Development and validation of a qPCR tool for the environmental detection of *Anguillicoloides crassus*, an invasive parasite in the American eel

Dr. Aaron Watson*, Maggie Jamison*, Dr. Steve Arnott*, Dr. Peter Kingsley-Smith*, and Dr. Isaure De Buron**

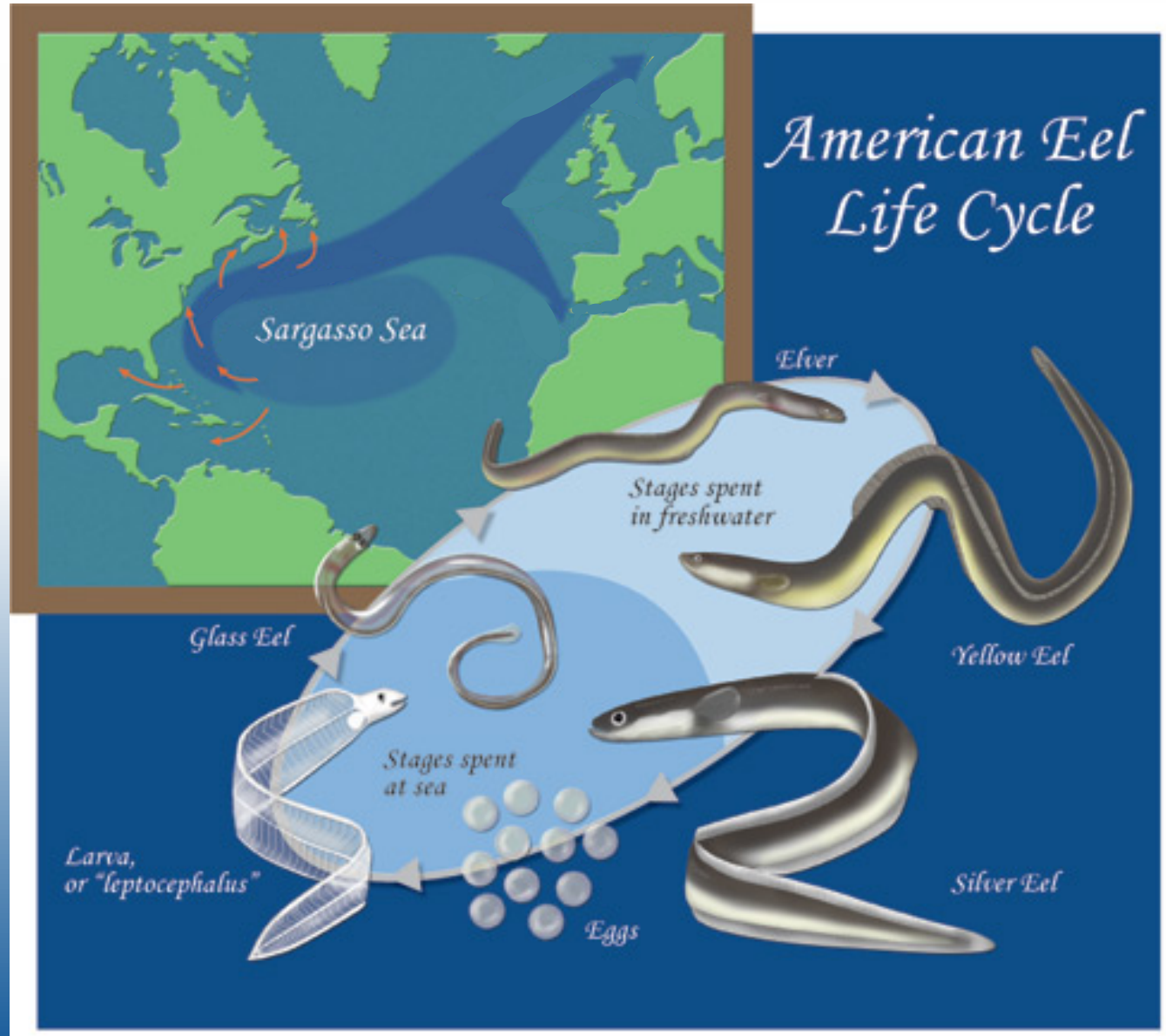
* SCDNR Estuarine Finfish Research Section
** College of Charleston

