

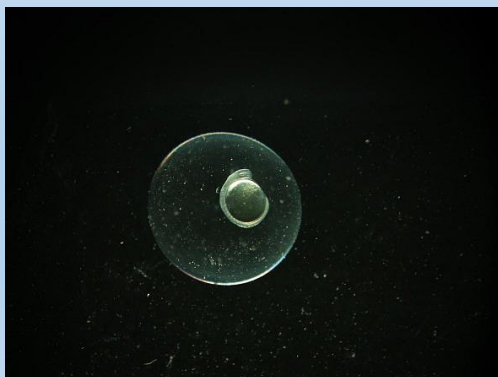
# Evaluation of a quantitative PCR screening procedure for rapid identification of invasive carp eggs and larvae in ichthyoplankton samples



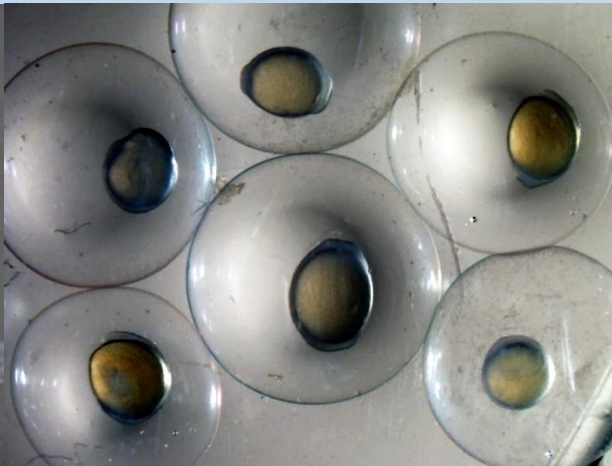
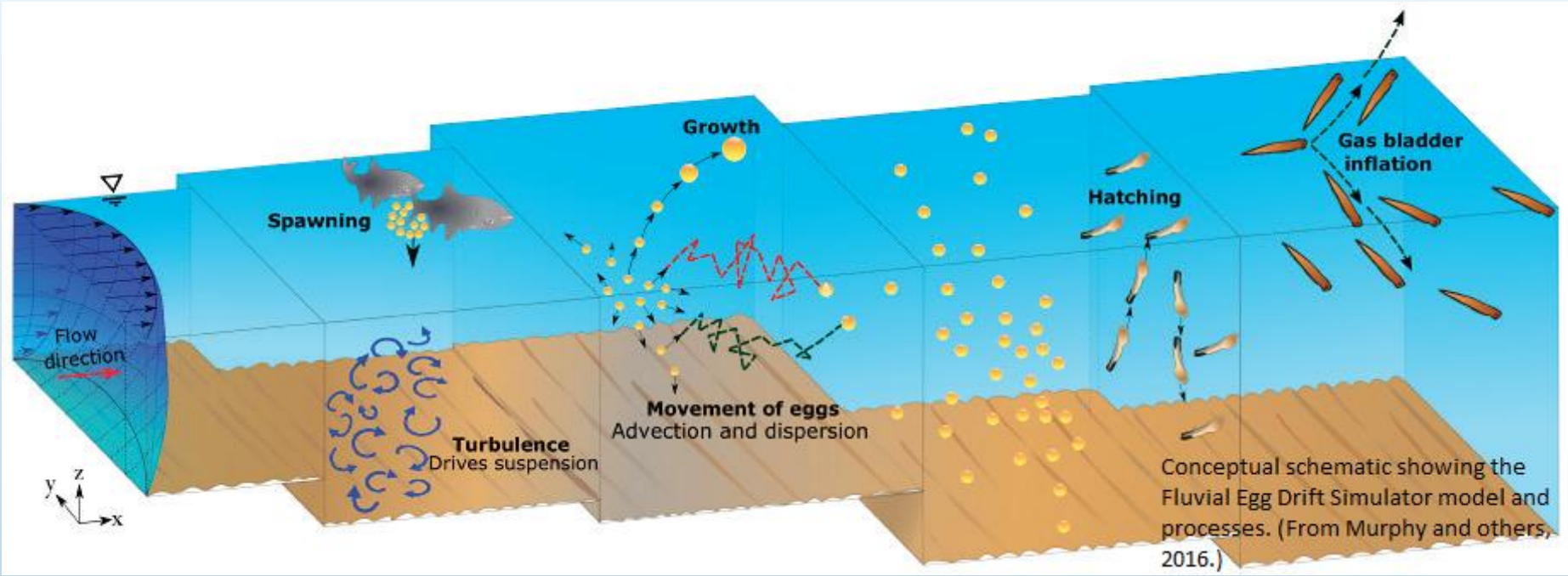
Steven E. Butler<sup>1</sup>, Nicholas J. Iacarus<sup>1</sup>, Mark A. Davis<sup>1</sup>, Zebadiah Woiak<sup>2</sup>,  
Patrick W. DeHaan<sup>2</sup>, Joseph J. Parkos<sup>1</sup>



<sup>1</sup> Illinois Natural History Survey, Prairie Research Institute, University of Illinois  
<sup>2</sup> U.S. Fish and Wildlife Service, Whitney Genetics Lab



# Invasive Carp Reproduction 101



# Sampling for invasive carp eggs and larvae



# Ichthyoplankton monitoring limitations

- **Time intensive: processing large number of samples can take weeks to months**
  - **High labor cost**
  - **Time lag in delivery of information**
- **ID limitations: coarse taxonomic resolution**
  - **Many taxa can only be identified to genus or family level using meristic and morphometric characteristics**
  - **Eggs may be difficult or impossible to ID even as invasive carp**
  - **Genetic analysis adds additional cost to monitoring program**



# **Ichthyoplankton monitoring limitations**

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**Can we determine whether invasive carp eggs or larvae occur in ichthyoplankton samples without expending the time and labor involved with manual sample processing?**









# Quantitative PCR (qPCR) method

Biol Invasions (2019) 21:1143–1153  
<https://doi.org/10.1007/s10530-018-1887-9>

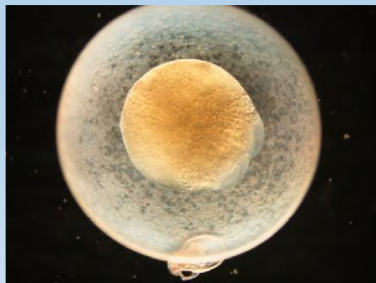


ORIGINAL PAPER

## Development of a quantitative PCR method for screening ichthyoplankton samples for bigheaded carps

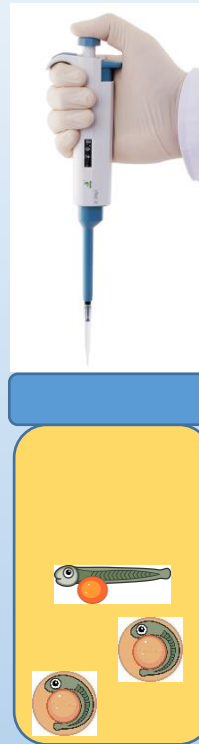
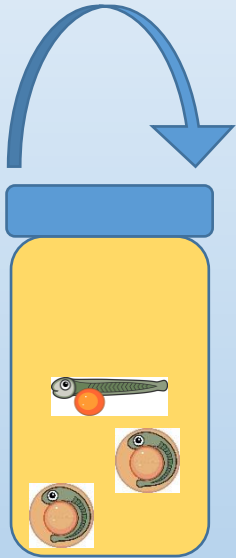
Andrea K. Fritts  · Brent C. Knights  · James H. Larson  · Jon J. Amberg  ·  
Christopher M. Merkes  · Tariq Tajjioui  · Steven E. Butler  ·  
Matthew J. Diana · David H. Wahl · Michael J. Weber  · John D. Waters

- Use qPCR to identify samples that have a high probability of containing invasive carp eggs or larvae based on the quantity of target DNA in a sample



# qPCR Screening Method

Ethanol Exchange



Draw replicate aliquots  
of sample preservative

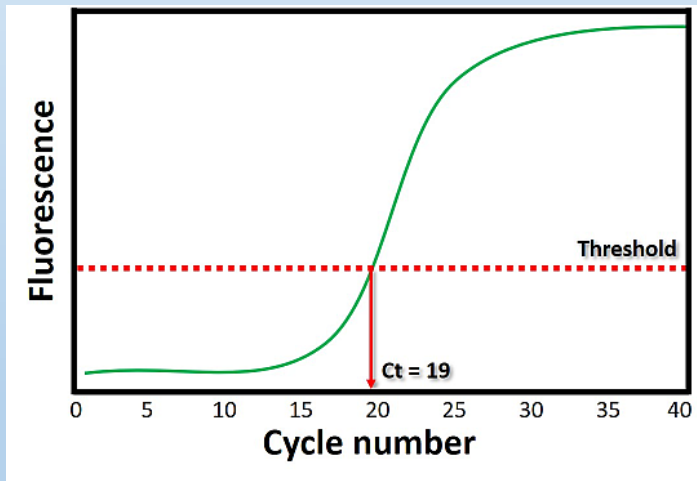
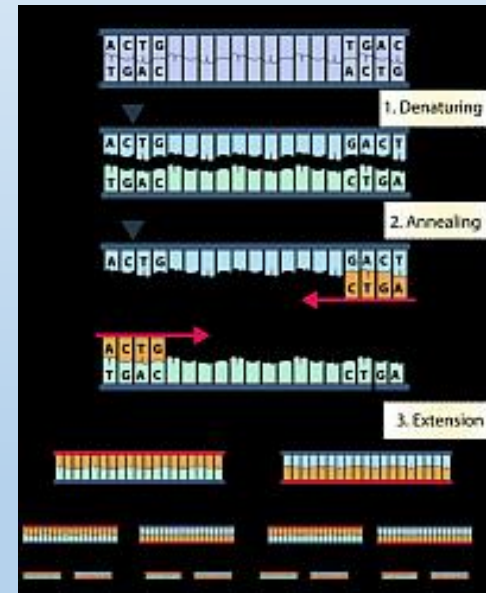


