Gulf and South Atlantic Regional Panel on Invasive Species
Houston, Texas, September 17th-19th 2014

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

Peter Kingsley-Smith[1], Steve Arnott[1], Isaure de Buron[2], Jennifer Hein[1], Tanya Darden[1], Aaron Watson[1], William C. Post[3] & William A. Roumillat[1].

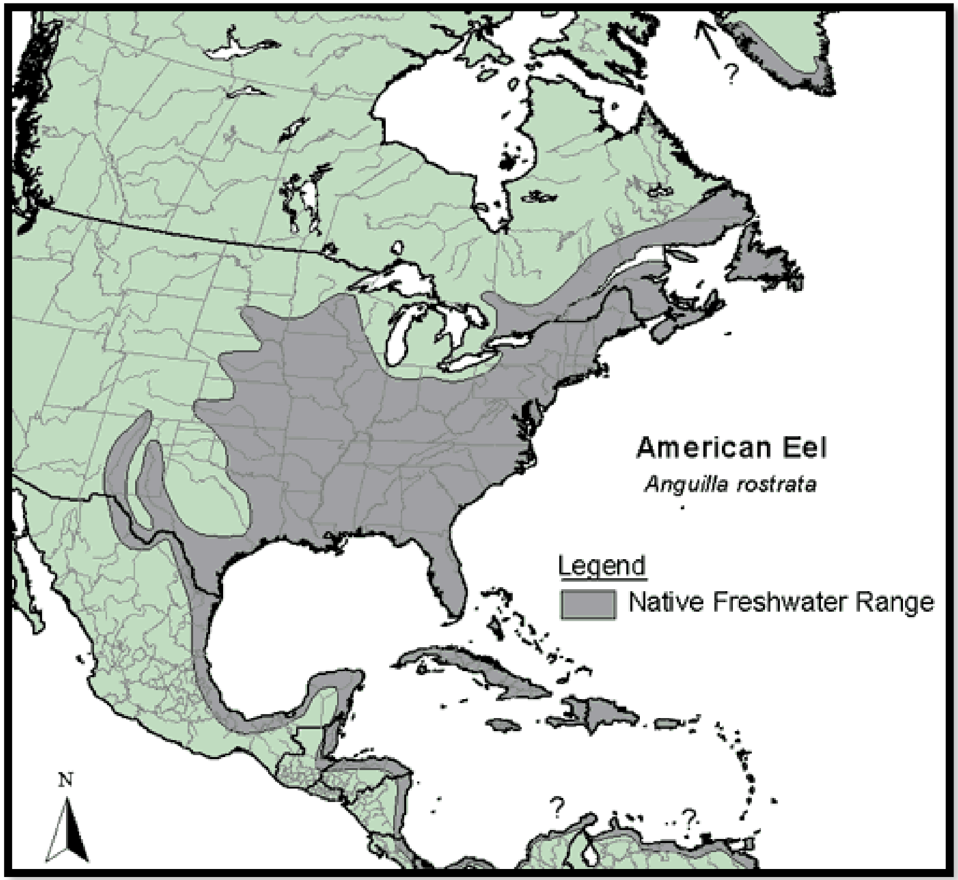
[1] South Carolina Department of Natural Resources Marine Resources Research Institute
[2] College of Charleston Department of Biology
[3] South Carolina Department of Natural Resources Diadromous Fishes Section

Adult *A. crassus* parasites in the swimbladder of an American eel.

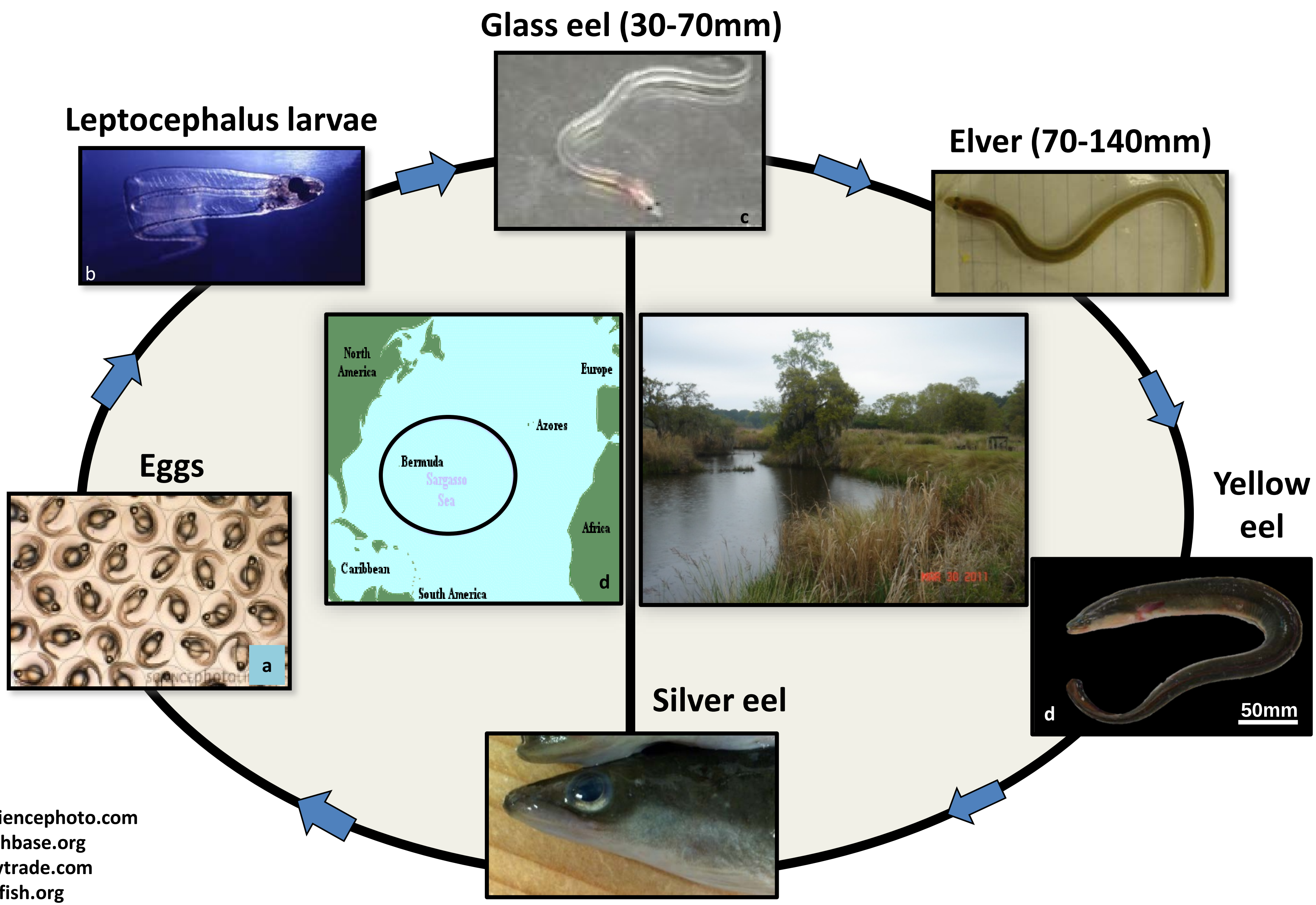
Photo by J. Hein

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

Eel vulnerability and decline

- **High age at maturity (~10-30 yrs, varies with latitude), only spawn once, and potentially very long-lived…**

