RECLAMATION

Managing Water in the West

Improving Detection of Quagga Mussels by Polymerase Chain Reaction

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Special thanks to Jamie Carmon
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Hydraulic Investigations & Lab Services

U.S. Department of the Interior
Bureau of Reclamation

DNA Detection Optimization

- **RDLES conducts research to optimize every step of the sample collection and analysis process**
  - **Field collection**
  - **Processing**
  - **Analysis**

- **Techniques can be used to monitor other invasive or endangered species**

DNA Sample Processing

-Implemented a barcode program to track samples

-Priority samples are analyzed by both microscopy and PCR

-Single suspects are also analyzed to PCR to confirm ID

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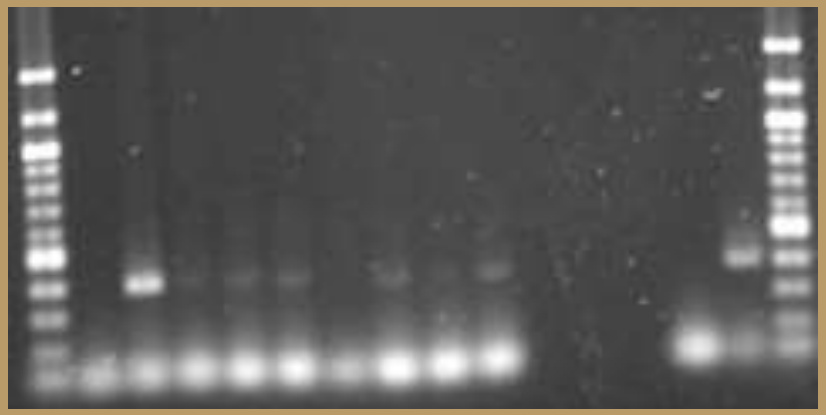
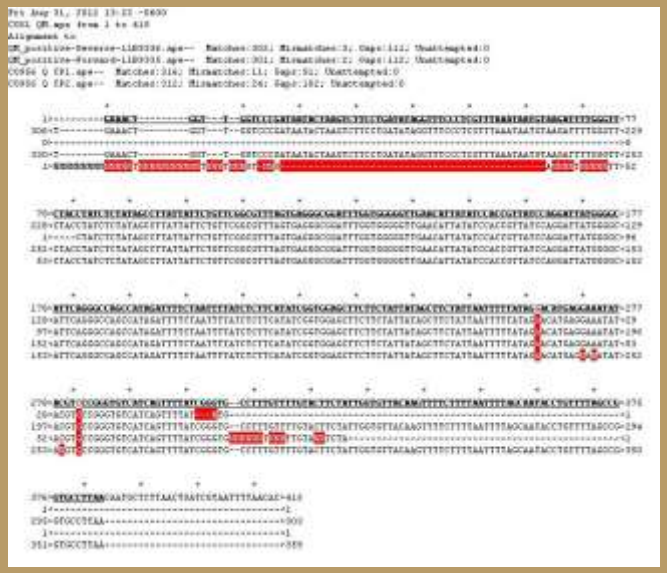
Analyzing Water Samples by Polymerase Chain Reaction





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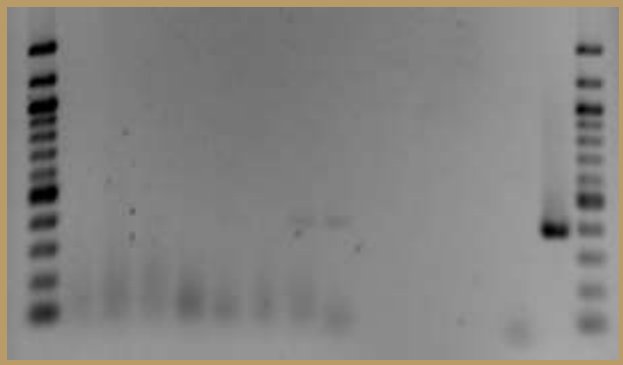
Optimization of PCR Analysis for Quagga Mussels

Areas of research

- PCR Optimization
- Veliger Integrity (eDNA)
- Sample preservation
  - Buffered vs Non-buffered
  - 0% vs 20 % Alcohol
  - Low vs High Inhibitors



