Performance of nonlethal methods of detecting Ranavirus infections in captivity and trade

Jesse L. Brunner
Anjulie Olson
Jeremy G. Rice
Mitchel J. Le Sage
Jennifer A. Cundiff
Caren S. Goldberg
Allan P. Pessier

Q: How can we minimize the movement and introduction of ranaviruses?

A: Improve our ability to detect them

especially in trade & aquaculture

What we want in a diagnostic test

Non-invasive & easy

Sensitive & well-validated

Affordable

What we have in diagnostic tests

	Validated?	Sensitive? Clinical/	Invasive?	Cost (# samples)
Tail/toe clips	Greer & Collins 2007 St-Amour & Lesbarrères 2007 Gray et al. 2012	Moderate/ ?	Moderate	~$25 × 30+
Swabs	Gray et al. 2012	Moderate/ ?	Low	~$25 × 30+
eDNA	No, but see Hall et al. 2016	?/?	Very low	~$35 × ??

Experiment #1

390 tadpoles

infected with one of three doses

sampled at one of ten time points (2-49d)

Used American bullfrogs (*Lithobates catesbeianus*)

Collected (in order)

- eDNA (125 or 250mL water)
- swab
- tail clip
- liver+kidney (gold standard)

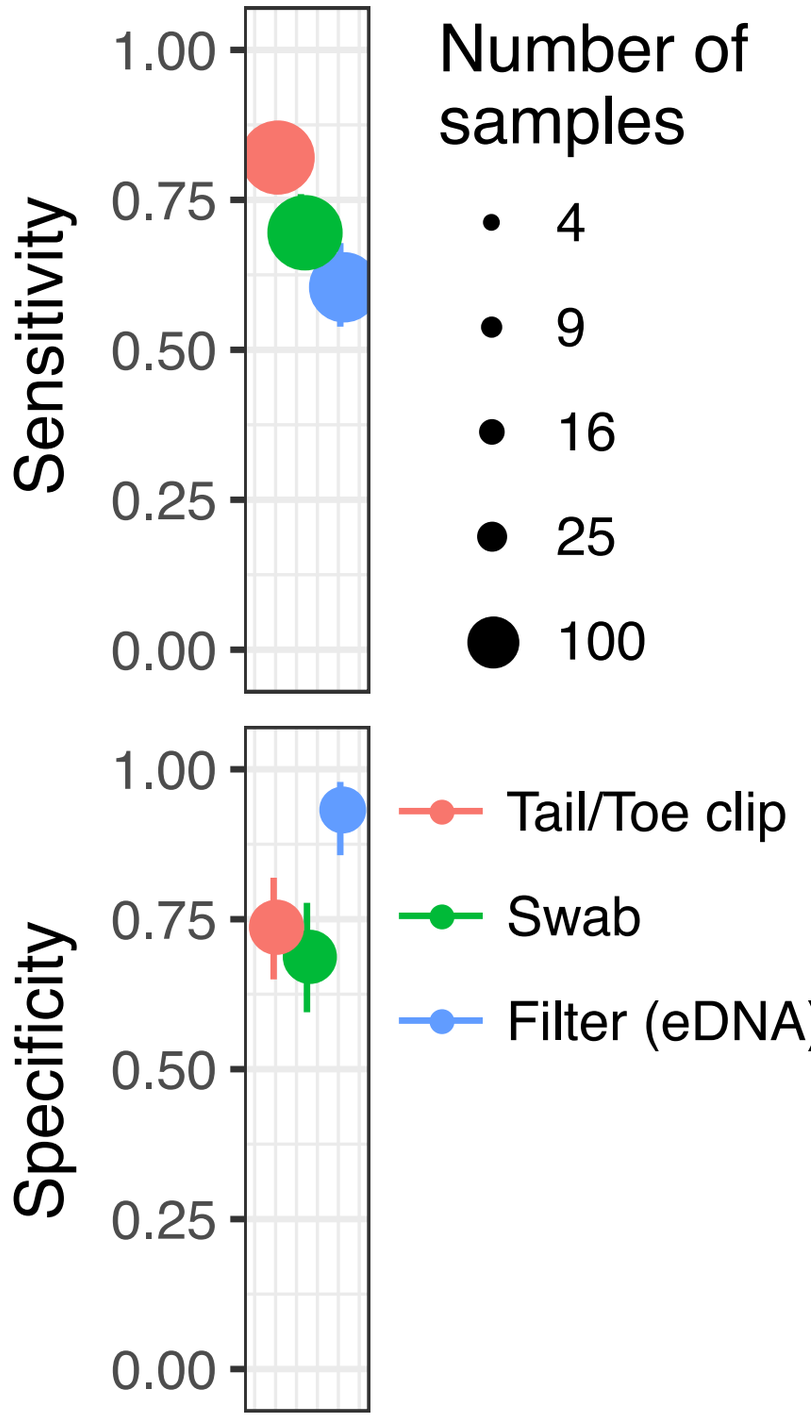
Screened with qPCR (Steckler & Waltzek)