Eel vulnerability and decline

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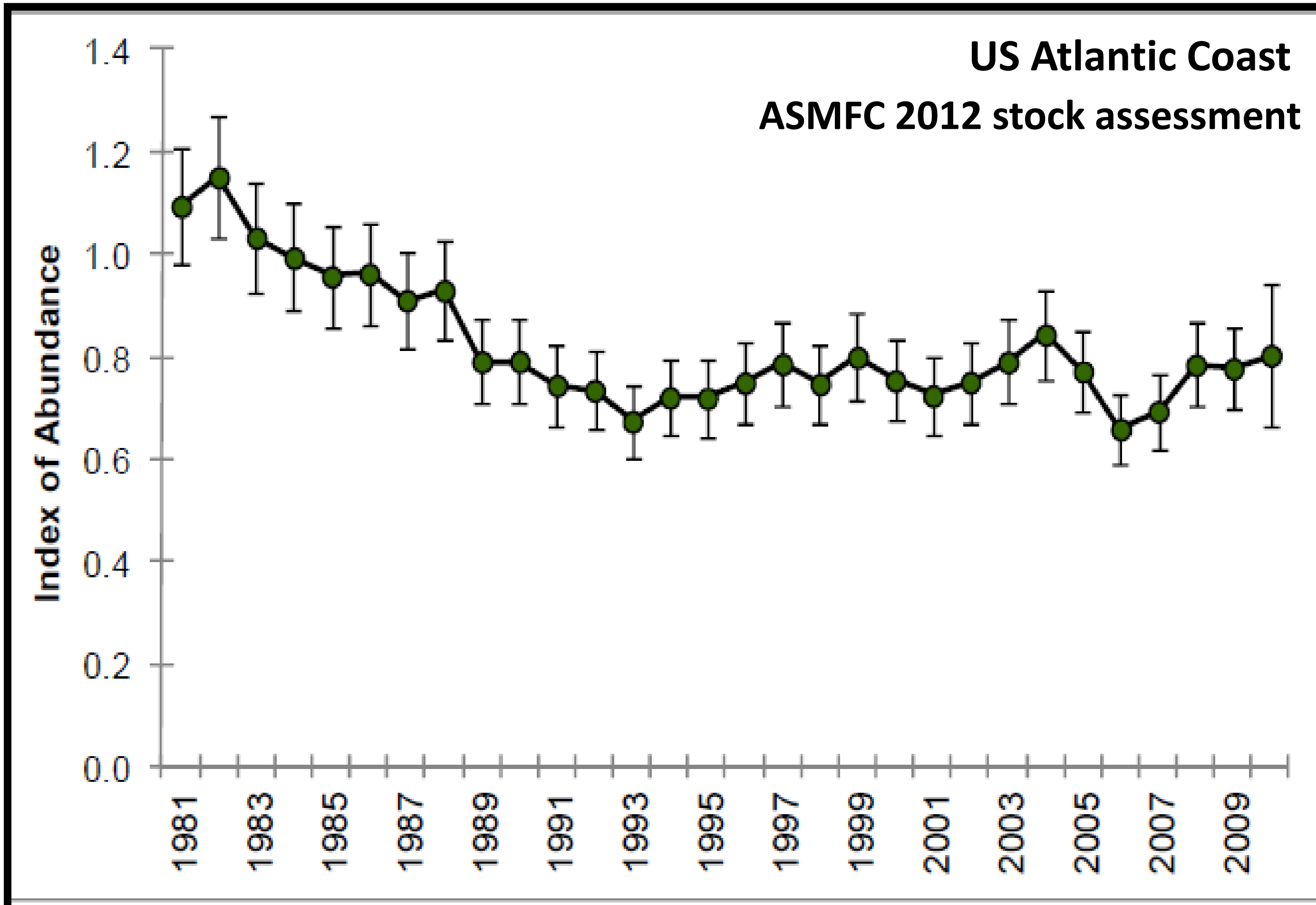
- American eels are harvested both commercially and recreationally throughout their range.
- Harvest peaked in 1979 at 3.95 million pounds and has been declining since, i.e., for past 30+ years.

American Eel Population Status



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

Eel vulnerability and decline

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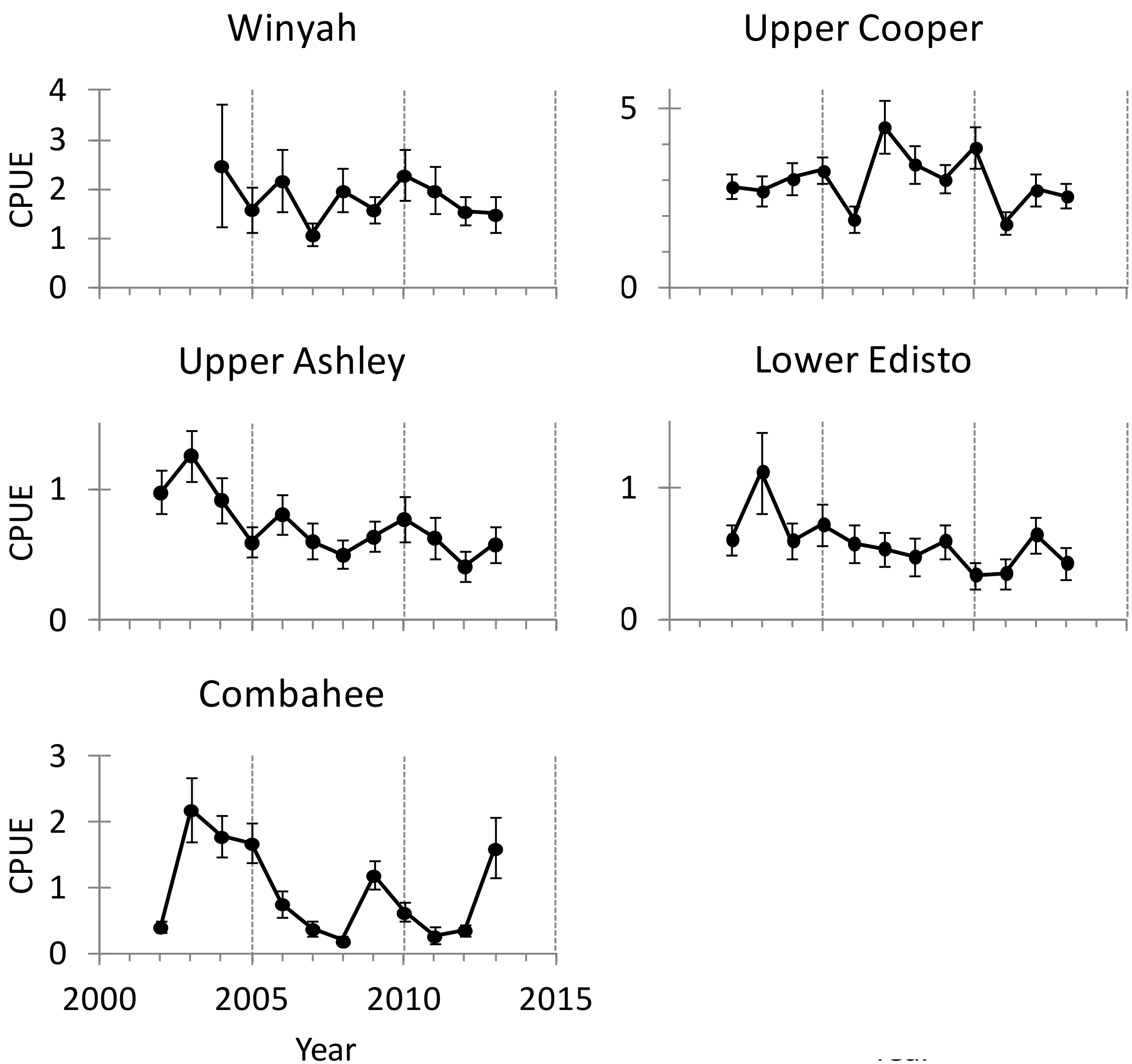
- **SCDNR electrofishing survey shows decline in eel populations since 2001.**

Annual Variation in Eel Catch Per Unit Effort



Delta models
Logistic : Probability of +ve catch
GLM: Number eels per 15 min set

Estuary: $p < 0.001$

Year: $p \leq 0.007$
(except Winyah, $p \geq 0.68$)

Year*Estuary:
$p \leq 0.003$

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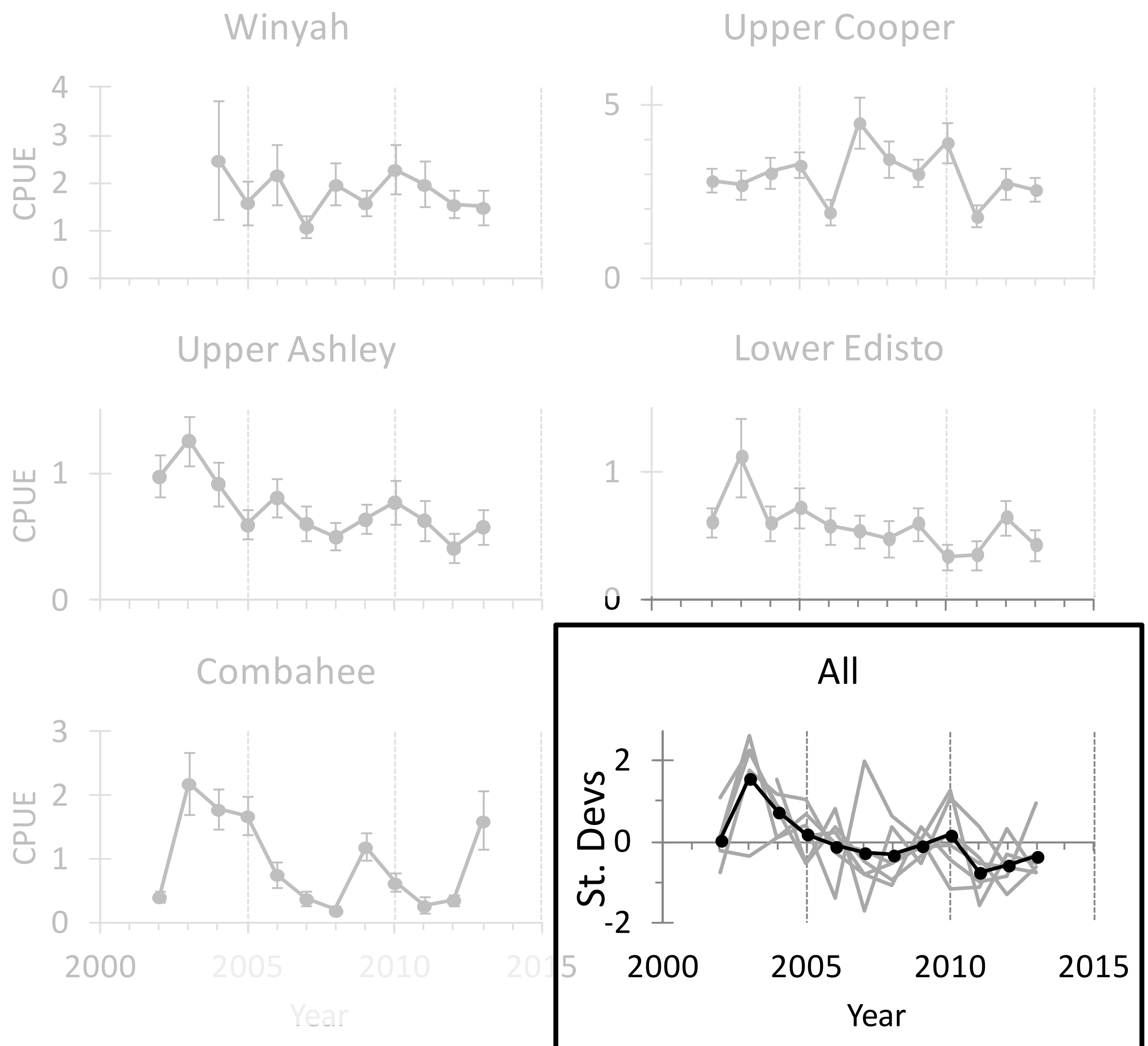