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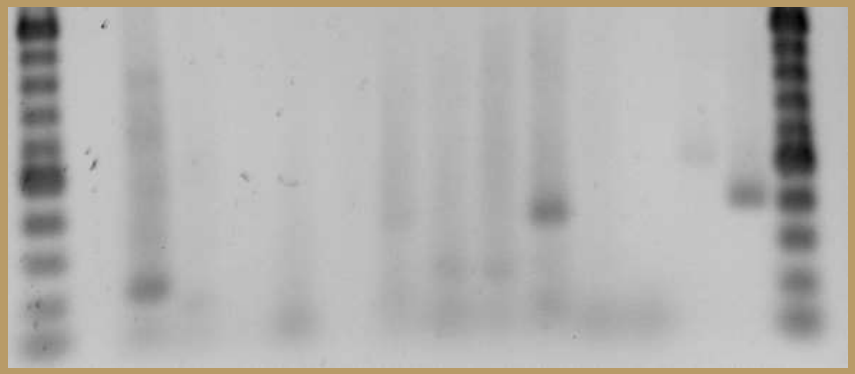
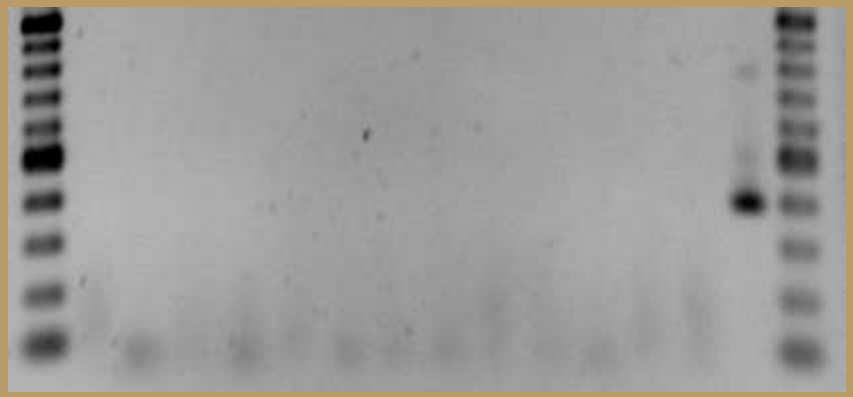
PCR Optimization