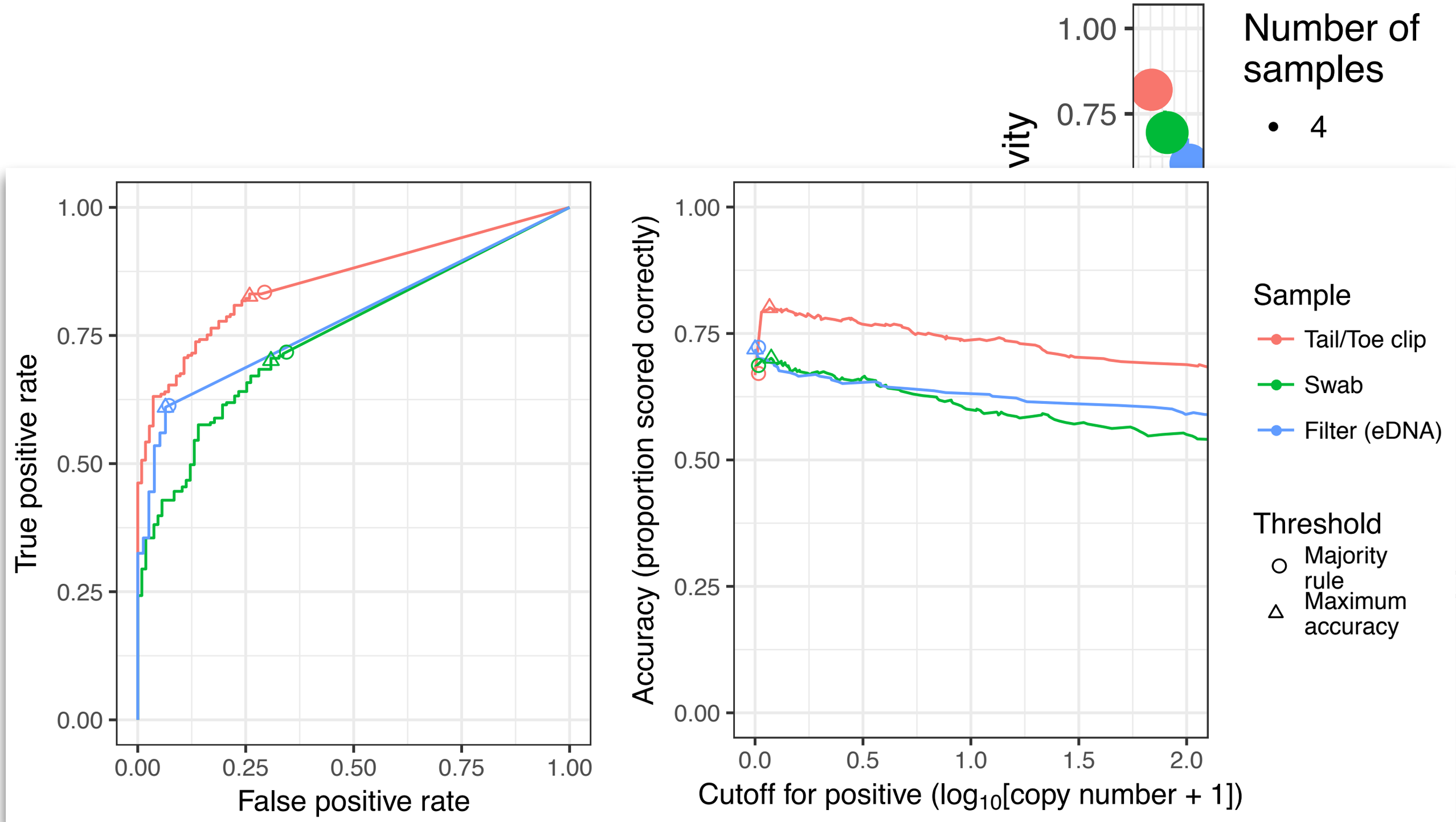
Results: Sensitivity & Specificity

With “majority rule”

(>1 well with clear amplification)