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Eel vulnerability and decline

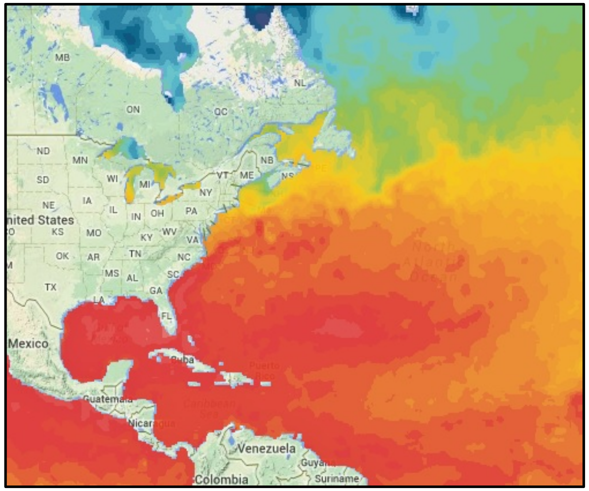
- High age at maturity (~10-30 yrs, varies with latitude), only spawn once, and potentially very long-lived…
- American eels are harvested both commercially and recreationally throughout their range.
- Harvest peaked in 1979 at 3.95 million pounds and has been declining since, i.e., for past 30+ years.
- SCDNR electrofishing survey shows decline in eel populations since 2001.
- **2004 & 2011: Petitions filed with US Fish and Wildlife and NMFS to list the American eel as an endangered species – still under review.**

Potential threats to eel populations

Harvesting

Barriers to migration

Turbine mortality



Environmental changes

Anguillicoloides crassus

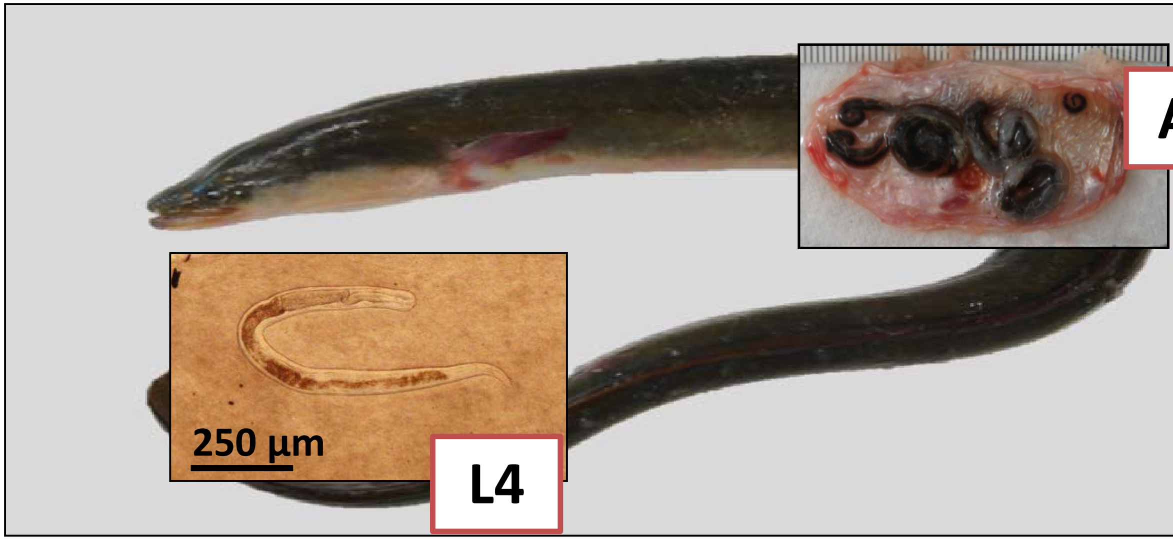
Anguillicoloides crassus

- **Nematode parasite - infects swimbladder lumen of anguillid eels.**
- **Endemic to East Asia; infects Japanese eels (*Anguilla japonica*) without causing serious pathology.**
- **Extremely pathogenic to non-native eel species (e.g., American eel, *A. rostrata* and European eel, *A. anguilla* (Knopf and Mahnke 2004; Taraschewski 2006).**
- **1982: *A. crassus* infections in the European eel (*Anguilla anguilla*) caused severe declines in eel numbers (Székely *et al.* 2009; Lefebvre *et al.* 2012).**
- ***A. crassus* has rapidly infected eel species in Europe, N. Africa, S. Africa and N. America - commercial movement of live eels.**
- **1995: First report of *A. crassus* in wild populations of *A. rostrata* (Winyah Bay, South Carolina, USA, Fries *et al*. 1996).**
- **From 1982-2010, 470 articles published on *A. crassus* since its first report outside of Asia, predominantly for European infections (Lefebvre *et al.* 2012).**

A. crassus life cycle

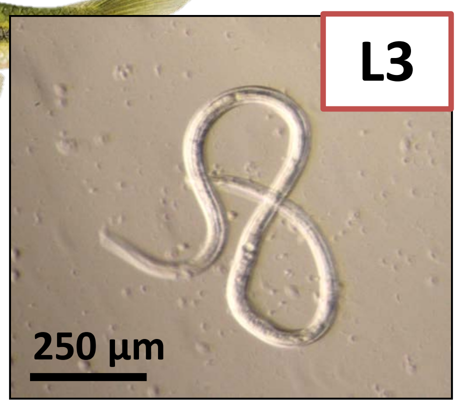
L4 molts to adult parasite in the swimbladder lumen

L3 stage molts to L4 stage in the swimbladder wall



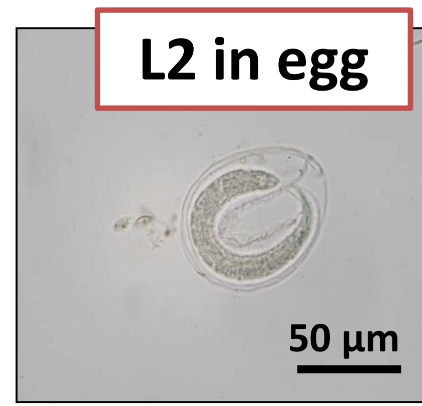
Parasite eggs are laid down in the swimbladder lumen, pass through the pneumatic duct, and exit the eel via its vent

[Paratenic host]



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

[+ Molluscs, ostracods and others in European studies]



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

- Understanding of life cycle is largely based on European studies; little research on life cycle in N. America; <u>however…</u>

Southeastern Society of Parasitologists (SSP) 2014 Meeting
April 9^{th}-11^{th} 2014, Dept. of Biology
Georgia Southern University, Stateboro, GA

Experimental infection of a potential cyclopoid vector of *Anguillicoloides crassus,* an invasive parasite of the American eel.

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[1]Department of Biology, College of Charleston
Charleston, SC.

[2]Marine Resources Research Institute, Department of Natural Resources Charleston, SC.

[3]Poseidon Taxonomic Services
Charleston, SC.

