Gulf and South Atlantic Regional Panel on Invasive Species
Myrtle Beach, SC Oct 6th-7th, 2015

Update on the invasive parasite, *Anguillicoloides crassus*, of the American eel, *Anguilla rostrata*

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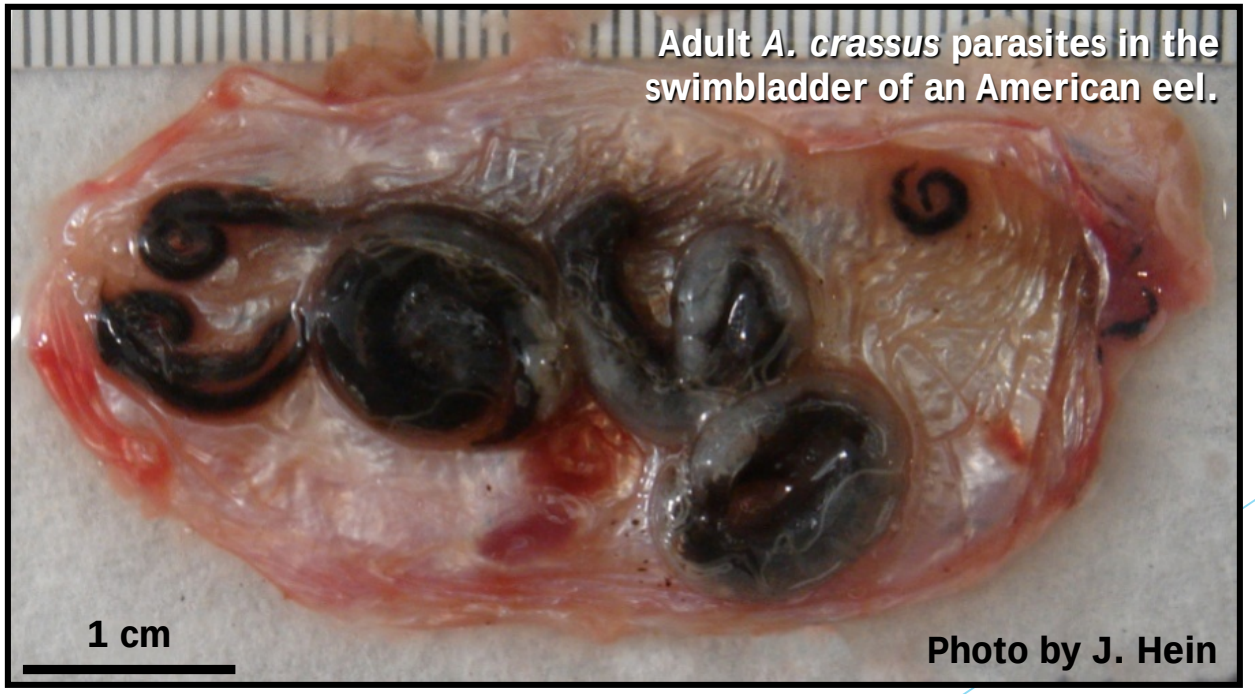
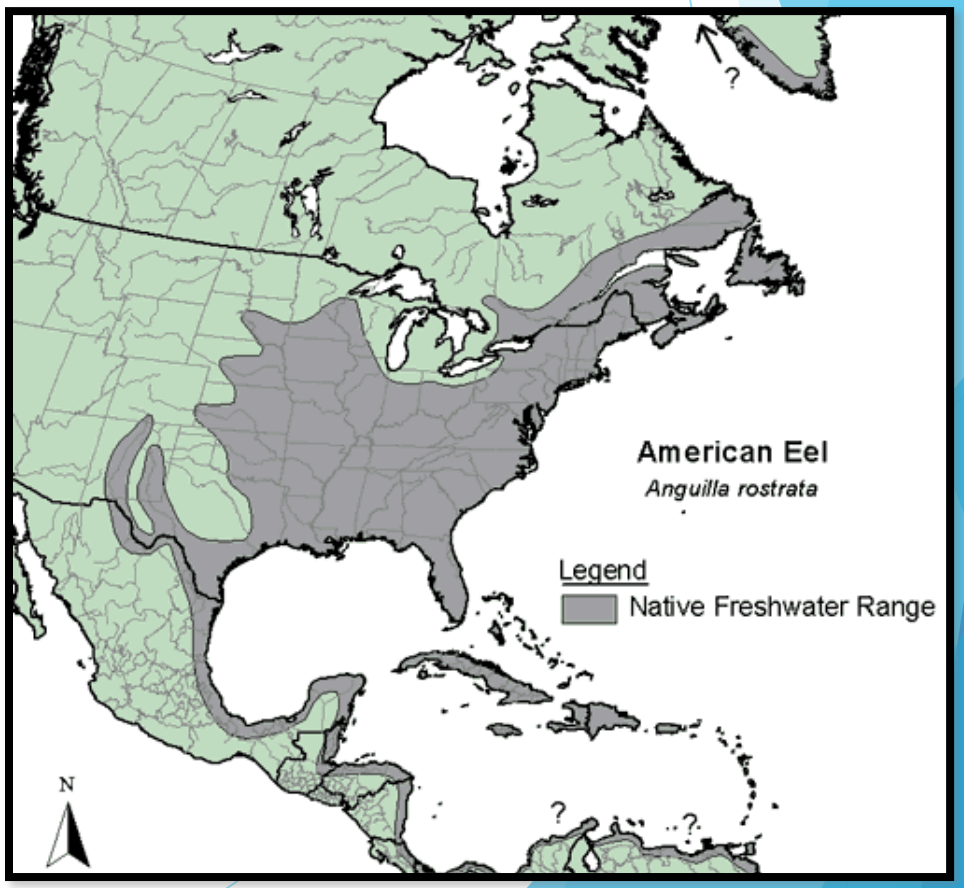
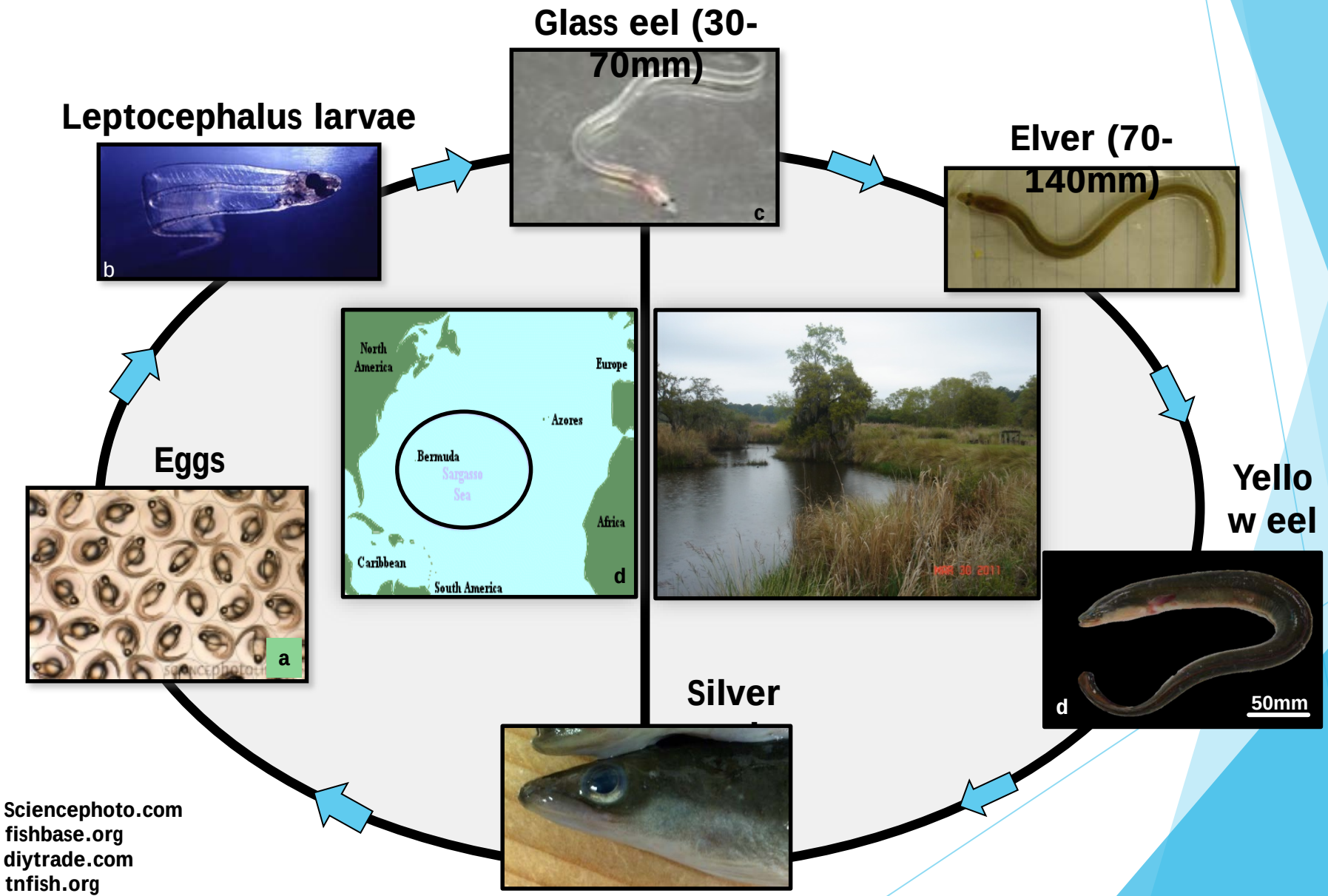