get moderate sensitivity



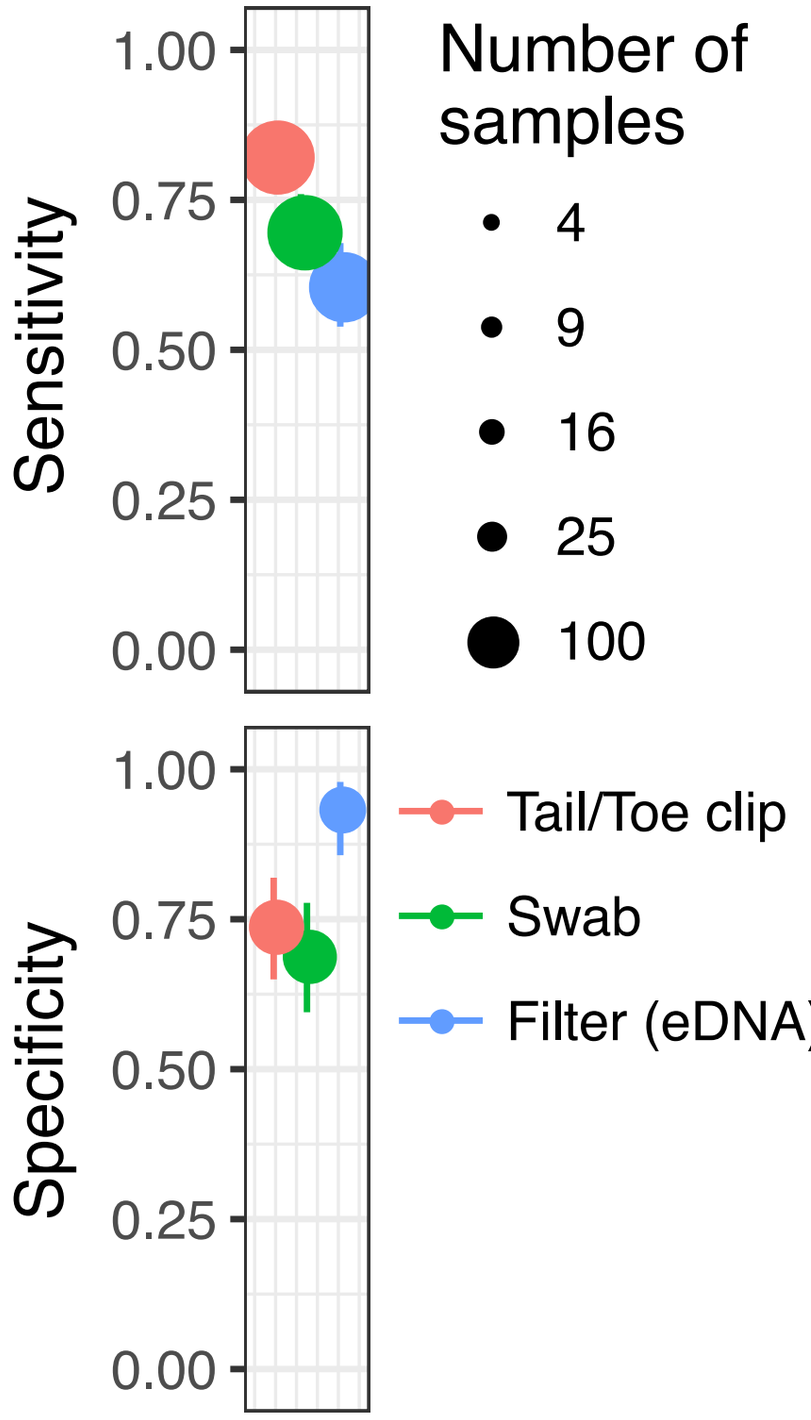

1.00
0.75
vity
Number of samples
• 4
True positive rate
False positive rate
Accuracy (proportion scored correctly)
Cutoff for positive (log_{10}[copy number + 1])