- **European studies have shown that cyclopoid copepods act as intermediate vectors for *A. crassus* infections; equivalent studies not previously conducted in N. America.**
- **Zooplankton were collected from Goose Creek Reservoir, South Carolina; copepods, ostracods and other invertebrates were exposed to *A. crassus* larvae.**

Dam at Goose Creek Reservoir: Sampling location for q-PCR work

- European studies have shown that cyclopoid copepods act as intermediate vectors for *A. crassus* infections; equivalent studies not previously conducted in N. America.
- Zooplankton were collected from Goose Creek Reservoir, South Carolina; copepods, ostracods and other invertebrates were exposed to *A. crassus* larvae.
- **Cyclopoid copepod of the genus *Acanthocyclops* selected for experimental infection:**
  - **Readily ingested larva and larvae moved into hemocoel**
- **Prevalence of infection for L_2 larvae at 21°C and 26°C was ~86% and ~89%, respectively.**
- **Prevalence of infection for L_3 at 21°C and 26°C was ~9 and ~7%, respectively; low numbers may reflect mortality post-infection.**
- ***Acanthocyclops* is a potential vector for larvae of *A. crassus***
  - **Common species at sampling site.**
  - **More research is needed on distribution and seasonality.**

Cyclopoid copepod genus *Acanthocyclops* infected by larval *A. crassus.*

Images by Ian Hubbard

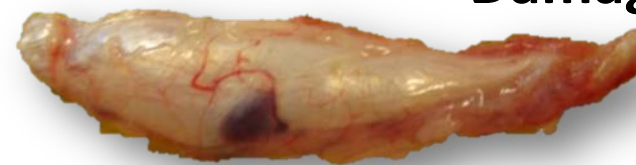
Cyclopoid copepod genus *Acanthocyclops* infected by larval *A. crassus.*

Video by Ian Hubbard

Significance of swimbladder damage

Healthy

Damaged



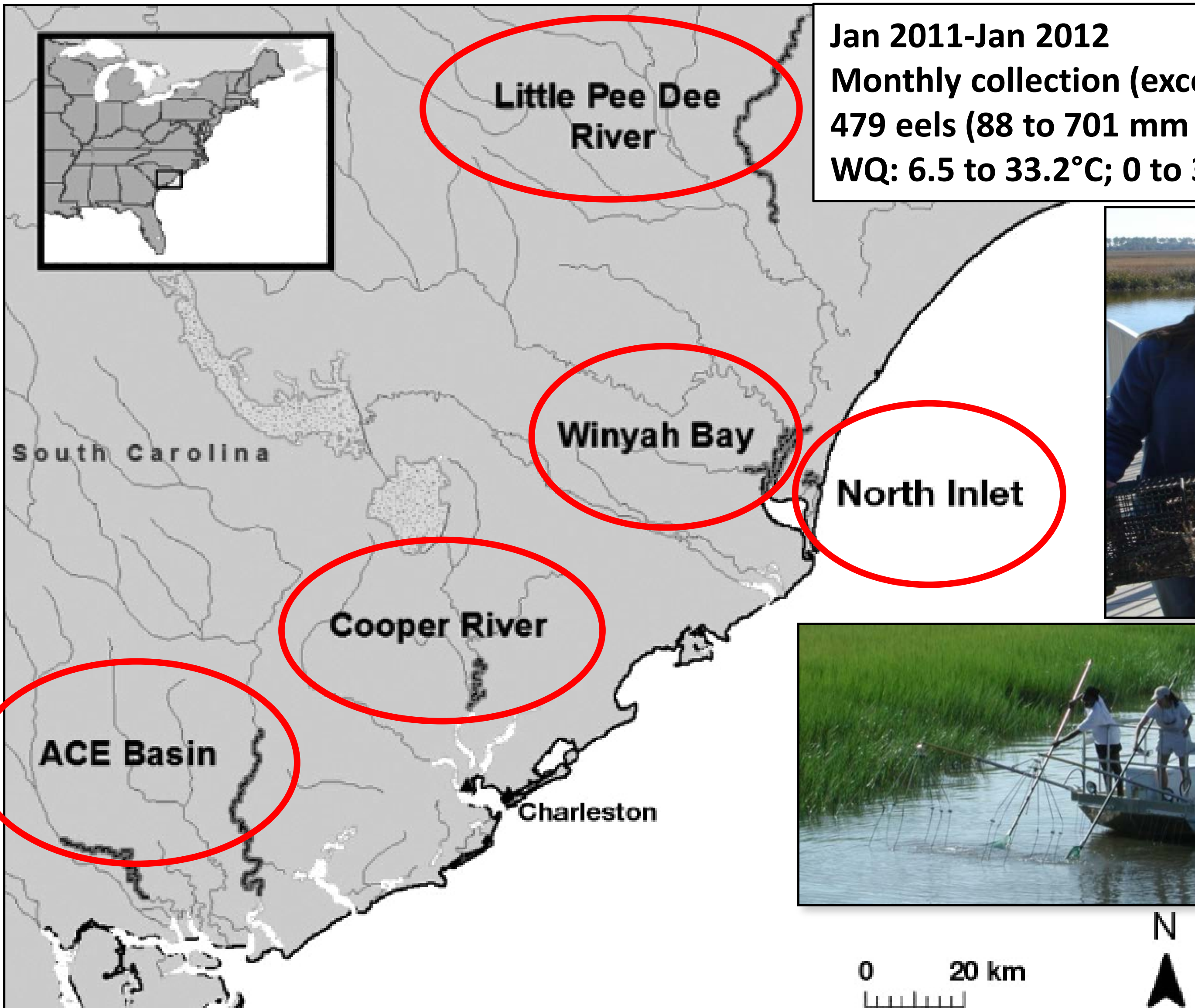
- **Irreversible damage is caused by the parasites feeding on the eel's blood (Molnár et al. 1995) and by larvae migrating through swimbladder wall.**
- **Consequences of swimbladder damage:**
  - **Damages to gas gland and cell function**
  - **Reduced O_2 content**
  - **Problems with buoyancy control (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.**

Yellow eel research (since 2011)

OBJECTIVES:

- **To quantify levels (abudance, prevalence and intensity) of *Anguillicoloides crassus* infections in *A. rostrata*.**
- **To determine the factors most closely associated with *A. crassus* infections in *A. rostrata* (locality, season, eel size).**
- **To compare findings with previous studies of *A. crassus* infections in South Carolina *A. rostrata* populations.**

Field collection of yellow eels



Jan 2011-Jan 2012
Monthly collection (except Pee Dee)
479 eels (88 to 701 mm TL)
WQ: 6.5 to 33.2°C; 0 to 36 ppt

Larval (L3/L4) parasite stages:

Prevalence: **29%** (137/471 eels)

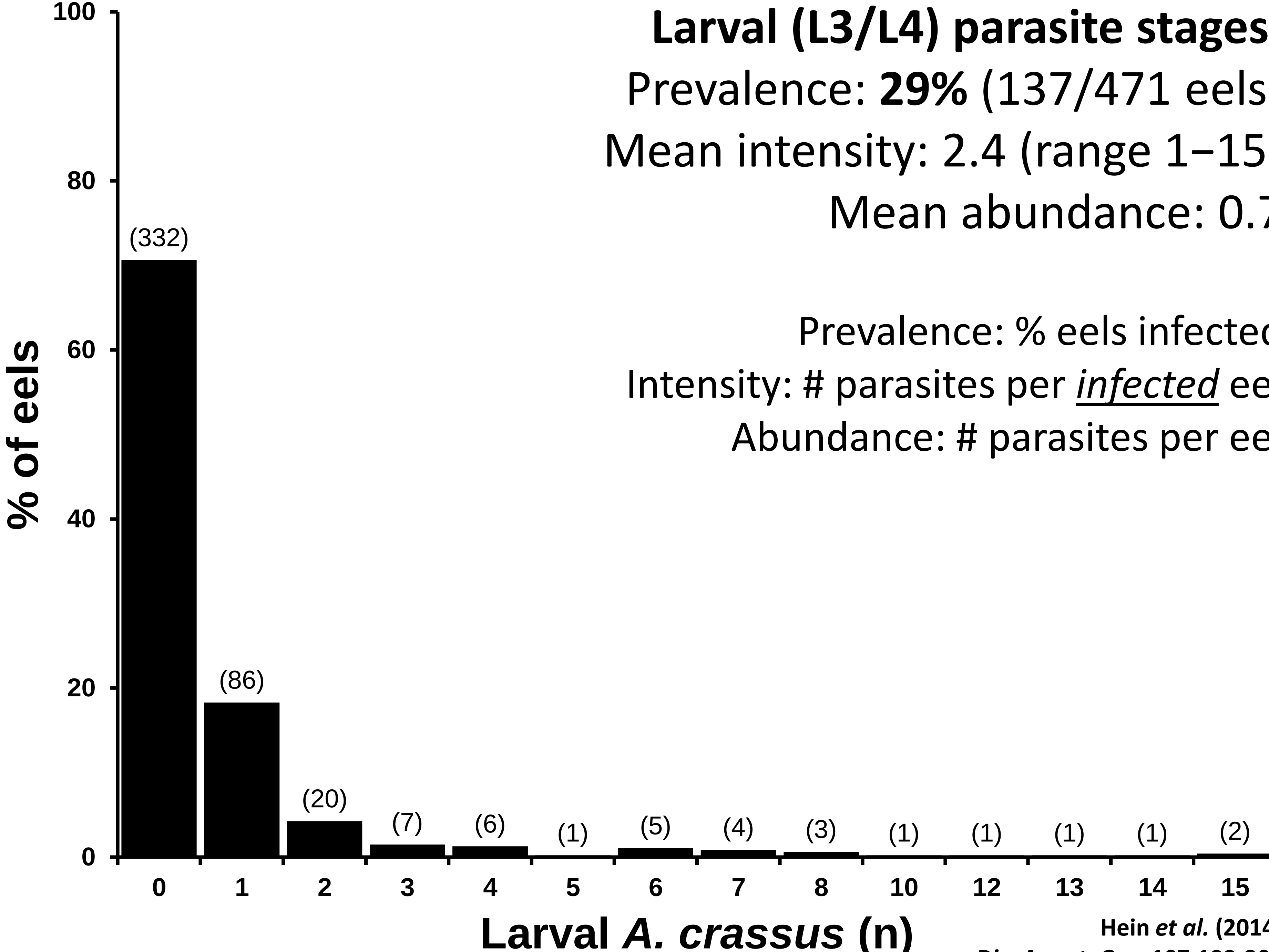
Mean intensity: 2.4 (range 1−15)