2012

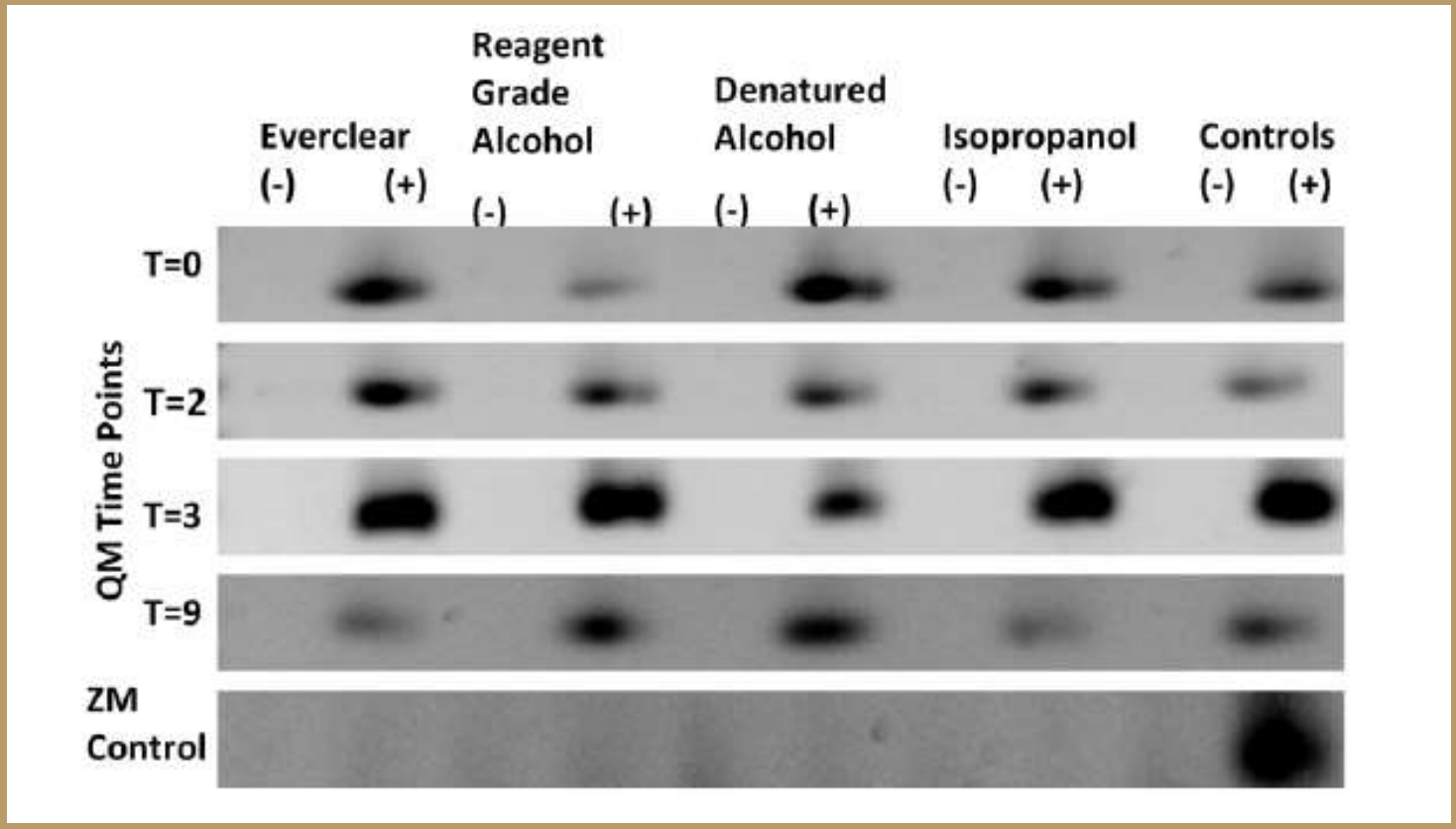


2016

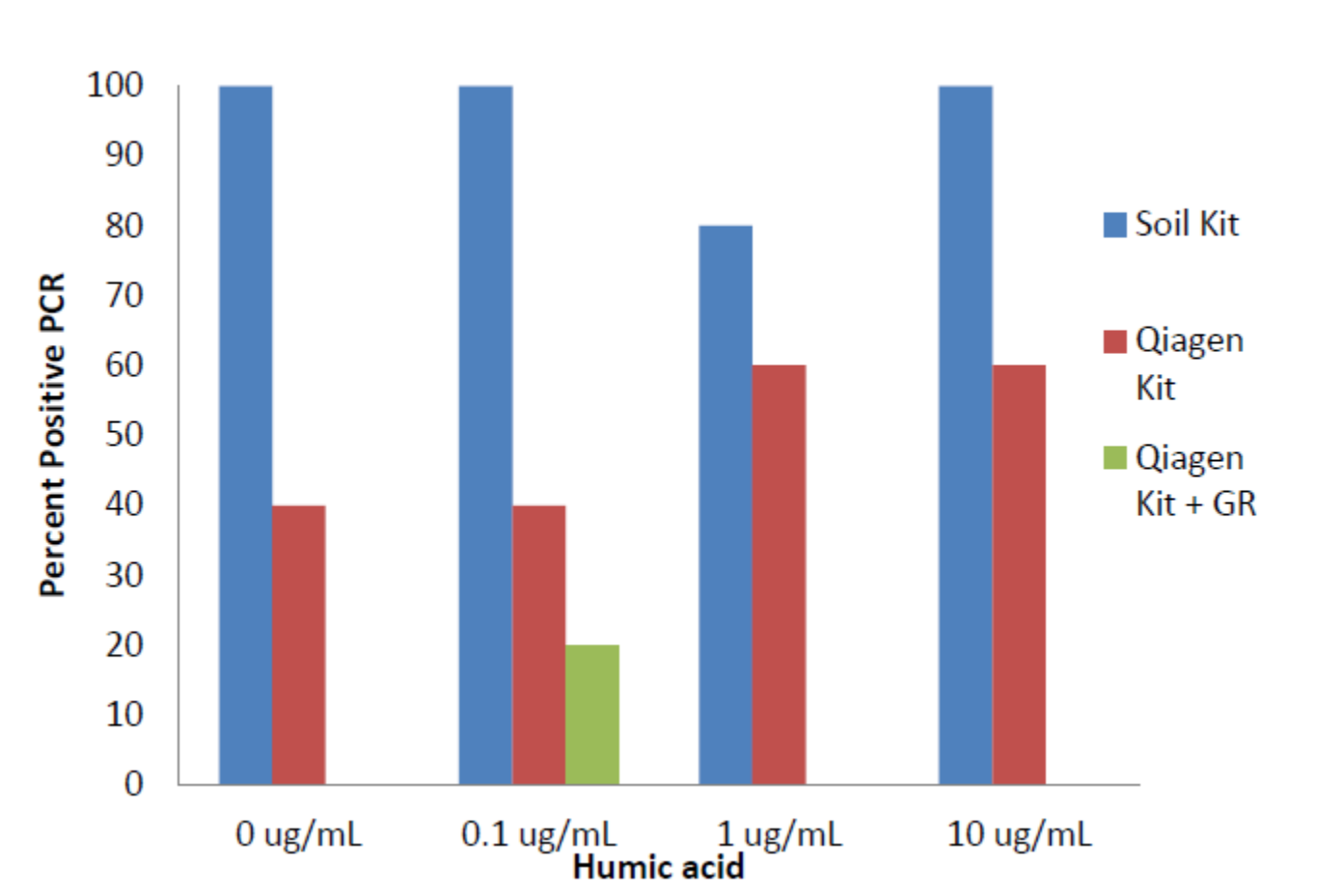


- **Extraction of QM DNA from raw water samples**
  - Best method
- **PCR Analysis**
  - Primer dimers
  - Magnesium chloride
  - Concentrations of reagents
  - Detection limits
- **DNA Sequencing**