Sample
Tail/Toe clip
Swab
Filter (eDNA)
Threshold
Majority rule
Maximum accuracy

Results: Sensitivity & Specificity

With “majority rule”

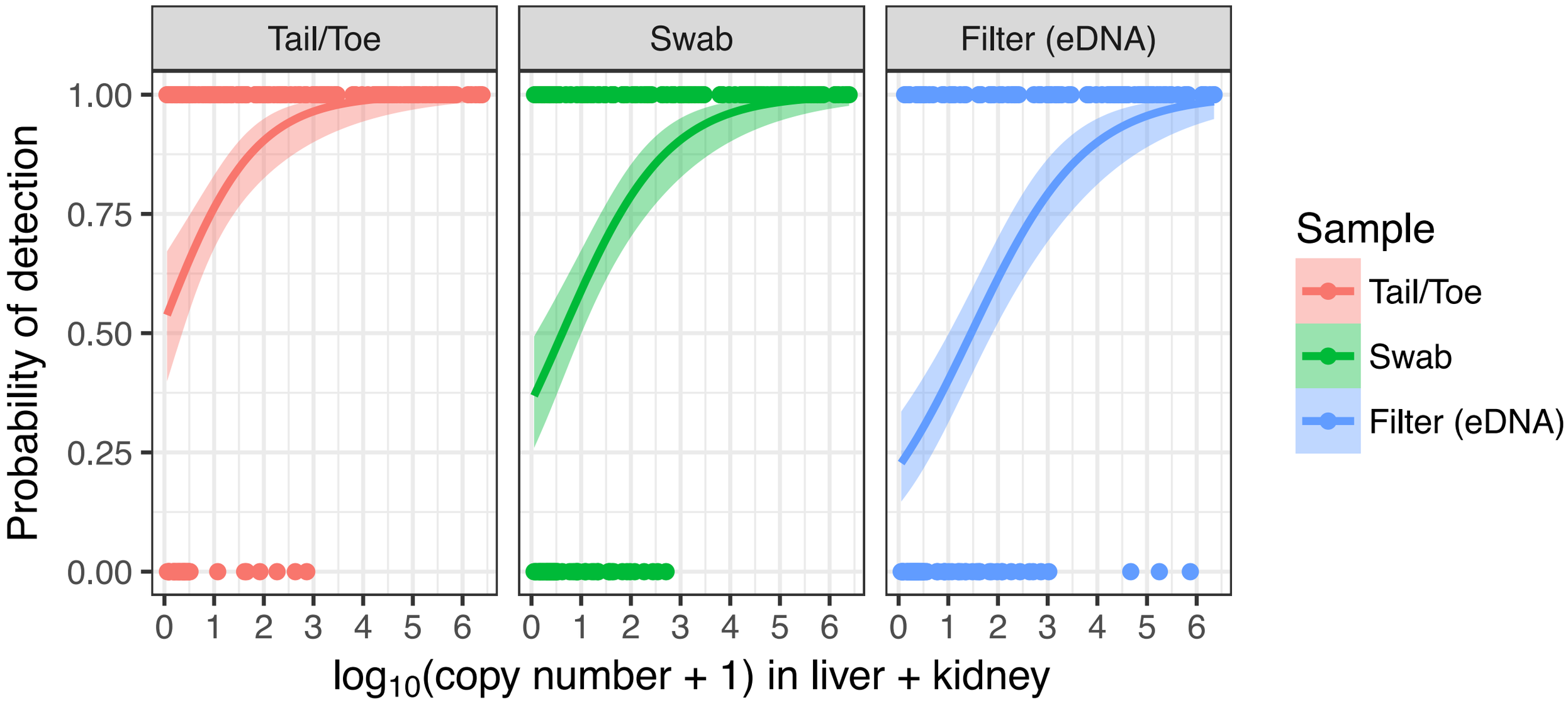
(>1 well with clear amplification)

