

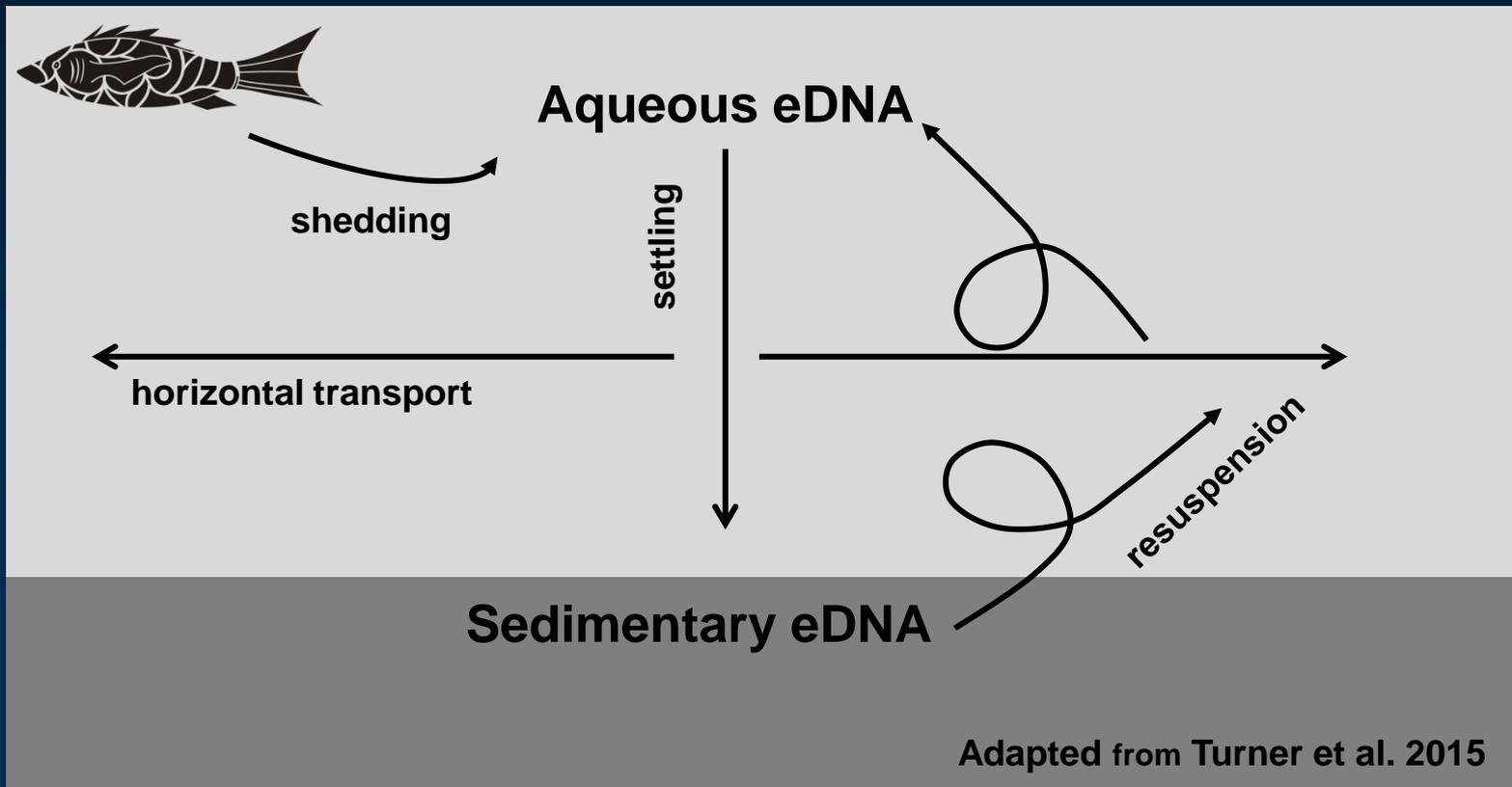
eDNA of Pacific Lamprey in Sediment: Controlled Laboratory Testing to Refine and Validate Field Sampling Methods

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Overview

- eDNA sampling in sediments is not common
- BUT...could be interesting



Why sediment for lamprey eDNA?