# qPCR Screening Method

DNA extraction



DNA amplification



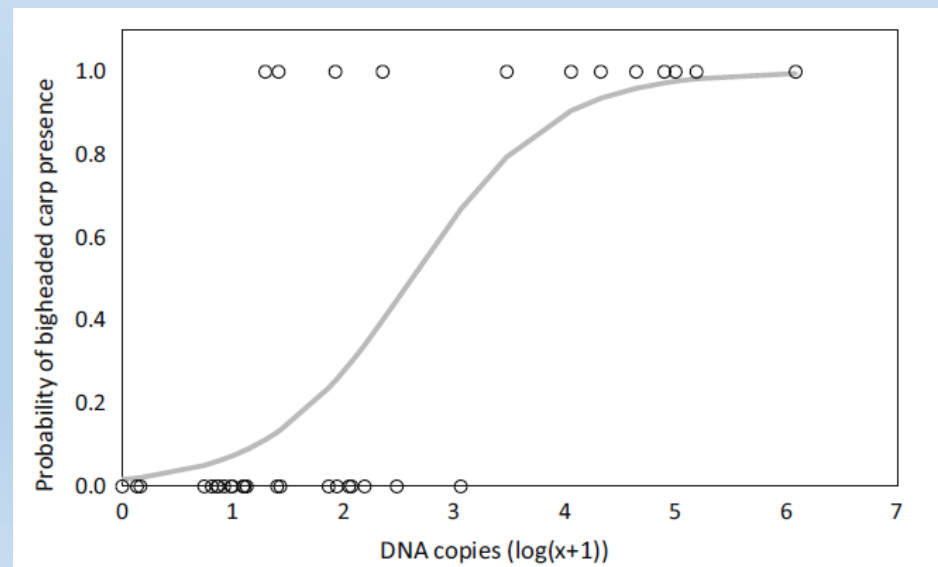
Monitor fluorescence increase per cycle;  
Identify number of cycles needed to reach threshold;  
Compare to standard curves of known number of DNA copies



# qPCR Screening Method

Fritts et al. provided proof of concept:

- Samples with  $> 10,000$  copies of DNA had 100% occurrence of bigheaded carp eggs or larvae
- Samples with  $< 10$  copies of DNA had 0% occurrence of bigheaded carp eggs or larvae
- 406 DNA copies  $\approx$  50% probability that a sample contains bigheaded carp eggs or larvae
- 15 DNA copies  $\approx$  10% probability



# Objectives

- Evaluate efficacy of qPCR screening procedure as part of sampling program monitoring for invasive carp reproduction
- Expand capabilities of qPCR methodology by screening for all 4 species of invasive carps
- Determine sensitivity, specificity of qPCR procedure
- Evaluate influence of organic debris on relationship between DNA copy numbers and presence of invasive carp eggs and larvae
- Evaluate the ability of the qPCR procedure to predict magnitude of egg / larvae abundance

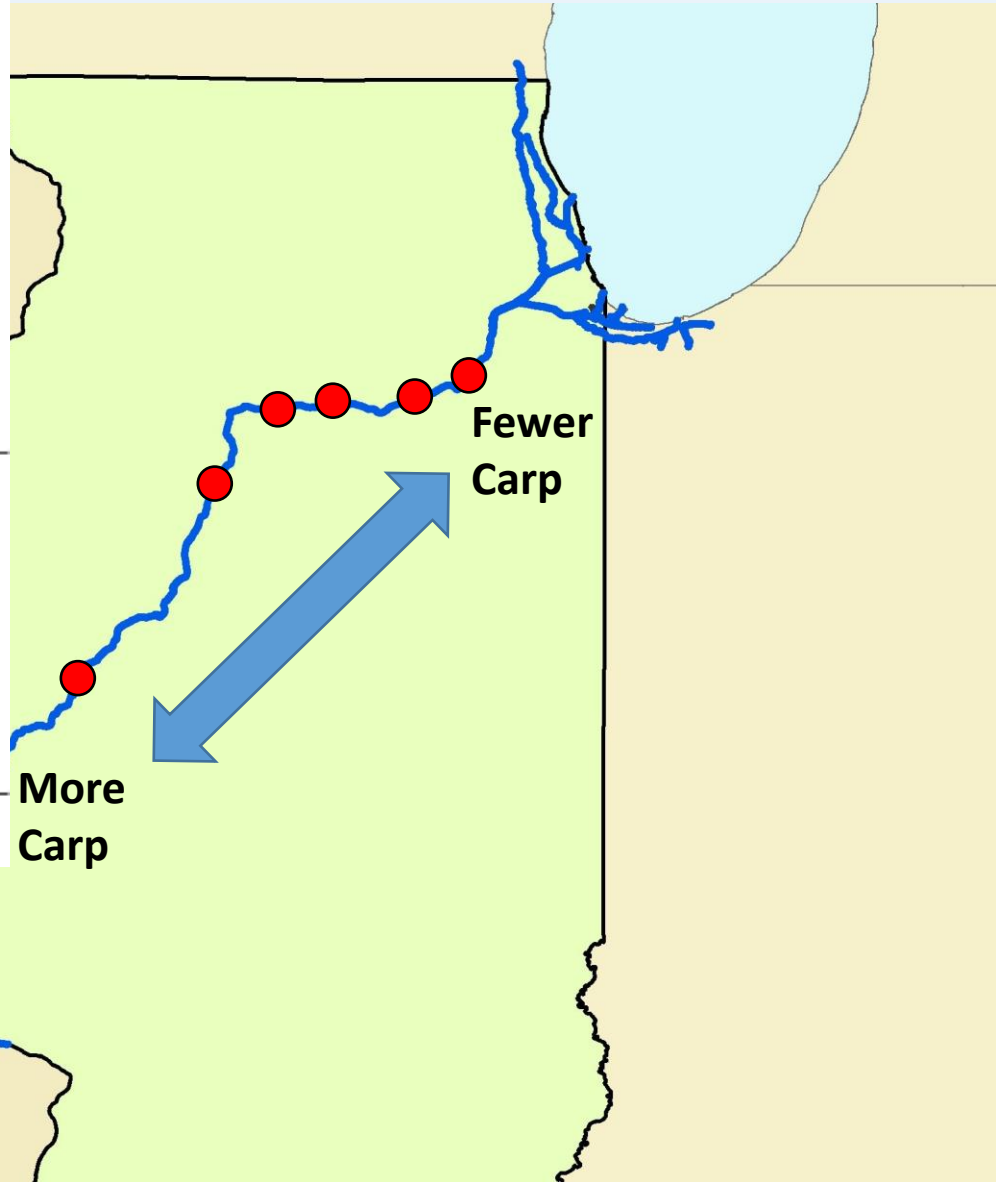
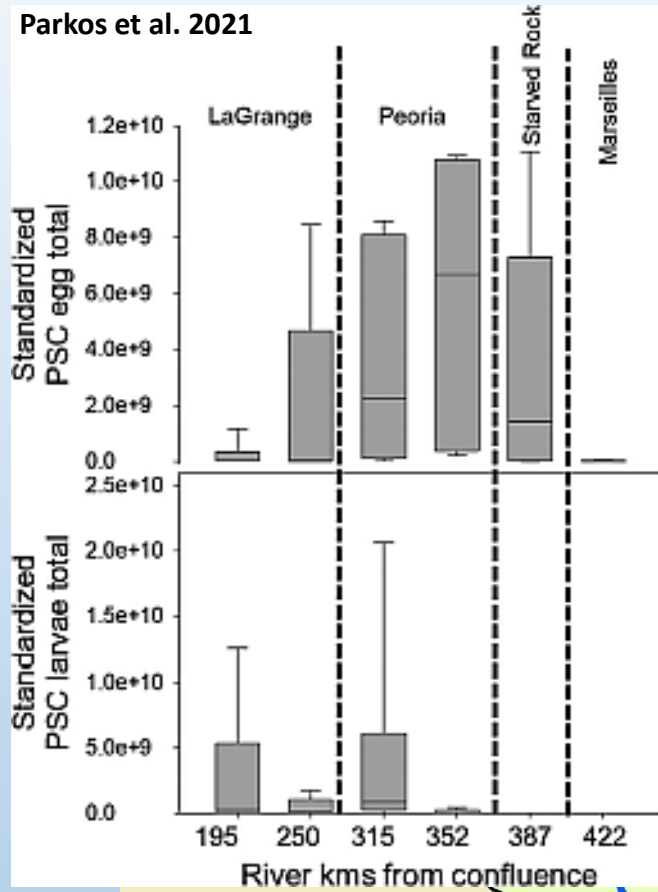


