Early Detection of a Highly Invasive Bivalve Based on Environmental DNA: Methods Development and Optimization

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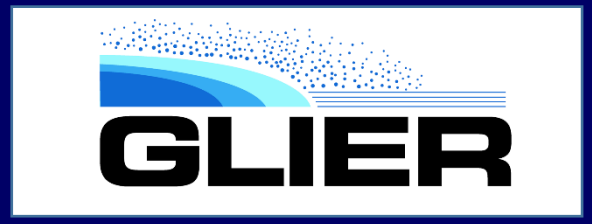
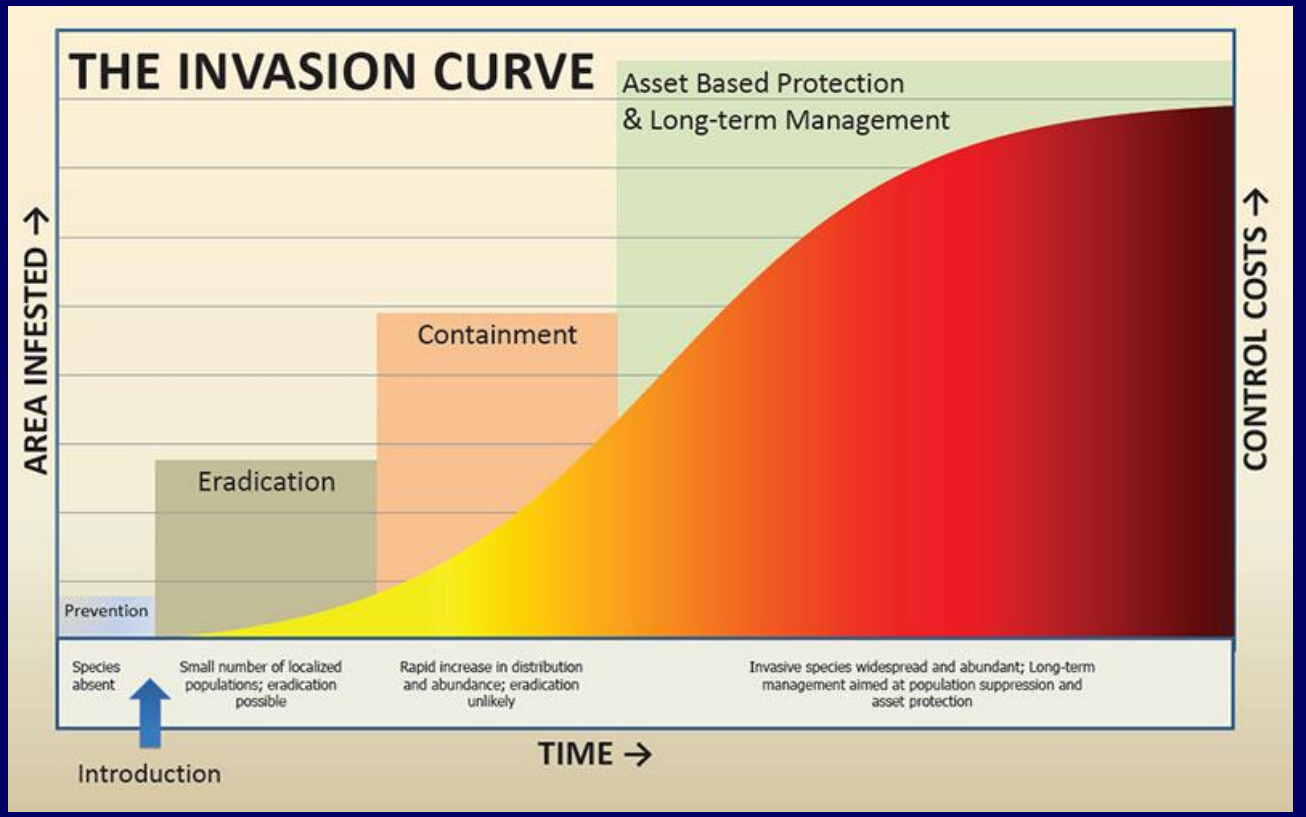


Early detection of non-indigenous species (NIS)