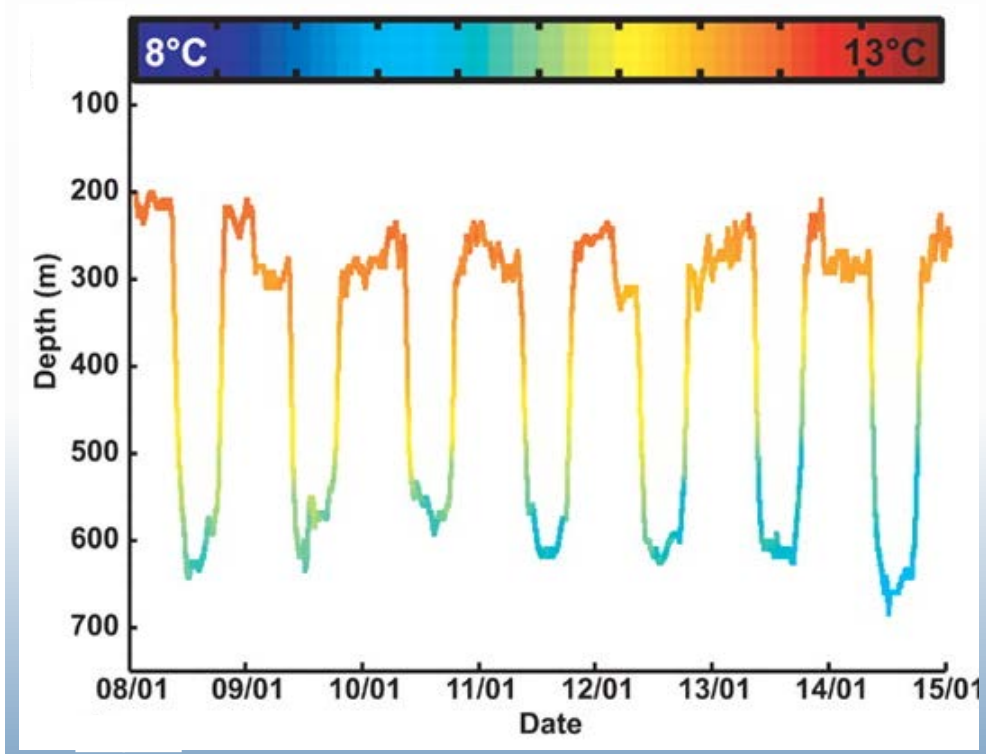
Multi-million dollar industry

Population in decline

Harmful invader

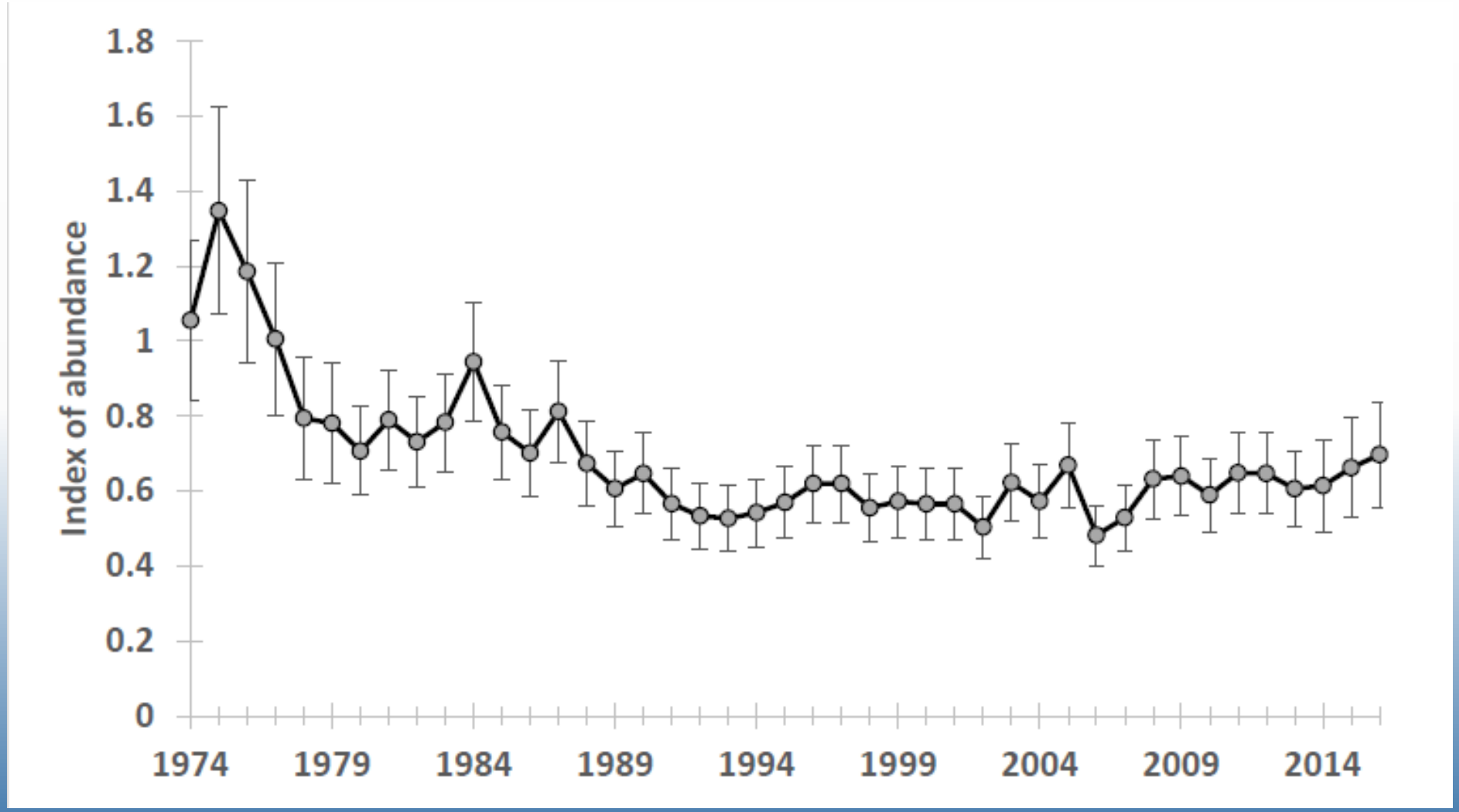
American Eel, *Anguilla rostrata*





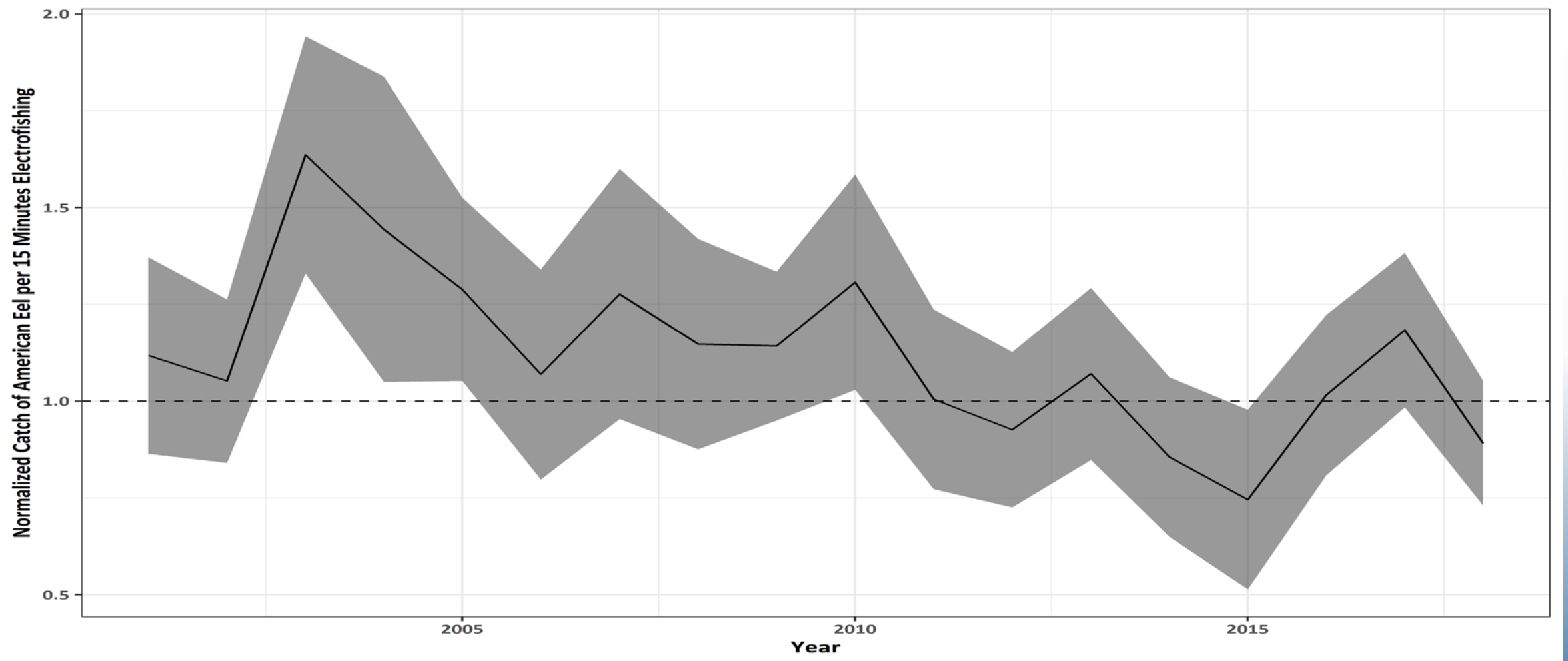
Aarestrup et al., 2009

American Eel Population Status



"Depleted" in US waters (ASMFC 2012 benchmark stock assessment); at or below historically low levels – no change in 2017 stock assessment update.

American Eel Population Status – South Carolina



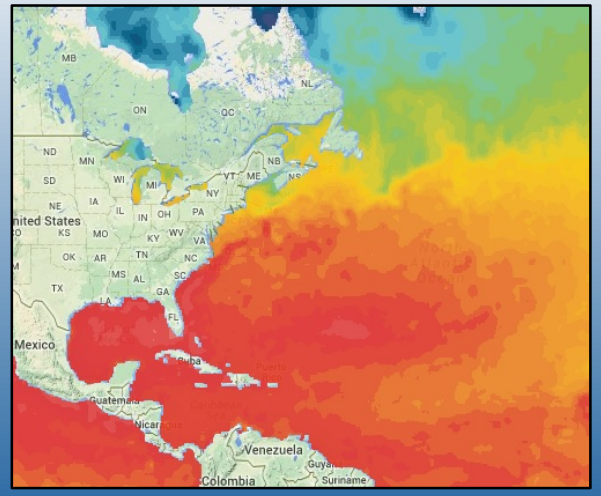
Relative abundance (± 95% CI; shaded area) of America Eel in the SCDNR Electrofishing Survey statewide trends. Relative abundance is presented as deviations from the 2004-2018 average catch of American Eel per 15-minute set in the Electrofishing survey statewide. Data source: SCDNR Inshore Fisheries Section.

Depleted Stock – Potential Causes

Harvesting

Barriers to migration

Turbine mortality



Environmental changes

Anguillicoloides crassus

Billion Dollar Industry

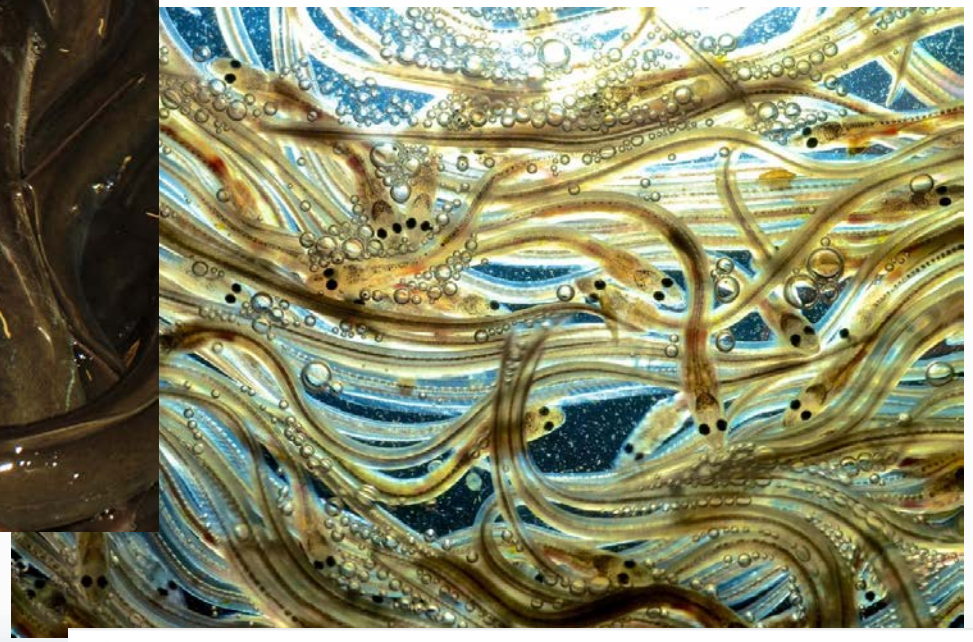
- Recreational – 9’’ limits and 10-25 eels/person/day (50 in DE)
- Commercial industry = $1.3 billion
- Licensed fishery in SC and ME – Glass eels
  - Limited entry fishery in SC
    - Illegal harvest lawsuit – SC, 2016
      - 400lbs valued at $740,000

https://si.wsj.net/public/resources/images/NA-CB074_ELVERS_P_20140506192735.jpg

TOP STORY

Men plead guilty to illegal Cooper River harvest of tiny eels

By Bo Petersen bopete@postandcourier.com Nov 28, 2016 (1)



Landings (in millions of pounds)

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

Price: $58.95 ($14.74 / ounce) + $12.95 shipping
Note: Not eligible for Amazon Prime.

