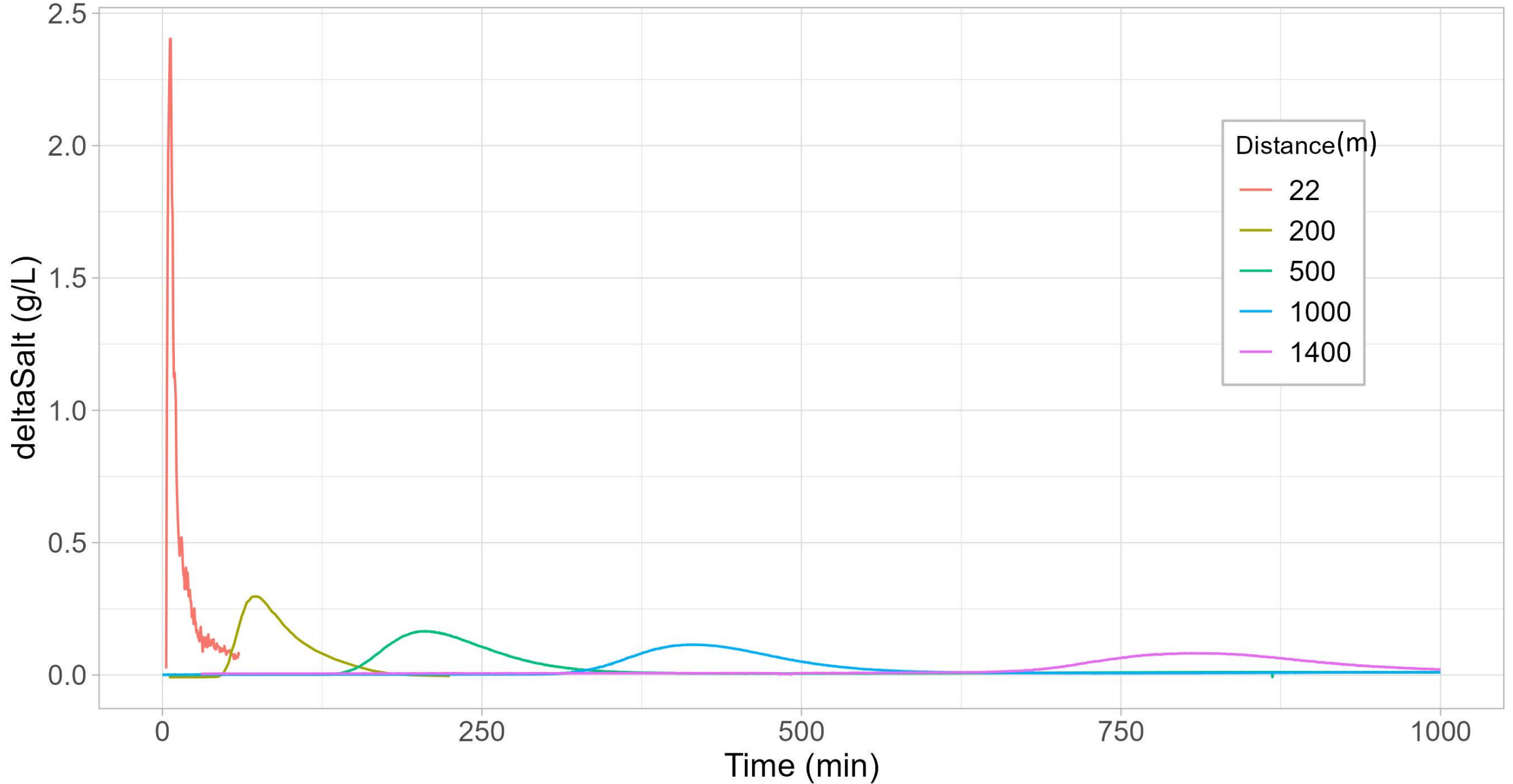


Tracking salt and flamingos

- 3 teams + 2 rovers
- 3 x YSI ProDSS
- 4 x solinst EC loggers
- 2 x autosamplers