get moderate sensitivity



Tail/Toe clip
Swab
Filter (eDNA)
Sensitivity
1.00
0.75
0.50
0.25
0.00
β = −0.037 ± 0.013
β = −0.064 ± 0.013
β = −0.041 ± 0.013
Number of samples
4
9
16
25
100
Specificity
β = 0.039 ± 0.016
β = 0.082 ± 0.02
β = 0.481 ± 0.405
0
10
20
30
40
50
Days post inoculation
Tail/Toe clip
Swab
Filter (eDNA)

Performance changes with time



Performance changes with *intensity*

Goal: use diagnostics to detect <u>at least one</u>

Find infection if present (not estimate prevalence)

Assume positive tests will be followed by other diagnostics

Goal: use diagnostics to detect <u>at least one</u>

Find infection if present (not estimate prevalence)

Assume positive tests will be followed by other diagnostics

<u>Individual samples</u>

$$\sum_{x=1}^{n} \frac{\binom{I}{x}\binom{N-I}{n-x}}{\binom{N}{n}} \left[1-(1-P_{detect})^{x}\right]$$

Prob sample (of size n) includes x infected individuals

Prob get at least one positive given x infected individuals tested

<u>eDNA</u>

$$1-(1-P_{detect})^{n}$$

Prob get at least one positive given n samples

Goal: use diagnostics to detect <u>at least one</u>

Find infection if present (not estimate prevalence)

Assume positive tests will be followed by other diagnostics

<u>Individual samples</u>

$$\sum_{x=1}^{n} \frac{\binom{I}{x}\binom{N-I}{n-x}}{\binom{N}{n}} \left[1-(1-P_{detect})^{x}\right]$$