In stock. Ships from and sold by La Tienda.

Qty: 1 Turn on 1-click ordering

Add to Cart

Add to List

Have one to sell? Sell on Amazon

Share

thrive
CULINARY ALGAE OIL

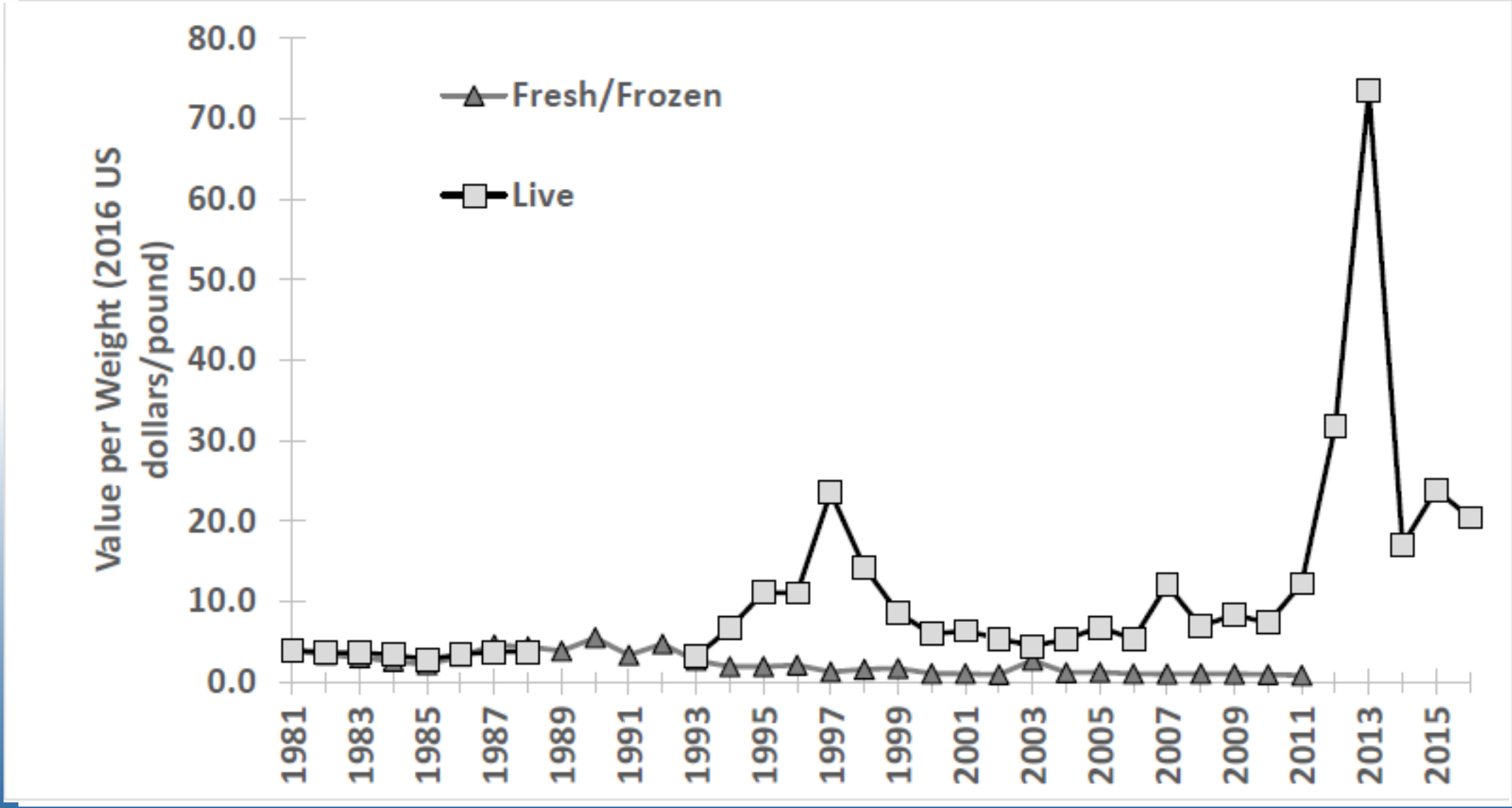
Healthy Cooking With Thrive Algae Oil

15% off coupon
on Thrive Culinary Algae Oil, 16.9 Ounce (Pack of 2)
Offer ends on or before 4/1/17

About the Product

- Real baby eels from Galicia
- Hand-prepared
- Packed in olive oil

U.S. Domestic Exports of Eels from Atlantic Coast



Eel Aquaculture Production

Quarantine

- Fungal infection
- Parasites
- Bacterial disease
- Viral disease

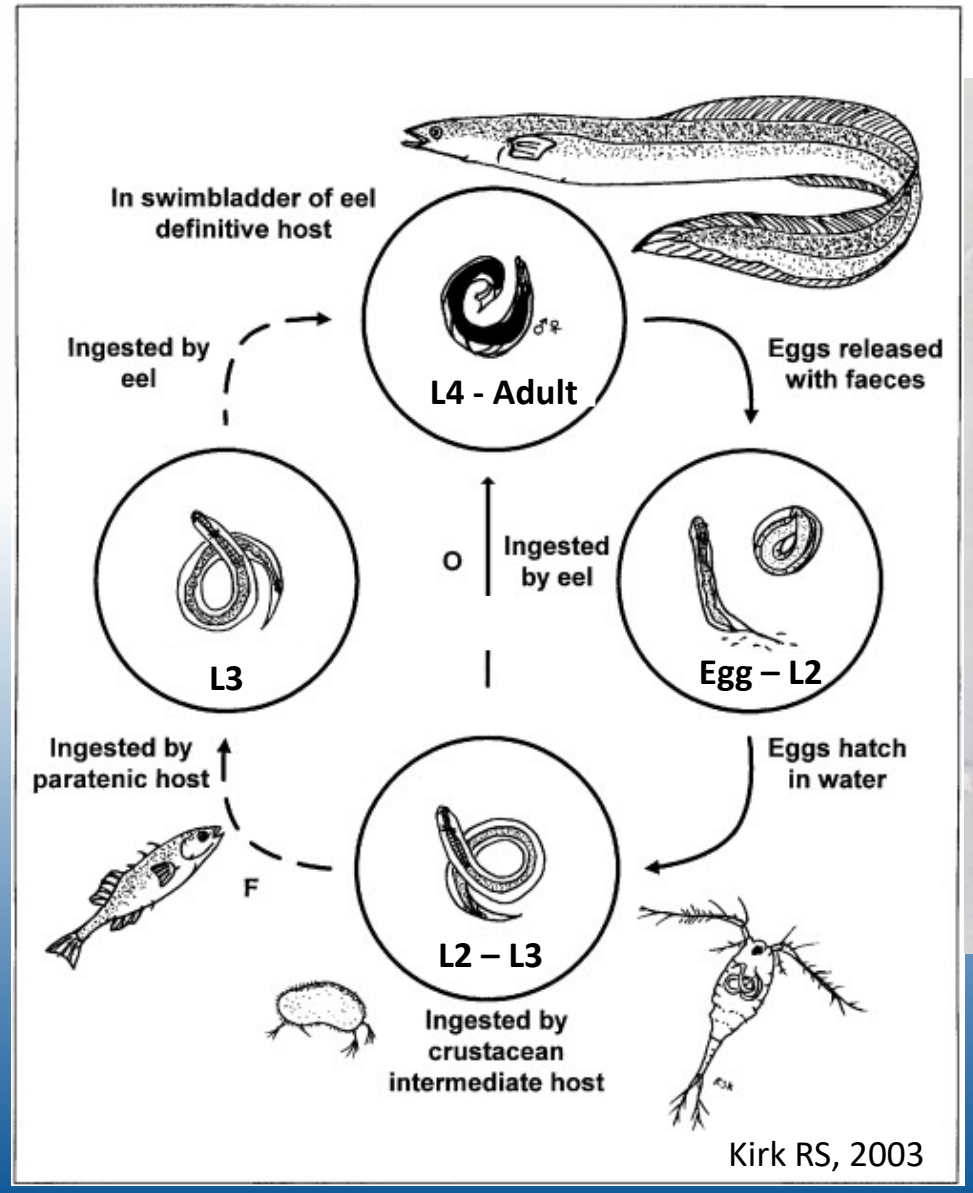
Grow out

Size Grading and Harvest

The Invader: *Anguillicoloides crassus*

- Introduced to Europe from Taiwan in the 1980's
  - Germany & Italy 1982
  - England 1987
- 1995: 1st detection in wild American eels
  - Winyah Bay, SC
- 2015 reported throughout American eel range
  - ~50% infection in SC in both yellow and glass eels
- *A. crassus* distribution was driven by long-range jumps along existing trading routes of live eels (Belpaire et al. 1989; Koops and Hartmann 1989; Kennedy and Fitch 1990; Fries et al. 1996)

