

eDNA-based detection and quantification to improve the management of the invasive American bullfrog in Belgium

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I. Background

American bullfrog

- *Lithobates catesbeianus*
- **Invasive** in ~40 countries
- > 500 km² in Belgium
- **Monitoring & eradication is challenging**
 - Cryptic life style
 - Adults are highly **mobile & fertile**
 - Density dependence

Environmental DNA

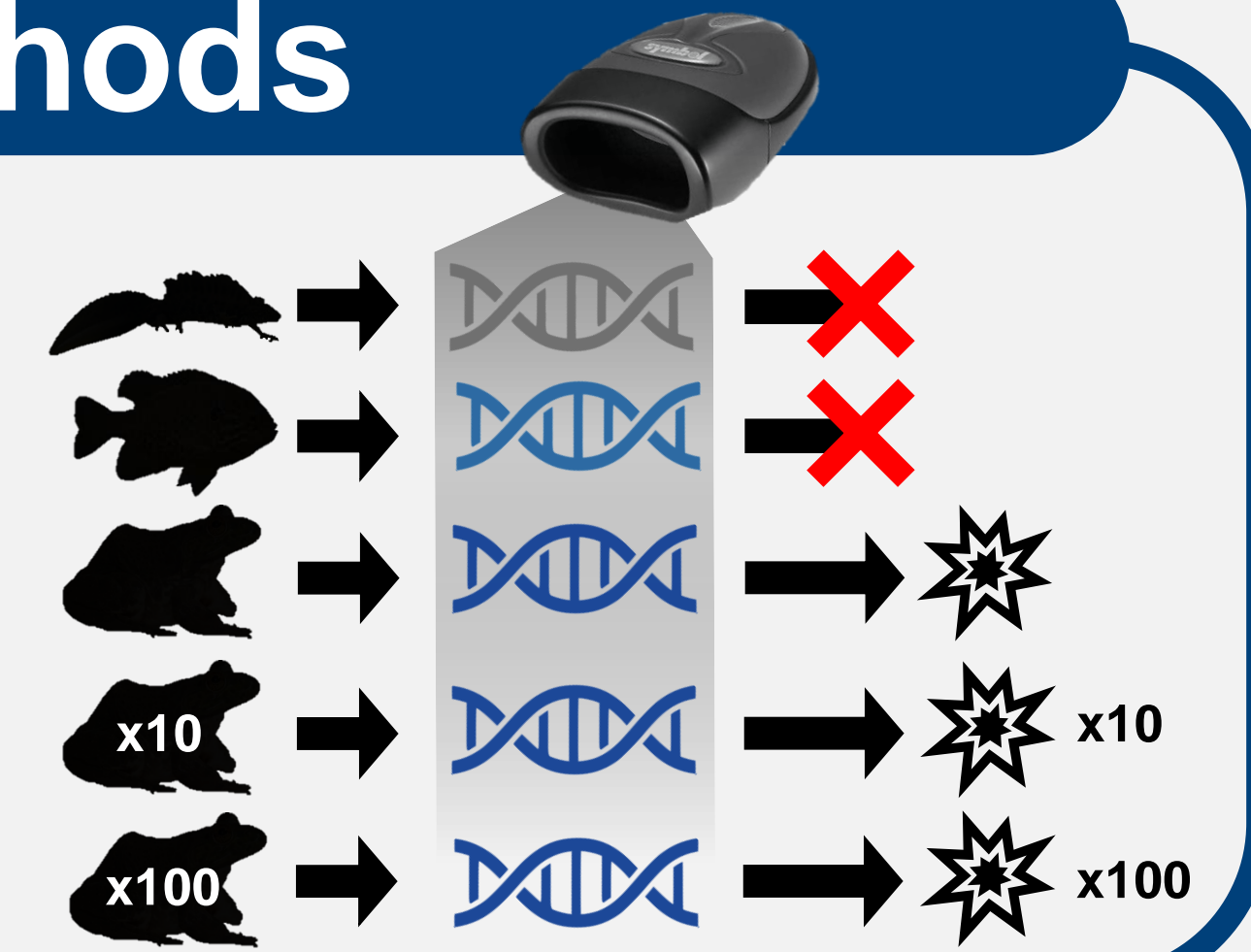
- Gametes, metabolic waste, shed tissue, etc. in the environment
- eDNA **barcoding** as a **tool to detect target species & quantify abundance**
- **Outperforms** conventional monitoring techniques