American eel, *Anguilla rostrata*

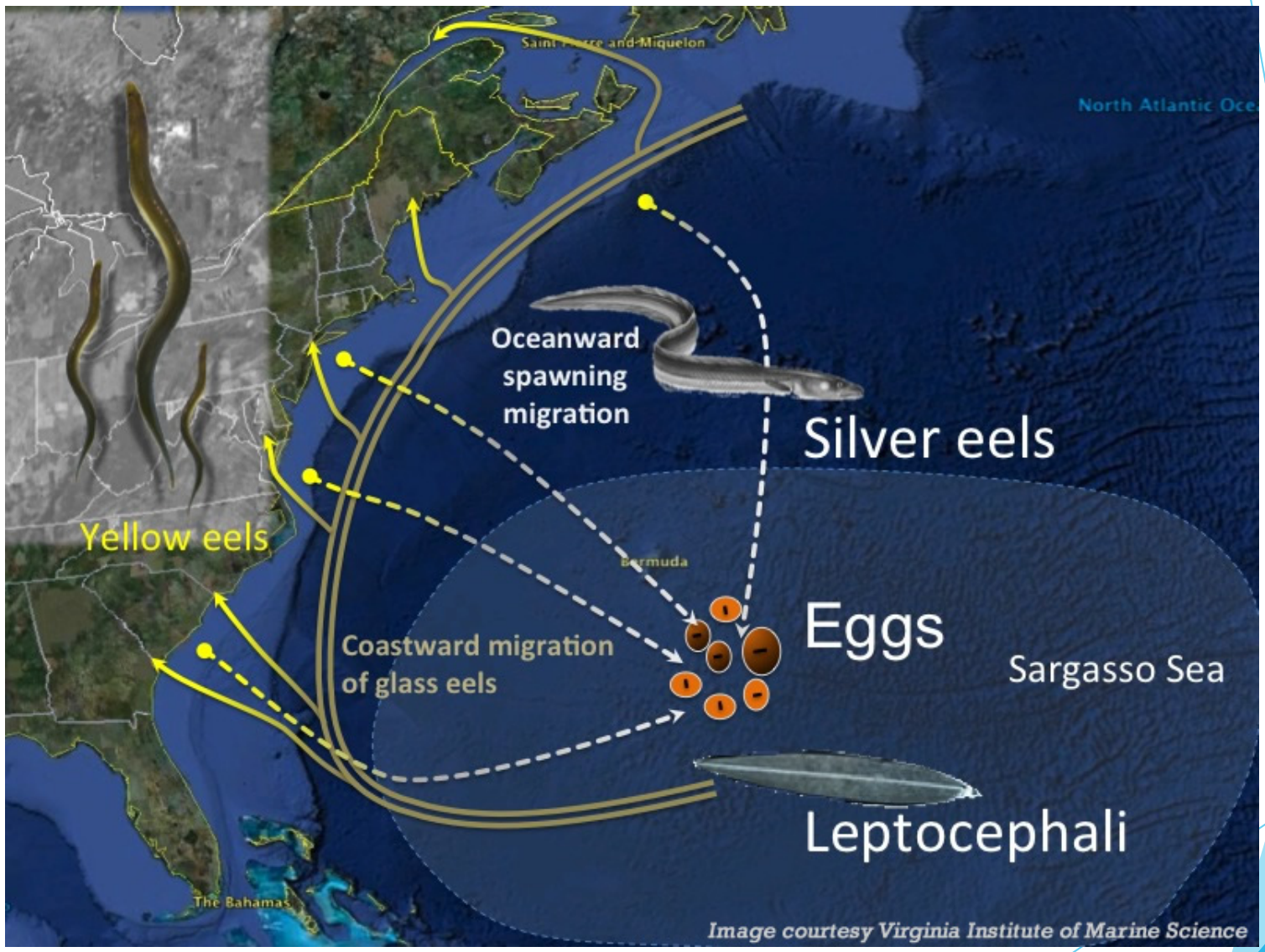
- **Range: Atlantic Coast, Greenland to South America.**
- **‘Catadromous’ – adults spawn in Sargasso Sea and juveniles develop in freshwater, brackish, and estuarine systems.**