The Invader: *Anguillicoloides crassus*



Kirk RS, 2003

Courtesy of SERTC

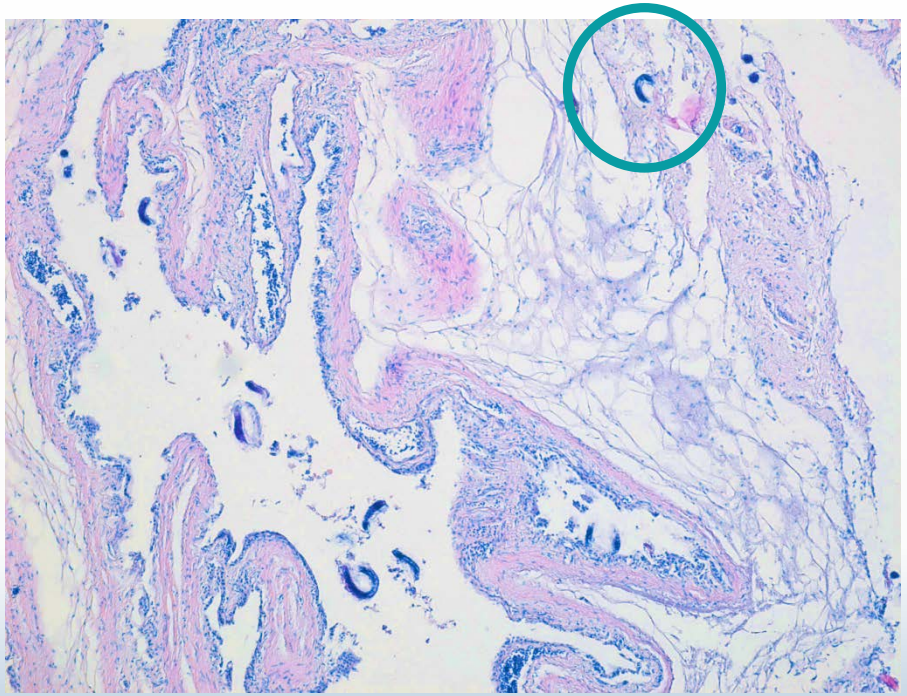
Anquillicoloides crassus

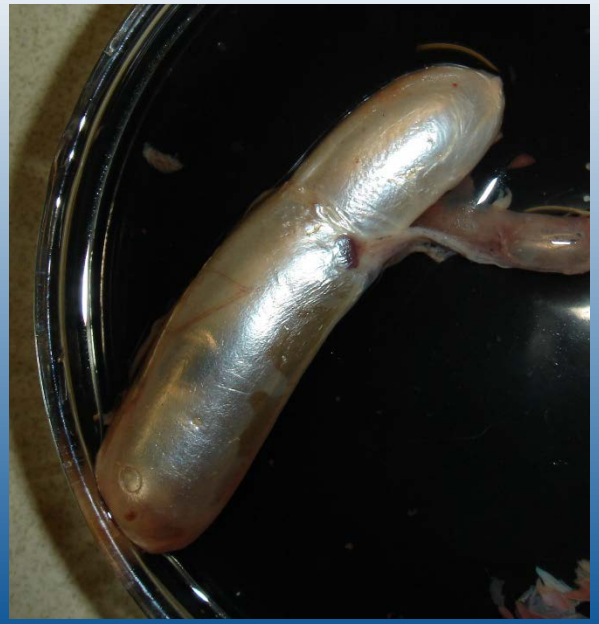

Courtesy of SERTC

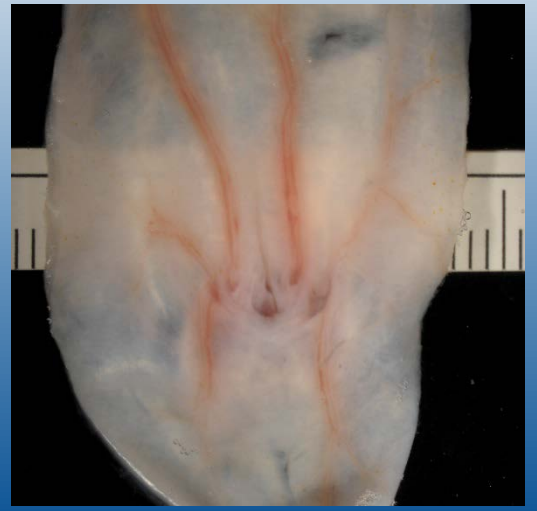
- Negative impacts on American eel survival
  - Anemia (Boon et al., 1990; Ooi et al., 1996)
  - Swimbladder damage (Molna´r et al., 1995; Lefebvre et al.,2002a, 2012a)
  - High mortality rates under stressful environmental conditions (Molna´r et al., 1991; Molna´r, 1993; Barusˇ and Prokesˇ, 1996)
- Sub-lethal effects?
  - Reduce survival in aquaculture
  - Reduce fitness and potential survival

Negative impacts on swimbladder function

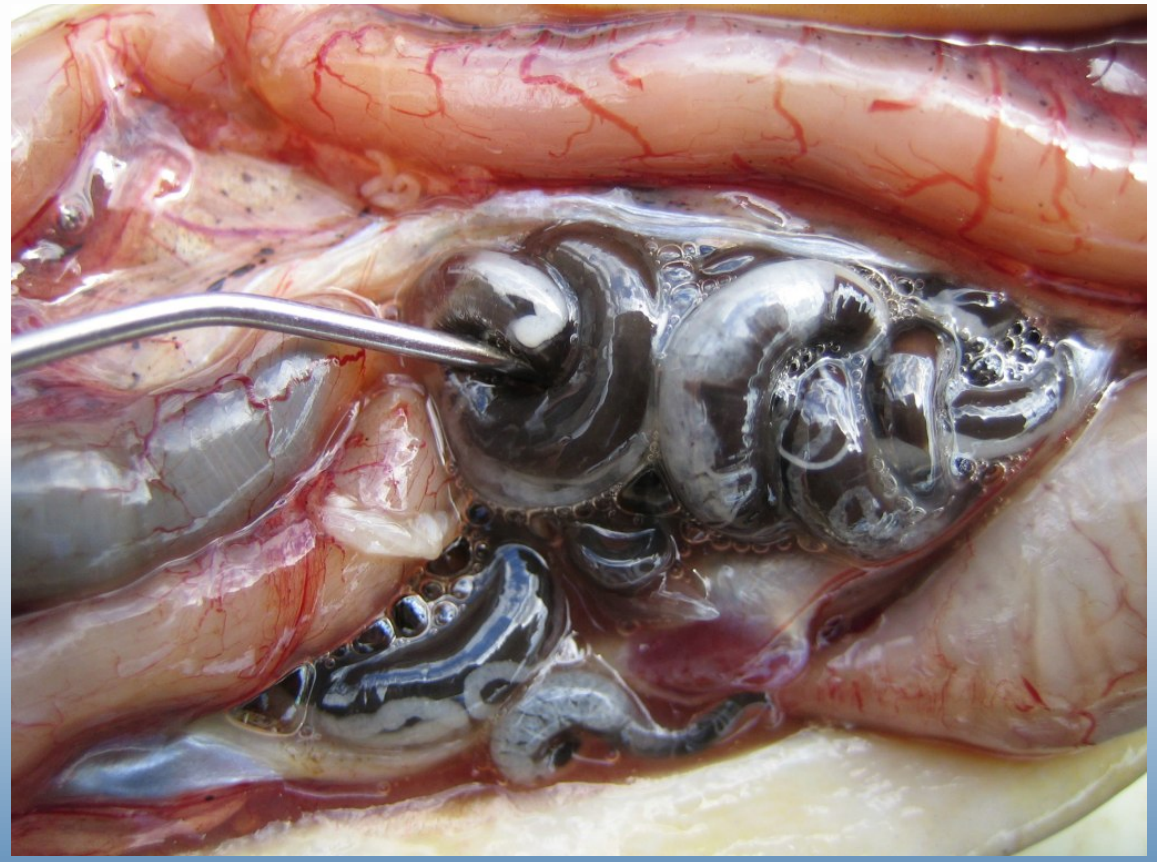
- Decreased survival in stressful conditions
  - Lesions and tunnel formations in swim bladder from migrating larvae
  - Epithelial lesions from bloodsucking adults
  - Degeneration and inflammation of swim bladder wall
    - “Cauliflower-like proliferation”