Salt tracking



Catching and counting flamingos

**Sample
water**



Filter



**Extract
DNA**



**Amplify
DNA**



Quantify DNA



**DNA
copies / L
river water**



**Volume
filtered**

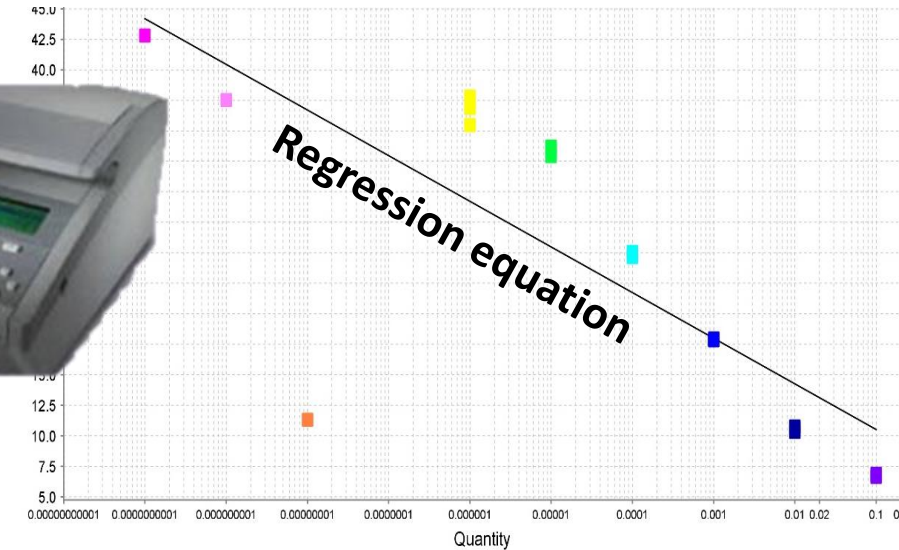


**Extract
volume**



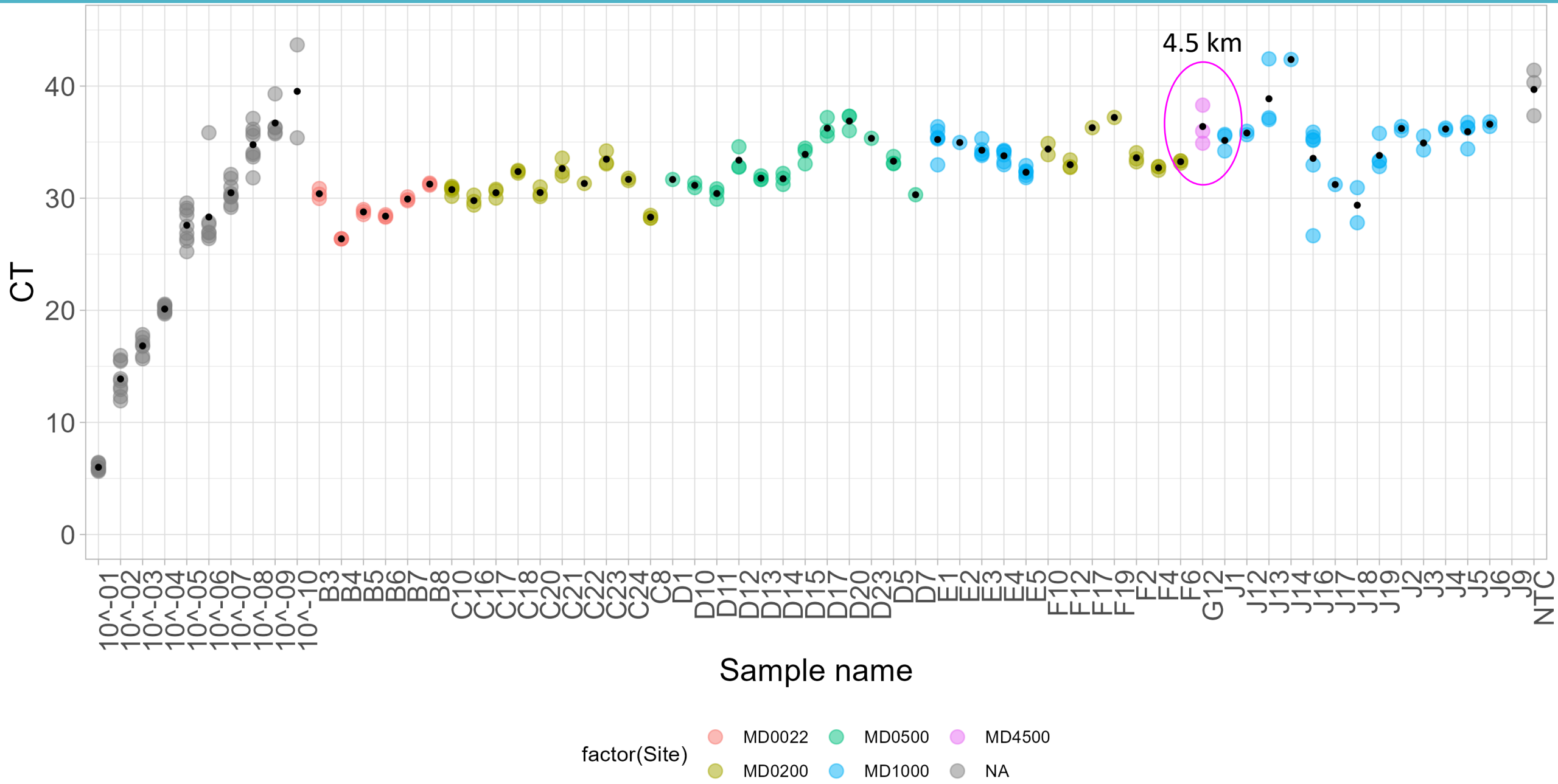