American Eel Life Cycle



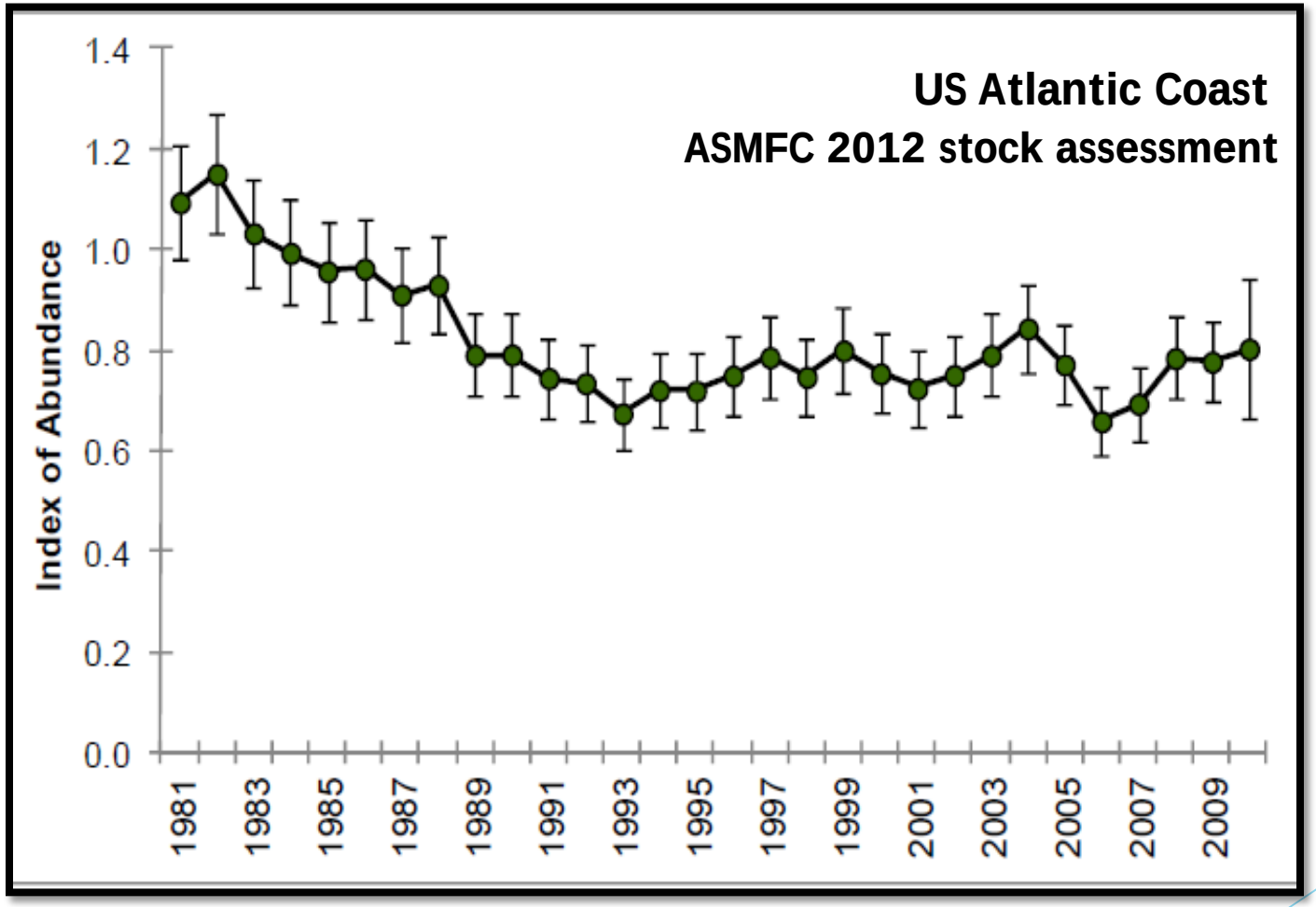
a. Sciencephoto.com
b. fishbase.org
c. diytrade.com
d. tnfish.org


Saint Pierre and Miquelon
North Atlantic Ocean
Oceanward
spawning
migration
Silver eels
Yellow eels
Bermuda
Eggs
Sargasso Sea
Coastward migration
of glass eels
Leptocephali
The Bahamas
Image courtesy Virginia Institute of Marine Science

Eel vulnerability and decline

- **High age at maturity (~10-30 yrs, varies with latitude), only spawn once**
- **American eels are harvested both commercially and recreationally throughout their range.**
- **Harvest peaked in 1979 at 3.95 million pounds and has been declining since, i.e., for past 30+ years.**

American Eel Population Status

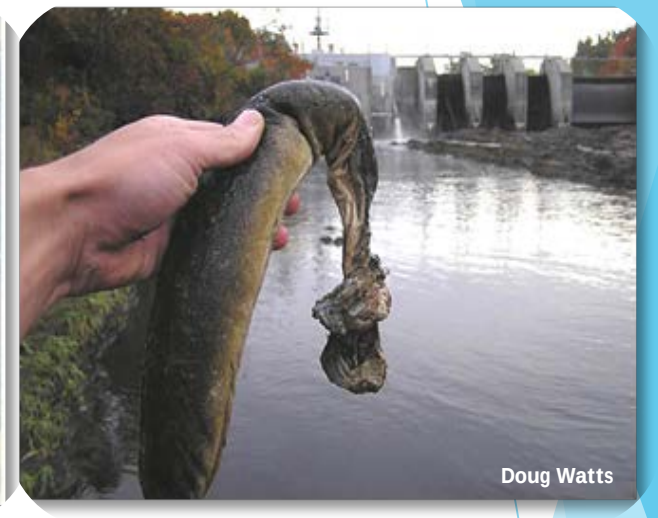


“Depleted” in US waters (ASMFC 2012 benchmark stock assessment); at or below historically low levels.

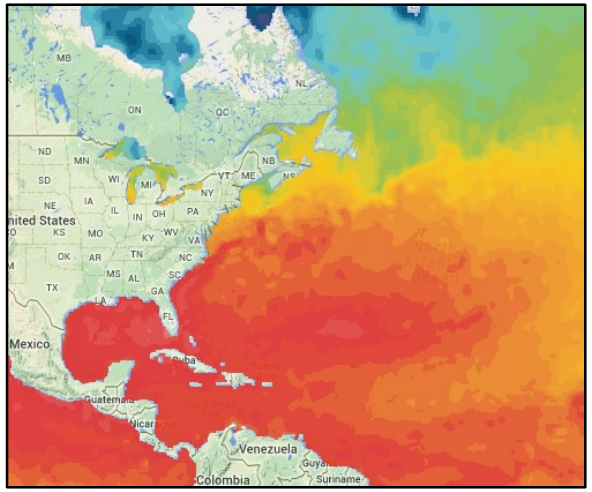
Potential threats to eel populations

Harvesting

Barriers to migration



Turbine mortality



Environmental changes

Anguillicoloides crassus

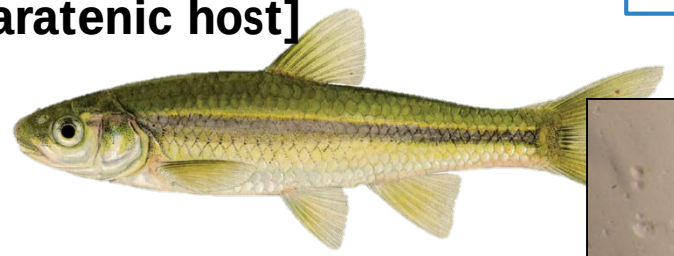
A. crassus life cycle

L4 molts to adult parasite in the swimbladder lumen

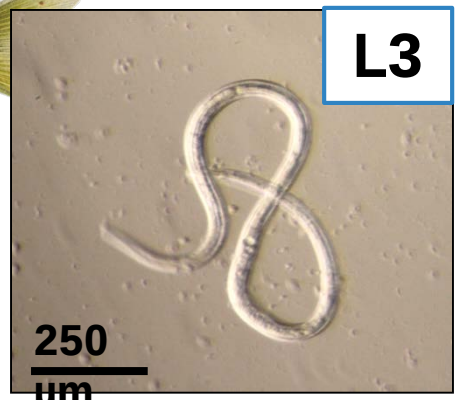
L3 stage molts to L4 stage in the swimbladder wall

Parasite eggs are laid down in the swimbladder lumen, pass through the pneumatic duct, and exit the eel via its vent

[Paratenic host]



L2 crosses gut wall of IH; L2 molts to L3, which are consumed by eel or paratenic host once in body cavity