Primary method of *A. crassus* identification is lethal



https://europeaneel.com/2015/03/26/endoparasites-affect-swimming-behaviour-in-eels/

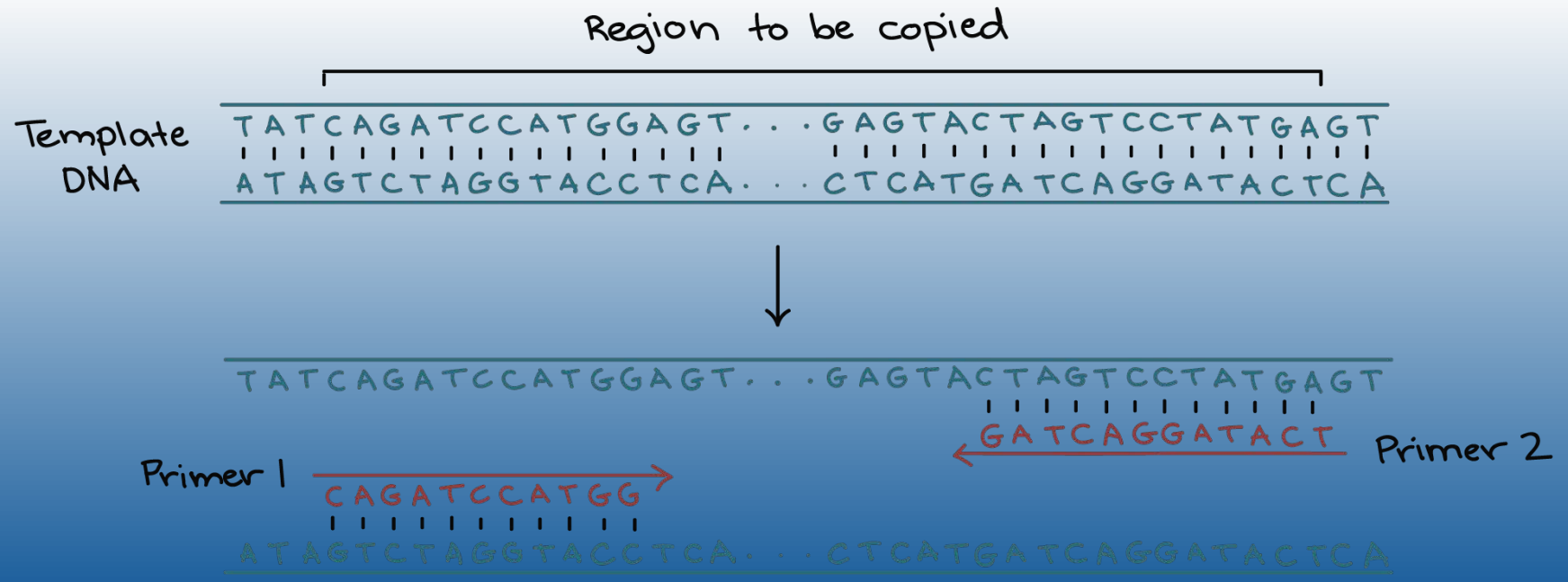
Objectives of Tool Development

Design a species-specific primer and probe for detection of *A. crassus* and apply it to a field setting

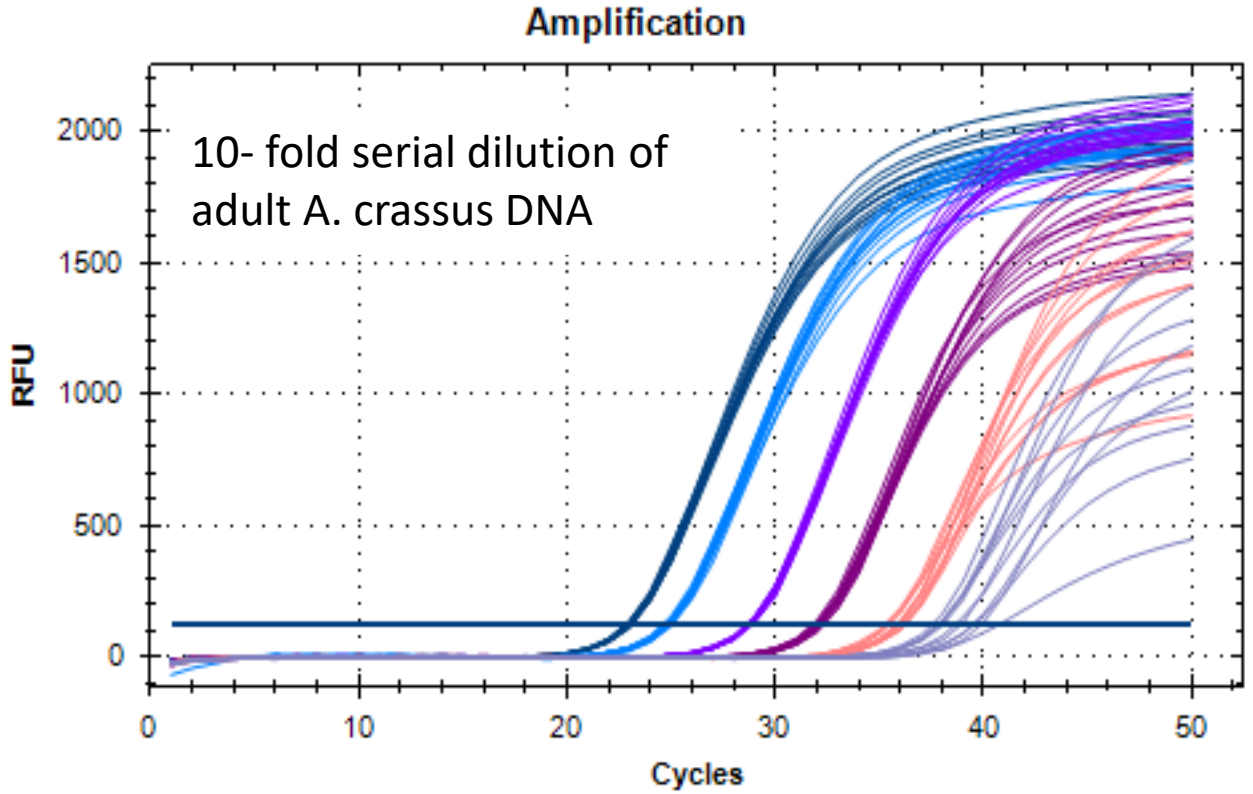
- Design an efficient compatible primer and probe set
  - Test specificity of primer and probe
  - Test the limitations in known L_2 and L_3 life stages
  - Validate tool against synthetic DNA
- Apply in a field setting
  - Optimize sampling protocol
  - Test for inhibition

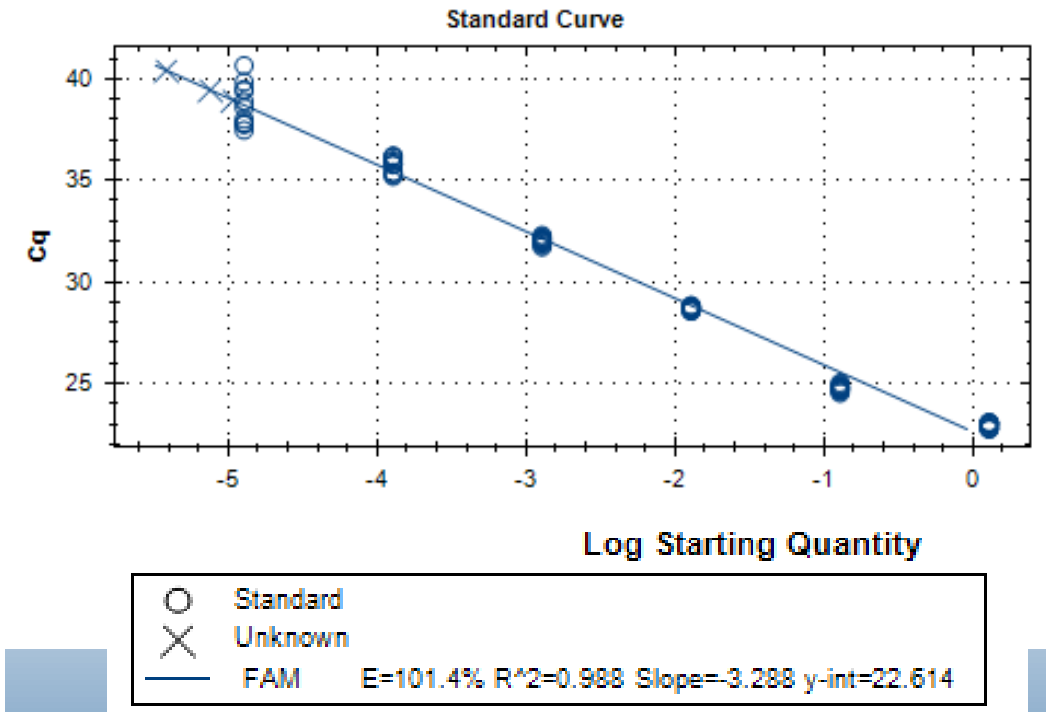
Design Primer and Probe Pair

- Primer and probe pairs were tested from a species-specific sequence with-in the COI region (Grabner et al., 2012)
- Primers were tested for self-complementarity (Primer3 and Oligocalc software)
- Identify similarities with closely related species (NCBI database) - <85% base similarity