- eDNA sampling strategy should be study specific
 - Know your study animal and system
 - Detection only describes target species, not the life stage or shed material (gametes, skin, feces)
- We were interested in ammocoetes....so sediment
- Sediments less mobile than water in flowing systems....improved spatial linkage
- eDNA can persist for long periods in sediment
 - Longer than in water
 - 4 months for carp, 5x longer than water (Turner et al. 2015)
- Its not limiting...could also detect adults or juveniles

Inference from eDNA



**Pacific Lamprey
eDNA detected**

Who was detected?

How many are there?

Are they right here?

Are they here now?

Study Approach

- Series of controlled laboratory tests to refine inference capability from eDNA detection

- Ammocoetes
- Sediment.....and water (not presented)



- What does an eDNA detection in sediment mean?

- **How many ammocoetes are present?**

- Evaluate relationship between eDNA and ammocoete biomass
- Goal was to distinguish low, medium, and high numbers of lamprey

- **How far away from the sample point are they?**

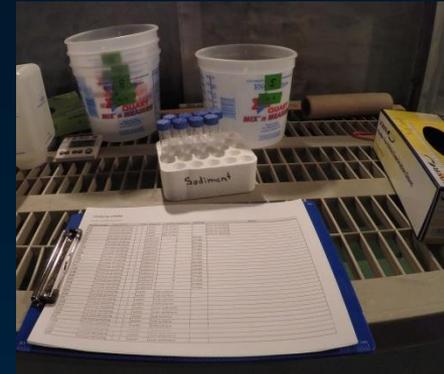
- Determine how far from a known source eDNA can be detected

- **How long ago might ammocoetes have been present?**

- Evaluate persistence of eDNA following removal of ammocoetes

General Methods

- Testing at USGS lab in Cook, WA
- Pacific Lamprey ammocoetes reared at lab
- New play sand for sediment
- Small tanks with steady flow (5 – 9 °C)
- Tested water, food, and sediment for lamprey eDNA and all were negative
- Clean sampling techniques using bleach, rinsing, and clean gloves
- *Entosphenus* specific qPCR assay using the ViiA 7 real-time qPCR system
 - Quantified eDNA for biomass experiment
 - Used presence – absence for other tests



Detection vs. Quantification

➤ Limit of Detection (LOD)

- Lowest concentration of DNA that can be reliably *detected*
- 1/8 replicates in standard curve amplified
- 1 copy per qPCR

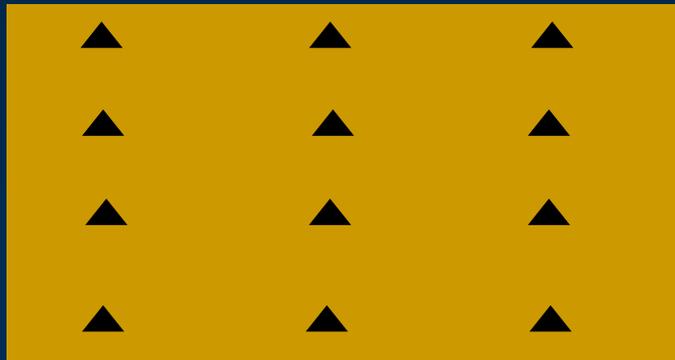
➤ Limit of Quantification (LOQ)

- Lowest concentration of DNA that can be precisely *quantified*
- 8/8 replicates in standard curve amplified
- 6 copies per qPCR

Sediment Sampling Methods

➤ Composite sampling

- 12 locations throughout tank
- Spoon inserted into sediment ~ 5cm
- Sediment combined and mixed for 1 minute
- Sample tubes filled from composite