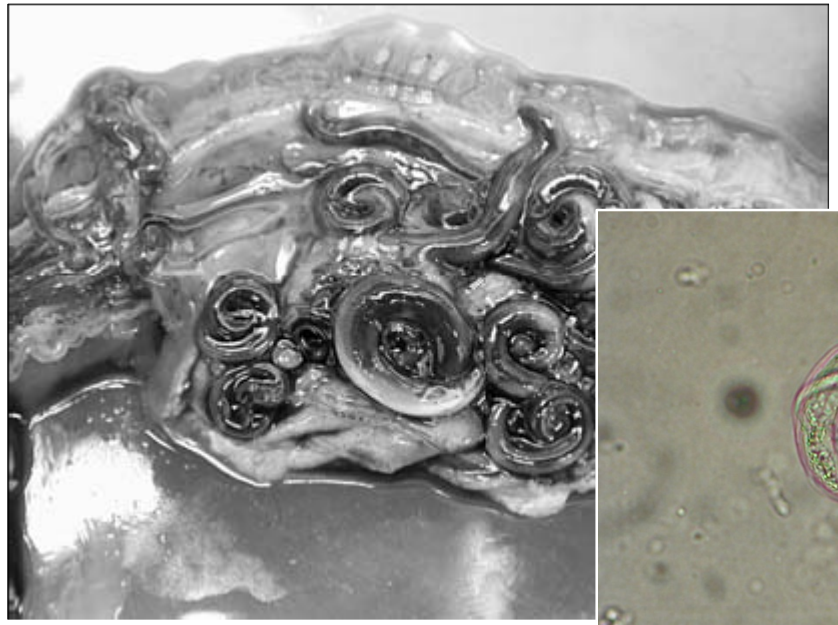


L2 hatch in the water and are ingested by an intermediate host (IH)

[+ Molluscs, ostracods and others in European studies]

- Understanding of life cycle is largely based on European studies; little research on life cycle in N. America

Infection of the invasive swim bladder nematode parasite *Anguillicoloides crassus* in South Carolina populations of the American eel *Anguilla rostrata*.



Sokowlowski & Dove 2006

Hatchlings (courtesy of D. Knott)

Adult (courtesy of D. Knott)

Juvenile parasites in eel swimbladder wall.
Photo courtesy of Jen Hein (College of Charleston).

Significance of swimbladder damage

Healthy

Damaged

- Irreversible damage is caused by the parasites feeding on the eel's blood (Molnár et al. 1995) and by larvae migrating through swimbladder wall.
- Consequences of swimbladder damage:
  - Damages to gas gland and cell function
  - Reduced O_2 content
  - Problems with buoyancy control (Wurtz et al. 1996)
  - Compromises swimming efficiency and survival in migrating eels (Molnár *et al.*, 1995; Palstra *et al.*, 2007; Clevestam *et al.*, 2011)
  - Mortality under stressful conditions (Molnár *et al.*, 1991; Barus and Prokes 1996; Lefebvre *et al.*, 2002)
  - Reduced swimming efficiency
    - Vulnerability to predation (Barse and Secor 1999)
    - Migration to Sargasso Sea (Sjoberg *et al.*, 2009); European eels are known to greatly reduce their gut while expanding their swimbladder to enable significant vertical oceanic migrations as they travel to the Sargasso Sea.

Gulf States Marine Fisheries Commission (GSMFC) Subcontract Award:

Detection of an invasive parasite of American eels using qPCR

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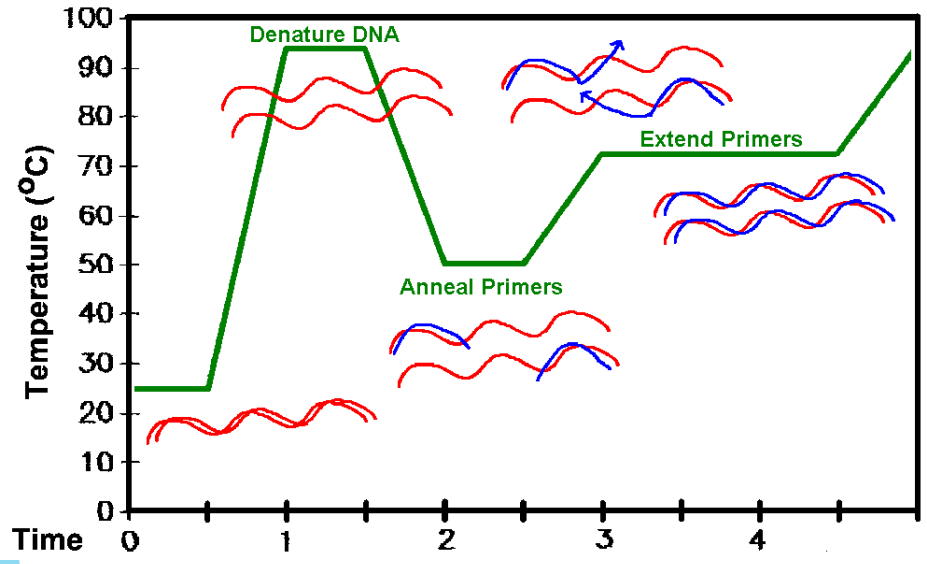
PROJECT GOALS:

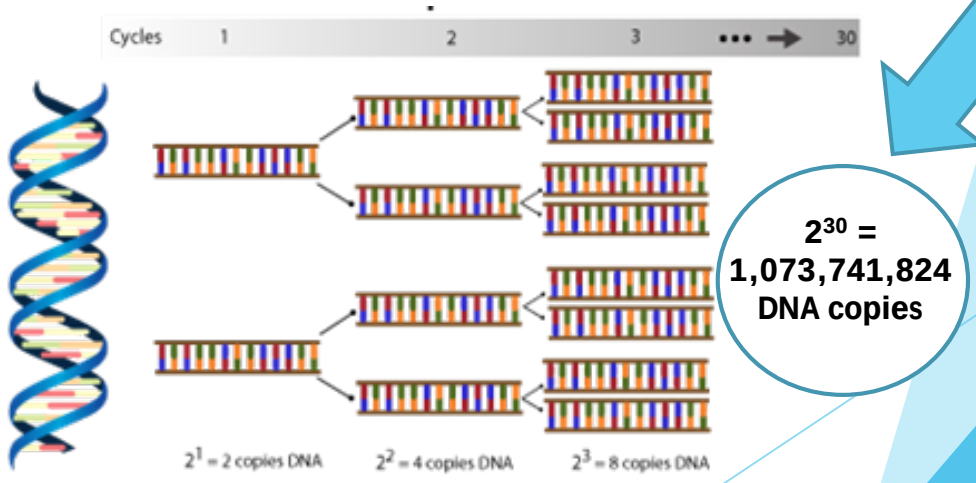
- **To test whether qPCR can detect *A. crassus* collected from the wild, through the collection of planktonic and benthic crustaceans at the Goose Creek Reservoir, South Carolina.**
- **To generate standard curves and establish limits of detection for qPCR through laboratory cultures and infections of intermediate hosts (i.e., copepods).**
- **To use data from qPCR standard curves to quantify parasite abundance and densities in the field.**

Modified Polymerase Chain Reaction (PCR) Project

- Protocol received from Canadian group was end-point PCR with an ~800bp product
  - Was not a species specific fragment
  - Not good for quantitative assays
- Re-designed primers for q-PCR (~150bp product)
  - Primers do not align with any closely related species (from available NCBI sequences)