II. Aim

How can eDNA-based detection methods facilitate bullfrog management?

III. Methods

- eDNA **barcoding** of pond water collected **before & after** bullfrog capture
- **Quantifying** eDNA concentrations using **Droplet Digital PCR**

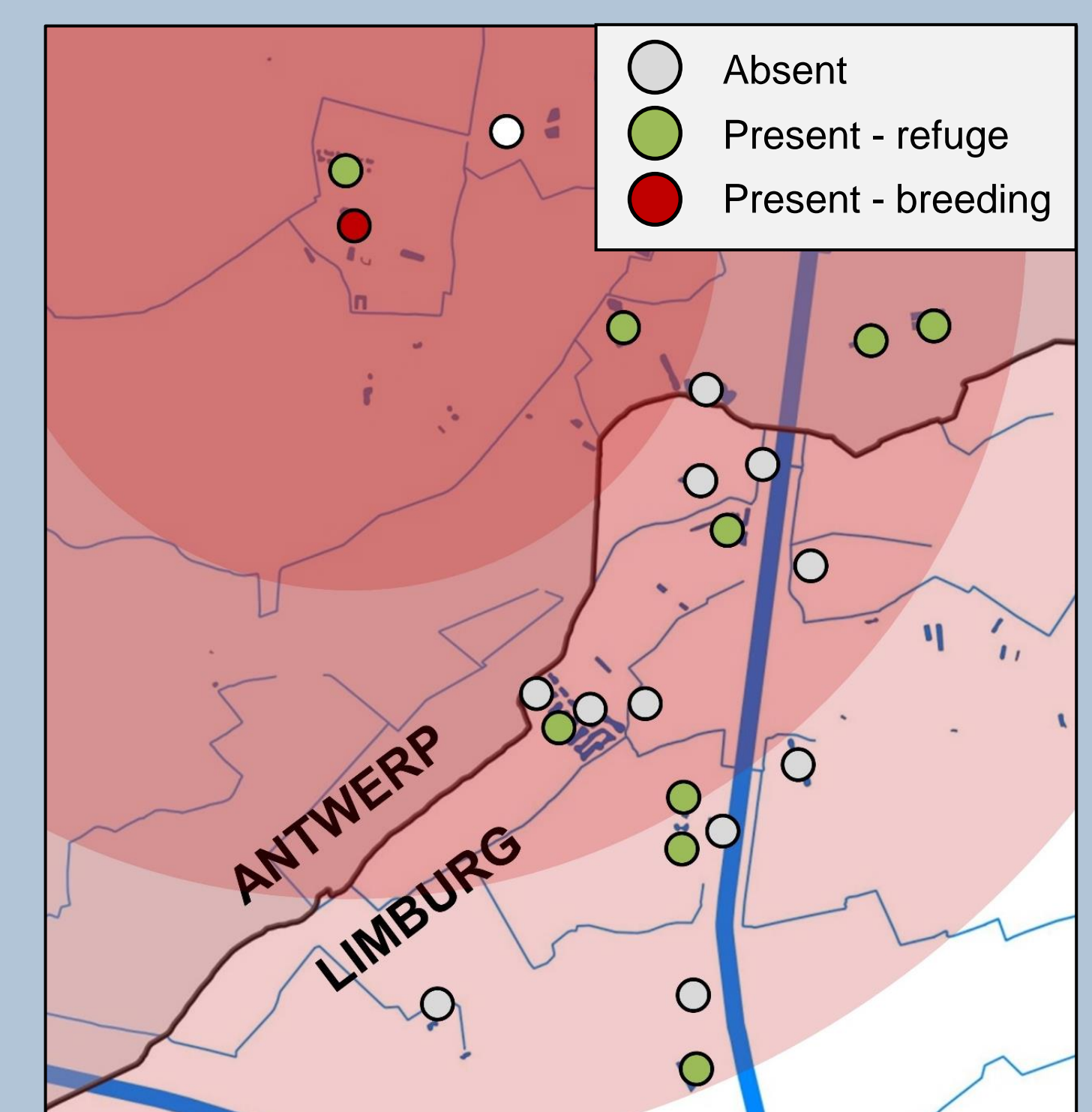
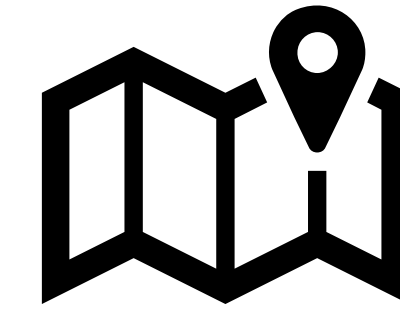