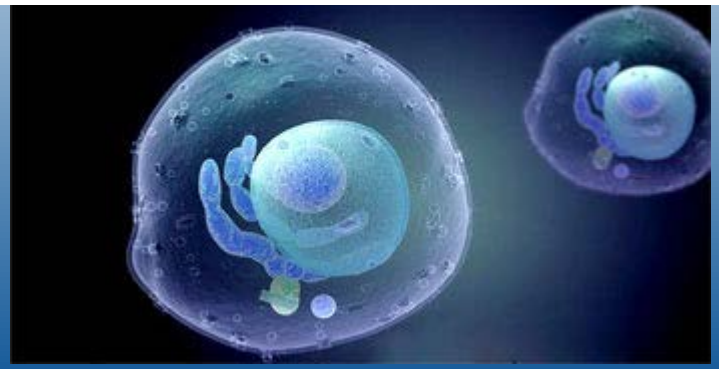


Assay Efficiency



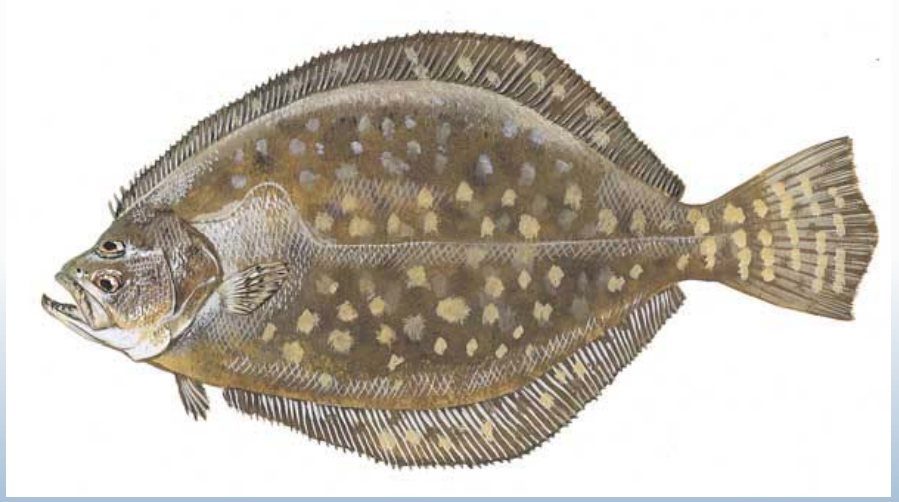


0.0000136 (µg/mL) with 100% detection
0.000000136 (µg/mL) still detectable



Specificity and Standard curves

- Various life history stages of *A. crassus*
  - Nematode species belonging to the closely related family Philometridae collected from various fish species
  - American eel tissue (swimbladder wall, pectoral fin)



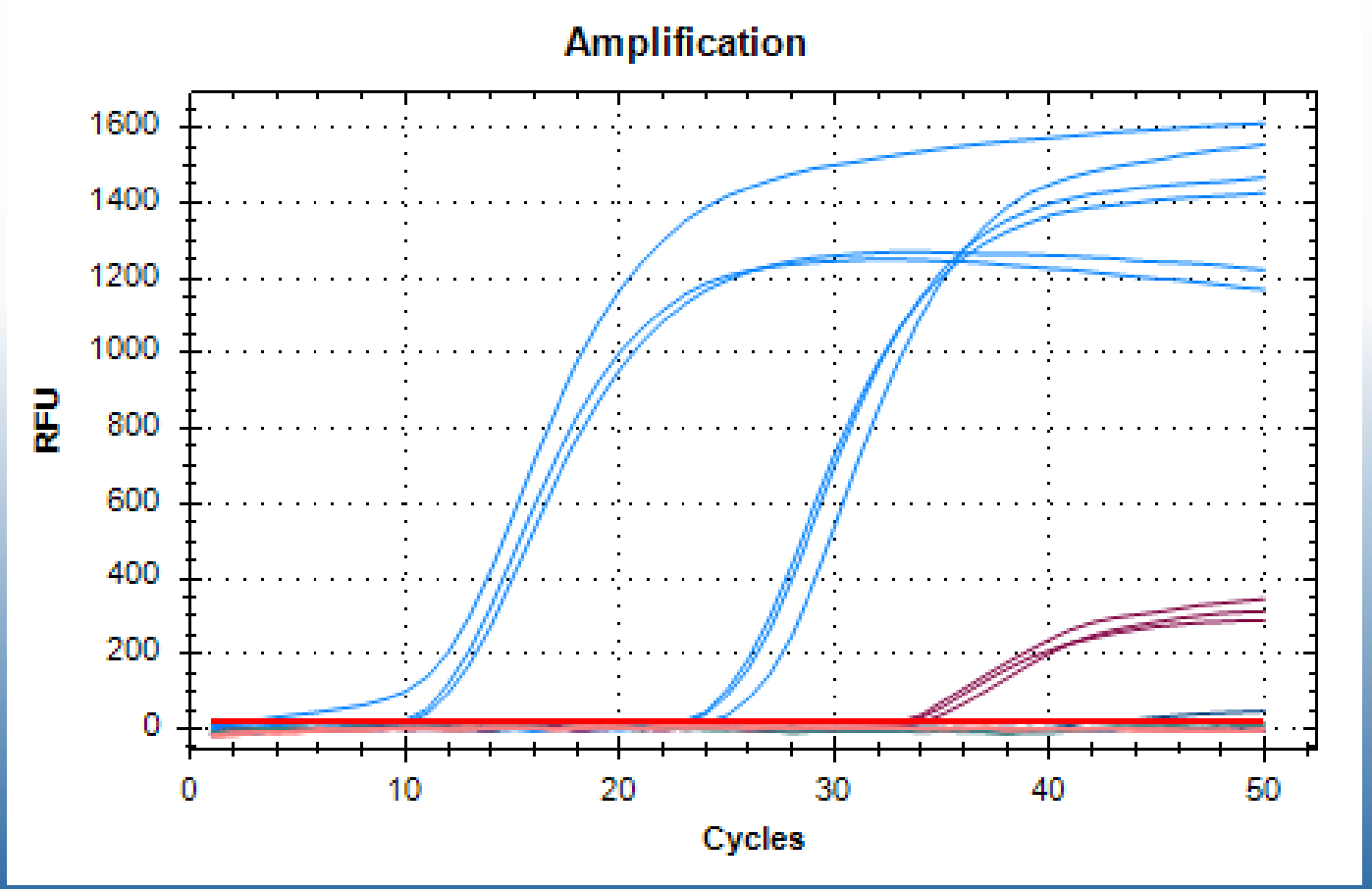
No amplification from non *A. crassus* samples collected locally

Synthetic DNA

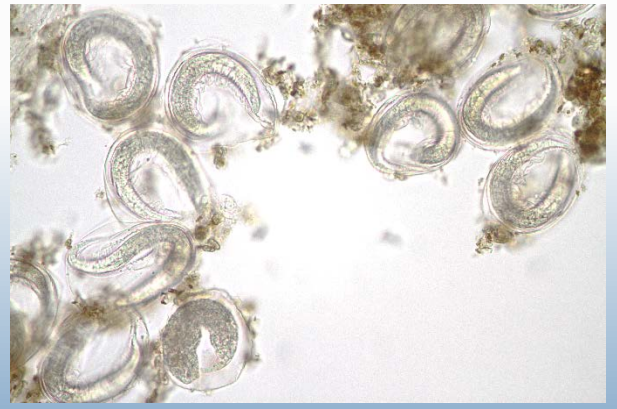
- Artificial DNA fragments of the other *Anguillicoloides* species:
  - *A. globiceps*
  - *A. australiensis*
  - *A. novaezelandiae*
  - *A. papernai*