2 uL

Standard curve : 10-fold dilution series

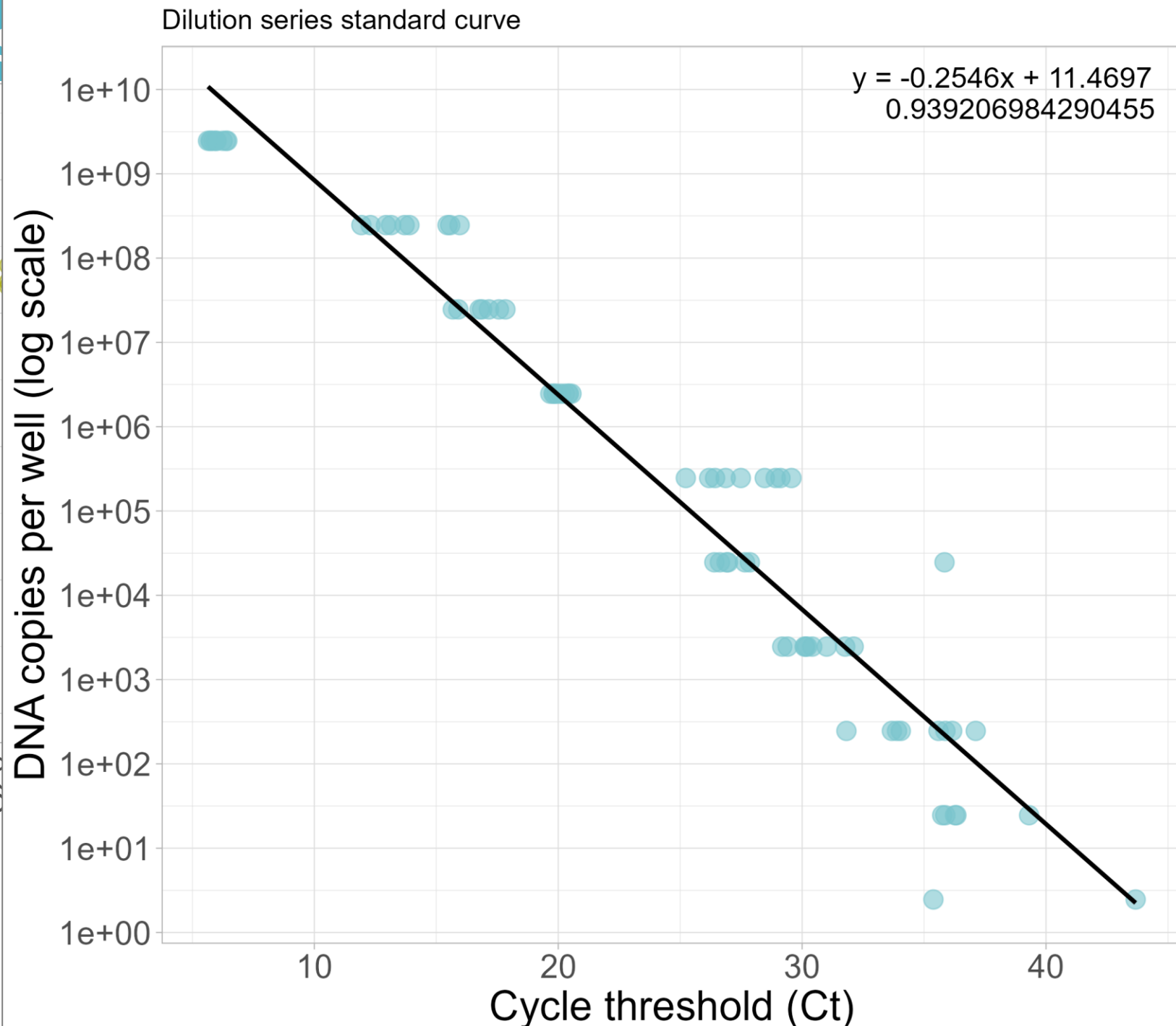
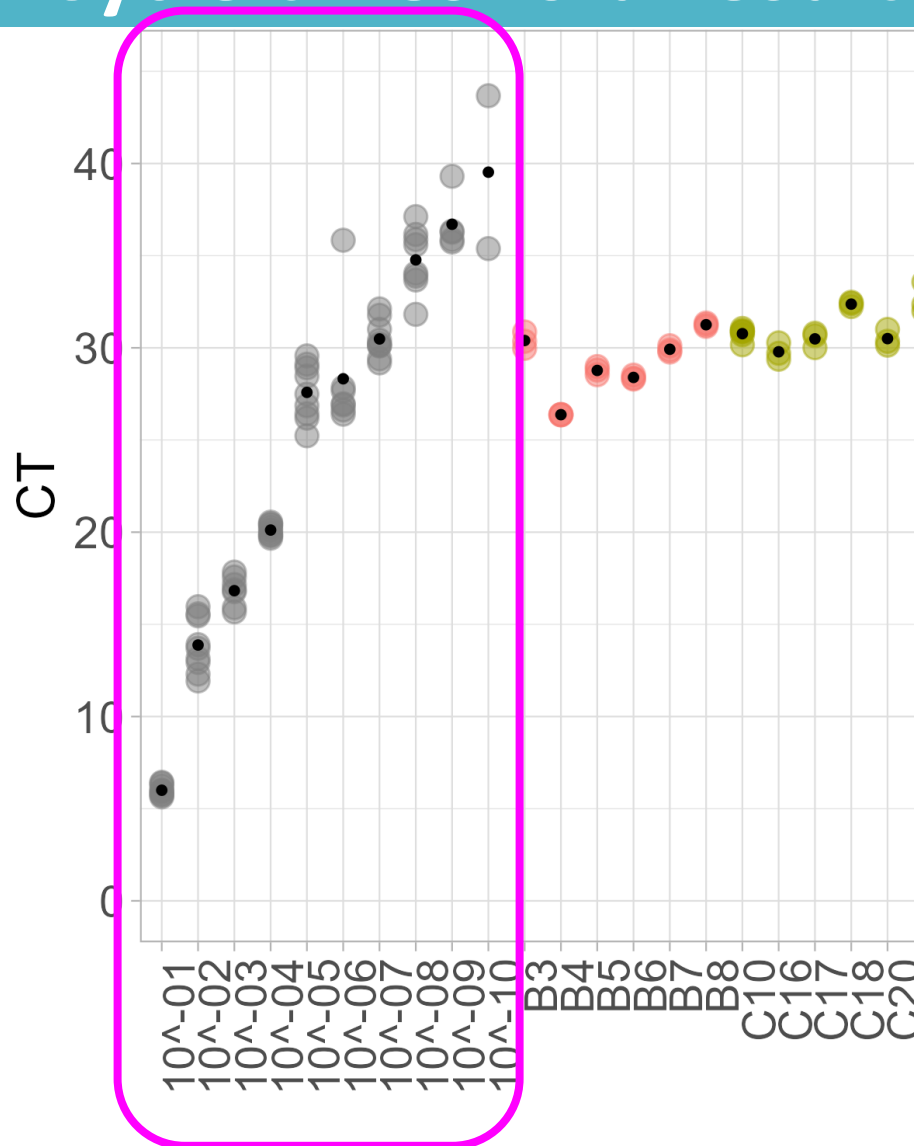