IV. Results & Conclusions

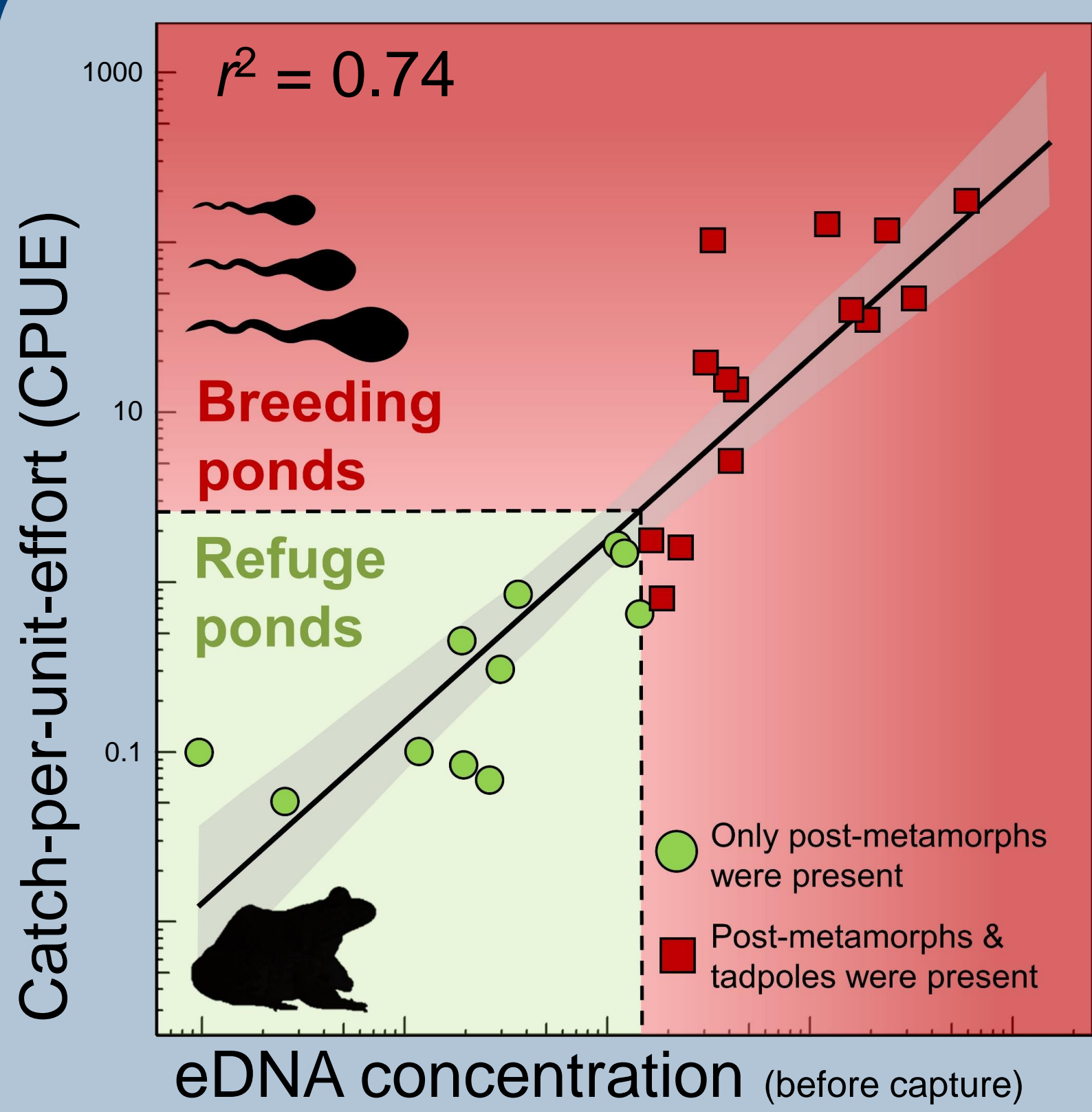
1 Identify breeding sites & forecast catch success