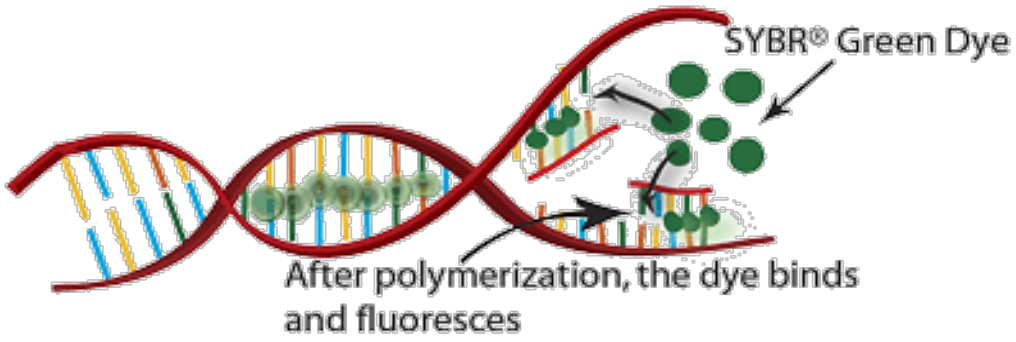
- Simulates natural cell DNA replication
- Allows for DNA detection/visualization
- Key is the precise thermal cycling
- DNA quantity doubles with each thermal cycle

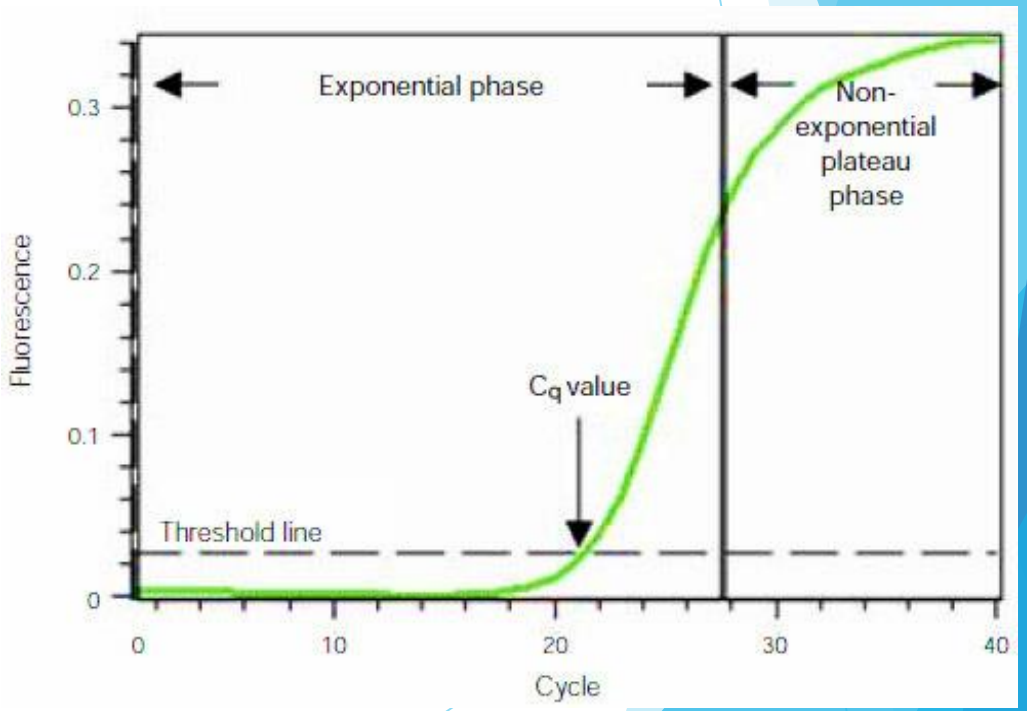




Quantitative PCR (q-PCR)

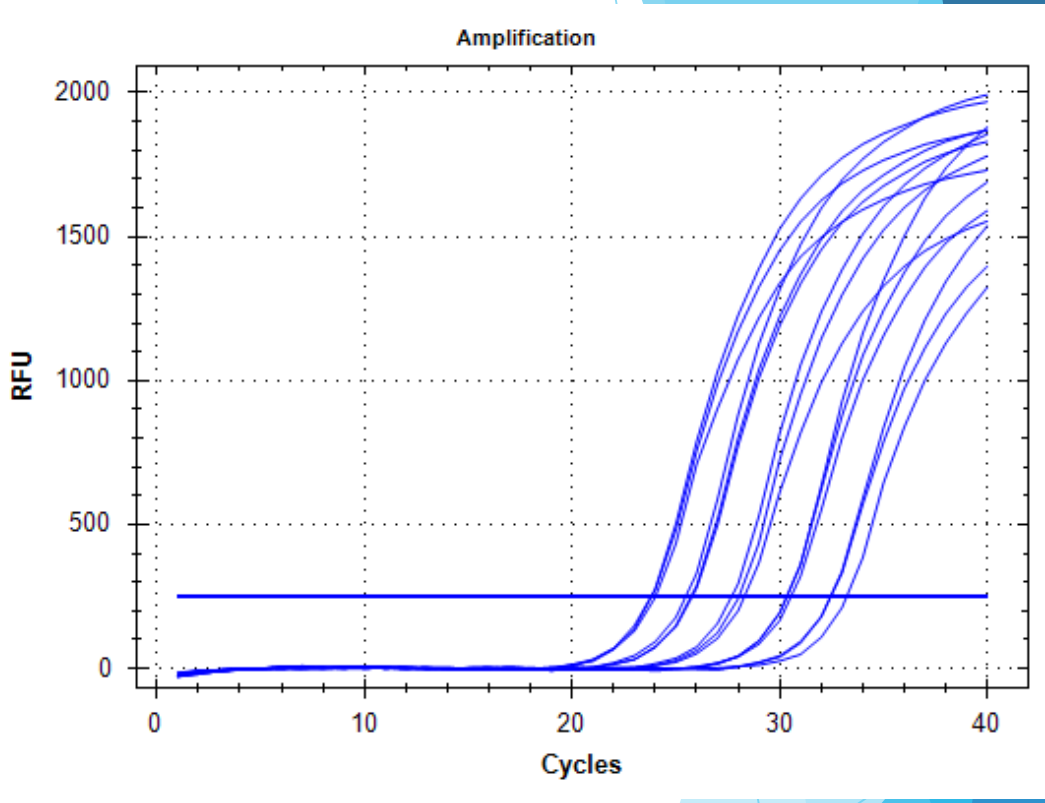
- **Same concept and process as end-point PCR**
- **Use fluorescent dye or targeted probes to visualize increase in product in real time**





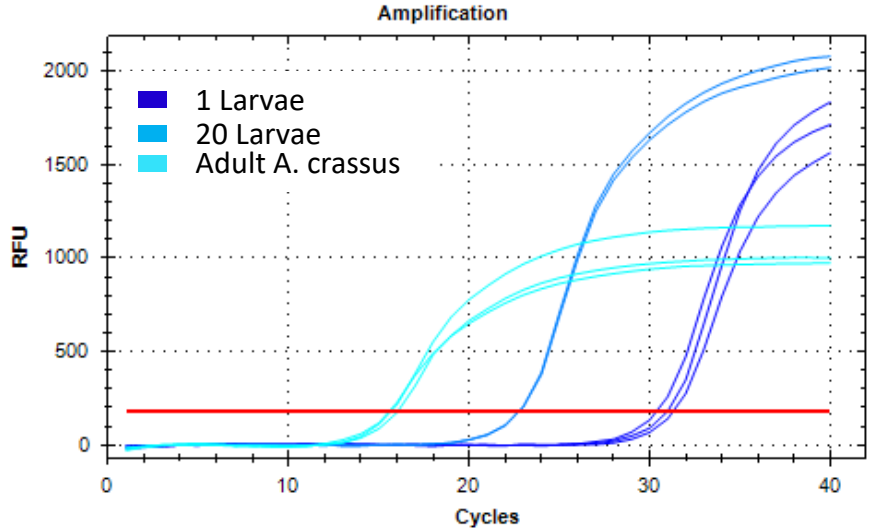
Quantitative PCR (q-PCR)