Different Types of Alcohol Used to Preserve the Samples

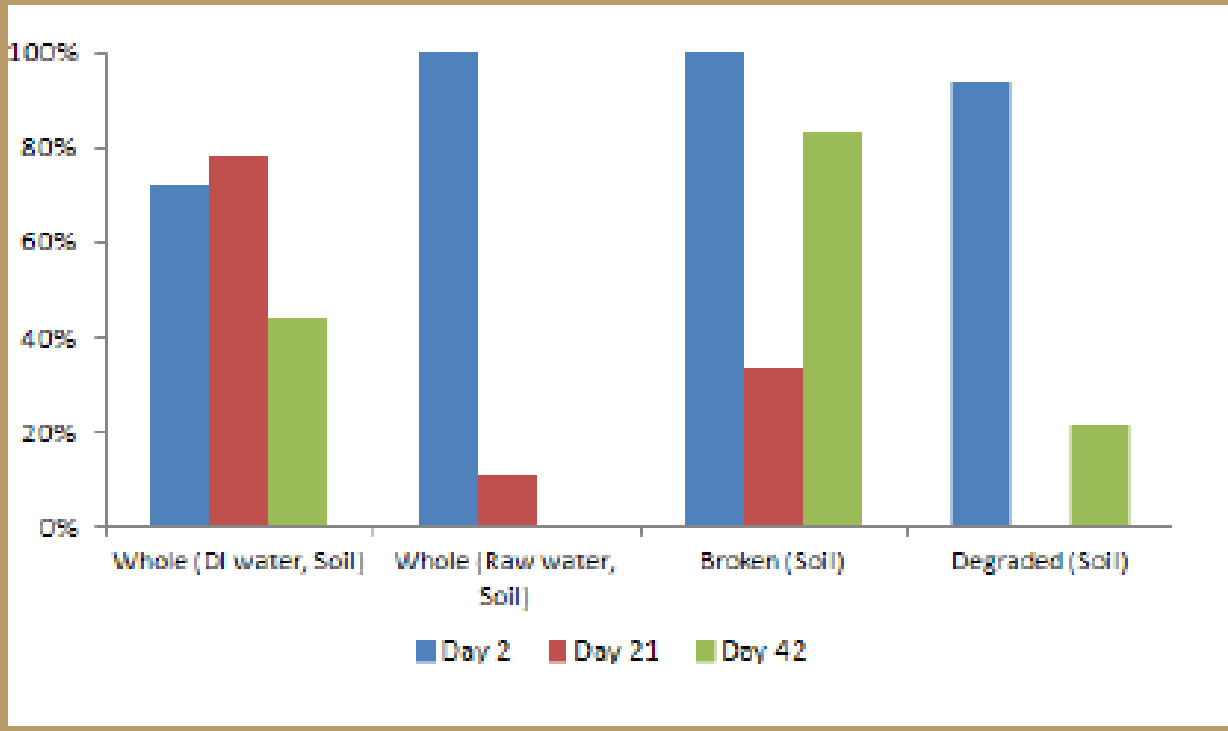


Impact of Inhibitors on PCR Detection



Impact of Veliger Integrity on PCR Detection

- **Impact of various factors on detection of DNA**
- **Sources of veliger DNA**



Soil Kit, Twenty-five veligers per sample

Sample Preservation Studies