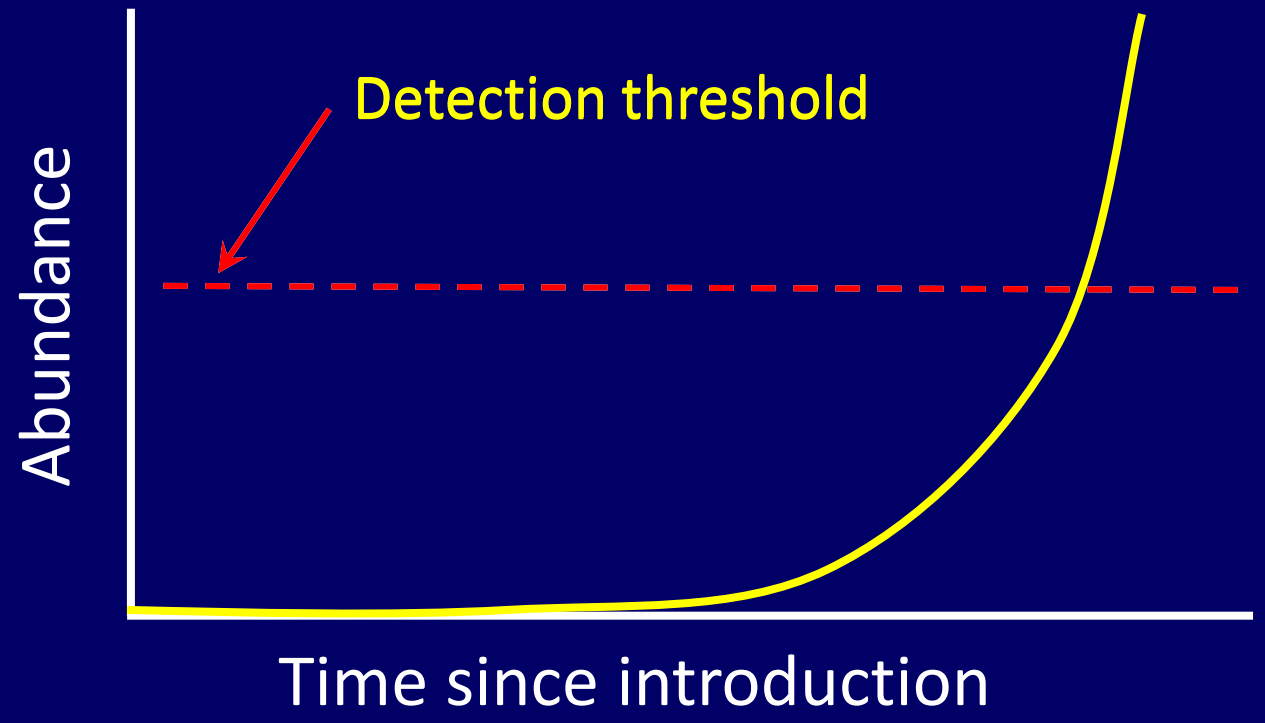
- When NIS are rare, they're easier to manage
  - Eradication (Rejmanek & Pitcairn 2002)
  - Containment/Control (Harrington et al. 2009)



North American Invasive Species Network (2013)

Early detection of non-indigenous species (NIS)

- They're also harder to find (Harvey et al. 2009)



Lockwood et al. (2013)

eDNA applications in species detection

eDNA： environmental DNA

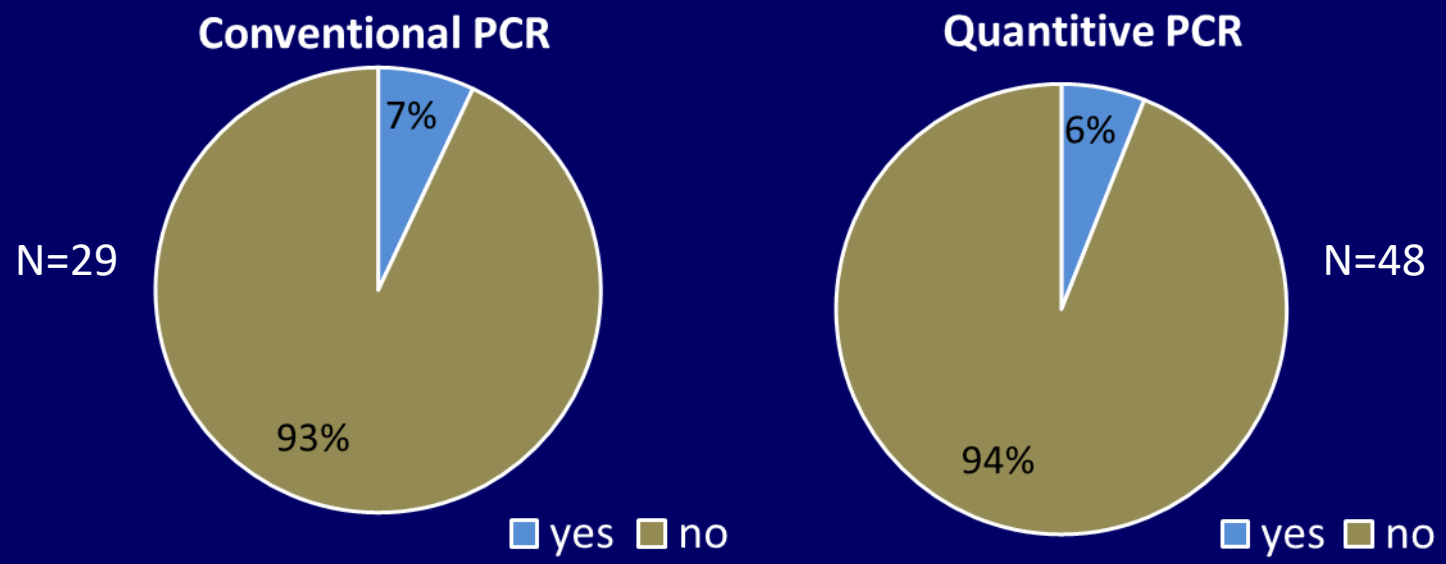
- Single species detection
- Community profiling

Fig. 1 Sampling for eDNA metabarcoding analysis in different environments. From top left, clockwise: collection of water samples, sediment cores of Arctic lakes, sediment cores of Alpine lakes and soil. Photo credits: C. Miaud, I. G. Alsos, M. Bajard, P. Deline. Photos kindly provided G. F. Ficetola.