2 Detect new invasions & expanding invasion fronts

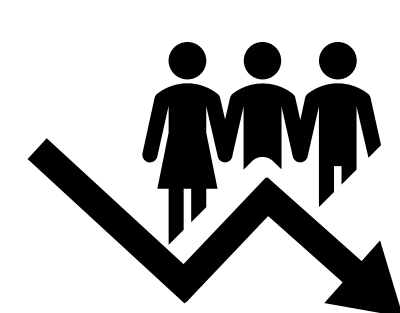


An eDNA barcoding study in the region between two provinces in Belgium revealed an **expanding bullfrog population** that recently crossed the provincial border. The observed eDNA concentrations suggested that these newly invaded ponds were **refuge ponds**. A nearby **breeding pond** on the original side of the border presumably served as a source population.

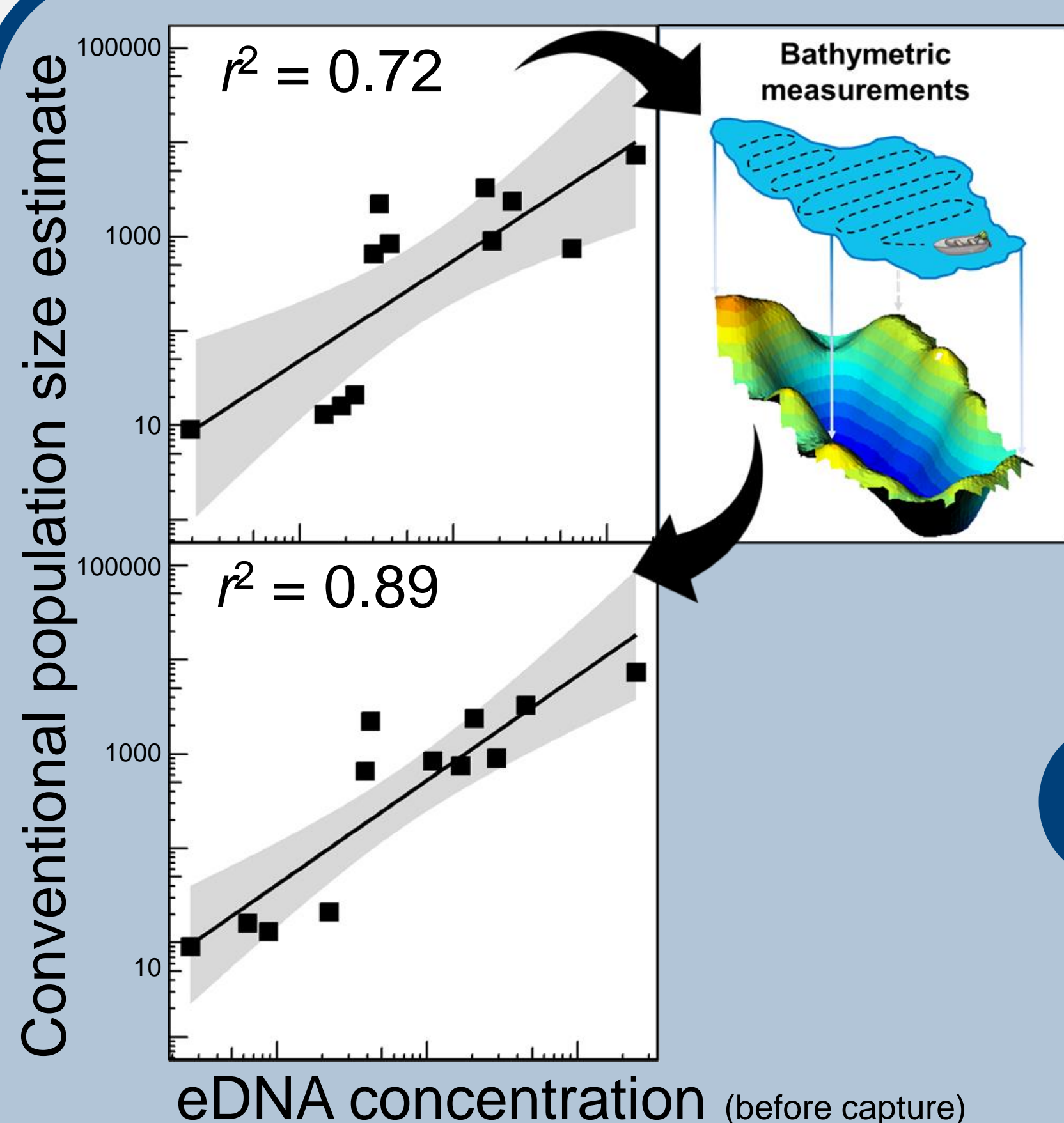
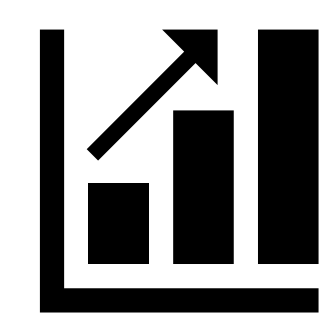


More bullfrogs could be captured in ponds where **eDNA concentrations before bullfrog capture** were higher. A clear threshold in eDNA concentrations (1.5 copies μL^{-1} ; black dotted lines) separated **refuge ponds** (only post-metamorphs were present) from **breeding ponds** (post-metamorphs and tadpoles were present).