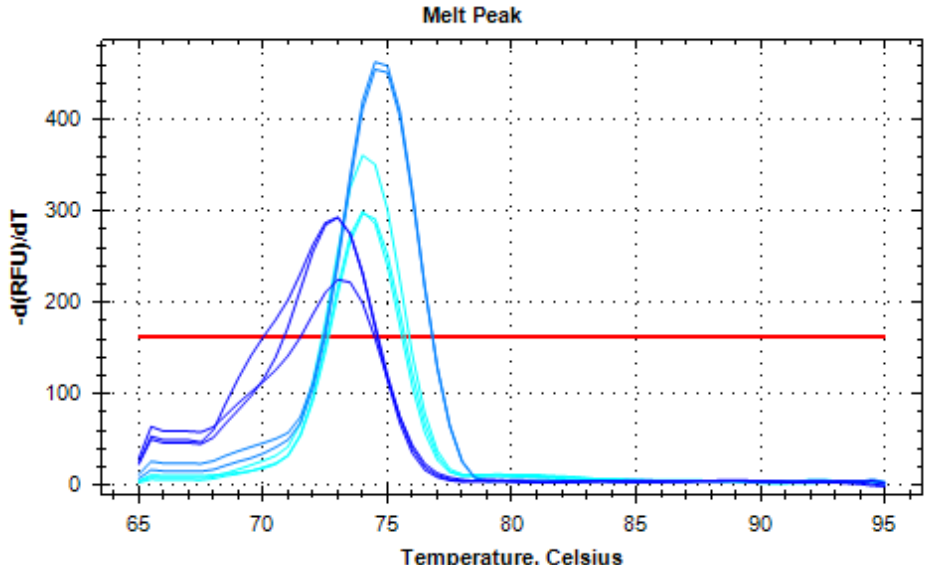
- S-shaped curves, as the reaction progresses, more substrate is generated, and the curve becomes logarithmic.
- Threshold line where all curves have begun the logarithmic amplification; earlier the curve crosses the threshold, the more starting DNA is present.

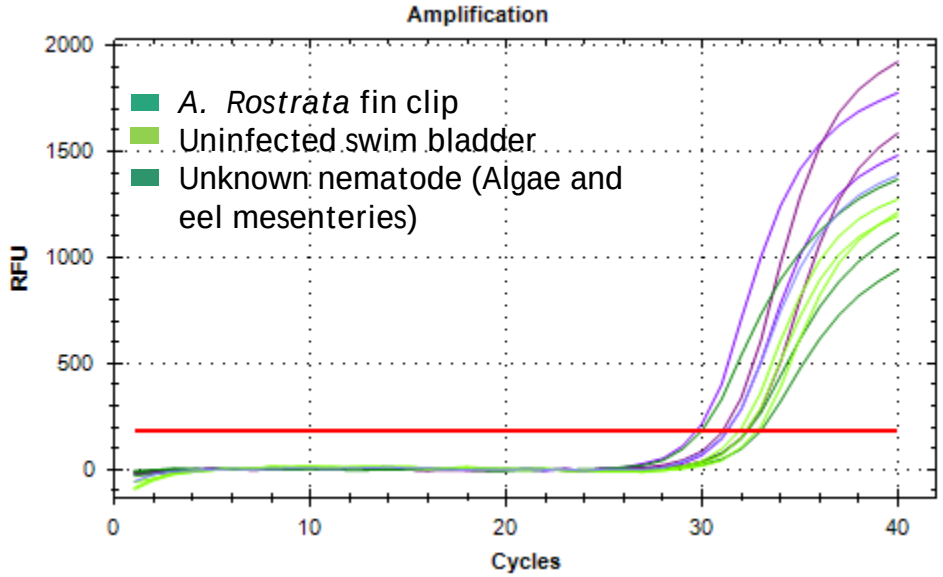


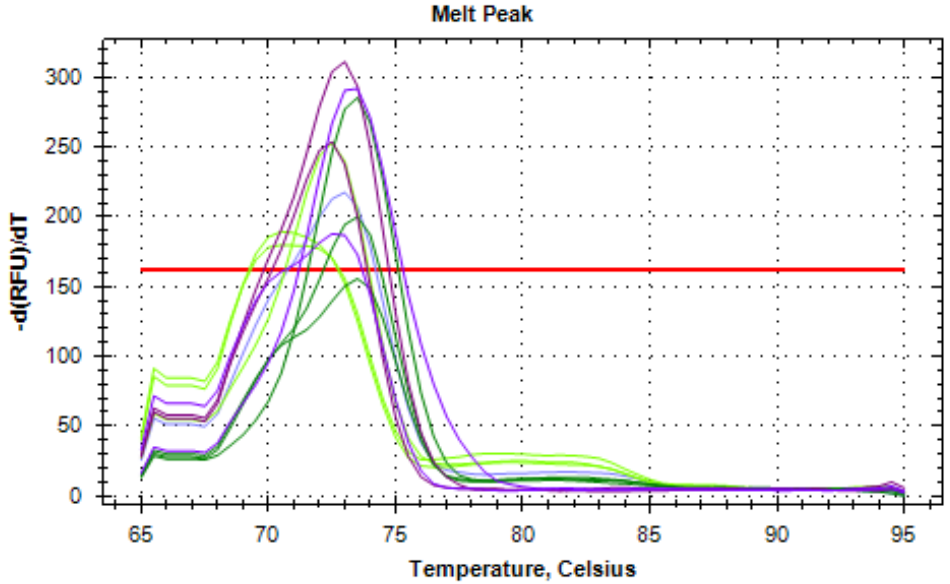
- Excellent method for quantifying relative differences in gene expression between species, tissue types, treatments, etc.
- Excellent method for detection of rare DNA in environmental samples (endangered species, invasive species, parasites, etc.)