# Methods



# Methods

Parkos et al. 2021

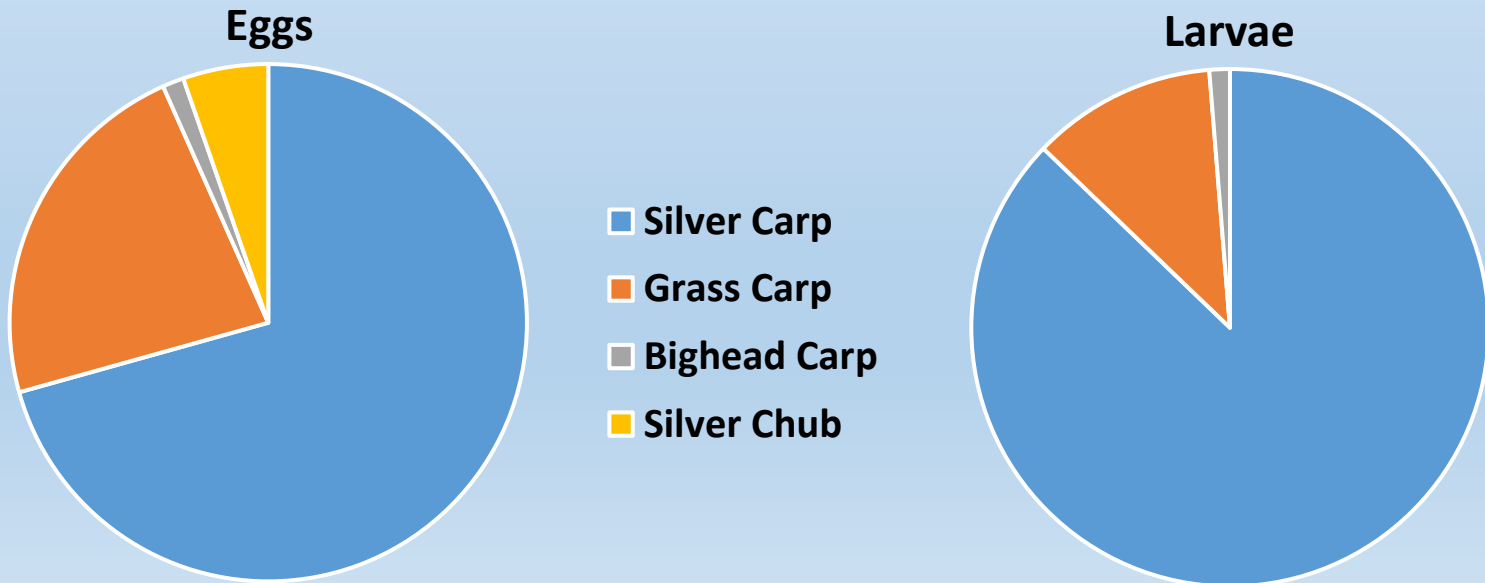


# Methods

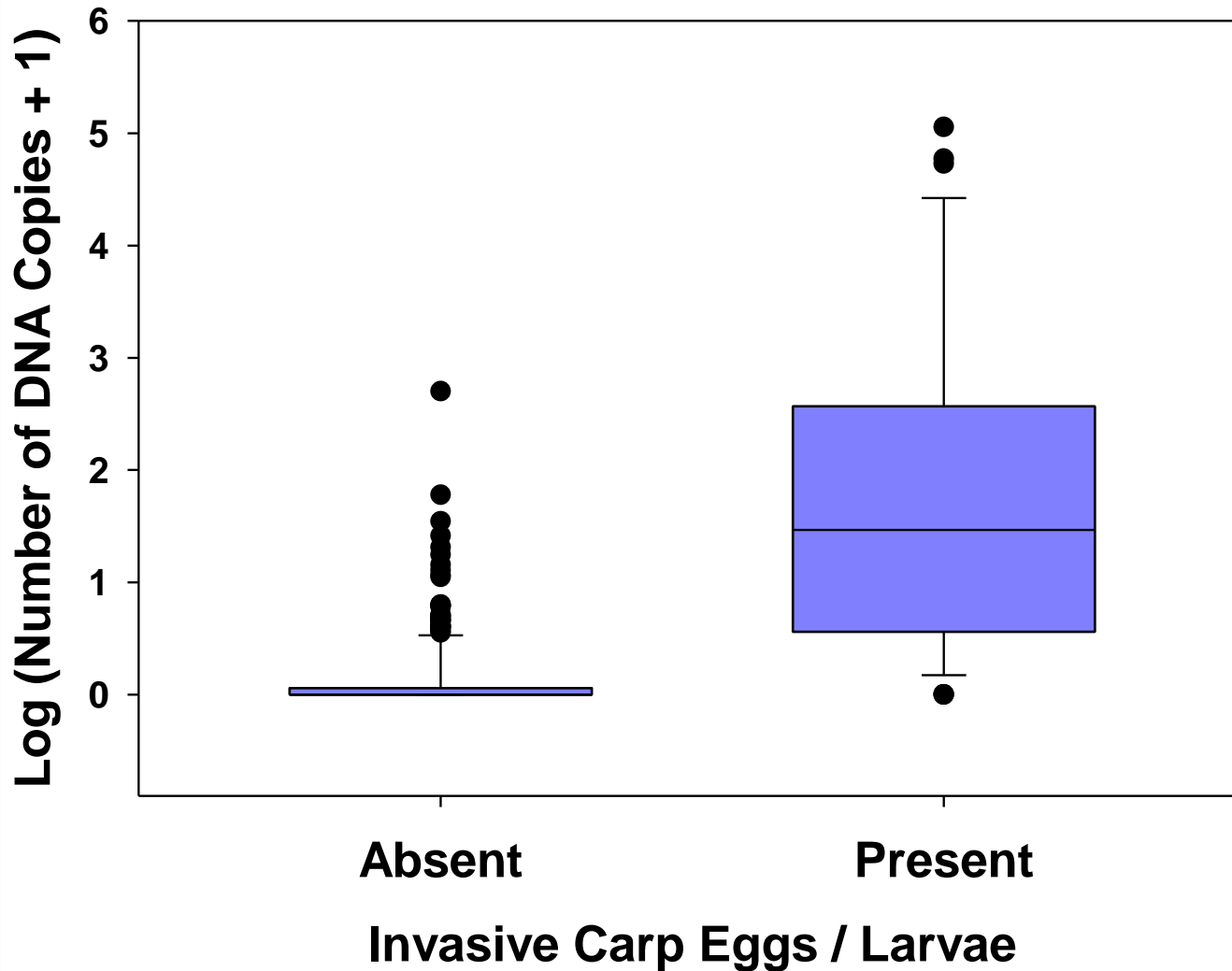
- **Ichthyoplankton samples collected from 6 sites in the Illinois Waterway during 2020 and 2021**
- **Preservative aliquots drawn from each sample following ethanol exchange; organic debris volume and mass in each sample measured; eggs and larvae visually identified and enumerated**
- **Egg and larvae identifications independently assessed by USFWS Whitney Genetics Lab**
- **All aliquots screened for quantity of DNA from all 4 species of invasive carp using qPCR reactions – INHS Collaborative Ecological Genetics Lab**
- **Relationships between DNA copy numbers, presence / absence of invasive carp eggs & larvae, quantity of organic matter in each sample assessed using generalized linear models**