Jerde & Mahon (2015)

Optimization of genetic markers remains a gap

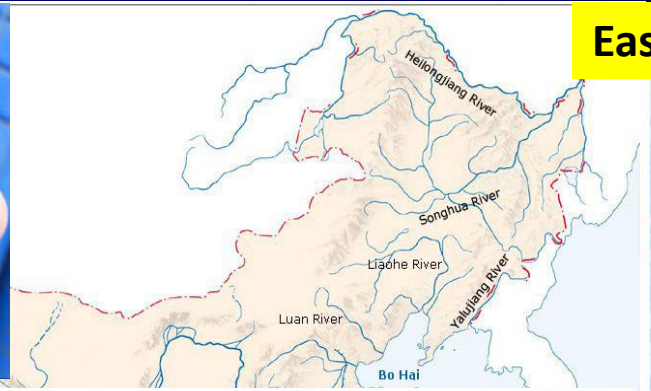
- Are primer pairs optimized to lower the detection threshold?



Data sources: 77 articles on aquatic species detection using species-specific primers

Xia et al. unpublished

Distribution and invasion history of golden mussel (*Limnoperna fortunei*)



East River Water Diversion Project, 1965

Xu et al. (2009)

South to North Water Diversion (SNWD) project

http://www.iwhr.com/zgskyww/index.htm

Middle route:

- 1276 km long
- 140 m altitude difference
- 9 billion m^3 per year

Opening of the SNWD should facilitate spread of Golden Mussel

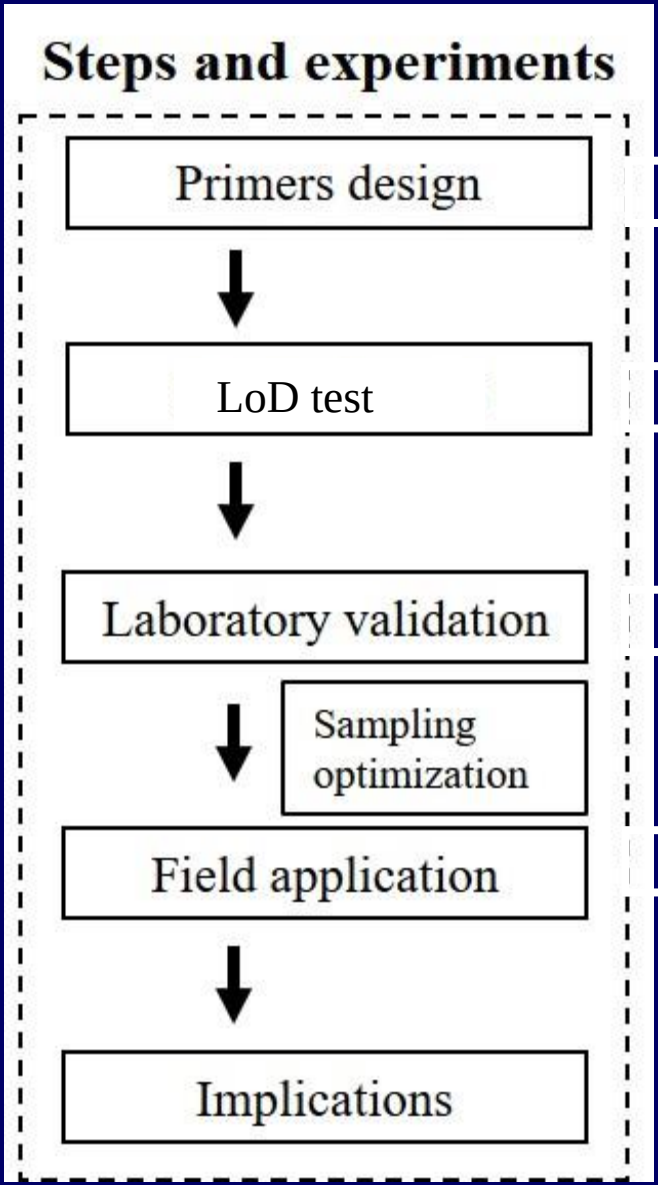
Objective

To develop and optimize early detection of golden mussel based on eDNA methods.

We hypothesized:

1. Primer screening can improve early detection of golden mussel from eDNA samples (experiment 1).
2. Quantitaive PCR should outperform conventional PCR (experiment 2).

Experiment 1: Early detection based on conventional PCR



NCBI Blast; 13 species specific-primers on COI; 127 ~299 bp; in-vitro test (5 common sympatric species).

Serial dilution & total genomic DNA PCR.

1, 5 and 15 ind. in 10L aquarium tank; 5 replicates; Detecting at different time-points.