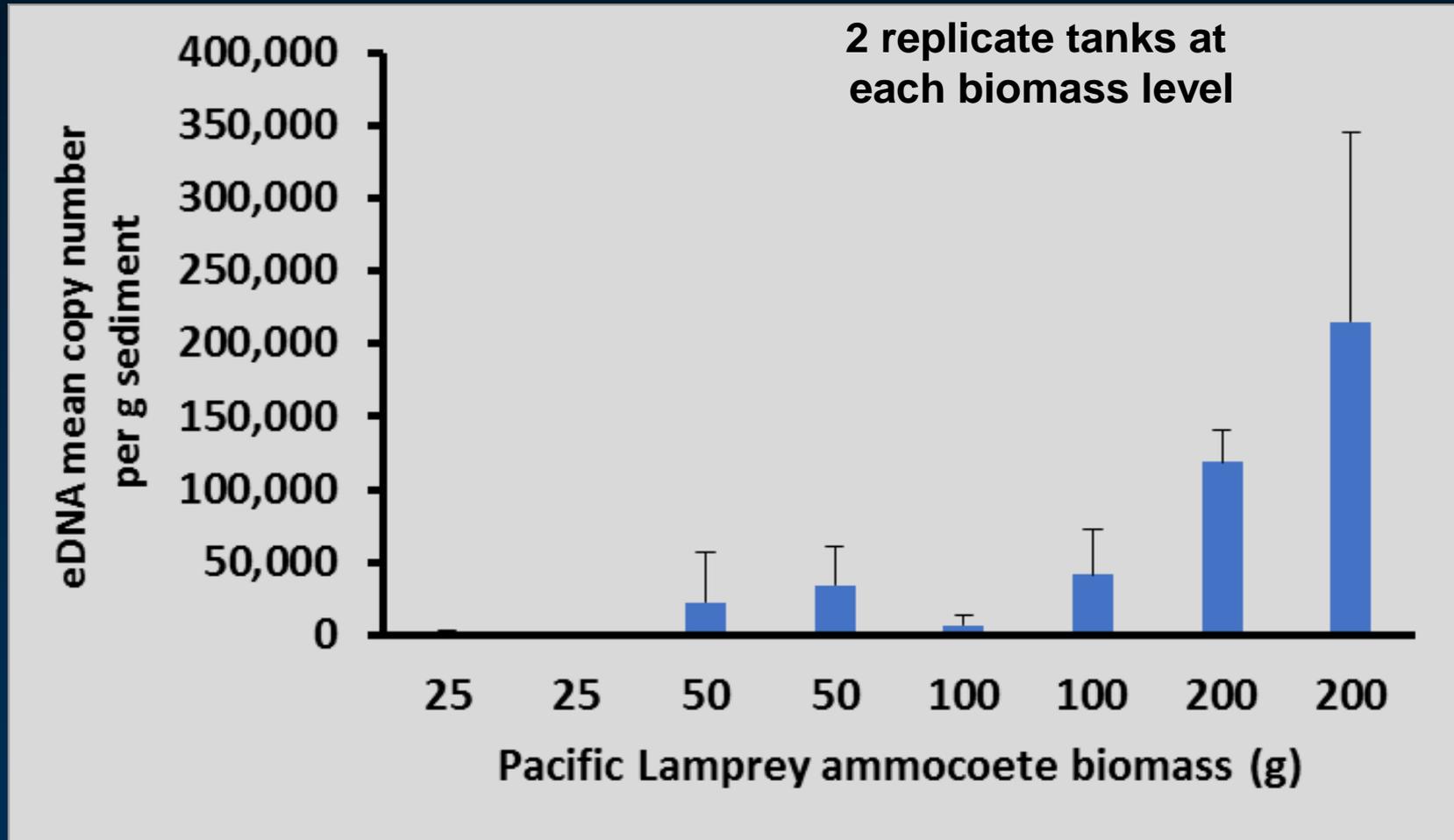


Biomass Experiment

- 4 biomass levels, each replicated in 2 tanks
 - 25 g (mean of 25 individuals)
 - 50 g (mean of 55 individuals)
 - 100 g (mean of 93 individuals)
 - 200 g (mean of 206 individuals)
- Mean fish size ~80 mm and ~1 g
- Sampled sediment 14 days after stocking
 - 3 composite samples per tank
- Estimated DNA concentration (number of copies) in 0.5 g sediment
 - 3 qPCRs per sample
 - LOQ