# Results

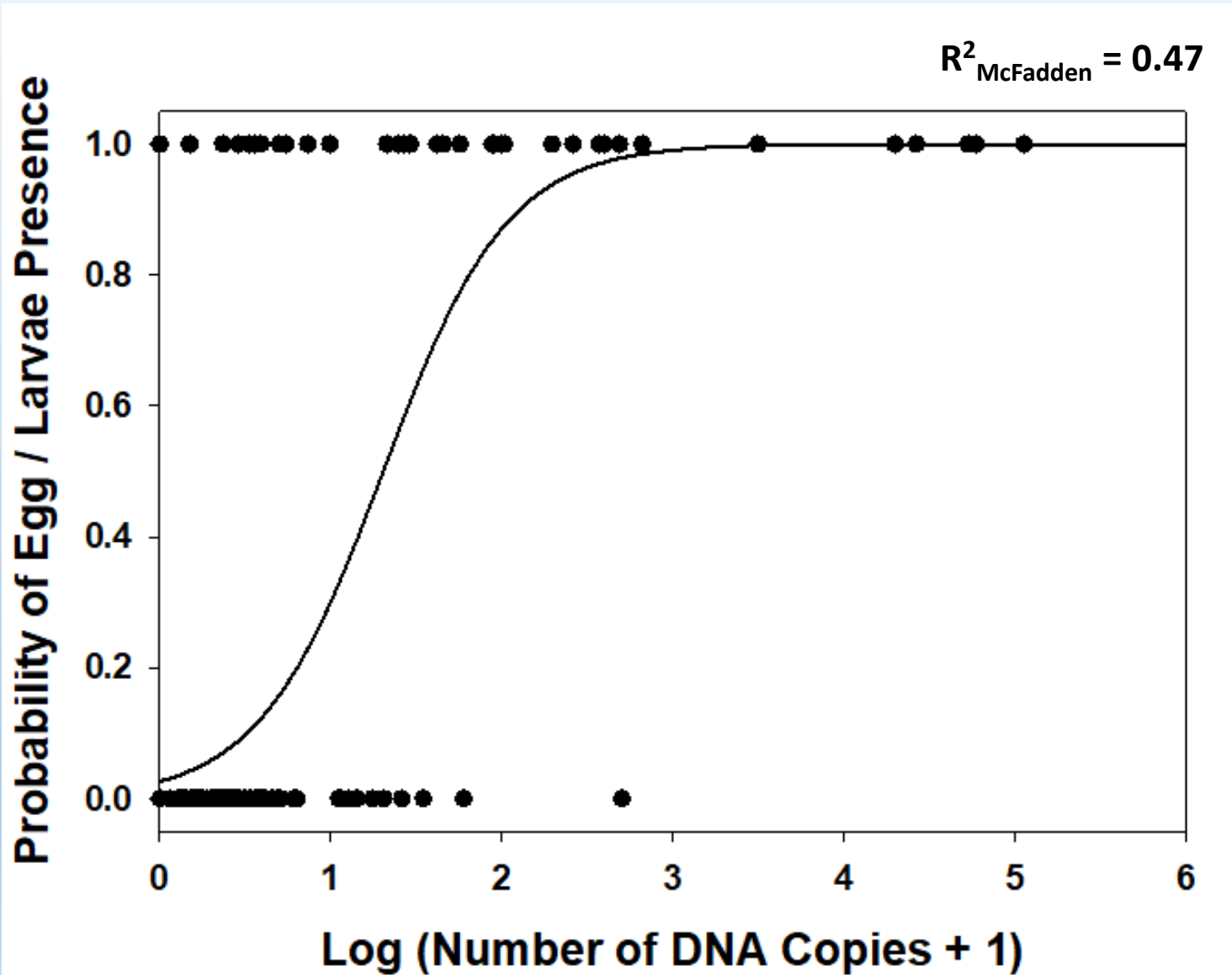
- 338 ichthyoplankton samples screened using qPCR procedure
  - 112 were found to contain at least trace invasive carp DNA (33.1%)
    - 94 Silver Carp
    - 41 Grass Carp
    - 6 Bighead Carp
    - 1 Black Carp
  - 39 contained invasive carp eggs and/or larvae (11.5%)
- 125 eggs / 169 larvae submitted for independent genetic identification