First Run @ 58˚ with full primer concentrations - Everything amplified

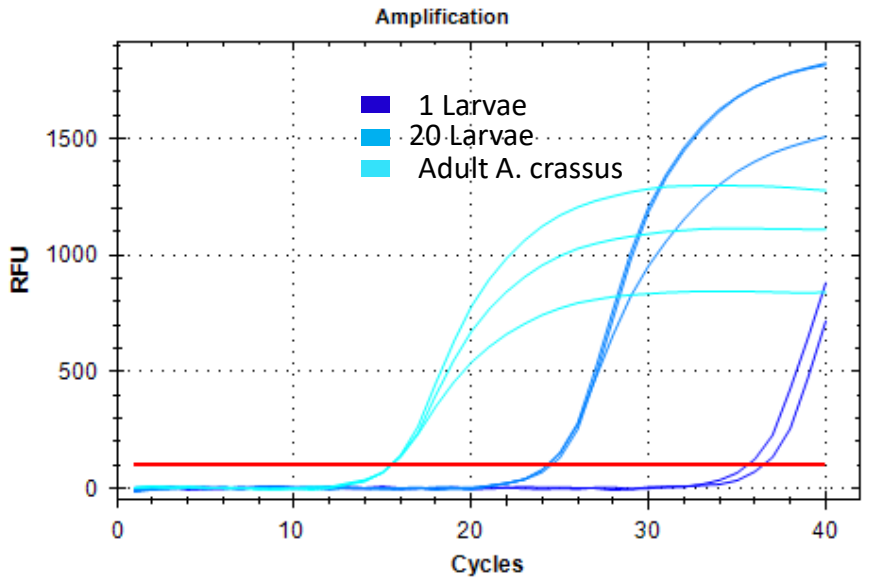


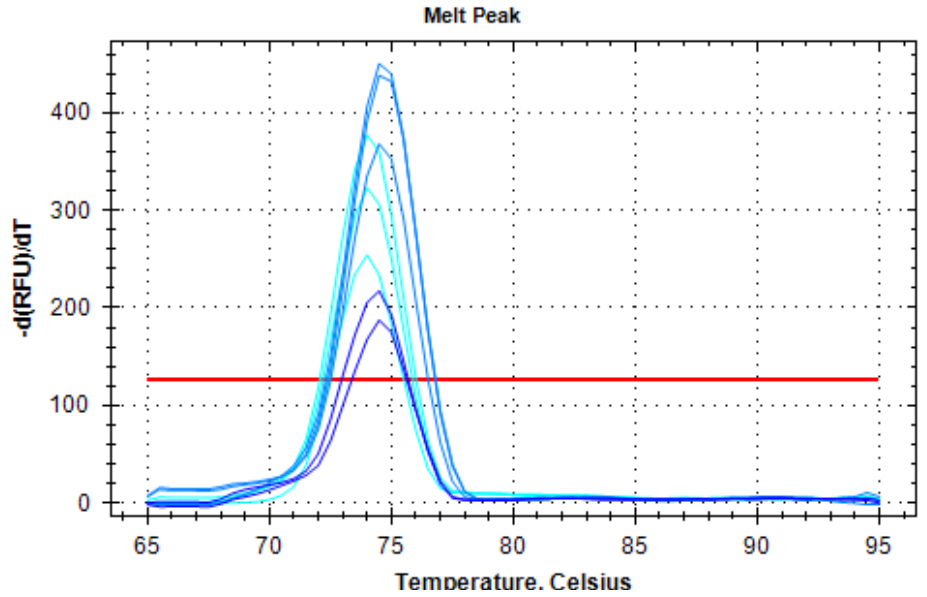


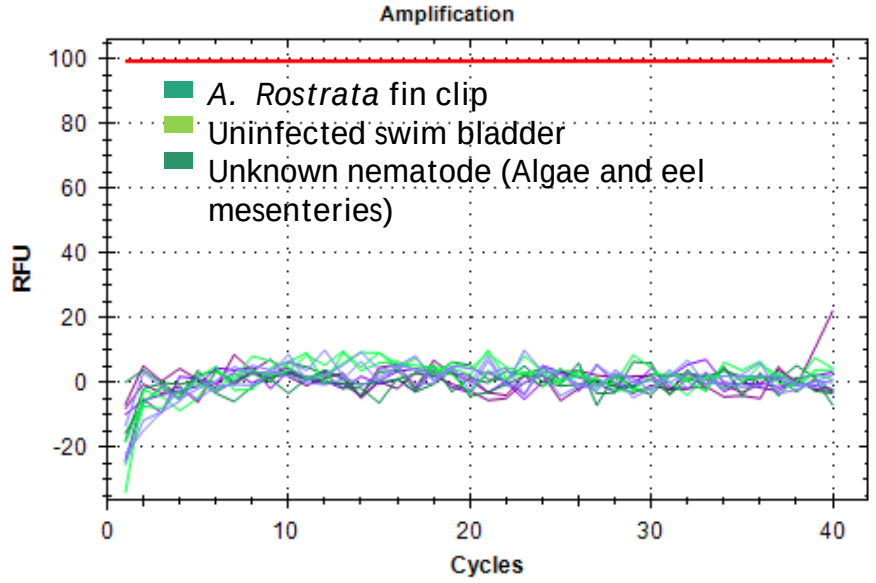


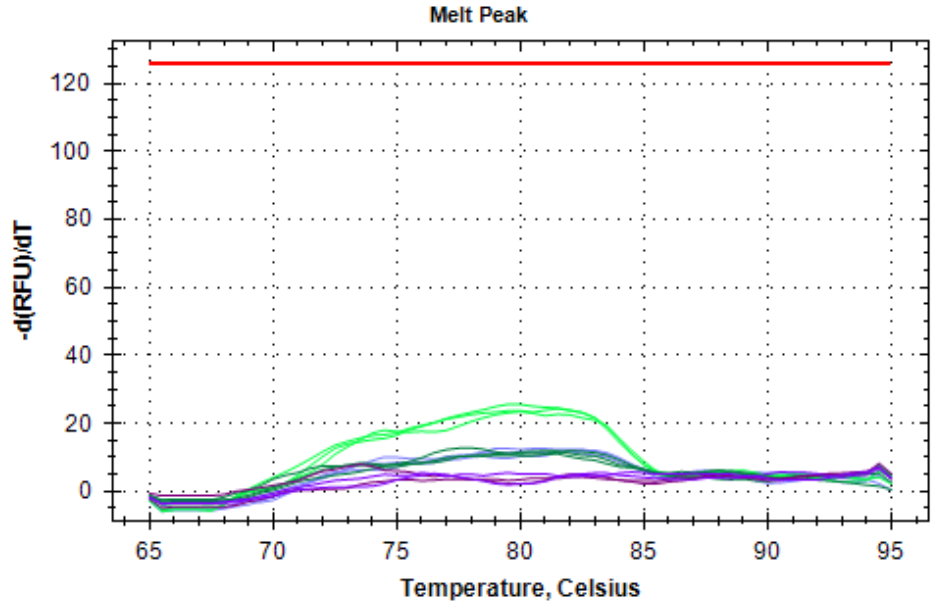


Optimized with ½ Primer concentration and 60˚ annealing temperature

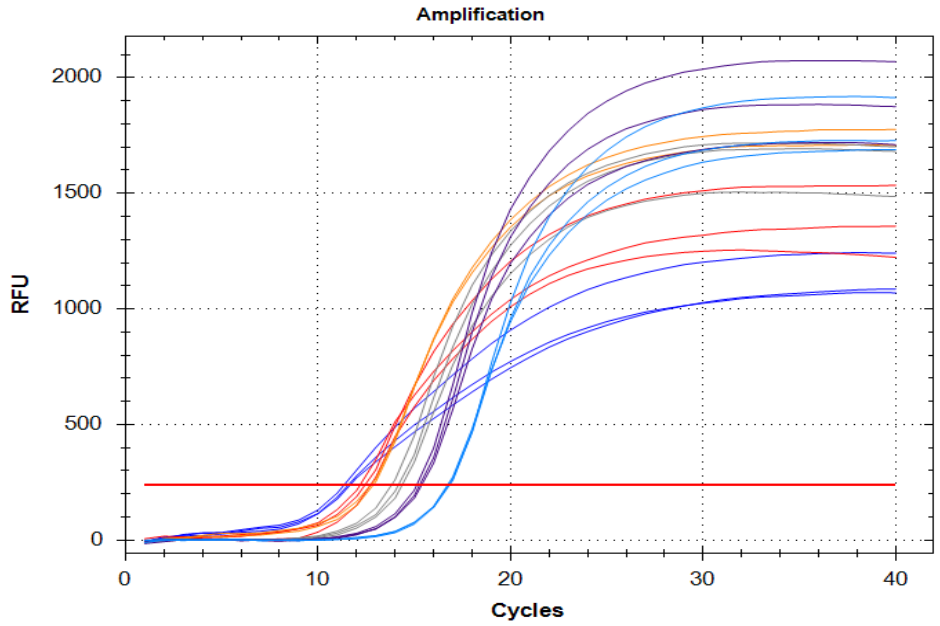


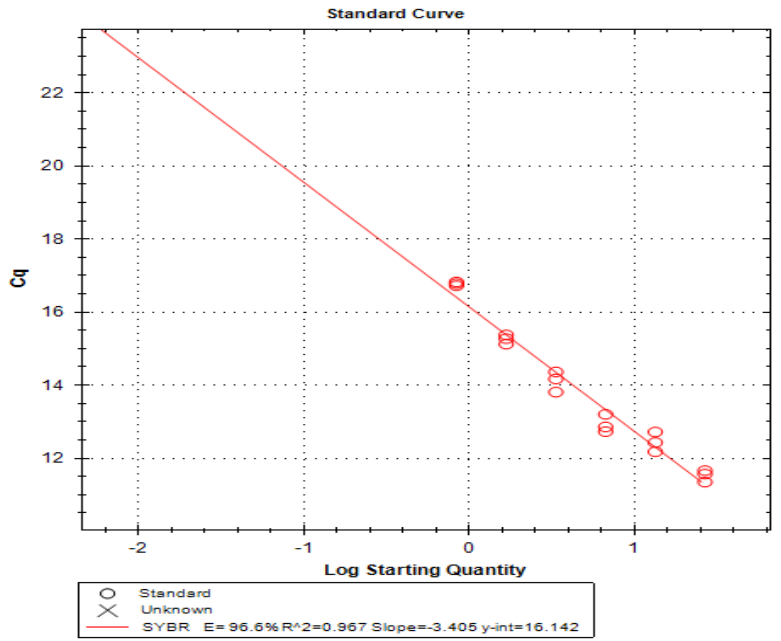


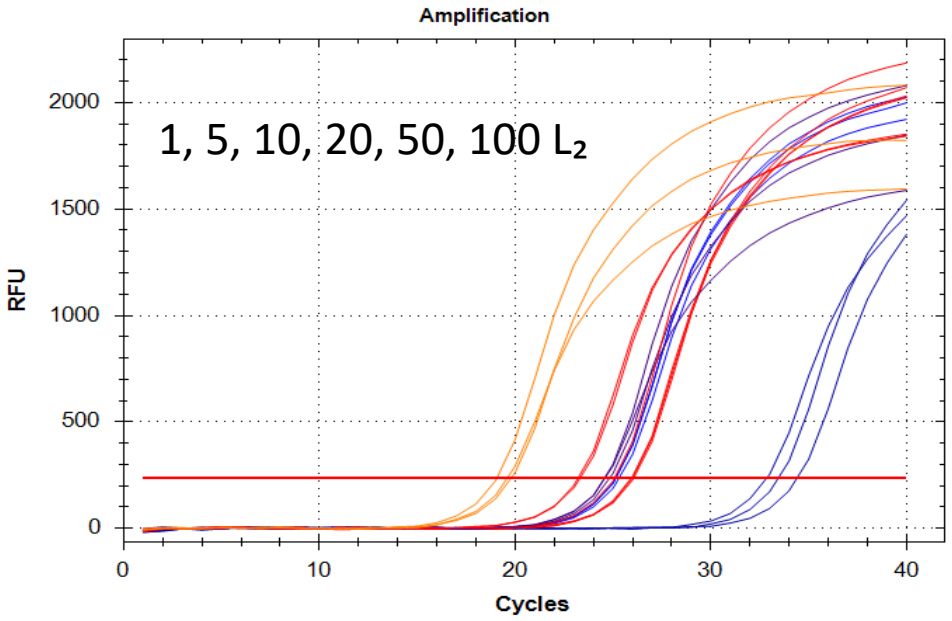




Establishing L_2 Standard Curve







- **Assays show acceptable efficiencies (~96.7%)**
- ***A. crassus* samples with low concentrations of DNA had high Cq levels, close to 35, warranting further research with sensitivity and DNA concentrations**
- **Overlapping ranges for quantification standards**

Expanding to Field Detection

- **Successful lab assay for detection and quantification of *A. crassus* DNA from L_2 stage**
- **However, eggs, L_2, and L_3 stages are all in the environment or intermediate hosts**
- **Potential inhibition of q-PCR in environmental samples**
- **Need to further test and validate assay for successful use as a field tool**
- **Increase specificity and reliability at low DNA concentrations**