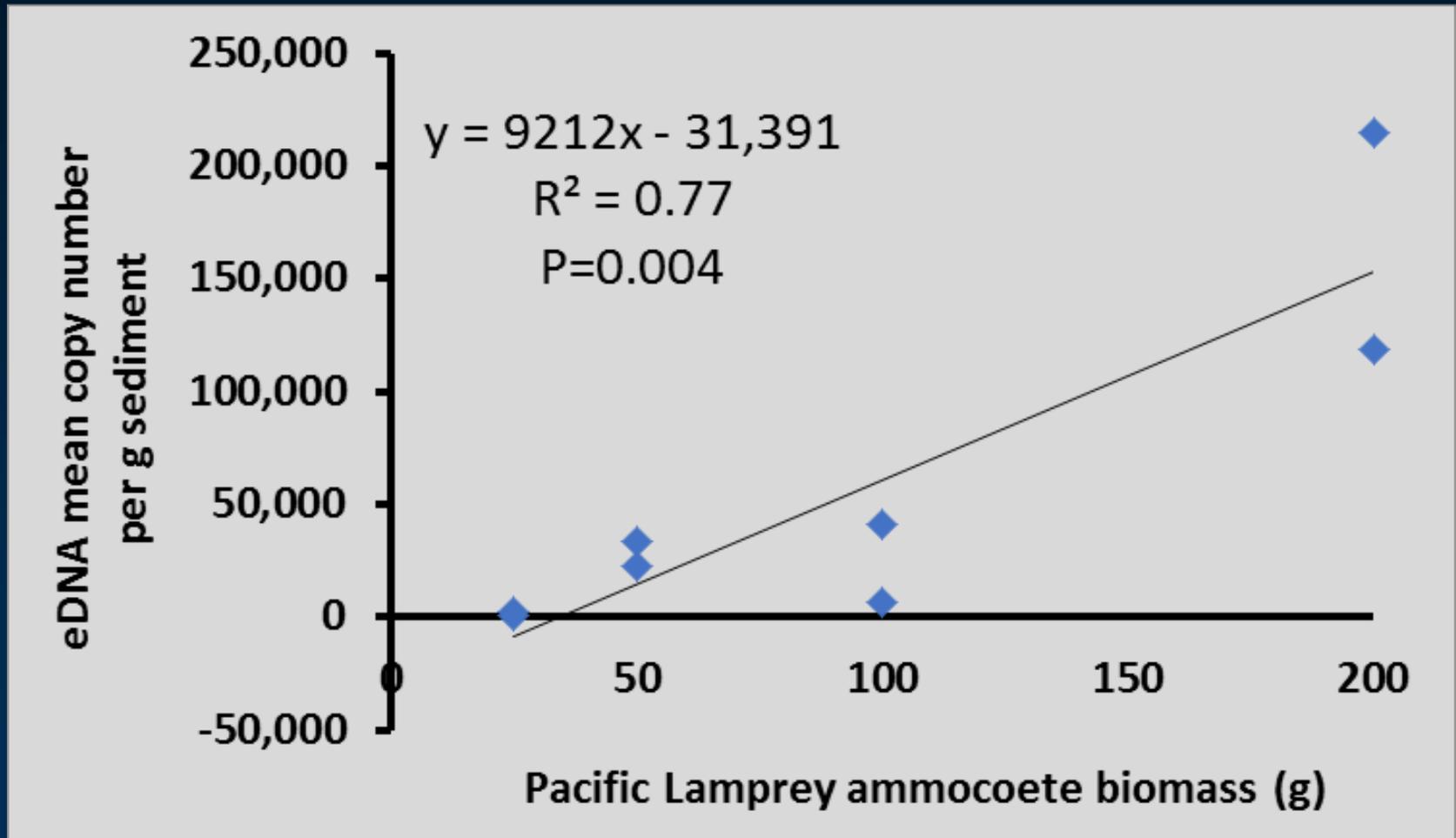


Biomass Results



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Biomass Results



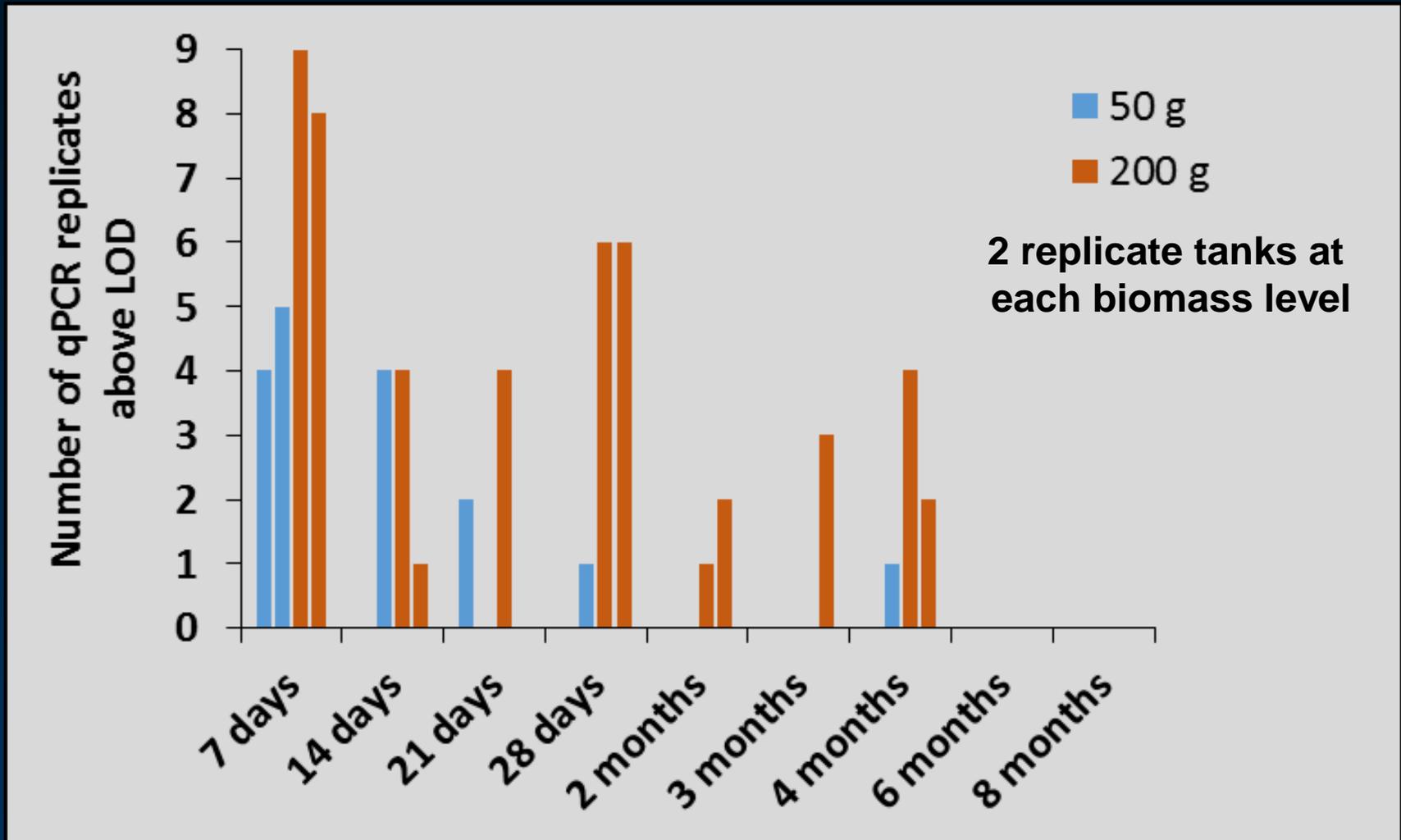
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Persistence Experiment

- Followed immediately after biomass experiment in same tanks
- Fish were removed from tanks
 - Mesh basket system
- Monitored 50 g and 200 g tanks
 - 100 g tank monitored for aqueous eDNA
- Sampled day 7, 14, 21, and 28 and then 2, 3, 4, 6, and 8 months
- Evaluated presence - absence of eDNA, without quantification
 - LOD