Inhabited waterbodies

Uninhabited waterbodies

Results: LoD of 13 primer pairs

Primer pair	A	B	C	D	E	F	G
LoD ($ng \cdot \mu L^{-1}$)	4×10^{-2}	4×10^{-6}	4×10^{-2}	4×10^{-2}	4×10^{-3}	4×10^{-3}	4×10^{-4}

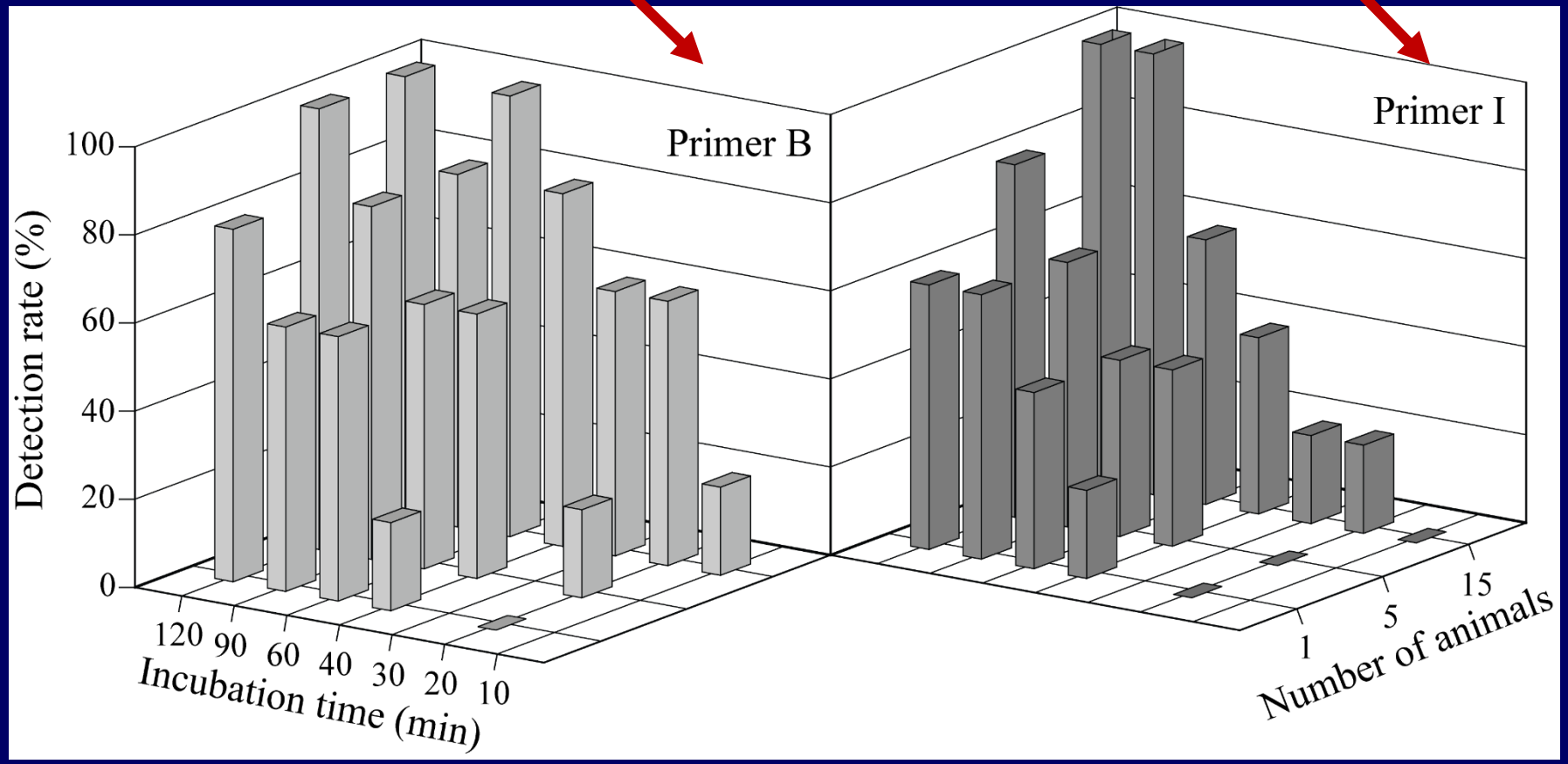
Primer pair	H	I	J	K	L	M
LoD ($ng \cdot \mu L^{-1}$)	4×10^{-3}	4×10^{-4}	4×10^{-4}	4×10^{-3}	4×10^{-4}	4×10^{-3}