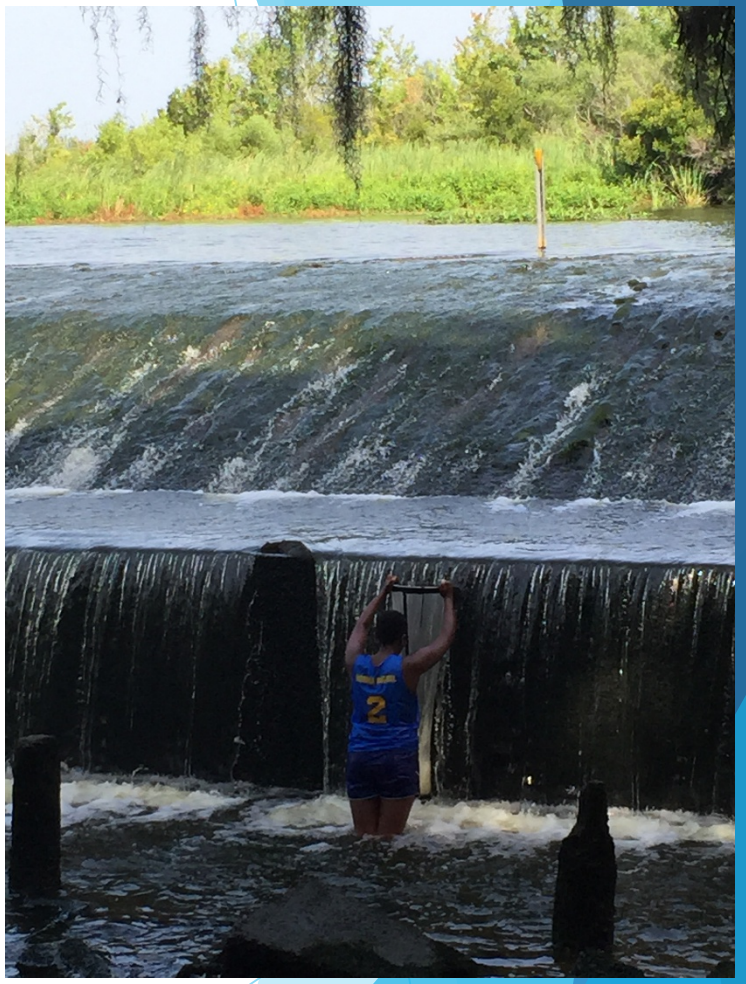
Current Progress

- **Designed and ordered a fluorescent probe to add to current assay**
  - **Increase specificity and decrease non-specific or late cycle products**

- **Sampling at Goose Creek Reservoir site**
  - **Summer (June/July) sampling**
    - **High prevalence of infected eels**
  - **Fall (October/November) sampling**
    - **New cohort of young eels recruits to the area**

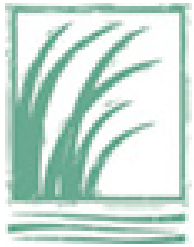
- **Sampling water, algal mat, sediment in middle of creek, and plankton**
  - **Sub-samples for DNA isolation and qPCR assay and zooplankton community ID**

- **Collecting adult eels and checking for infection**
  - **Dissecting out gravid *A. crassus* adults from eel bladders and collecting parasite eggs**
  - **Exposing copepod cultures to *A. crassus* eggs to establish supply of L3 stage parasites**
  - **Plan to establish standard curve of L3 life stage similar to L2 curve already established**



Acknowledgements – Funding Sources

DNR



NATIONAL ESTUARINE RESEARCH RESERVE SYSTEM

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Acknowledgements – People

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