# Results

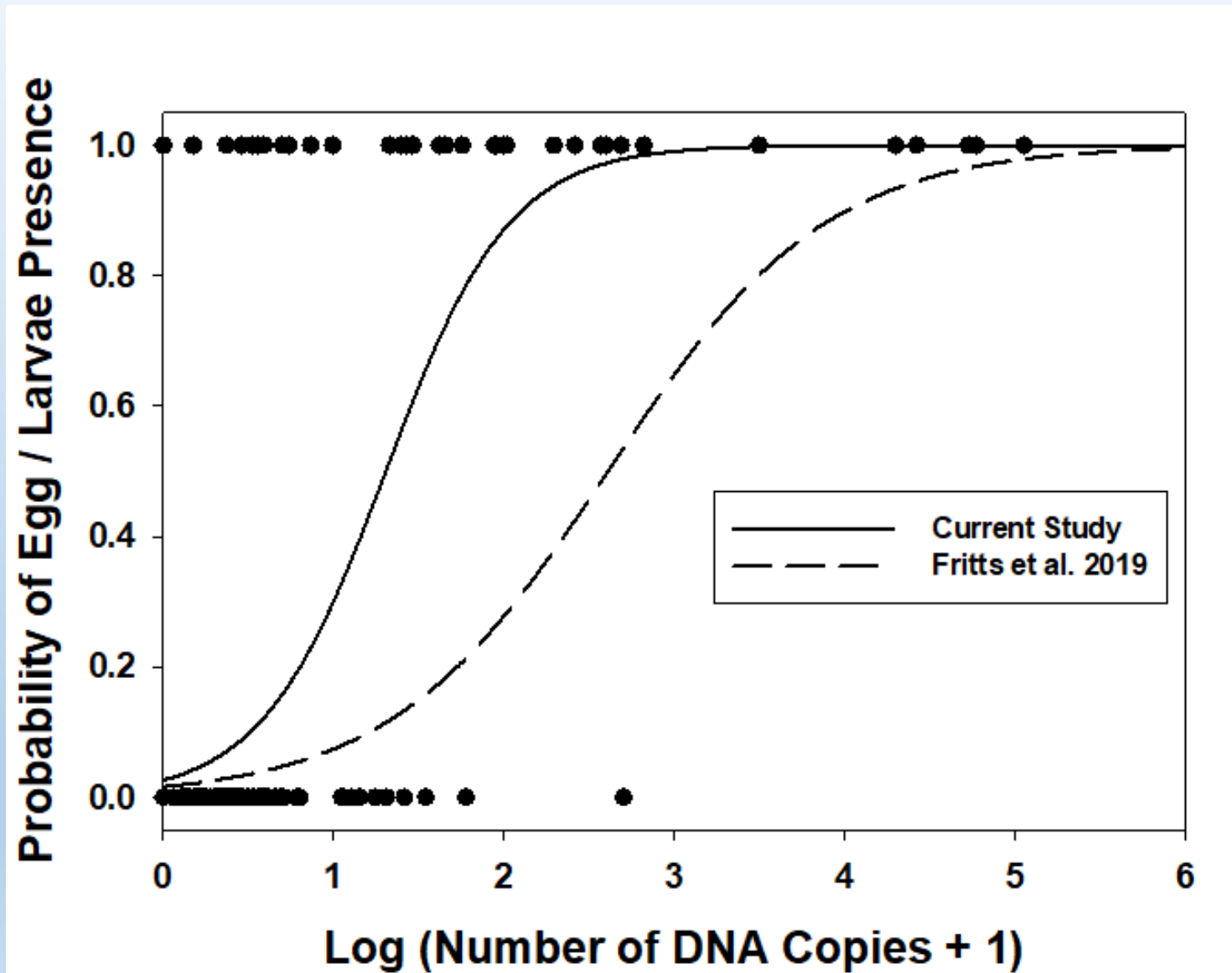


# Results

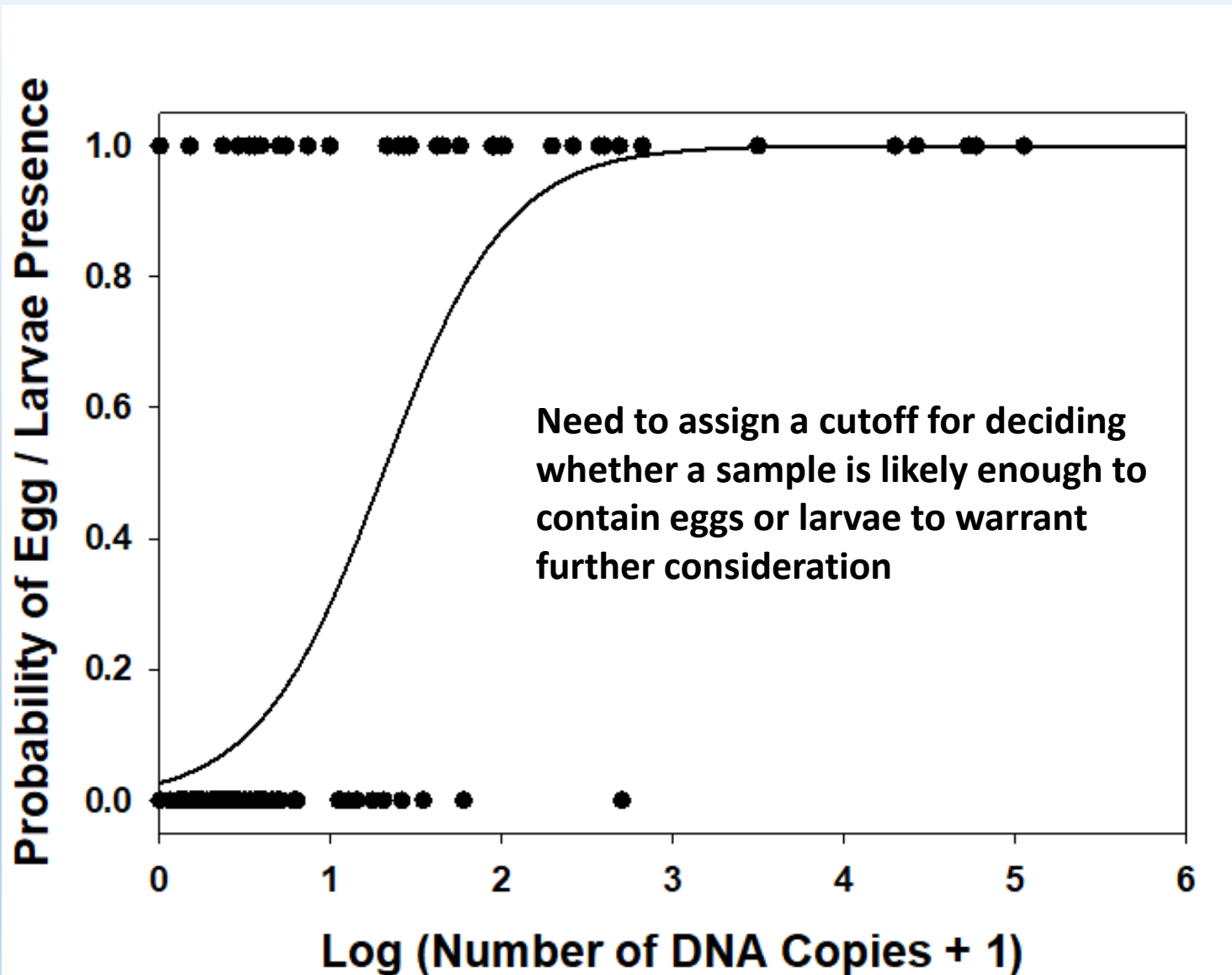




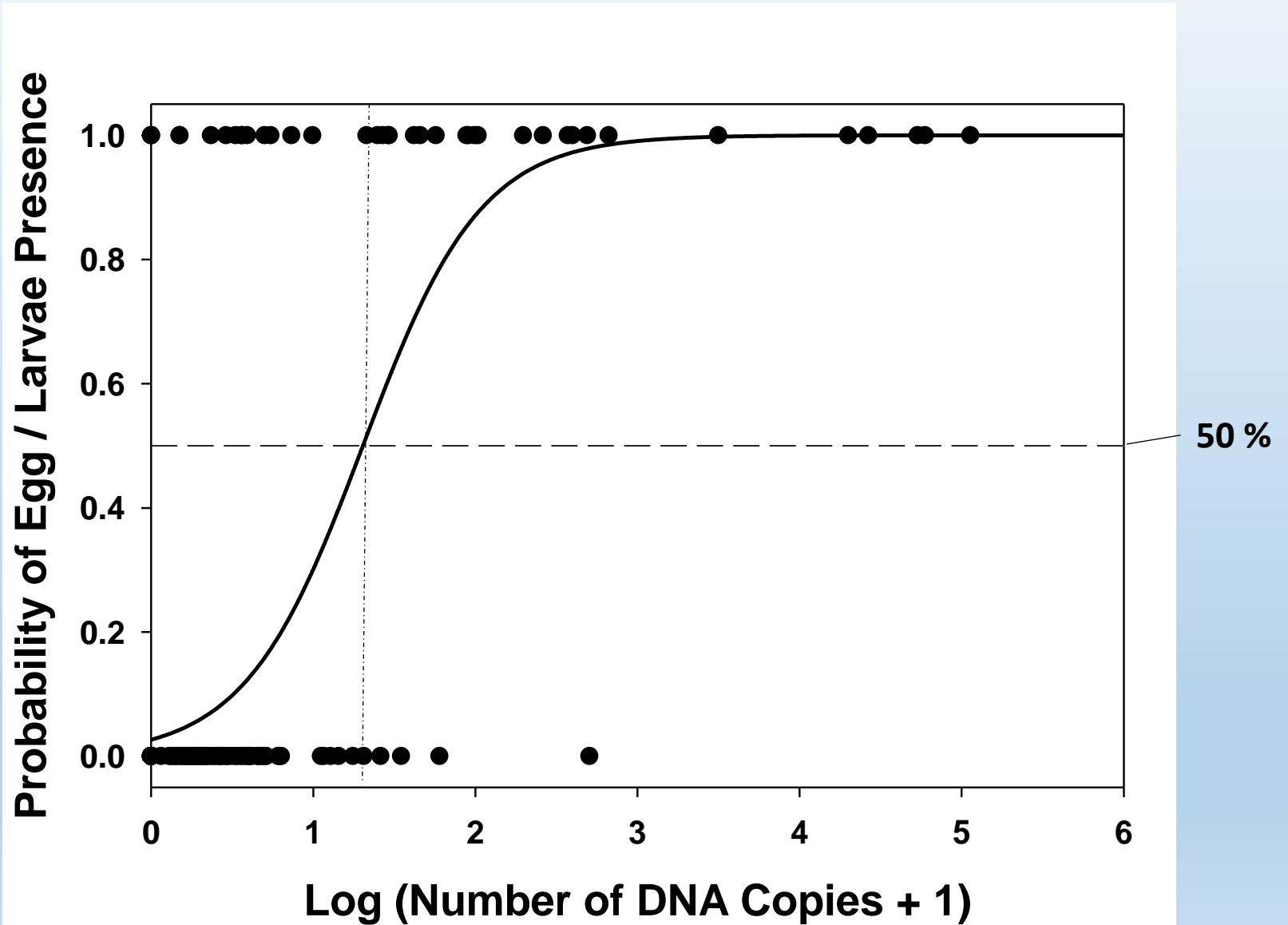
# Results



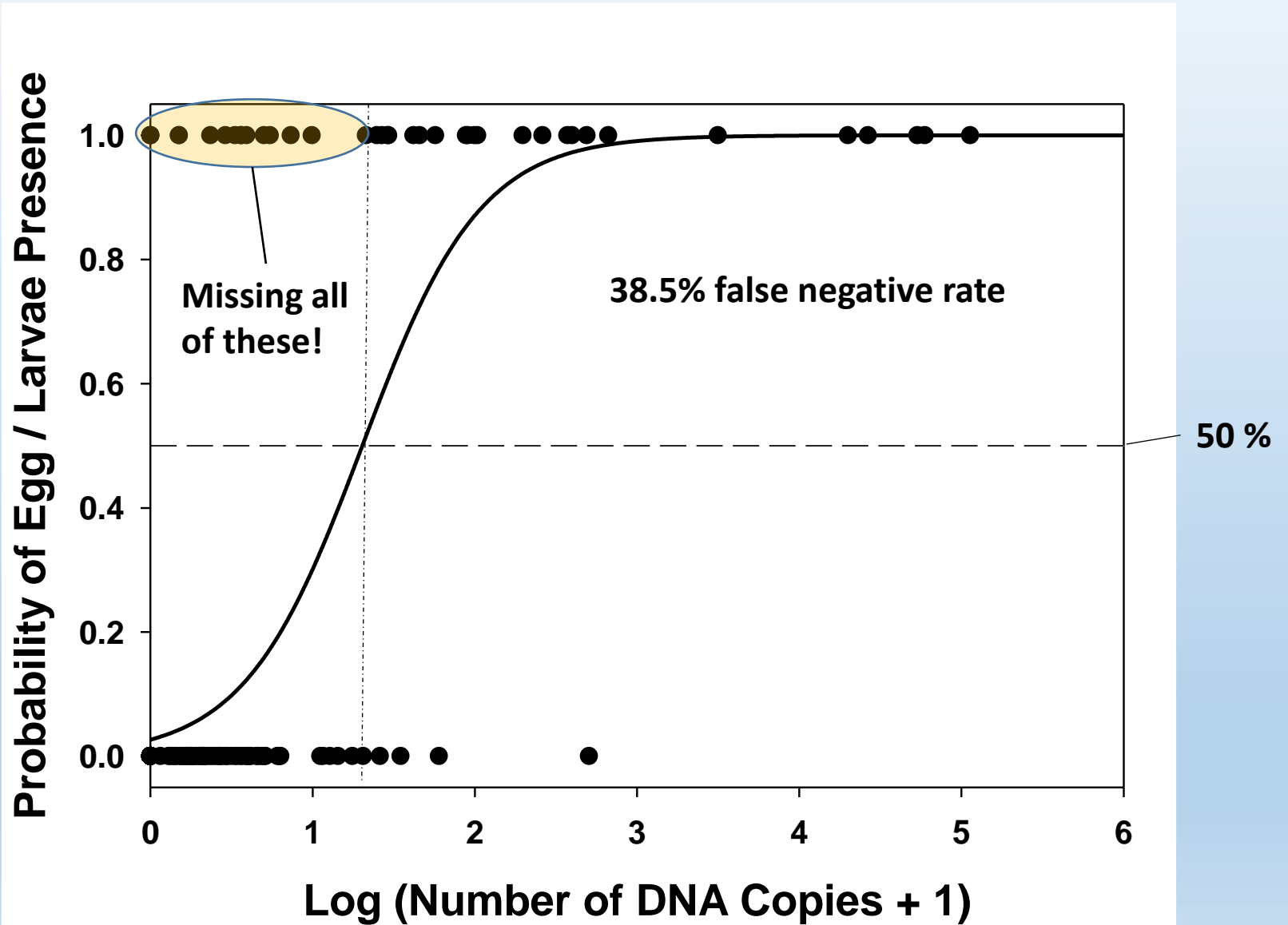