Xia et al. (2017) *Biol. Invasions*

Results: validation in laboratory aquarium

LoD: 4×10^{-6} ng·µL^{-1}

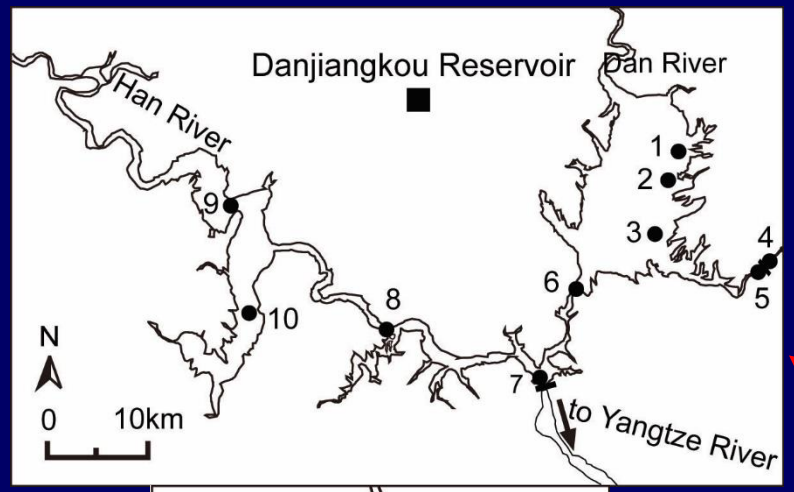
LoD: 4×10^{-4} ng·µL^{-1}

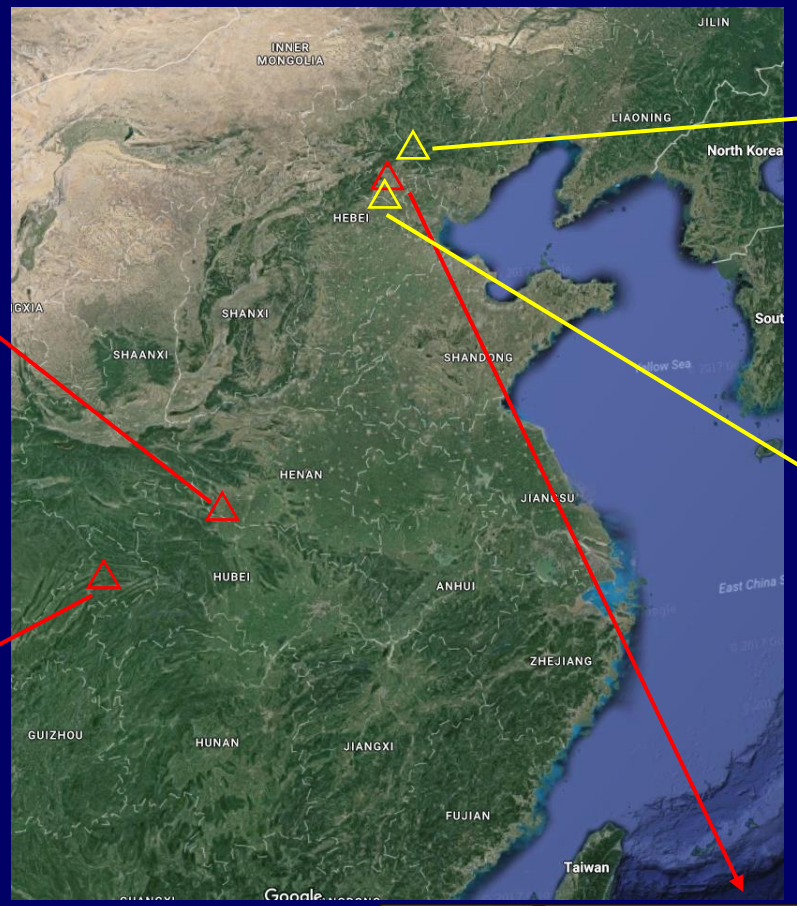


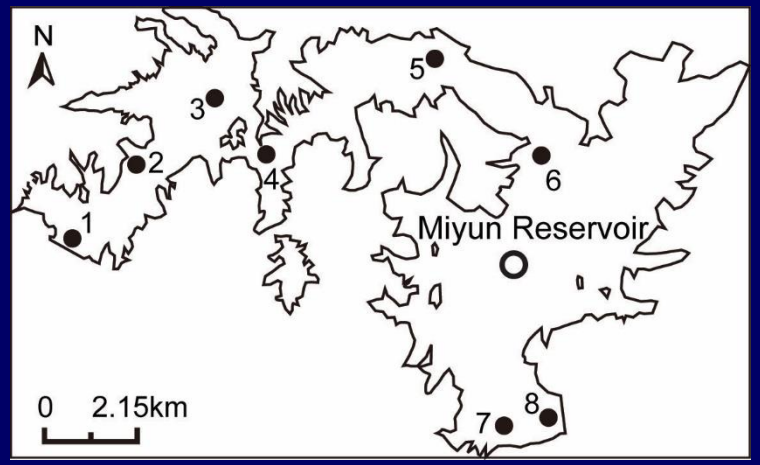
Lower LoD allows for earlier detection and at lower abundance