**Ct → DNA copies
in well**

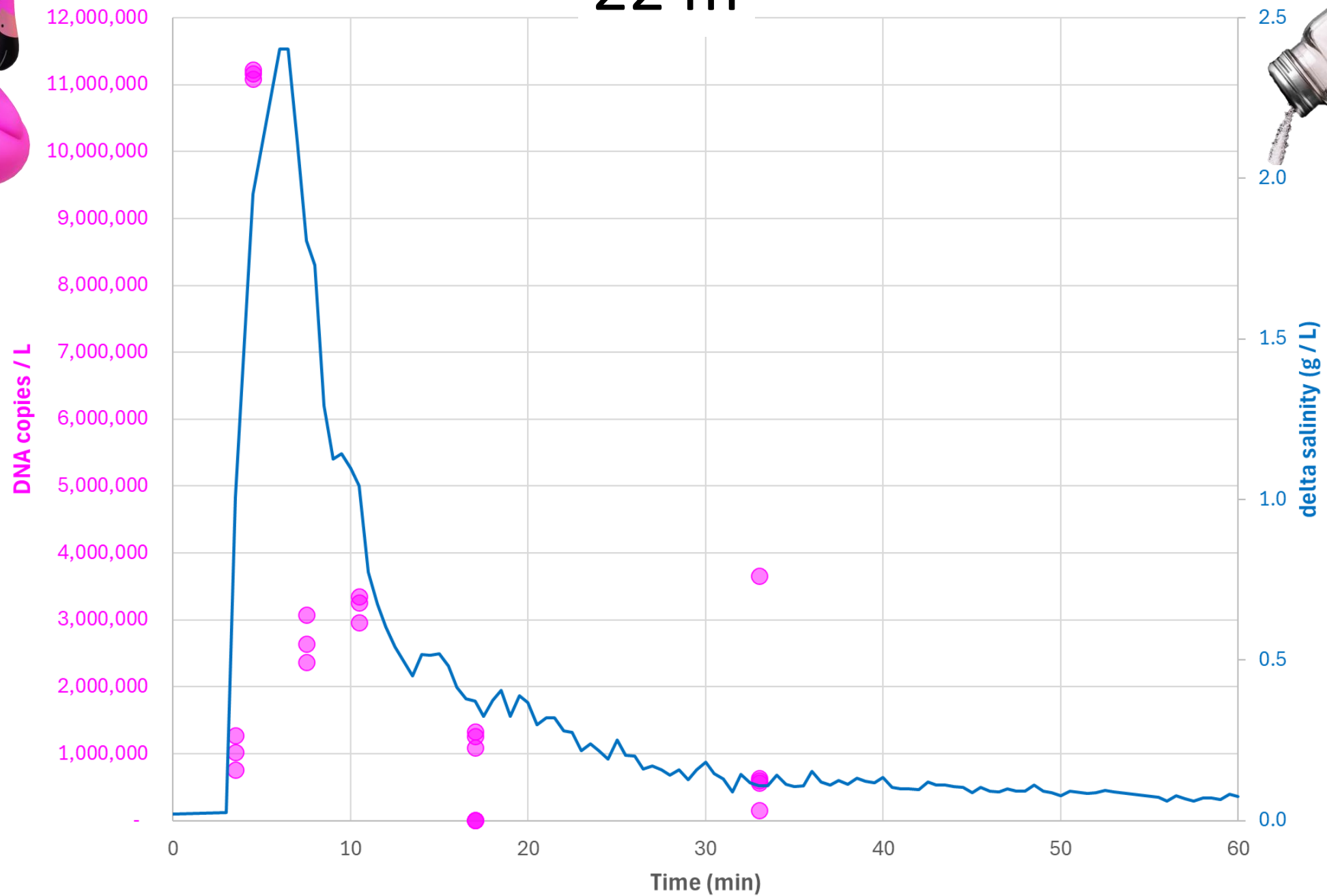
Cycle threshold results



Cycle threshold results

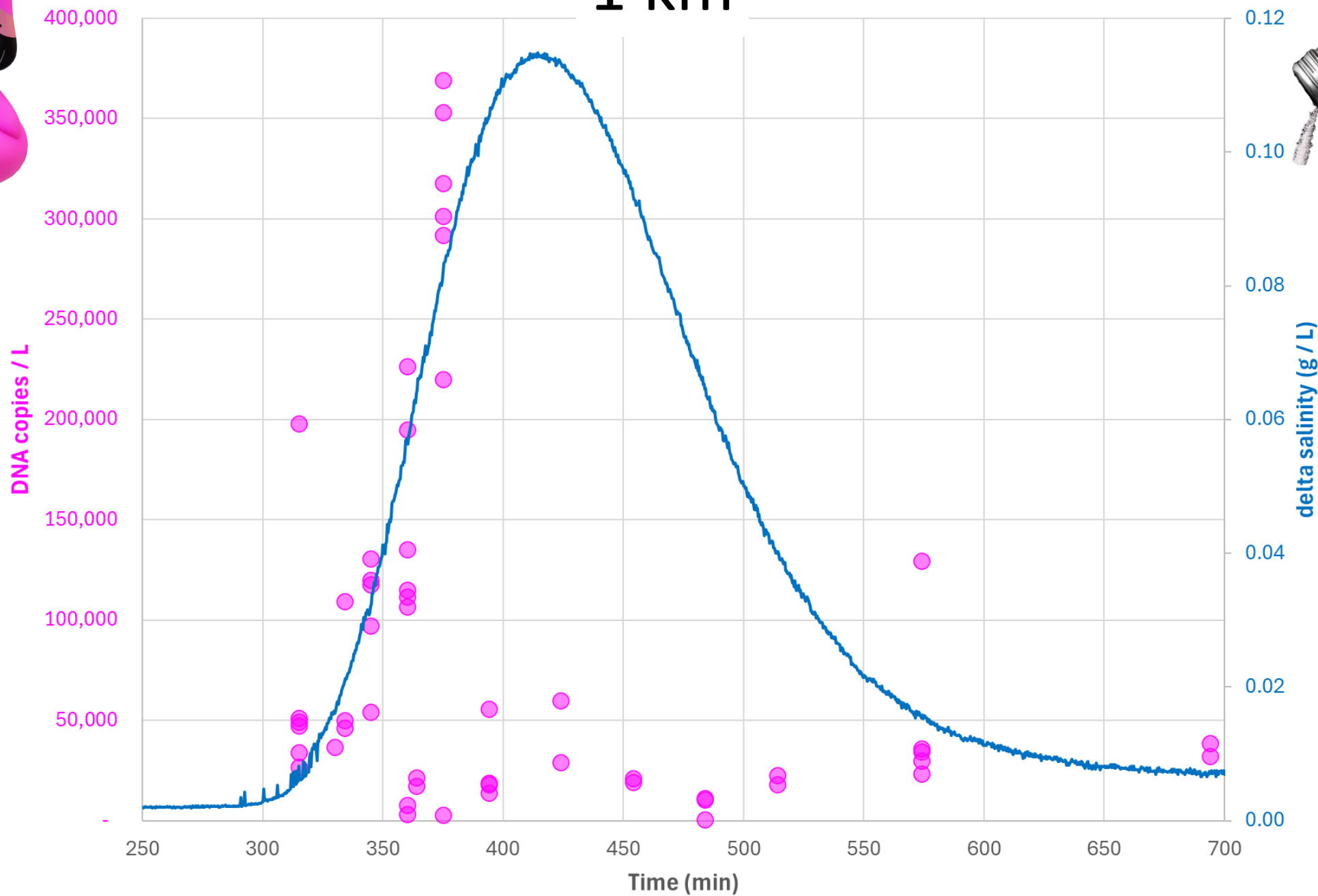


22 m





1 km



Key findings to date:

DNA is highly variable

- Further investigation into CT analysis and treatment of variation between replicates.

DNA dispersal differs to salt

- Will investigate potential causes

DNA detected 4.5 km downstream

- where salt was detected at near background salinity.
- Indicates that DNA may be a more sensitive hydrological tracer.

Next steps

Reactive Transport model

ReacTran in R :

One-dimension model

- diffusion and advection



Acknowledgements

Supervisors : Dr Gavan McGrath (DBCA)
Dr Anna Hopkins (ECU)
A/Prof Annette Koenders (ECU)
Dr Josephine Hyde (Biologic)

Special thanks

Tom Ryan (DBCA) in-field equipment management
Tina Berry (eDNA Frontiers) guidance in eDNA and primer design
Field crew: (ECU) Rachele Bernasconi, Alecia Gorbould, Bec Quah,
Jasmine Leighton
Greg Thompson: equipment manufacture