Mean abundance: 0.7

Prevalence: % eels infected

Intensity: # parasites per *infected* eel

Abundance: # parasites per eel



Hein *et al.* (2014)
Dis. Aquat. Org. 107:199-209

Hein *et al.* (2014)
Dis. Aquat. Org. 107:199-209

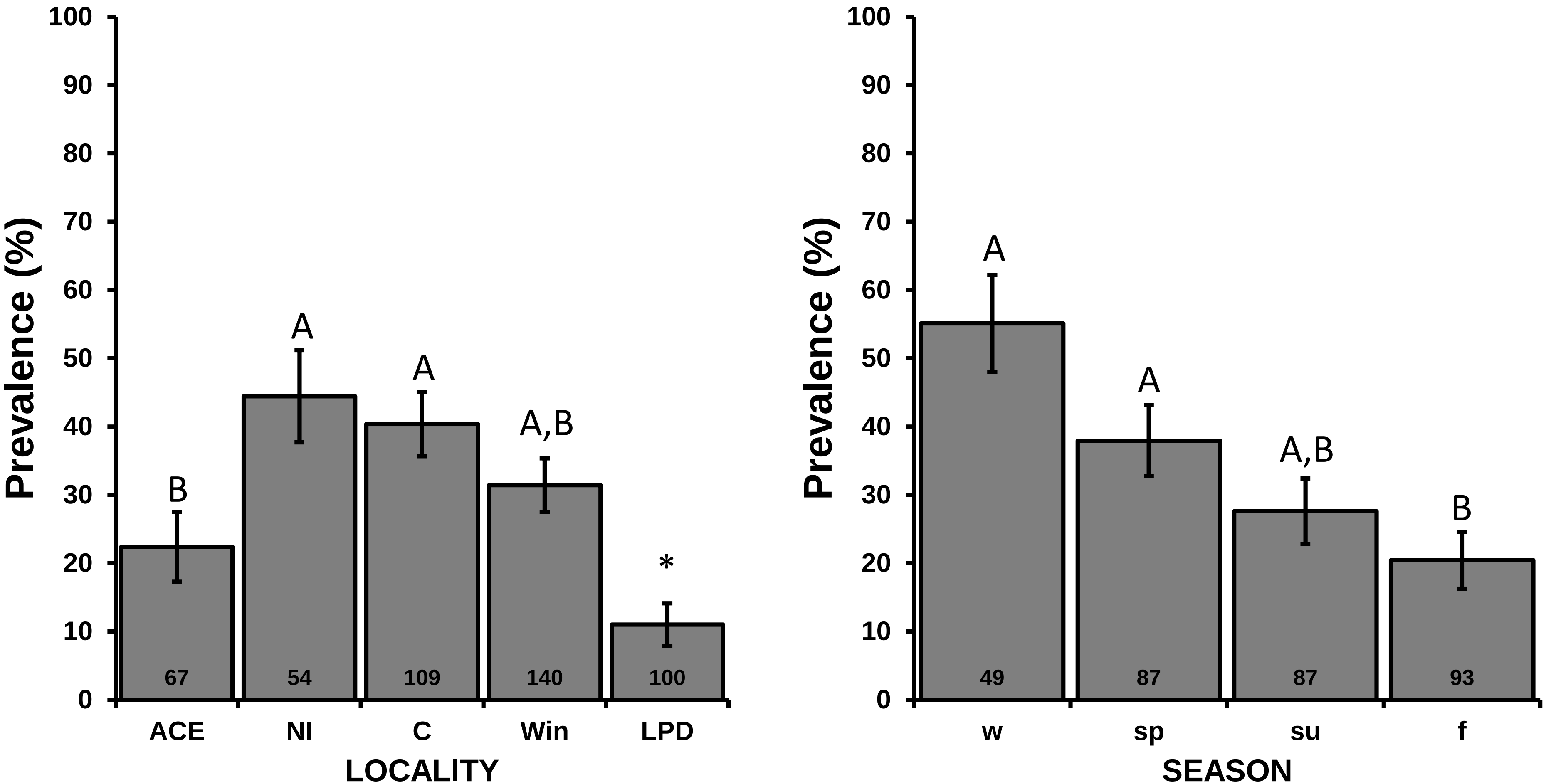
Prevalence - Larval (L3/L4) parasites:

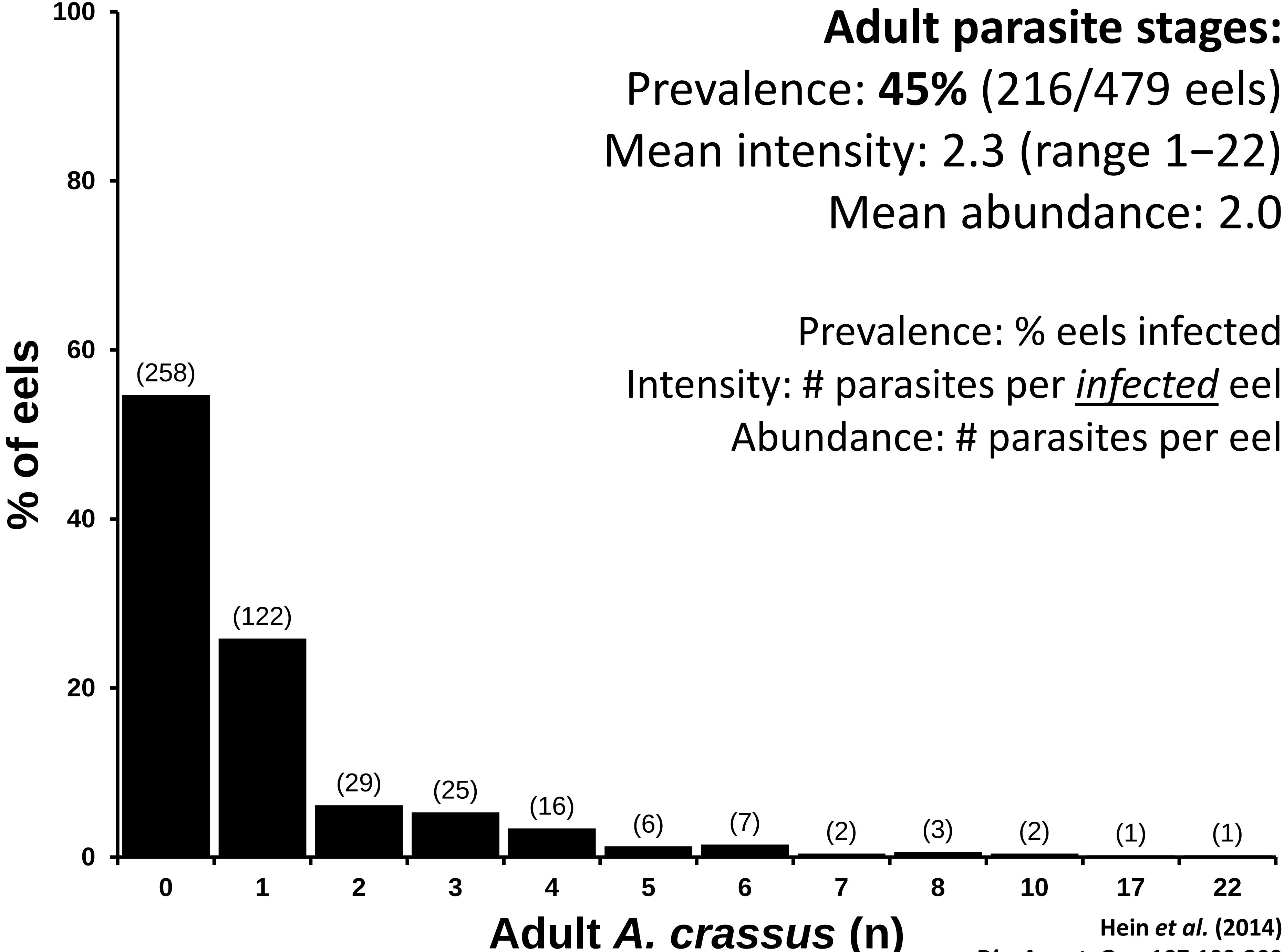
Locality, p=0.02

Season, p=0.02

Highest at North Inlet and Cooper River; during winter

[Intensity not significantly different btw locations or seasons].