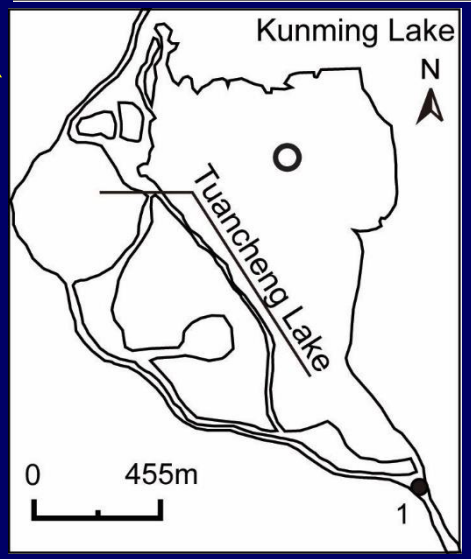
Xia et al. (2017) *Biol. Invasions*

Results: field application in china

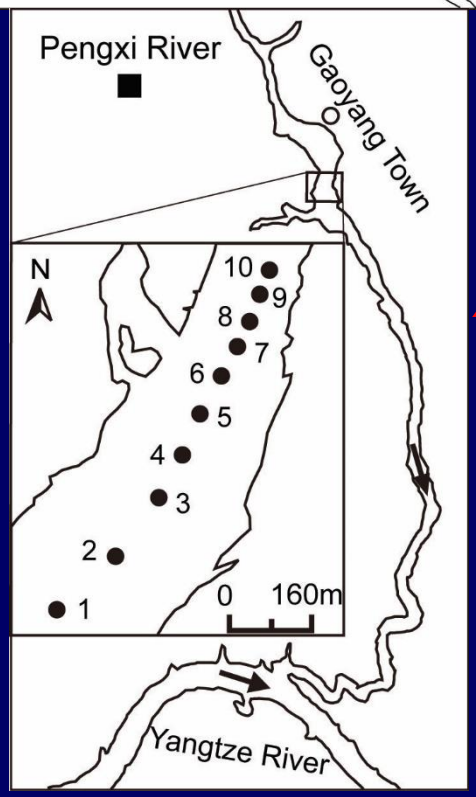




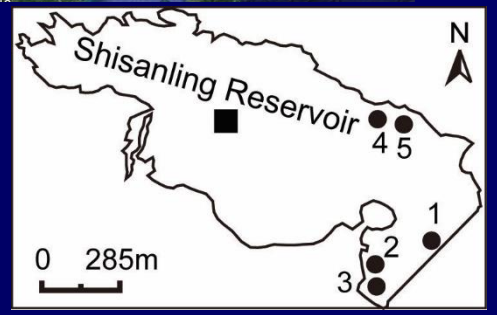




Uninhabited:
9 sites

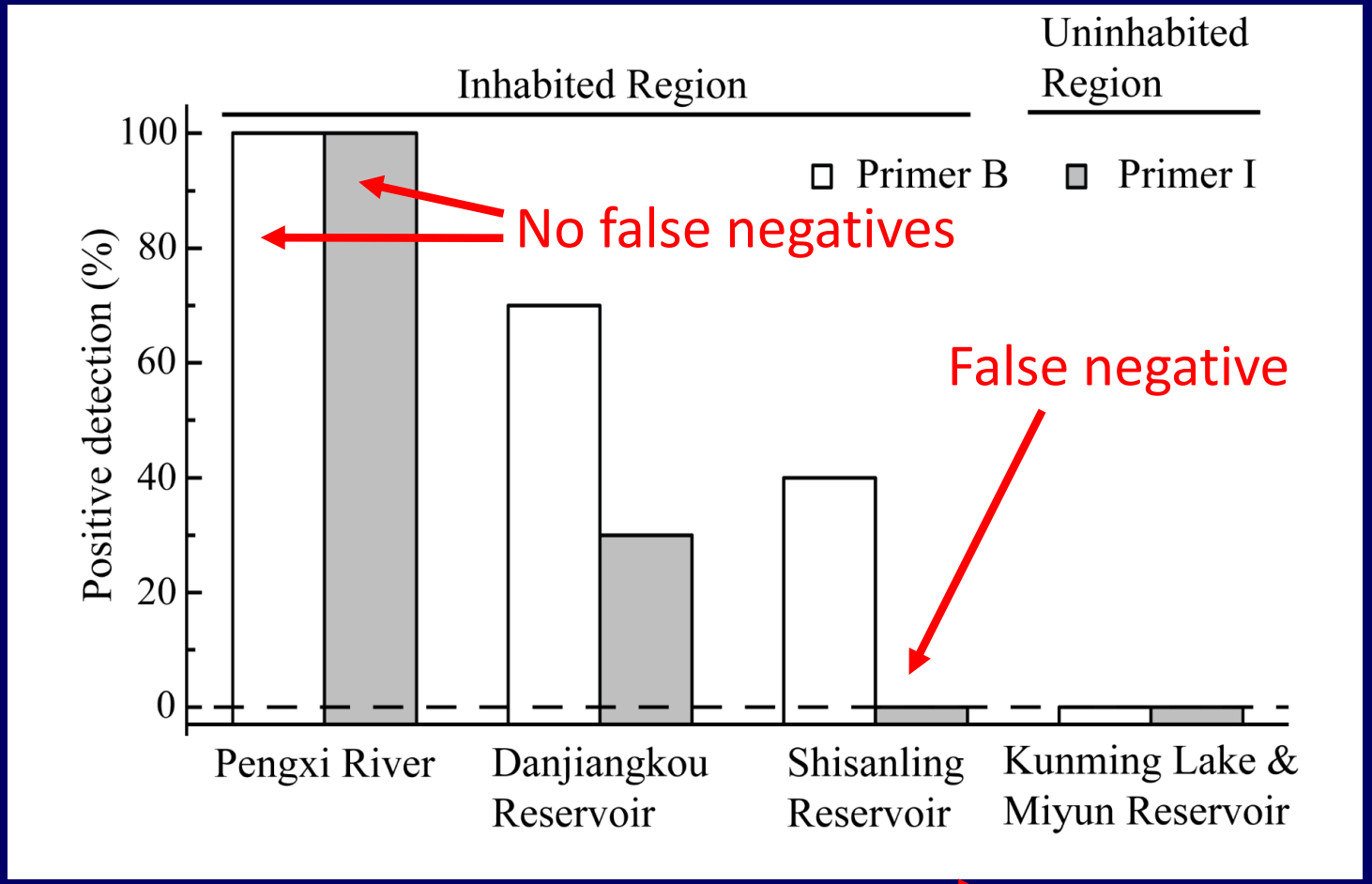


Inhabited:25 sites



Xia et al. (2017) *Biol. Invasions*

Results: detection rate of primer pairs B & I



Xia et al. (2017) *Biol. Invasions*

Results: re-suspended samples had higher detection probability

Sample source	Surface layer	Mixed water column
Aquarium tank (replicate 1)	+ (10^{-2})	+ (10^{-4})