# Results



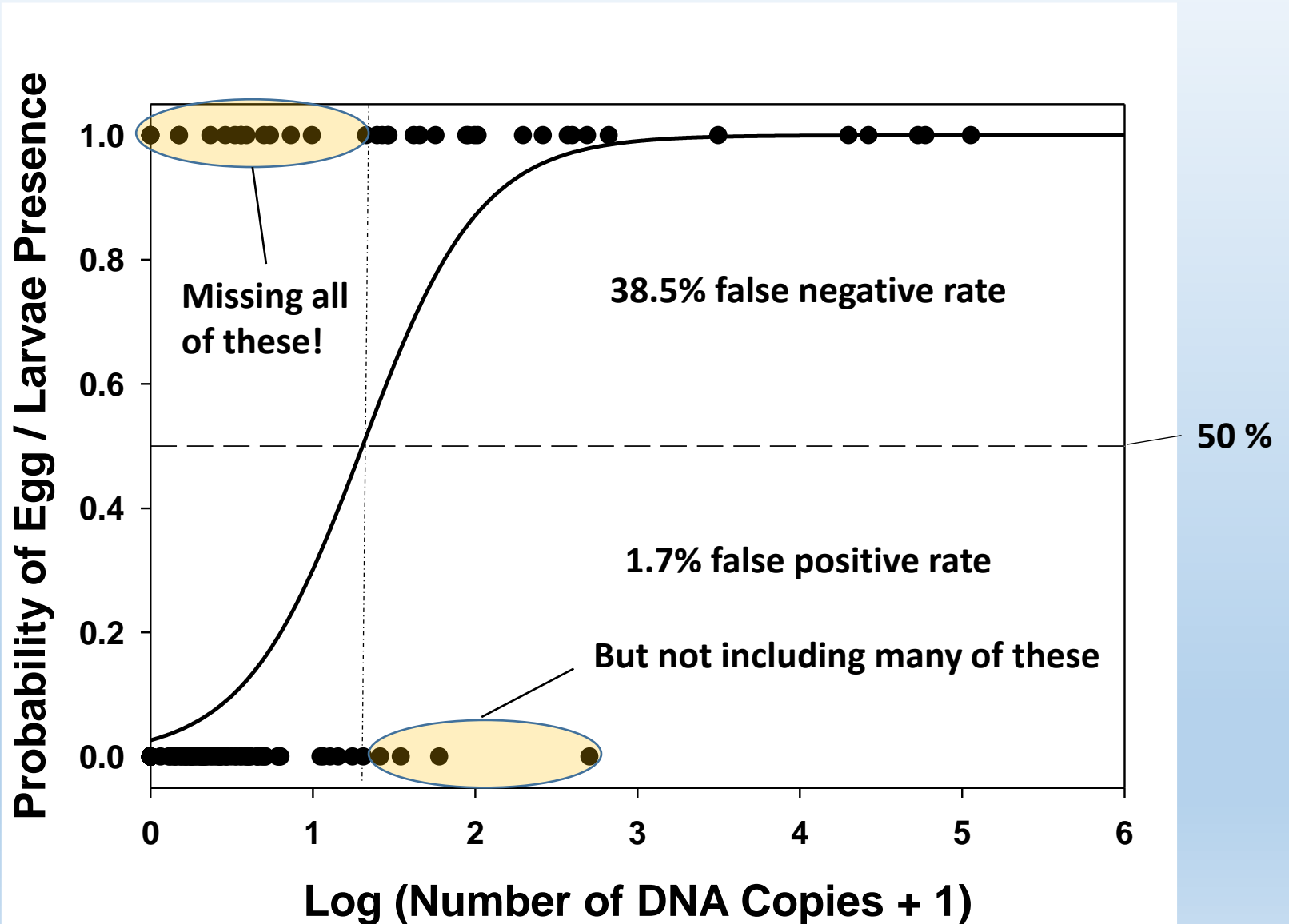
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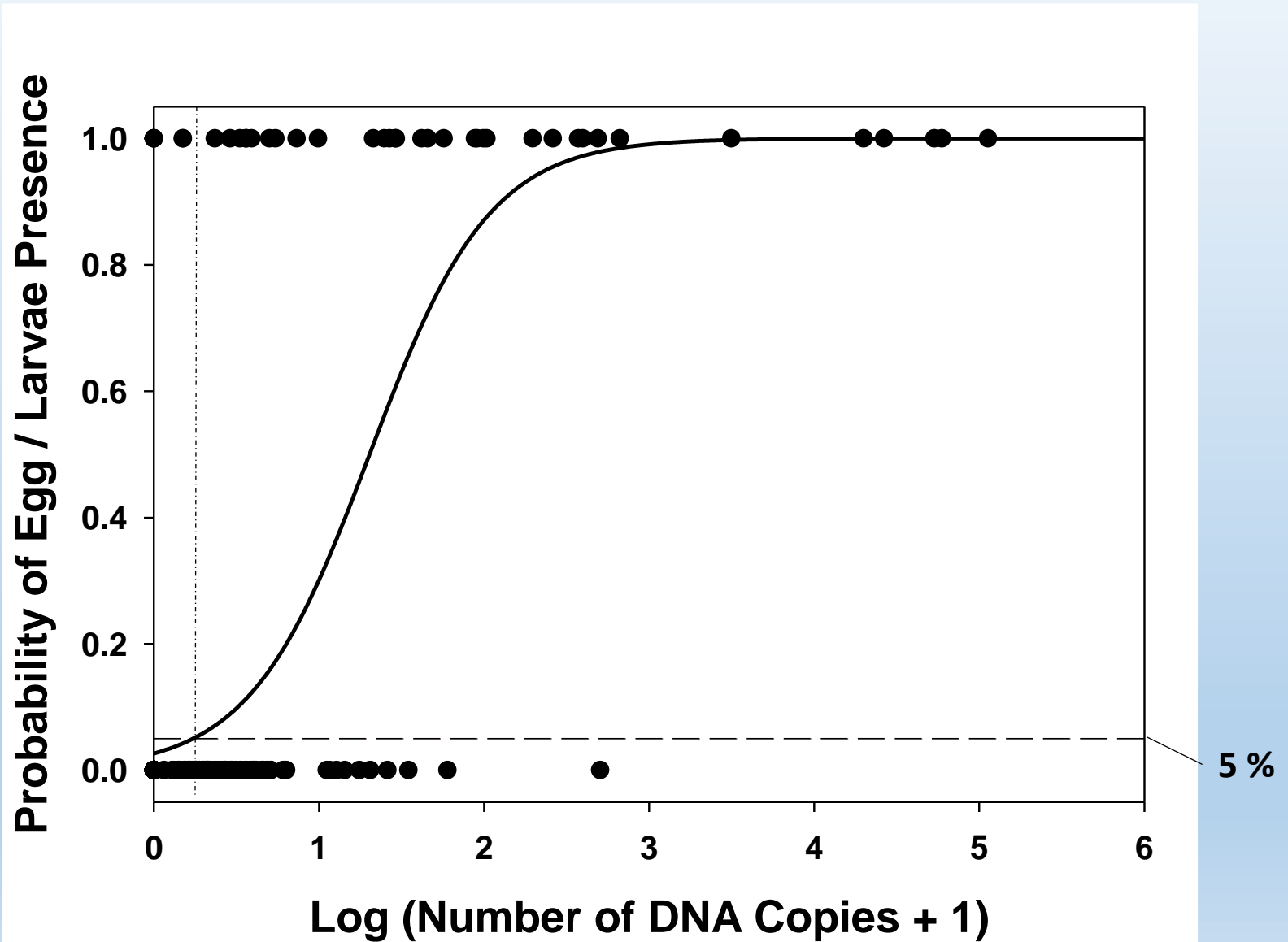
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