Blue: Synthetic and natural *A. crassus*

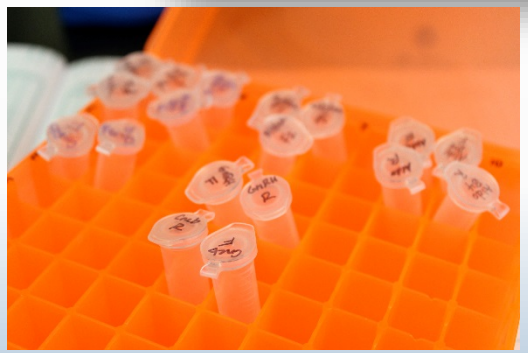
Purple: Synthetic *A. globiceps*



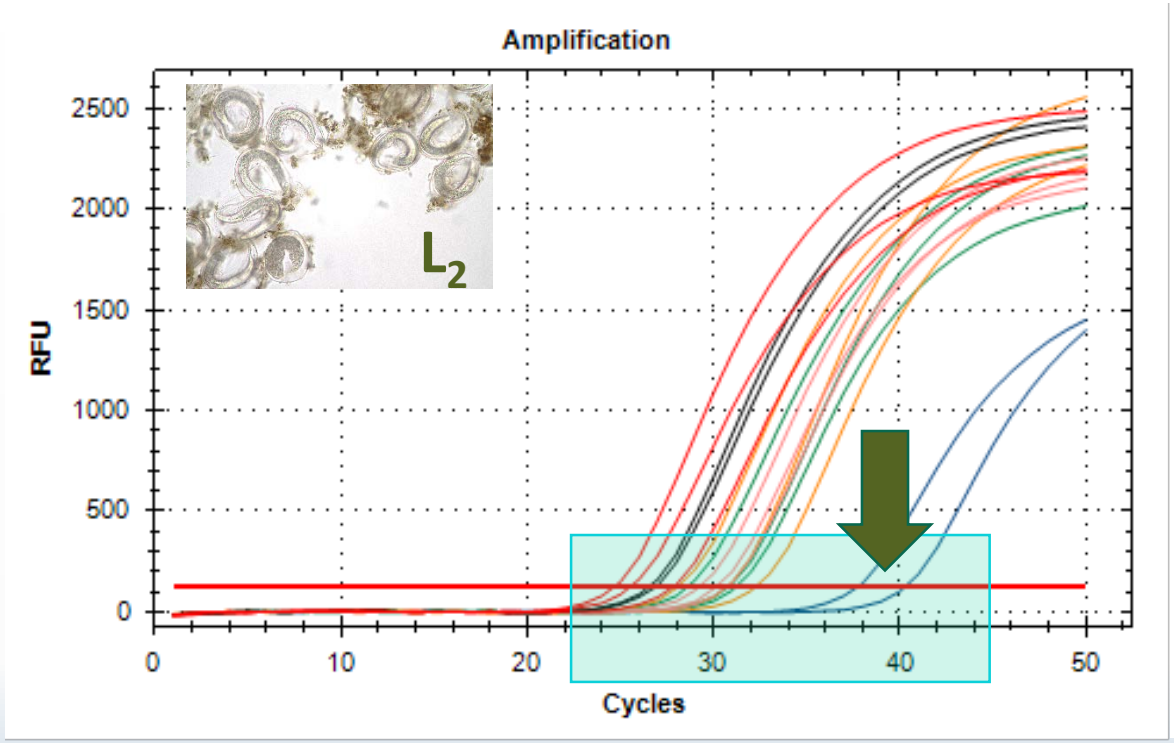
Can we differentiate the life stage present based on DNA concentration?