Aquarium tank (replicate 2)	+ (10^{-2})	+ (10^{-5})
Aquarium tank (replicate 3)	+ (10^{-2})	+ (10^{-4})
Shisanling Reservoir (site 1)	- (ND)	- (ND)
Shisanling Reservoir (site 2)	- (ND)	+ (0.2)
Shisanling Reservoir (site 3)	-(ND)	+ (0.1)

Lower dilution fraction

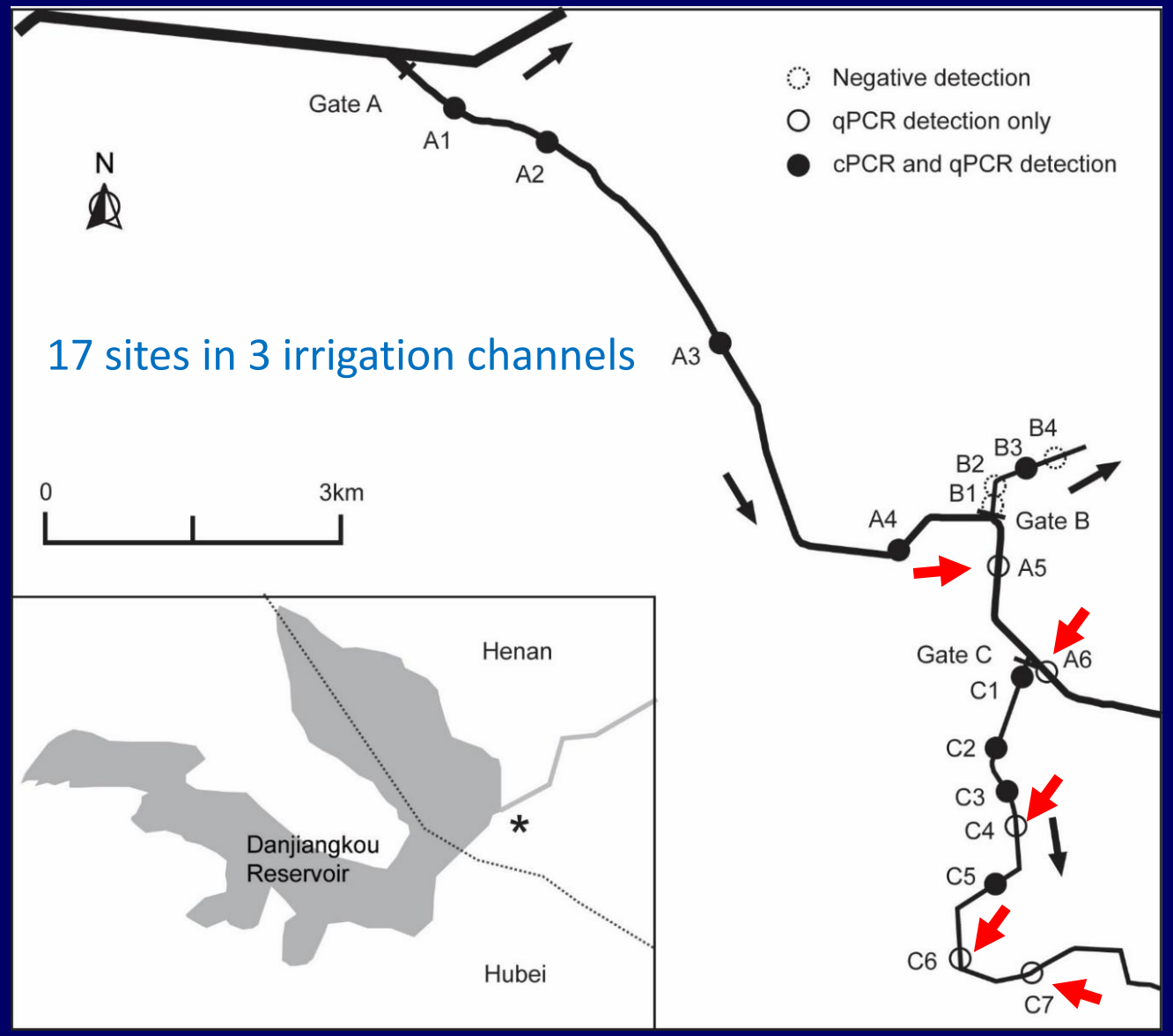
Higher probability

+: positive amplification; -: negative amplification; numbers in brackets refer to the lowest dilution fraction from the original eDNA extracts that could be successfully amplified by primer B; ND: not detected