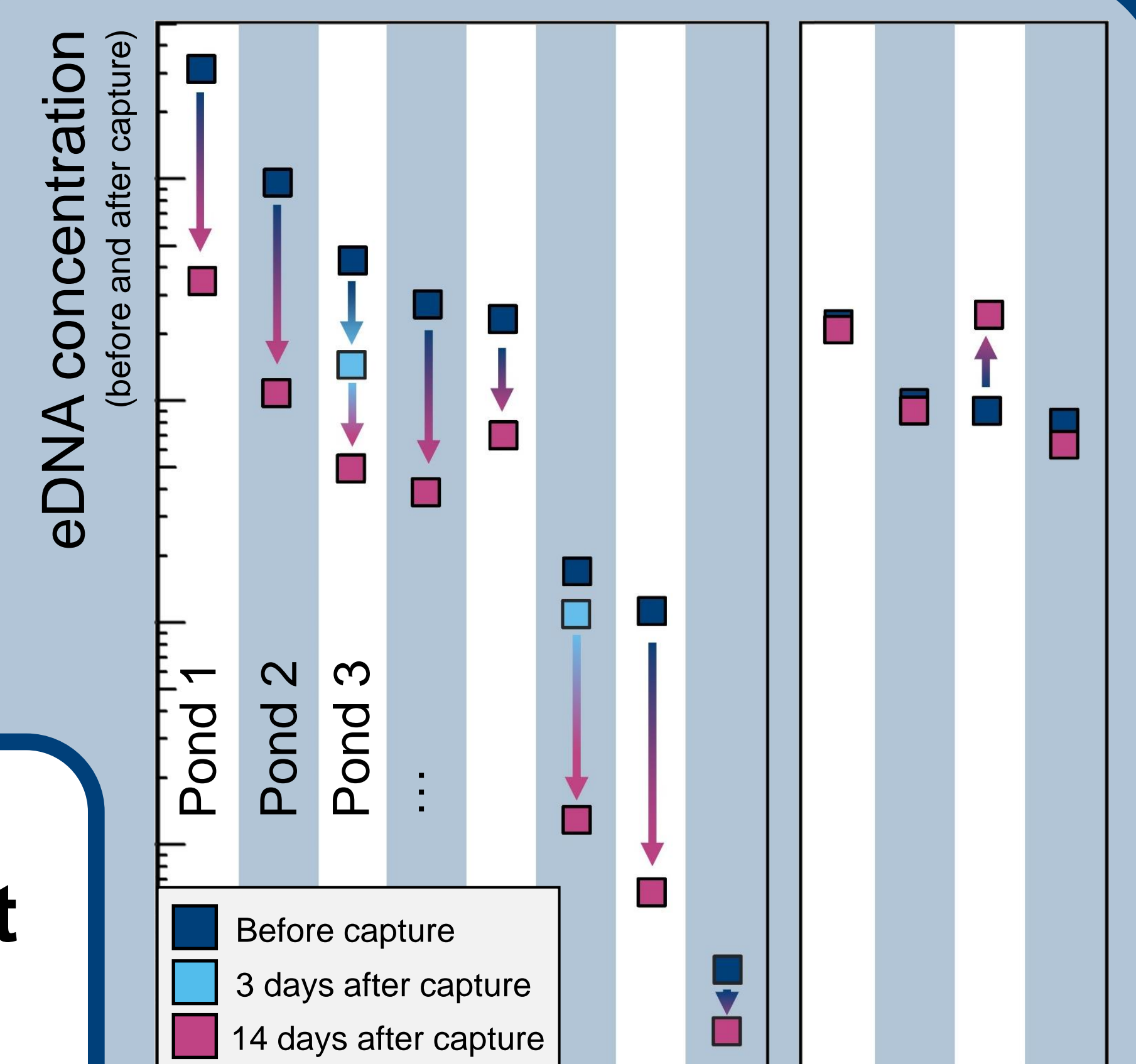
3 Quantify population size



4 Evaluate management efficiency



Bullfrog **eDNA concentrations before bullfrog capture** were strongly correlated with conventional population size estimates (obtained by **depletion sampling**), particularly when eDNA concentrations were **corrected for varying pond volumes**.



Overall, eDNA concentrations **after bullfrog capture** were lower than **before bullfrog capture**. These decreases in eDNA concentrations were proportional to the total catches (data not shown).

Further reading:

- Everts et al. (2021) Accurate detection and quantification of seasonal abundance of American bullfrog (*Lithobates catesbeianus*) using ddPCR eDNA assays. *Scientific Reports* 11, 11282.
- Everts et al. (2022) Using quantitative eDNA analyses to accurately estimate American bullfrog abundance and to evaluate management efficacy. *Environmental DNA* (in press).
- Everts, Van Driessche, Brys (2022) The American bullfrog wreaks havoc in Belgium - How eDNA can help control its spread [In Dutch]. *Hippocampus* 288, 54-57.

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