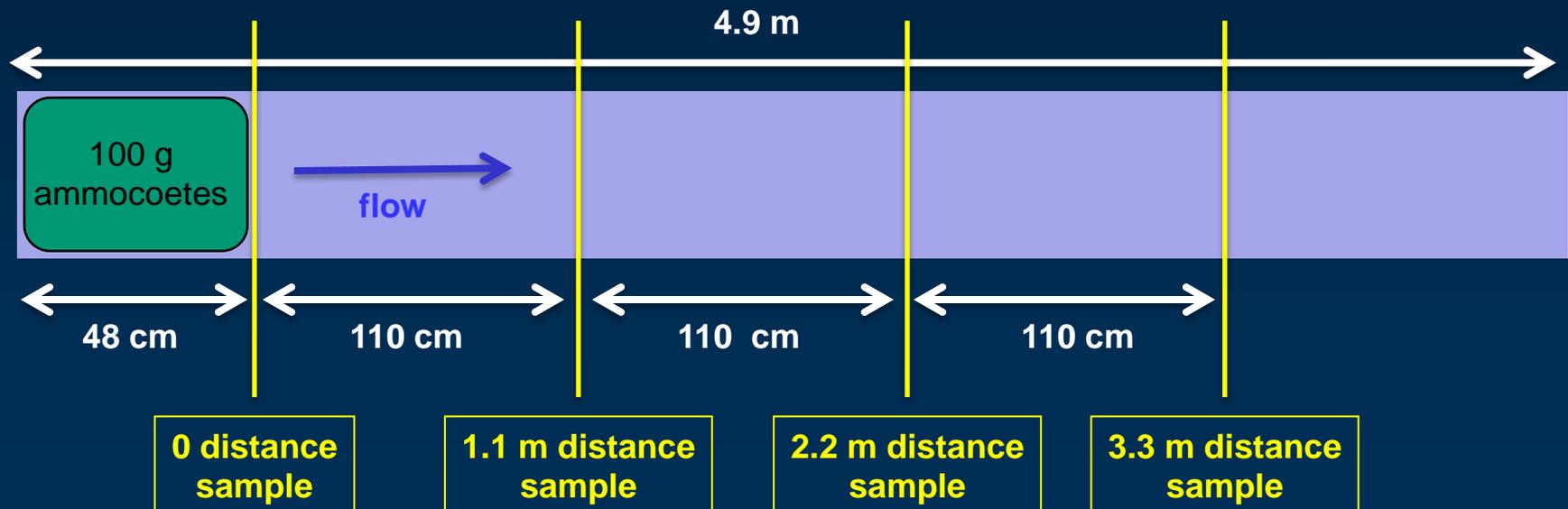


Persistence Results

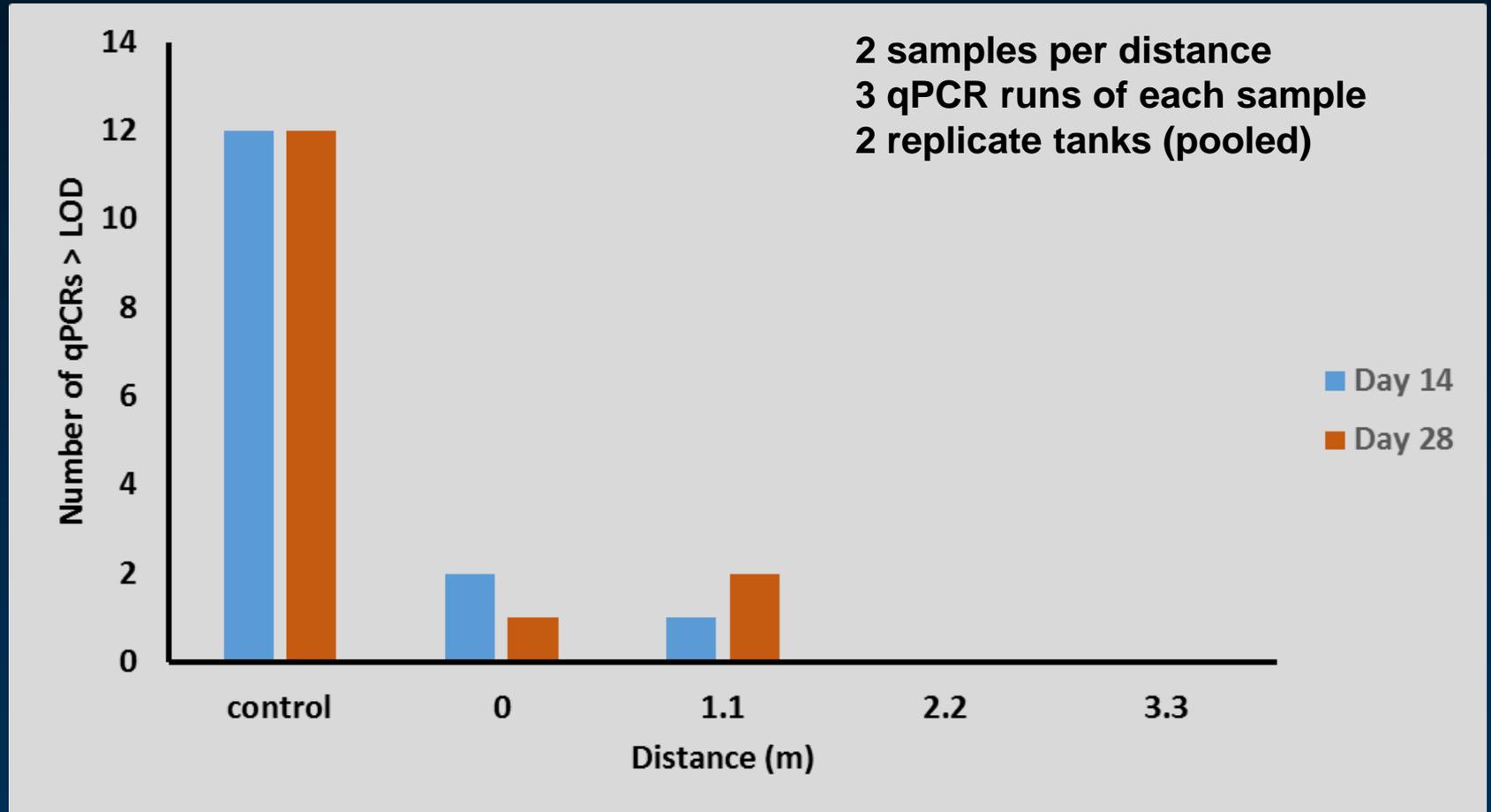


Distance Experiment

- Used 100 g biomass level, sampled at 14 d and 28 d
- Experimental set up:
 - Fish restricted to basket within 4.9 m long tank (2 replicate tanks)
- Samples
 - Within basket (positive control)
 - 4 distances form source (downstream sampled first)



Distance Results



Summary

➤ What does a lamprey eDNA detection mean?

➤ How many?

Relationship between eDNA copy number and lamprey biomass
Can estimate biomass from eDNA copy number....better by category

➤ How far?

Less than 2 m (max detection at 1.1 m)