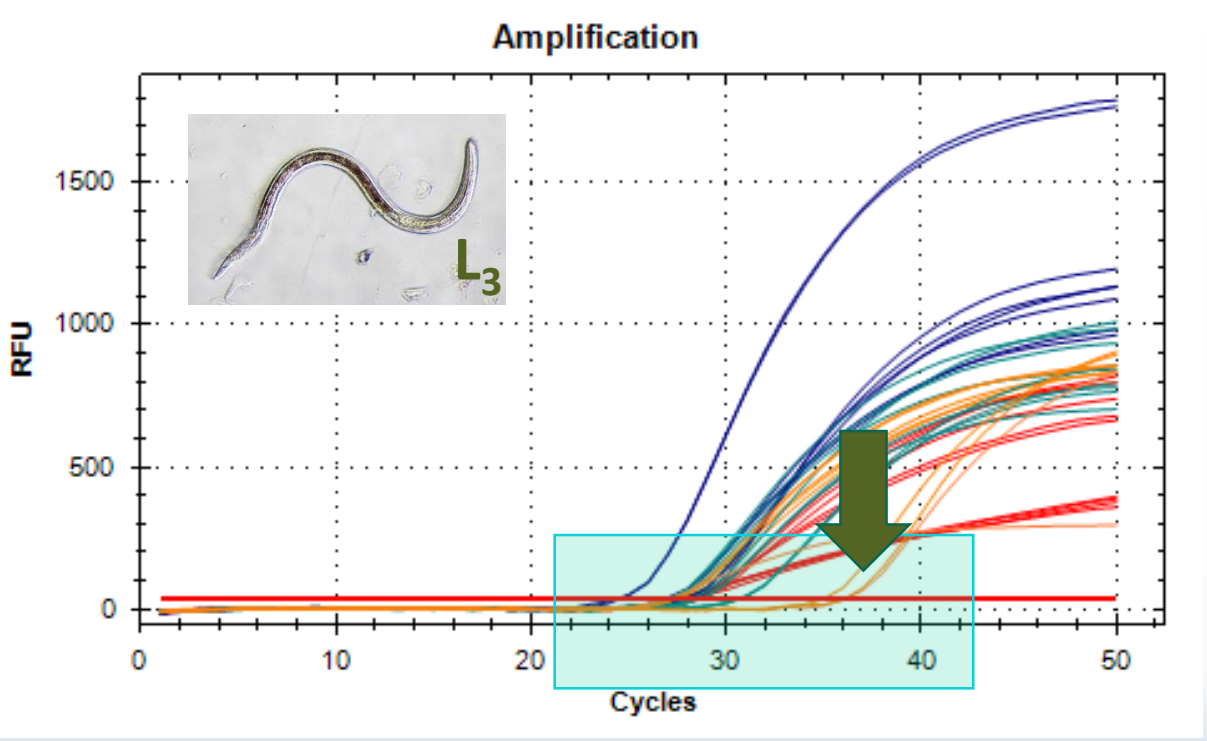


Second Life Stage – L2



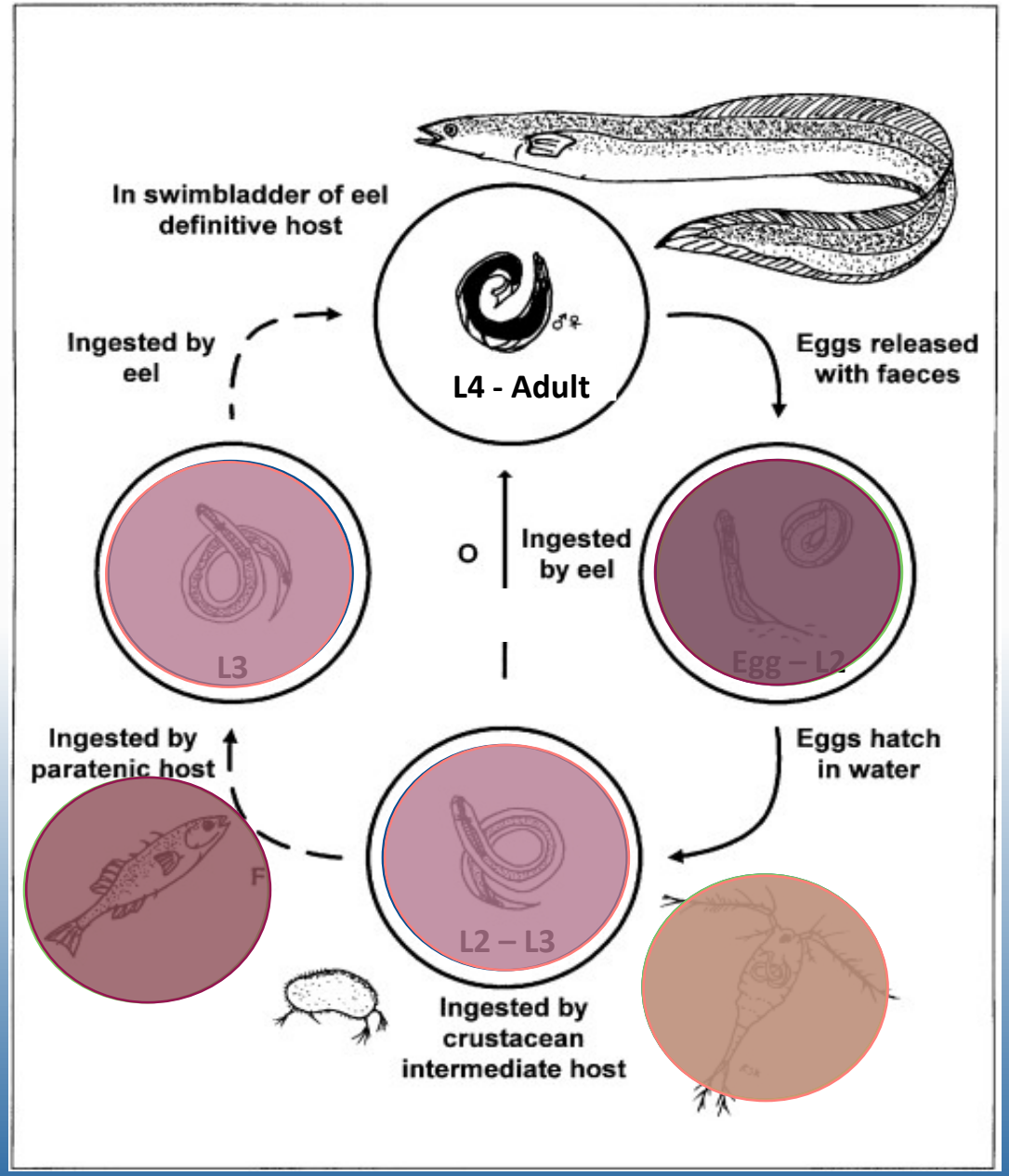
Third Life Stage – L3





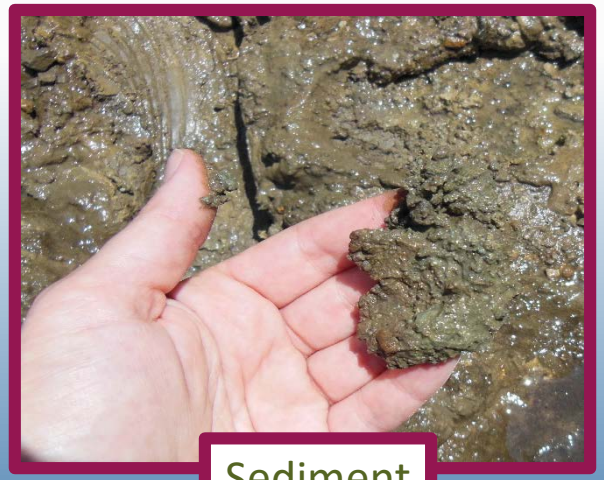
- Overlapping DNA concentrations for L2 and one L3
- We can detect one L2 and one L3

We can detect <u>one</u> parasite of either life stage



Filtered water

Algal mats



Sediment

Plankton

Field Sampling Results

No detection

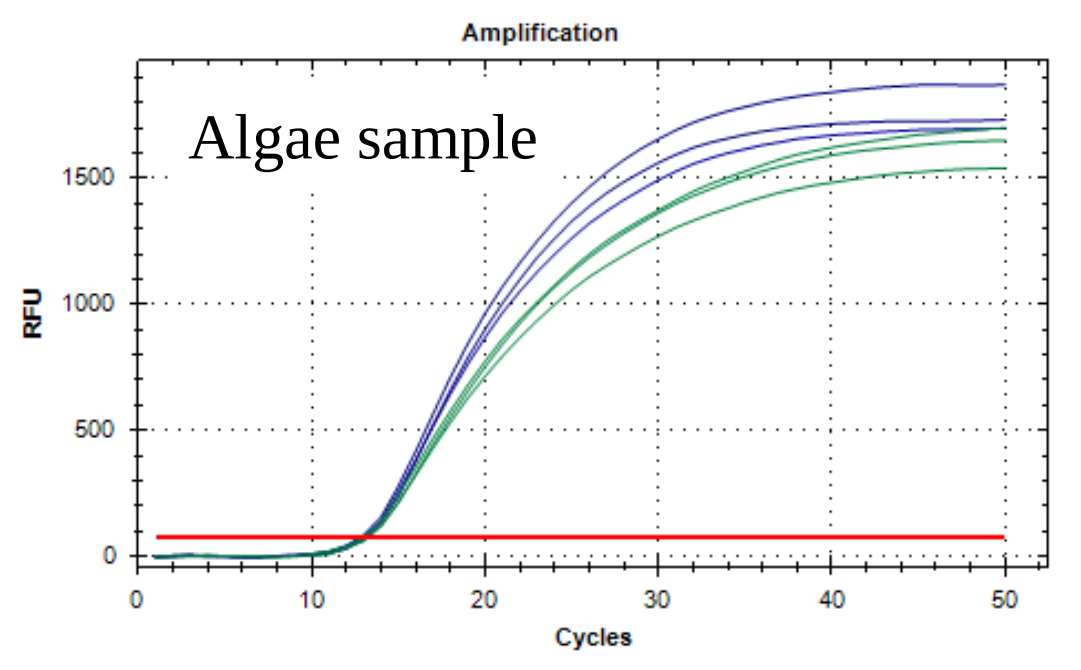
No detection

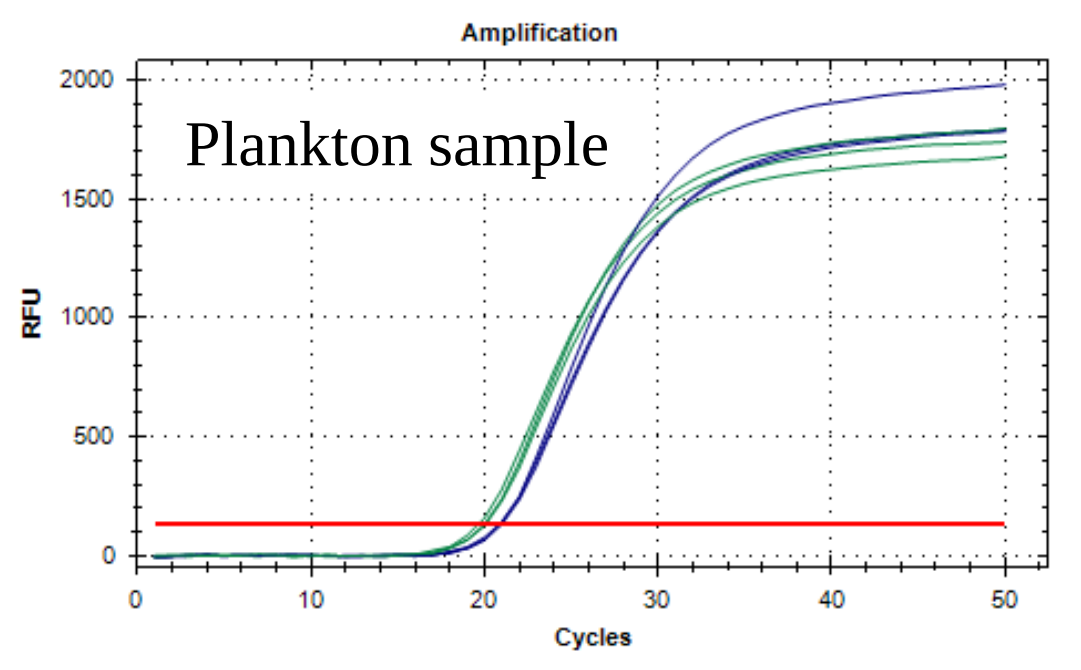
No detection

Positive detection

Inhibition Potential

- Chemicals which inhibit PCR are common in environmental samples
  - DNA extraction and isolation methods are designed to remove most impurities
    - Tannic and humic acids persist in the final DNA isolation





Blue – *A. crassus* DNA
Green – sample spiked with *A. crassus* DNA

Summary

- Assay is efficient, species-specific and unaffected by inhibition in the Goose creek area
  - *A. globiceps* may also amplify depending on region of world tool used
- Validated the use of the assay with field collections and the positive detection in the plankton sample
  - Copepods are known intermediate hosts, not surprising positive ID came from concentrated copepod collections
    - 100% detection with 2 mL of plankton
      - 2 mL is an appropriate sample volume
  - Potential for false negative among non-plankton field samples
    - May not have collected the appropriate volume
    - The appropriate volume for these samples may not be cost-effective for this method

Future work and Management Implications

- Future application of the assay to the aquaculture and stock enhancement practices
  - Aquaculture industry can reduce import of infected eels
  - Natural resource managers could limit transfer of eels to mitigate the spread of the invasive species
    - Sample numerous American eel elvers at once rather than dissecting individuals
- Assess temporal and spatial distribution of *A. crassus* in the south eastern United States
  - Non-invasive way to identify if *A. crassus* is present in an area

Acknowledgements

SCDNR Diadromous section

- Allen Hazel
- Elizabeth Miller

NSF REU MIMES

- Rachel Buissereth

SCDNR EFRS Inshore

- Brock Renkas
- Patrick Biondo
- Jessica Johnson

CofC Molecular Lab

- Kristy Hill-Spanik

SCDNR Population Genetics Lab

- Tanya Darden
- Beth Cushman
- Daniel Farrae
- Matt Walker
- John Robinson
- Tim O'Donnell

Photo credits:

- Jen Hein
- Ian Hubbard

U.S. Fish and Wildlife Service: State Interstate Aquatic Nuisance Species Management Plan Program

U.S. Fish and Wildlife Service Region 4 AIS Program – Small Grants Program