Prob sample (of size n) includes x infected individuals

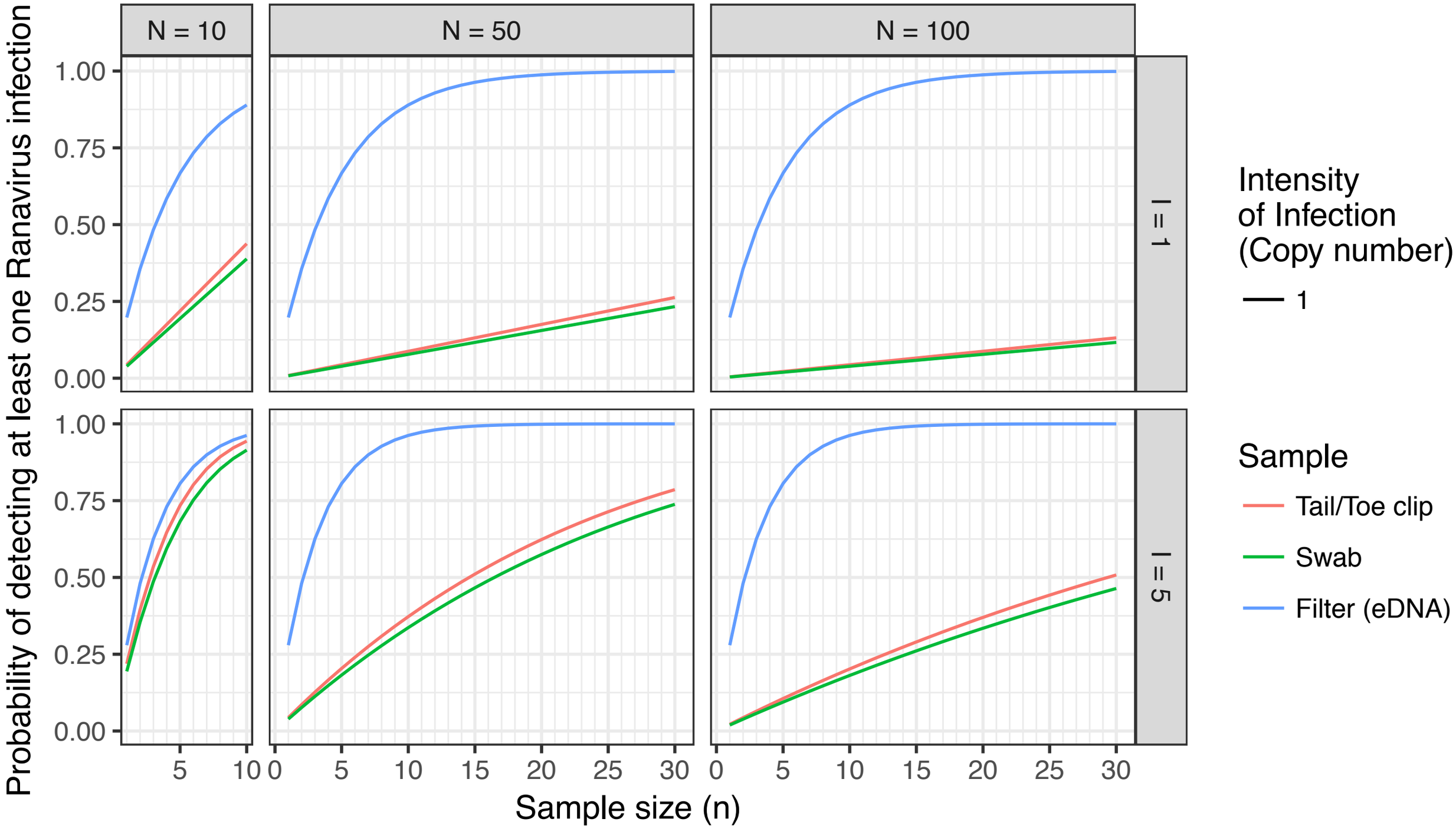
Prob get at least one positive given x infected individuals tested