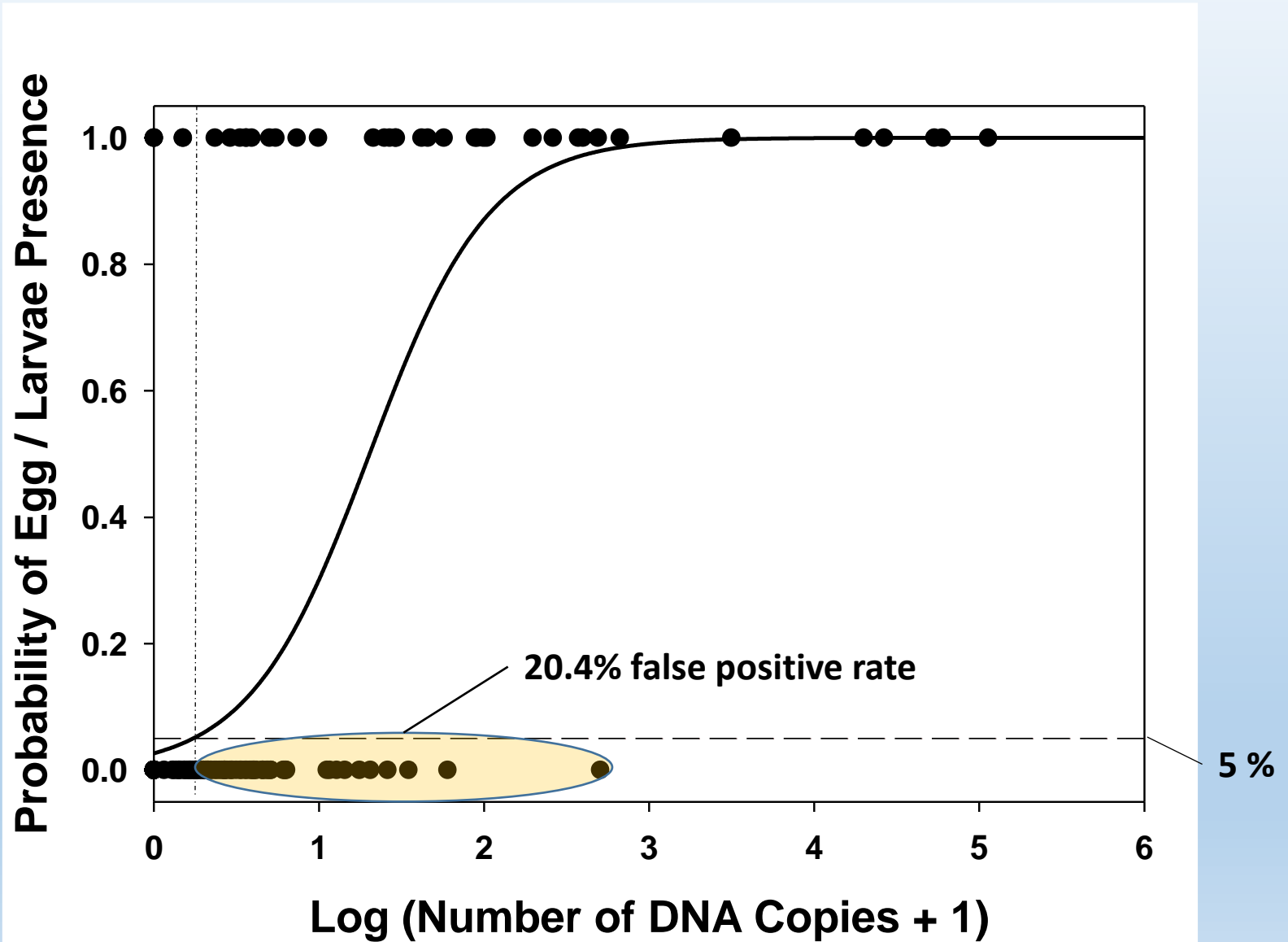
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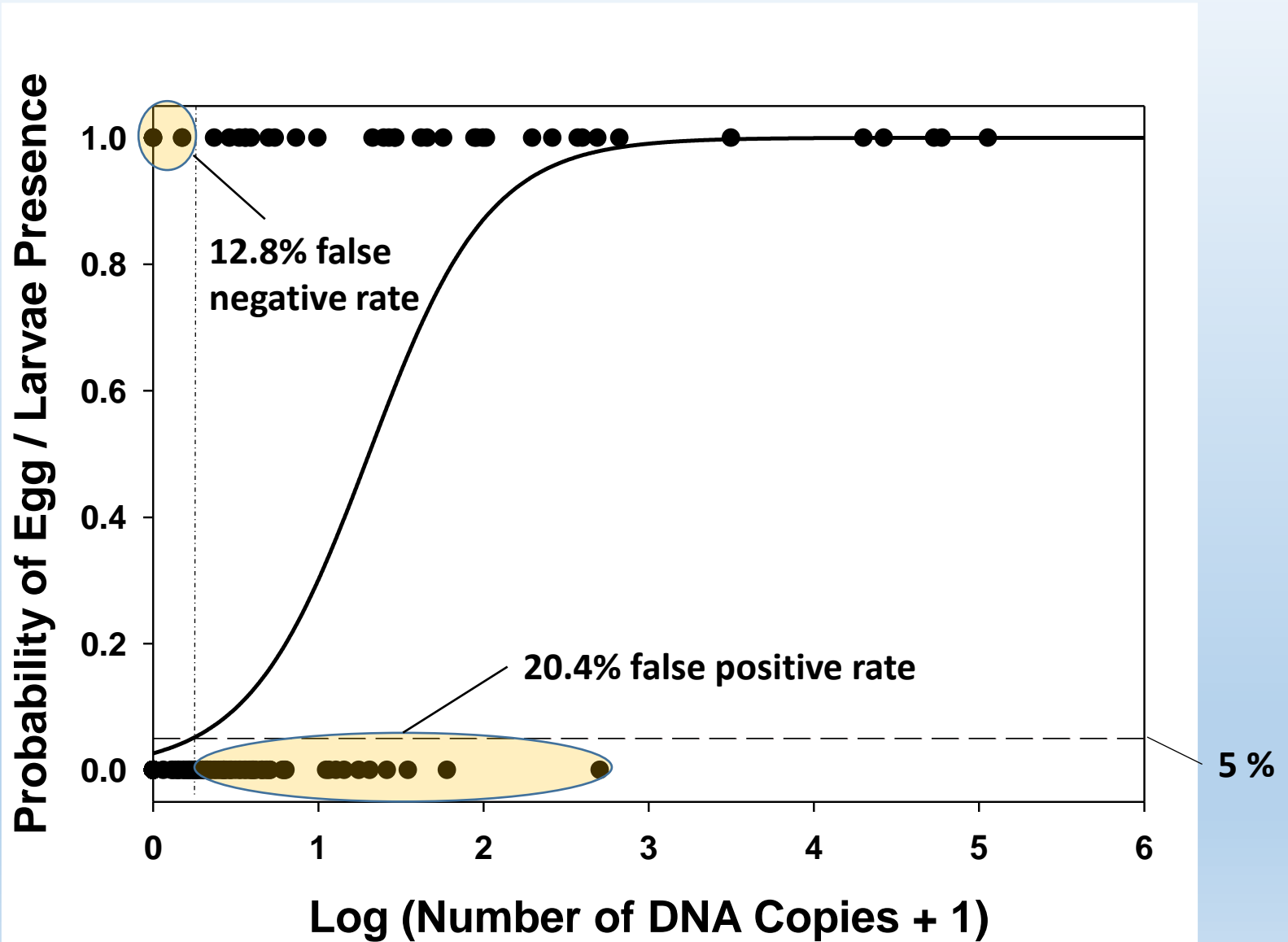
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# Results