Sample preservation impacts detection of veligers by PCR

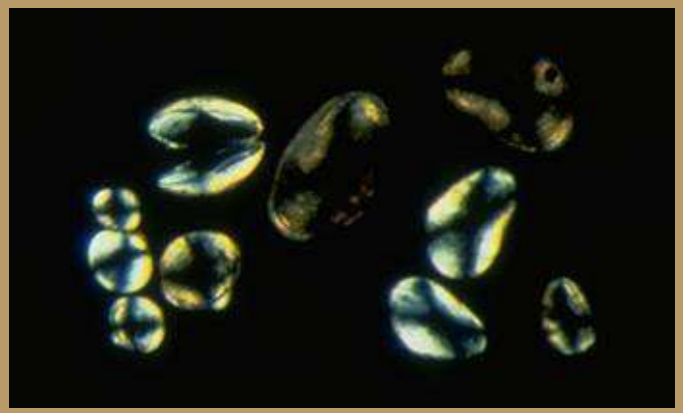
- **Samples with acidic pH**
- **Negative microscopy but positive PCR**

Tested detection after 1, 6, 21, & 42 days

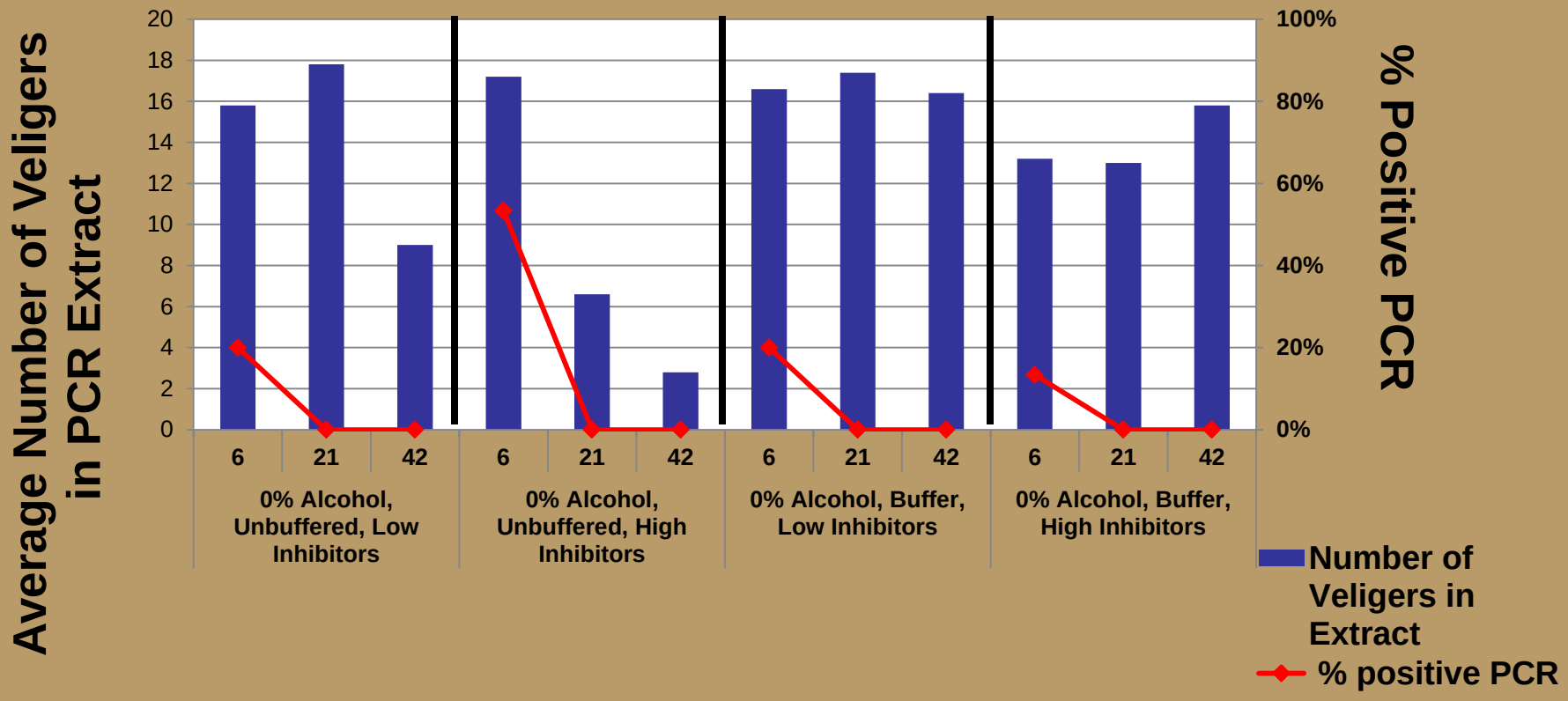
- **Alcohol concentration**
- **Buffered vs. unbuffered**
- **Zooplankton concentration**