<u>eDNA</u>

$$1-(1-P_{detect})^{n}$$

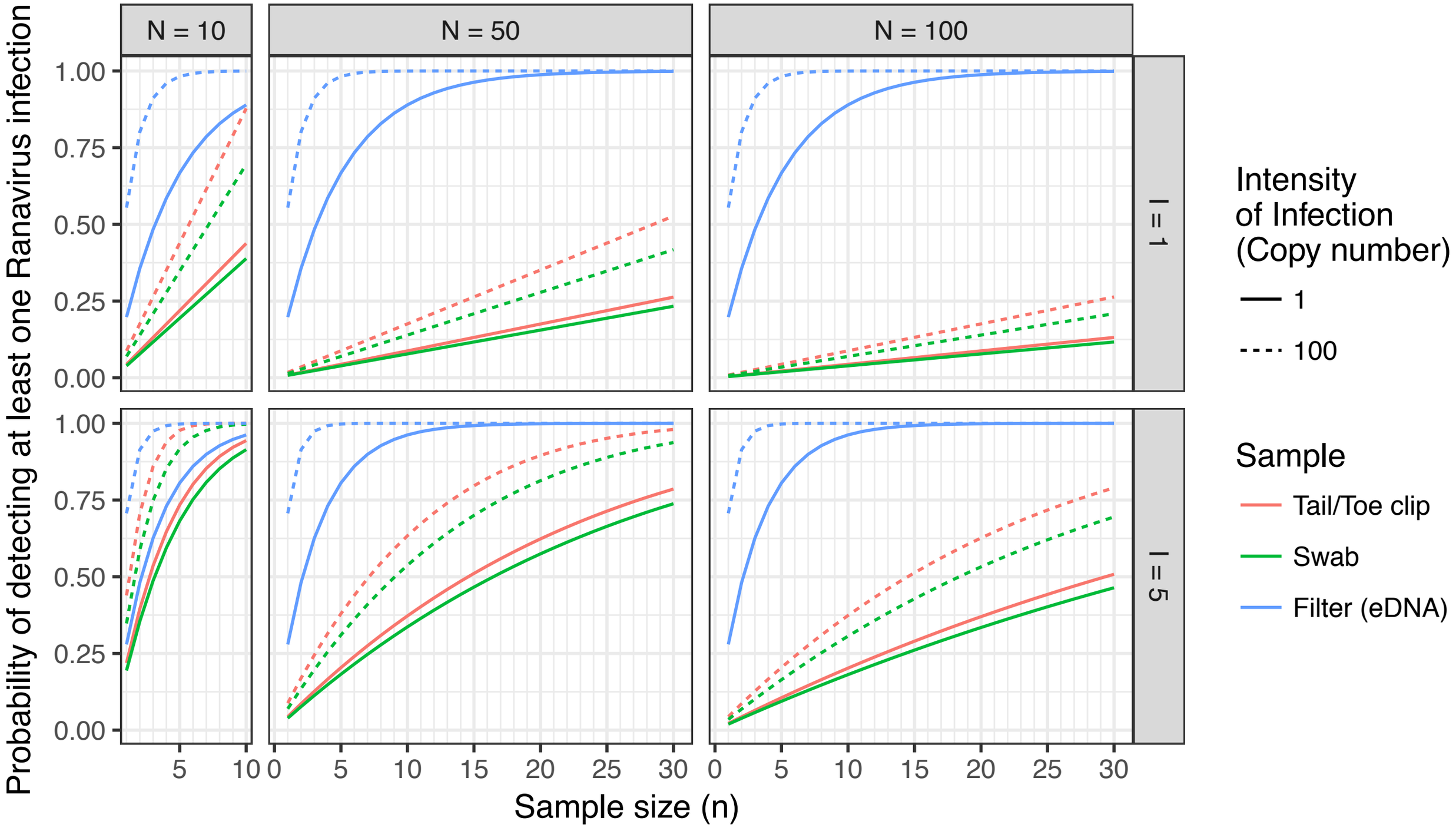
Prob get at least one positive given n samples

$$P_{detect} \sim \text{intensity}$$



eDNA can detect rare infections with fewer samples

Closed populations
Well-mixed water



eDNA can detect rare infections with fewer samples

Closed populations
Well-mixed water

Experiment #3

Screened

29 larvae

13 metamorphs

from five suppliers

Supplier	Stage	Kidney + Liver	Swab	Tail/ Toe	eDNA
Carolina Biological	L	0/7	0/7	0/7	0/3
LiveKoiForSale.com	L	0/9	0/9	0/9	0/3
Sugar Creek Fishery	L	**5/13**	**2/13**	**4/13**	**3/3**
Rana Ranch	M	0/8	***1/5****	0/8	0/3
Connecticut Valley Biological Supply	M	**3/5**	**5/5**	**3/5**	**3/3**

* false positive

Guidance and Considerations

Control/minimize contamination

- 50% bleach, strong UV, or flaming until metal turns blue
- Use dedicated room for *or* PCR hood in room without PCR products

Negative controls at each step

- Filter negatives (filter clean water alongside samples)
- Extraction controls (water blank and clean filter)
- No template controls

Synthetic positive control (e.g., gBlock with other target to look for contamination)

Use exogenous internal positive controls (exoIPCs) to check for PCR inhibition

Try to empirically evaluate P_{detect} for species of interest

How you will use the results? (Pessier & Mendelson 2010)

Pessier, A. P., and J. R. Mendelson. 2010. A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs. IUCN/SSC Conservation Breeding Specialist Group

Acknowledgements

Animal care and sample prep

Anjulie Olson

Jeremy Rice

Kai Wang

Sarah Meiners

Mitch Le Sage

Jenn Cundiff

Funding

Association of Zoo & Aquariums / Disney Conservation Fund

American Association of Zoo Veterinarians