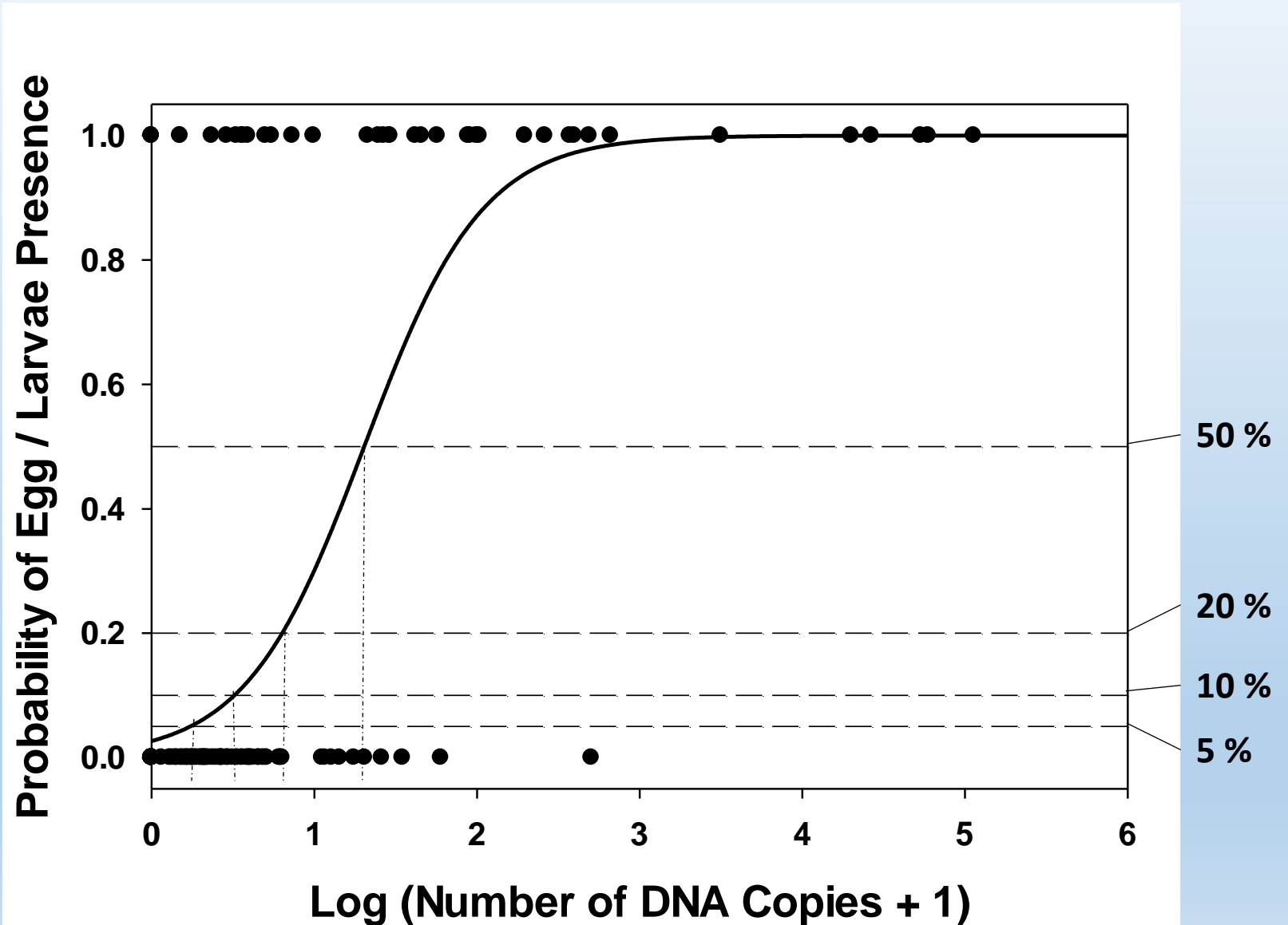


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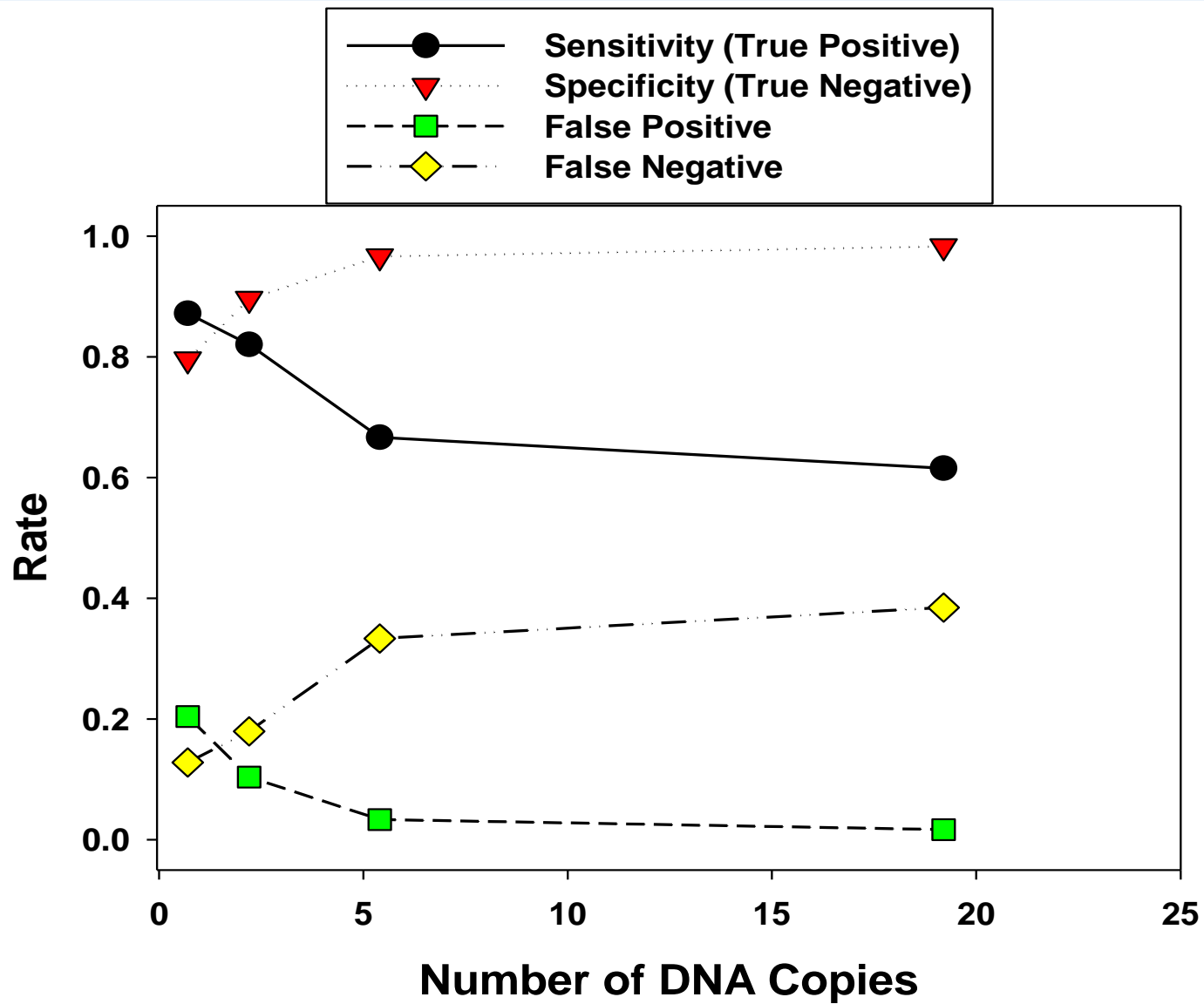




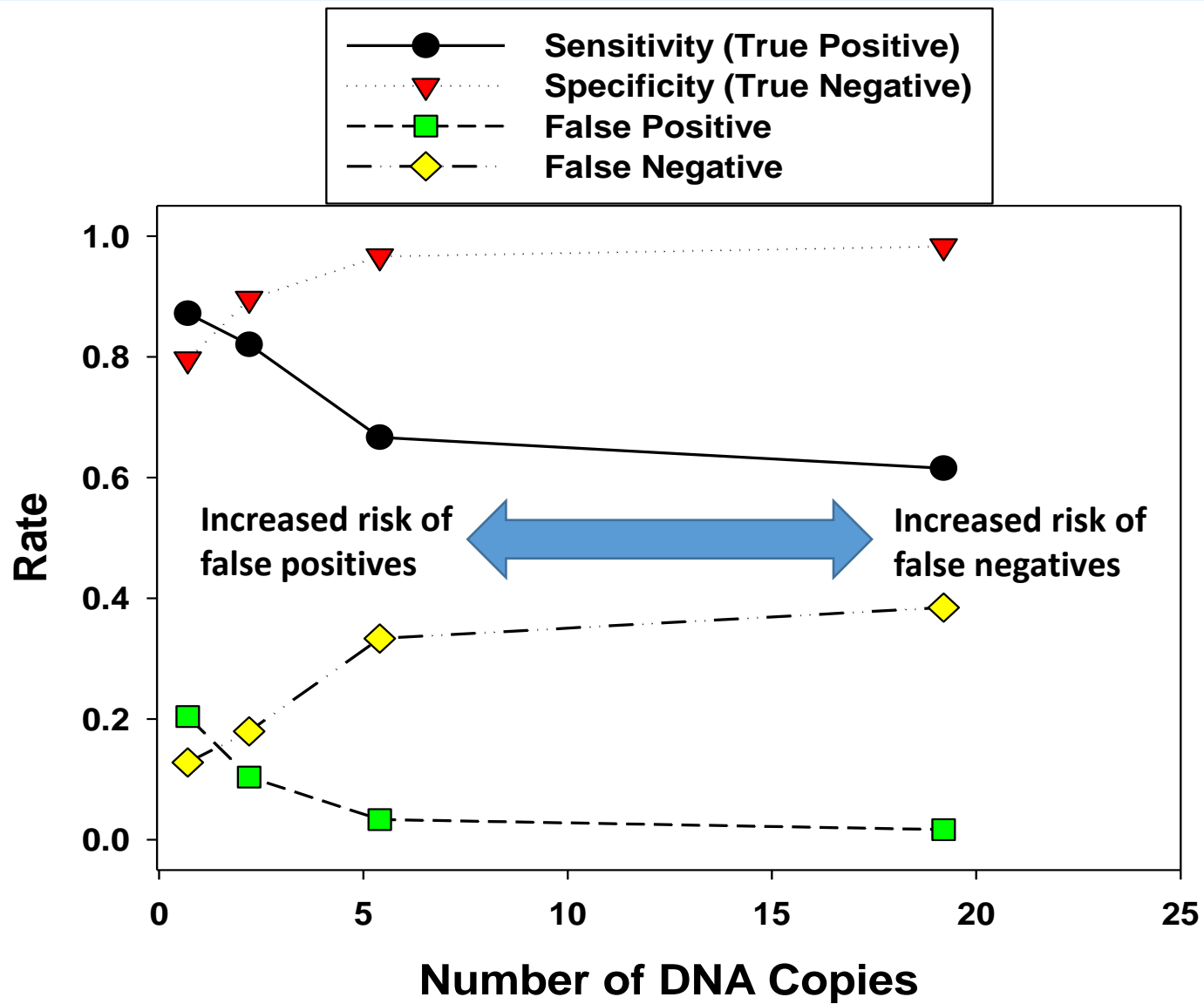
# Results



# Results



# Results



# Results

- 39 of 338 samples actually found to contain invasive carp eggs and/or larvae

## Comparing Thresholds:

- ANY invasive carp DNA
  - 112 samples (33.1%) flagged as potentially containing invasive carp
  - False Positives = 73
  - False Negatives = 3
- 5% probability = 0.7 DNA copies
  - 95 samples (28.1%) flagged as potentially containing invasive carp
  - False Positives = 61
  - False Negatives = 5
- 10% probability = 2.2 DNA copies
  - 63 samples (18.6%) flagged as potentially containing invasive carp
  - False Positives = 31
  - False Negatives = 7

# Results

- **Organic matter variables were not significant terms when added to the model**
- **Inclusion of any organic matter variable in logistic models had no effect on sensitivity, specificity, false positive, and false negative rates**







# Conclusions

- **Number of invasive carp DNA copies present in a sample is a significant predictor of the probability that a sample will contain invasive carp eggs and/or larvae**
- **Quantity of organic debris in a sample does not appear to affect the relationship between number of DNA copies and probability of egg/larvae presence**
- **Accuracy, sensitivity, and specificity of the qPCR screening procedure depend on the threshold probability of egg/larvae presence**
  - **Tradeoff between risk of false negatives and false positives**
- **Number of invasive carp DNA copies present in a sample is positively related to the overall number of invasive carp eggs and larvae, but poor predictive power**





# Future Directions

## Cost estimates:

- Cost of supplies, reagents, labor associated with qPCR screening
- Cost reduction associated with processing fewer samples

## Controlling for sources of error:

- False Positives: Minimizing DNA contamination
- False Negatives: Identify sources of PCR inhibition

## Species-specificity:

- Can we assign independent probabilities to each invasive carp species?

## Faster results:

- In-Field qPCR (30-60 minute results)

## Expand capabilities:

- Metabarcoding
- Other species – Invasives, T&E species



# Acknowledgements



- Staff of INHS Kaskaskia Biological Station, Collaborative Ecological Genetics Lab
- University of Illinois Graduate Students
- Ichthyoplankton ID Ninjas: Guy Schmidt  
Rafael Davila



Questions?