Best Preservation Method

- **20% alcohol per volume**
- **0.2 g baking soda per 100 mL**



No Alcohol, Buffered vs Non-Buffered, Low vs High Inhibitors



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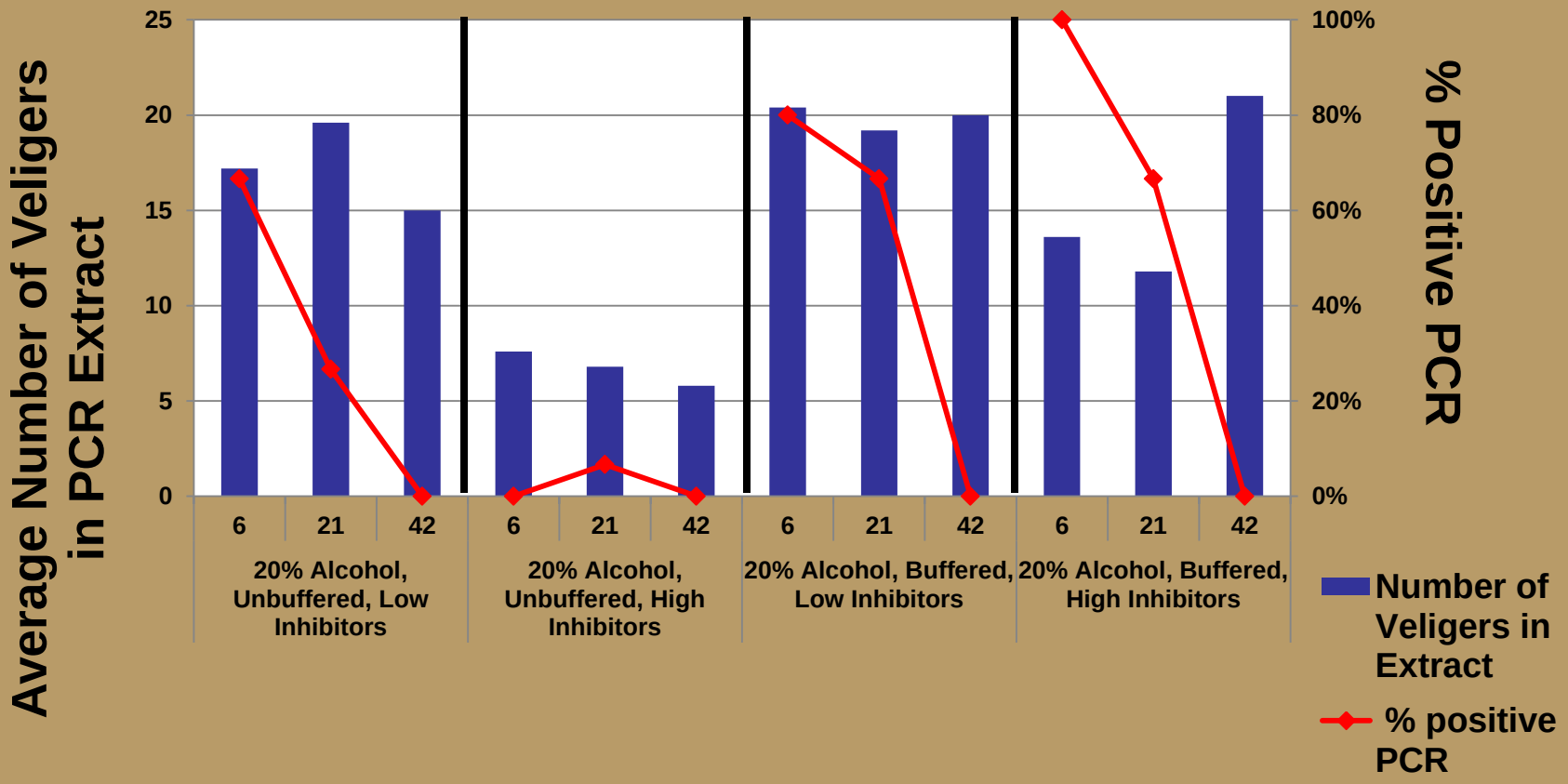
20% Alcohol, Buffered vs Non-Buffered, Low vs High Inhibitors