Xia et al. (2017) *Biol. Invasions*

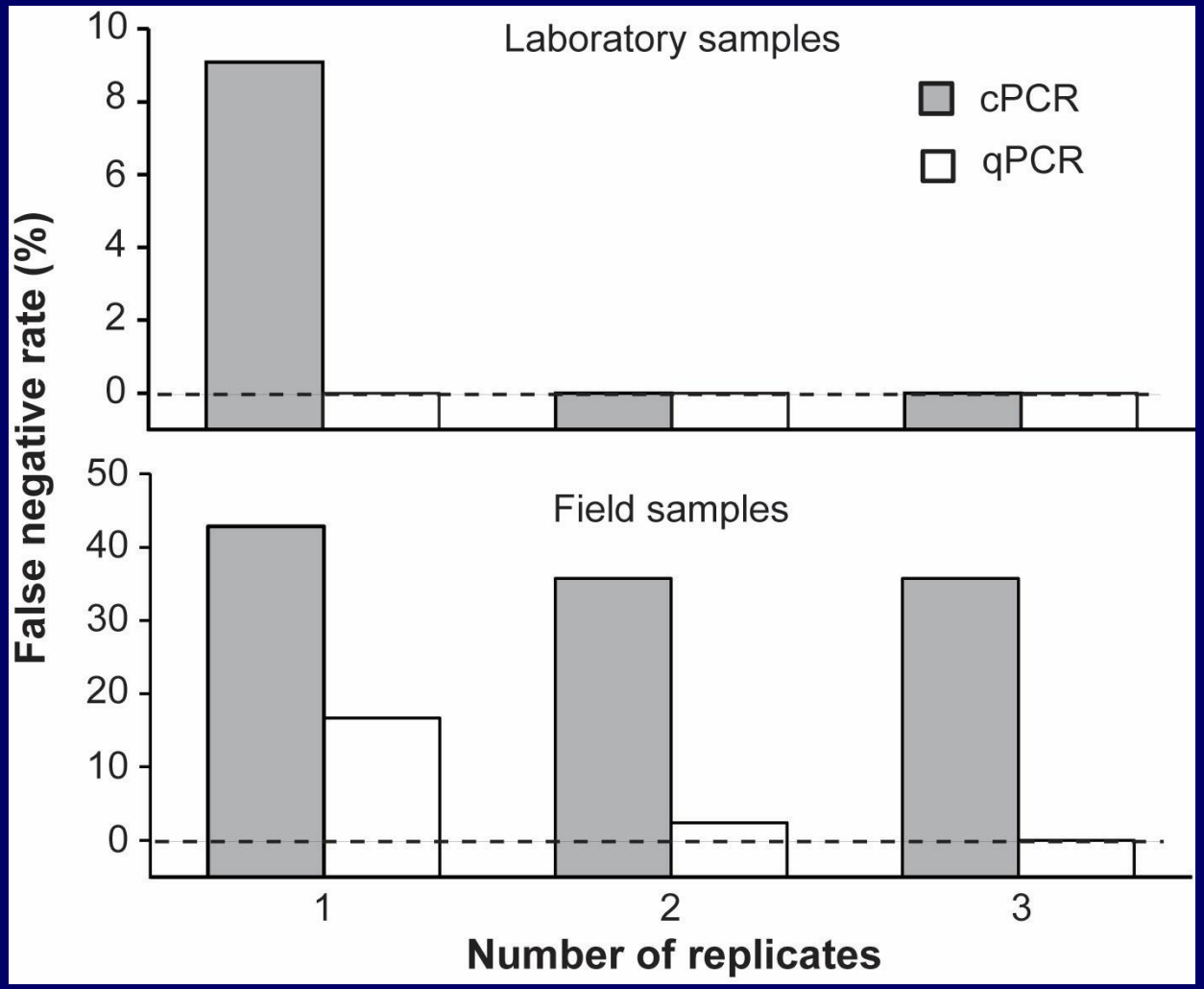
Experiment 2: conventional PCR *vs* qPCR test

qPCR had higher detection rate



Xia et al. *Mol. Ecol. Resour*. (under review)

Results: false negative rate under different number of sample replicates



qPCR had lower false negative rate

Use of replicate samples reduced false negative rate

cPCR: conventional PCR

Xia et al. *Mol. Ecol. Resour*. (under review)

Conclusions

1. Judicious selection of robust primers based on tested detection limit can improve early detection for eDNA samples, resulting in fewer false negatives.

2. qPCR exhibits higher detection capacity for eDNA samples than conventional PCR, resulting in lower false negative rate.

3. Proper sampling schemes (e.g., location) and sampling efforts (e.g., replication) are critical to maximize detection probability of NIS from eDNA samples, and they should be considered when designing field sampling programs.

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Thank you!

Any questions?