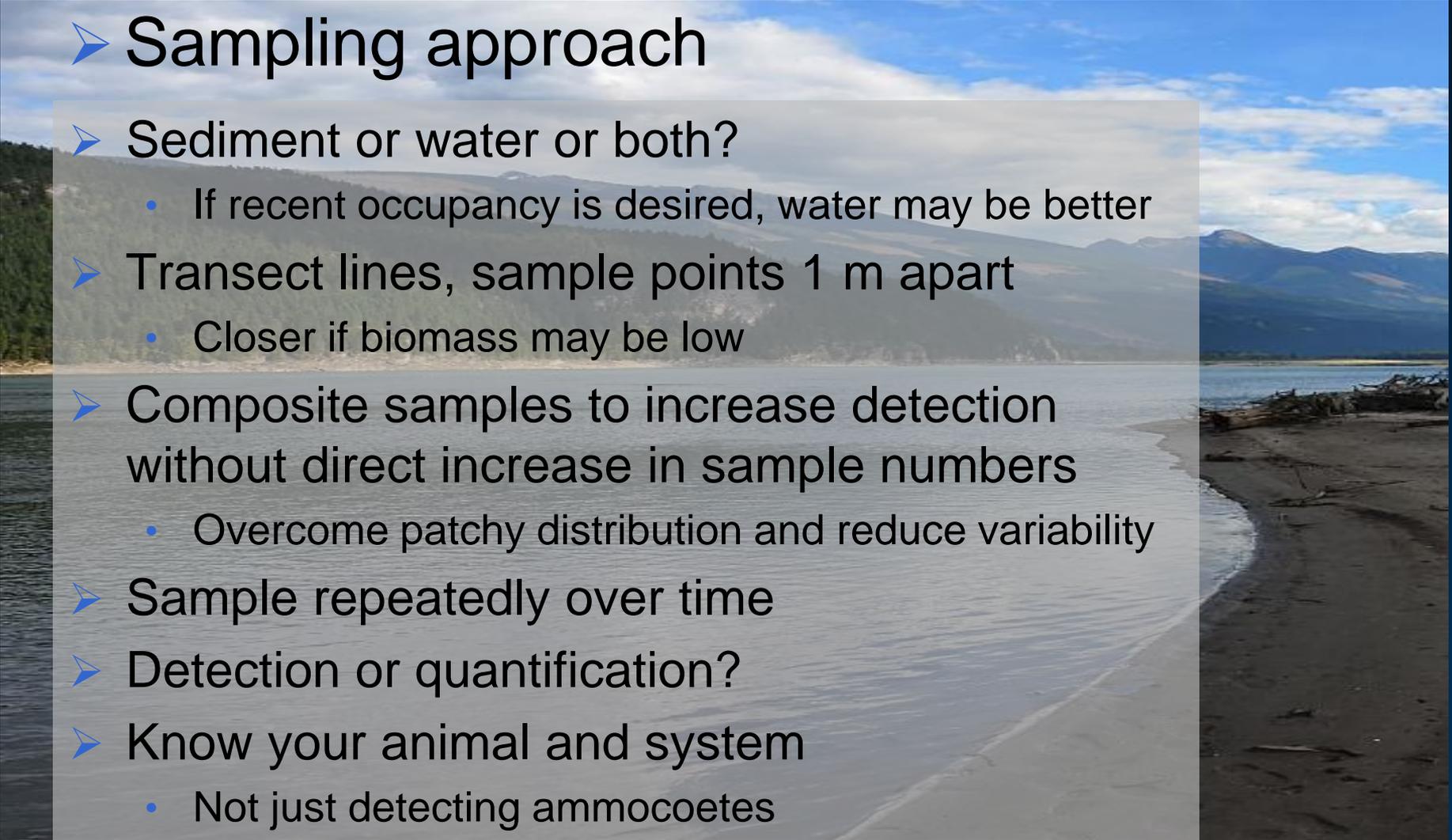
Detection distance likely influenced by biomass and maybe sediment

➤ How long?

Low biomass (50 g) - 28 days

High biomass (200 g) - 4 months

From Lab to Field

- 
- Sampling approach
 - Sediment or water or both?
 - If recent occupancy is desired, water may be better
 - Transect lines, sample points 1 m apart
 - Closer if biomass may be low
 - Composite samples to increase detection without direct increase in sample numbers
 - Overcome patchy distribution and reduce variability
 - Sample repeatedly over time
 - Detection or quantification?
 - Know your animal and system
 - Not just detecting ammocoetes

Future Directions

- Preparing manuscript on sediment and water findings
- Testing sediments for eDNA in the field to:
 - Assess impacts of dredging operations
 - Evaluate risk at water diversions (dewatering)
 - Advance understanding of distribution
- Field validation studies
 - Sediment vs. water vs. electrofishing
- Refining findings in laboratory
 - Different sediments (more coarse, or more organic matter)
 - Testing additional biomass levels to refine relationship
 - Testing relationship between biomass and detection distance



Lamprey eDNA Resources

Name	Affiliation	Email
Jon Amberg	USGS Upper Midwest Environmental Sciences Center	jamberg@usgs.gov
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Questions?