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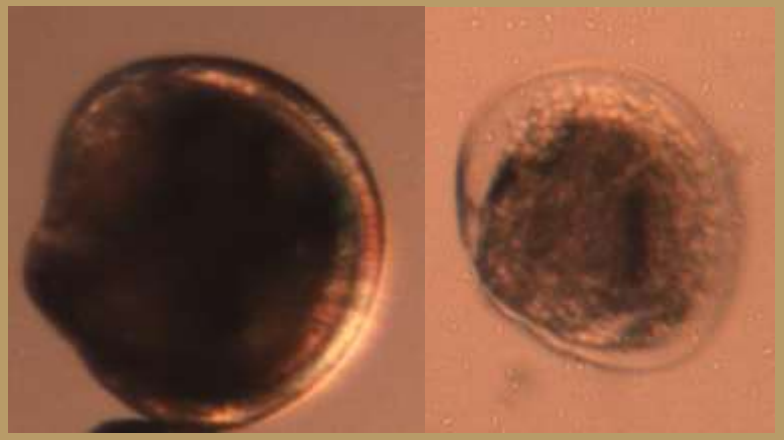
Importance of Storage Conditions for QM Detection

- Stability of QM DNA detection decreases over time when stored improperly

- Best Storage Conditions
  - 20% Alcohol, Buffered

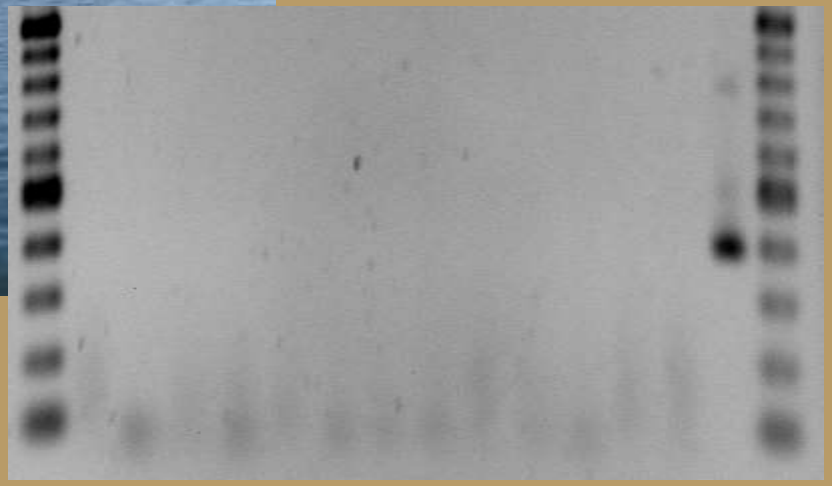


Veliger preserved properly after 21 days (left) Veliger not preserved properly after 21 days (right) (Photos by Jamie Carmon)

Optimization of PCR Detection Methods for Invasive Mussels

- **RDLES modified existing PCR methods to increase sensitivity by approximately 100x**
- **RDLES continues to conduct research to understand the limits of PCR in early detection**
- **RDLES applies this knowledge to additional organisms of interest**

Questions?



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