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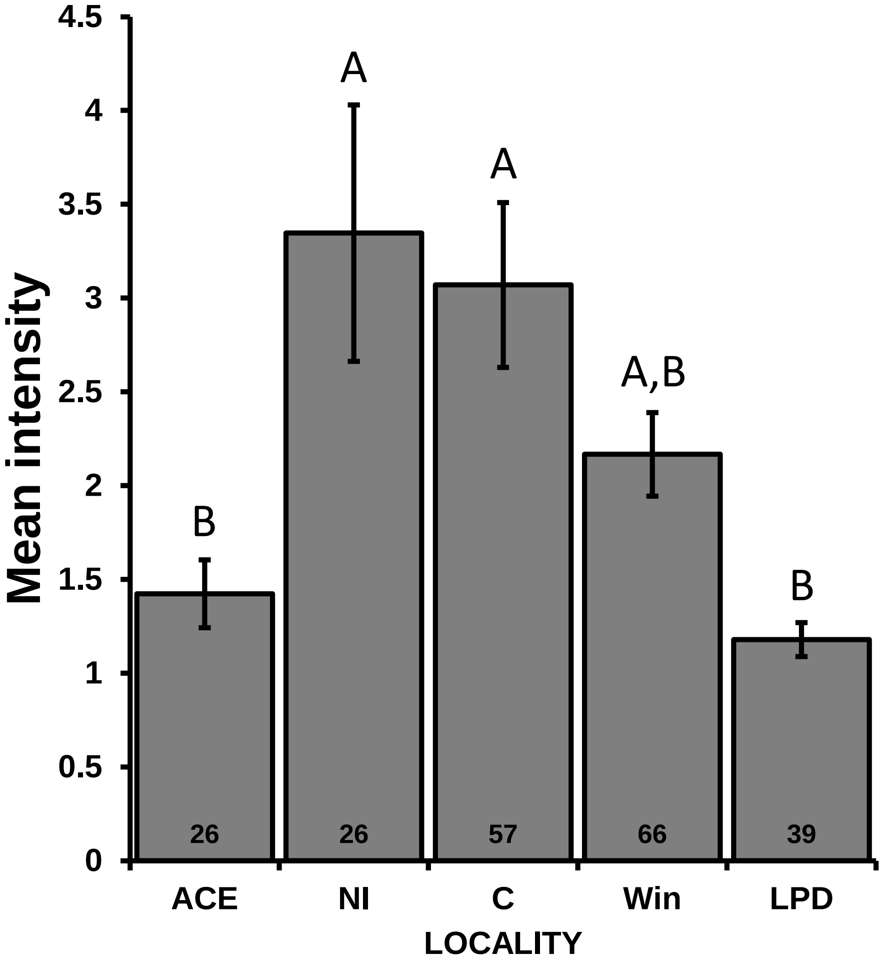
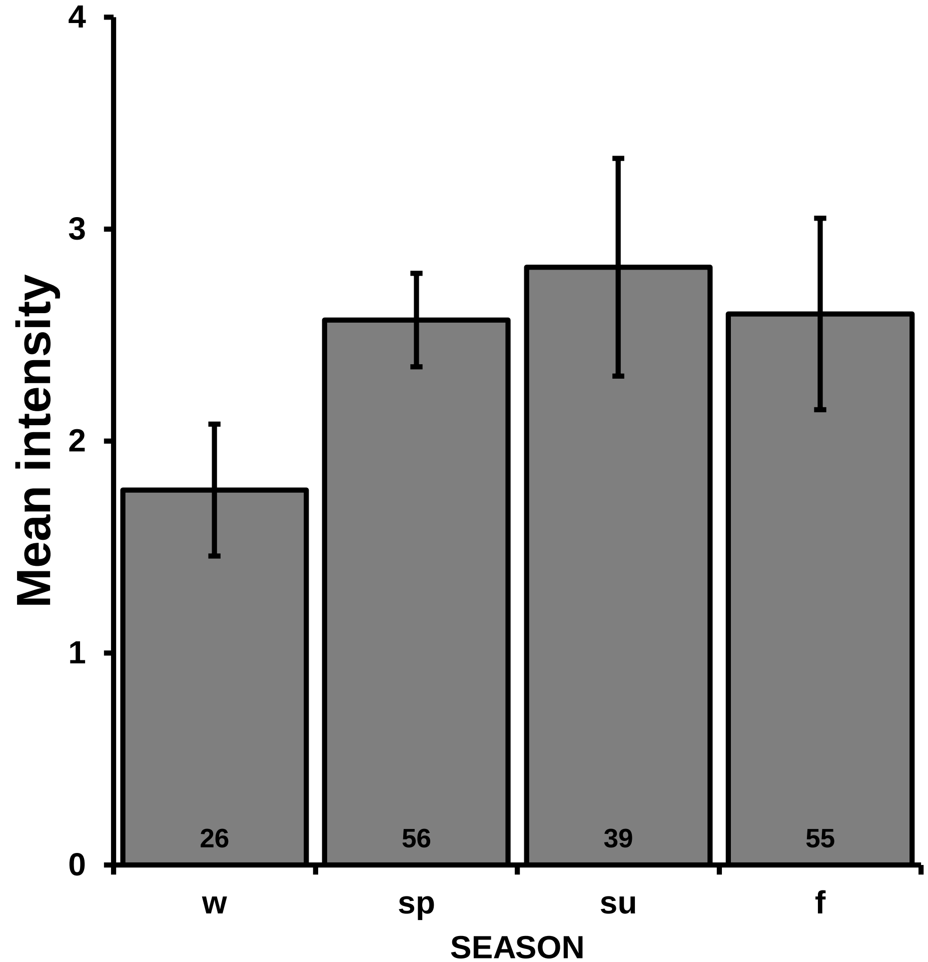
Intensity - Adult parasites:

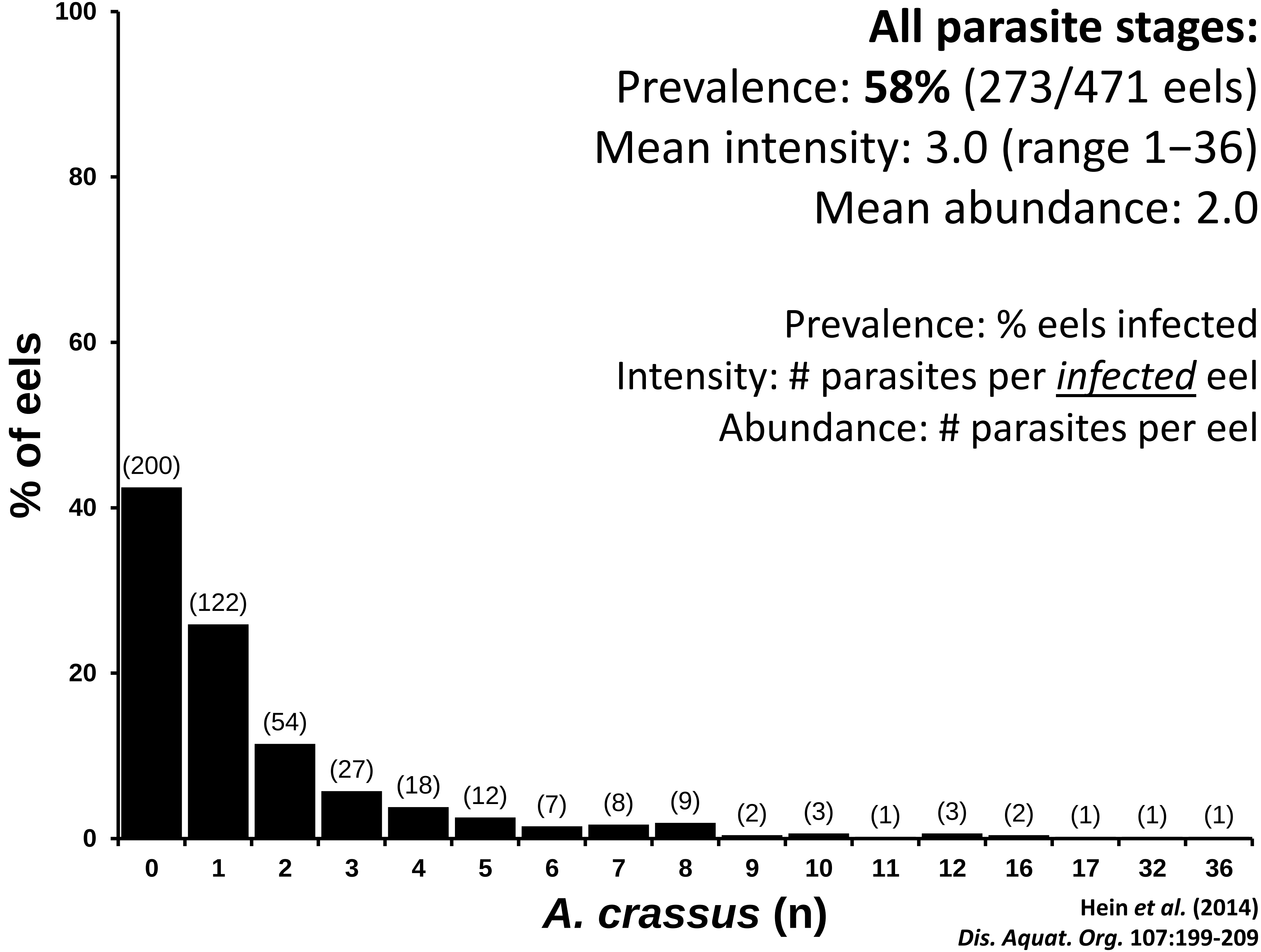
Locality, p=0.001

Highest at NI and Cooper, lowest at ACE Basin and LPD

Season: No significant differences






All parasite stages:
Prevalence: 58% (273/471 eels)
Mean intensity: 3.0 (range 1−36)
Mean abundance: 2.0
Prevalence: % eels infected
Intensity: # parasites per infected eel
Abundance: # parasites per eel
% of eels
100
80
60
40
20
0
(200)
(122)
(54)
(27)
(18)
(12)
(7)
(8)
(9)
(2)
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A. crassus (n)
Hein et al. (2014)
Dis. Aquat. Org. 107:199-209

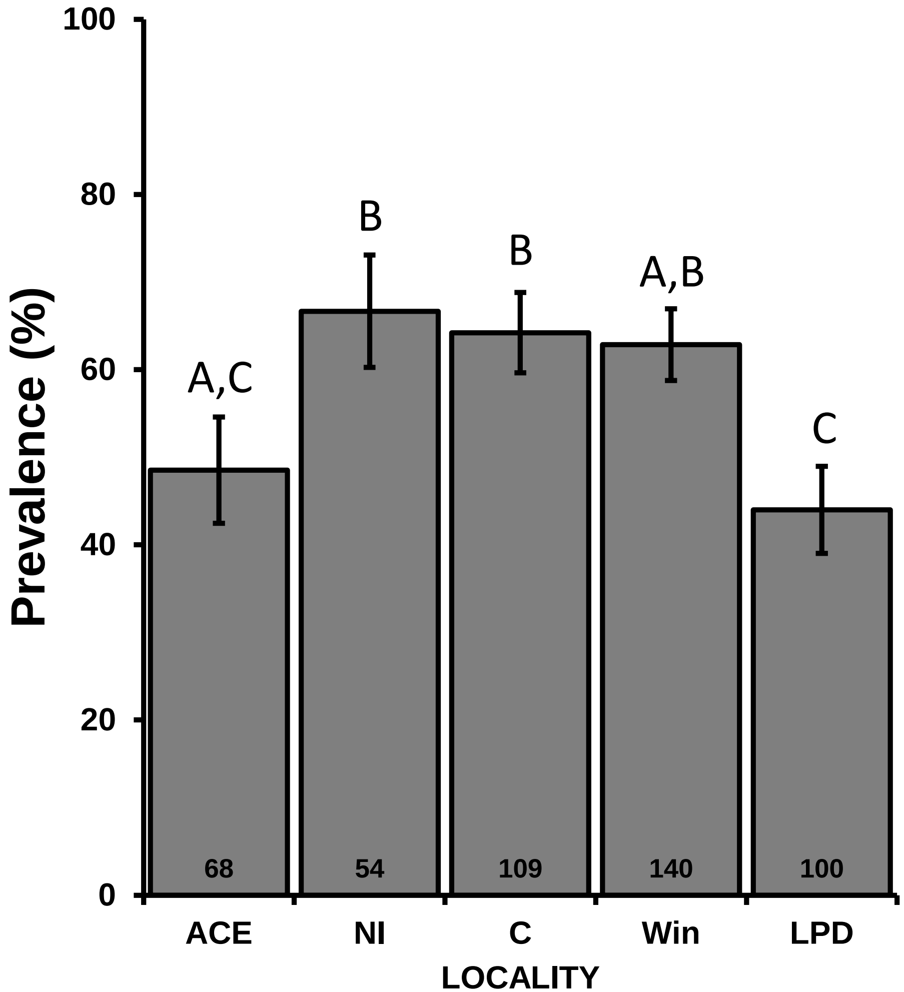
Hein *et al.* (2014)
Dis. Aquat. Org. 107:199-209

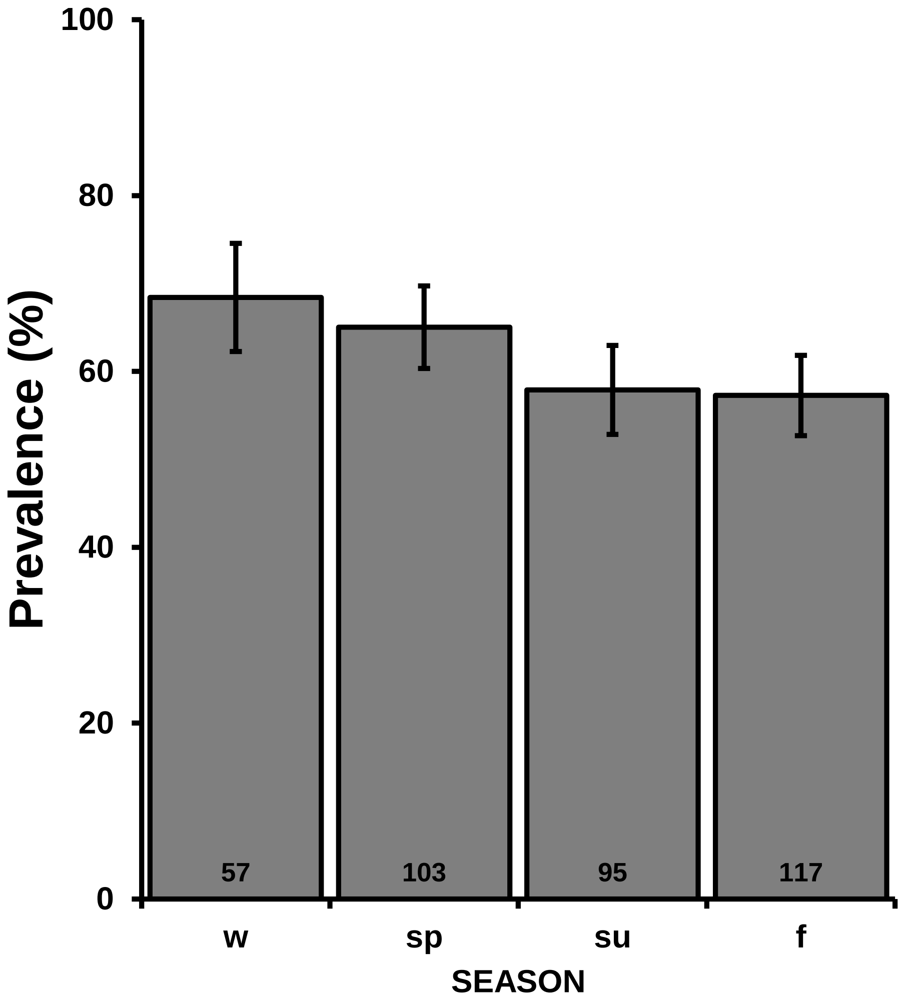
Prevalence - All parasite stages:

Locality, p=0.04

Highest at NI and Cooper, lowest at ACE Basin and LPD

Season: No significant differences

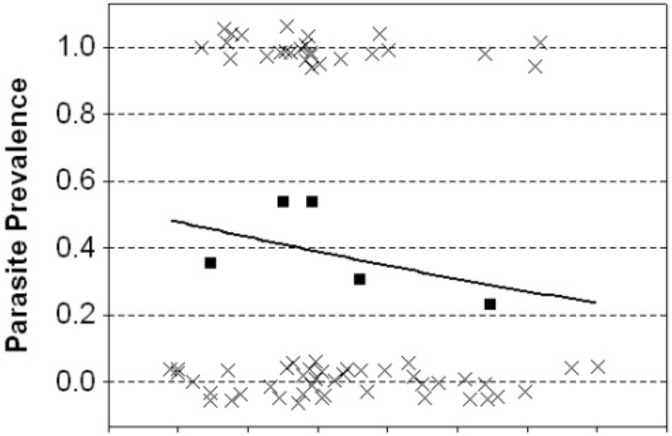




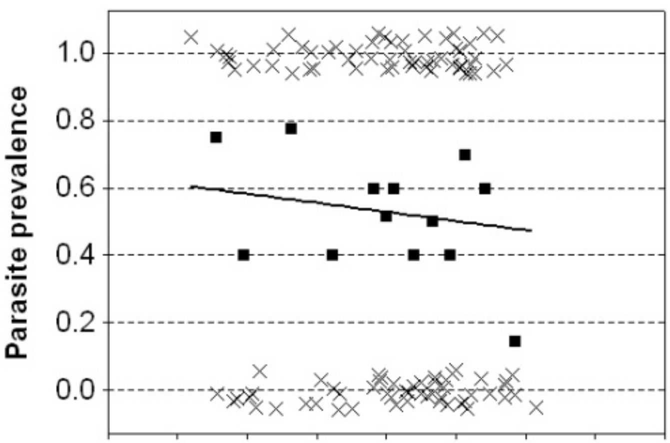
Prevalence - Adult parasites:

Locality, p=0.02

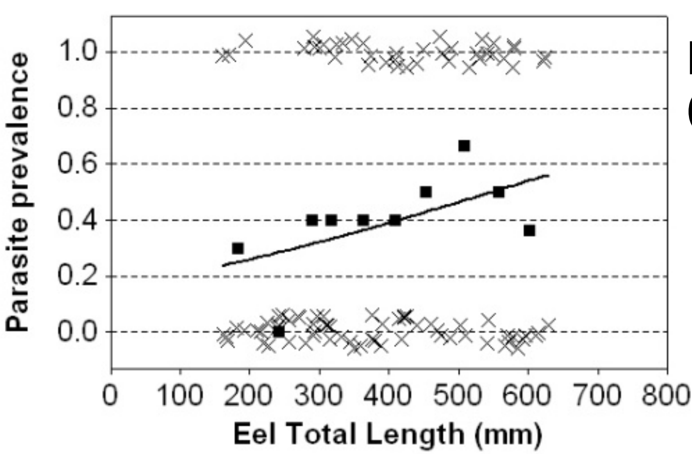
TL*Locality, p=0.03



ACE Basin
(left)

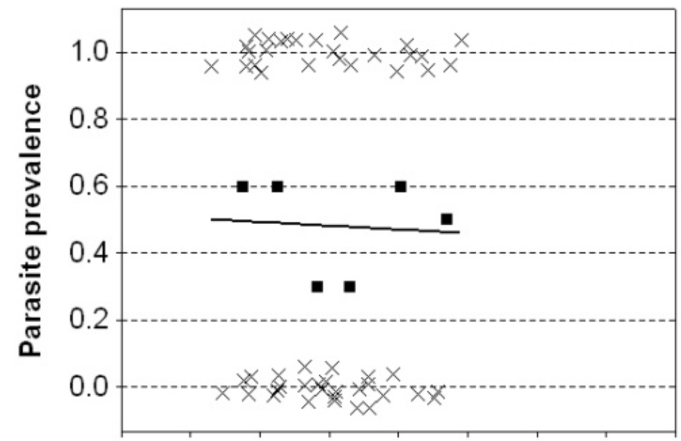


Cooper River
(left)

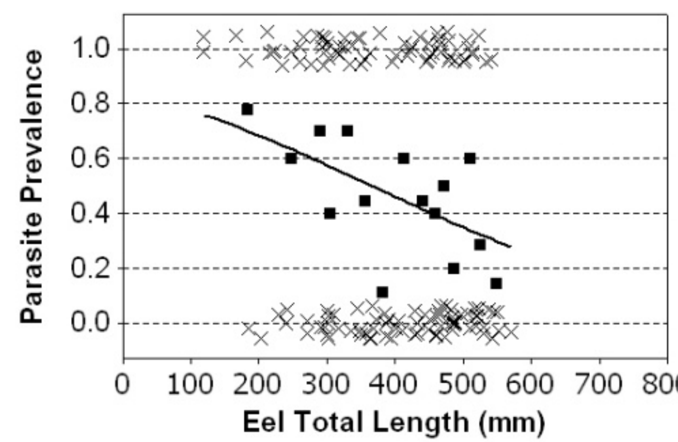


Little Pee Dee
(left)

North Inlet
(right)



Winyah Bay
(right)



Hein *et al.* (2014)
***Dis. Aquat. Org.* 107:199-209**

Summary Statistics

Parasite Stage	Measure	MODEL FACTORS			
		TL	*TL*Locality*	*Locality*	*Season*
Larval	Prevalence	-	-	$P < 0.05$	$P < 0.05$
	Intensity	-	-	-	-
Adult	Prevalence	-	$P < 0.05$	$P < 0.05$	-
	Intensity	-	-	$P < 0.001$	-
All	Prevalence	-	-	$P < 0.05$	-
	Intensity	-	-	-	-

Swim bladder damage

Swimbladder Degenerative Index (Lefebvre *et al.* 2002)

Category	Rank		
	0	**1**	**2**
Opacity	transparent	intermediate	opaque
Pigmentation & Blood	none	either	both
Thickness of swimbladder wall	<1mm	1-3mm	>3mm or no lumen

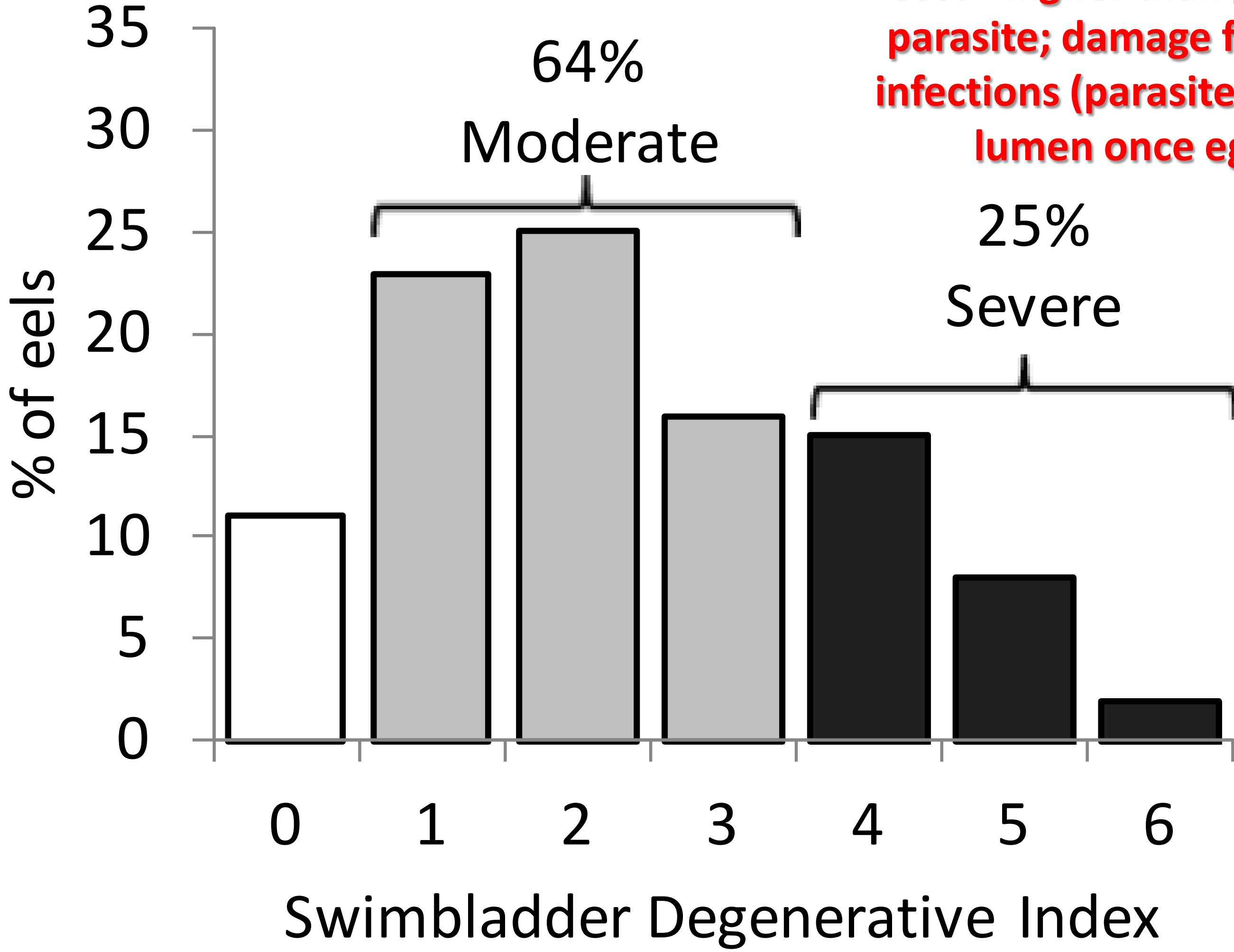
0 undamaged / 1-3 moderate damage / 4-6 severe damage

Healthy

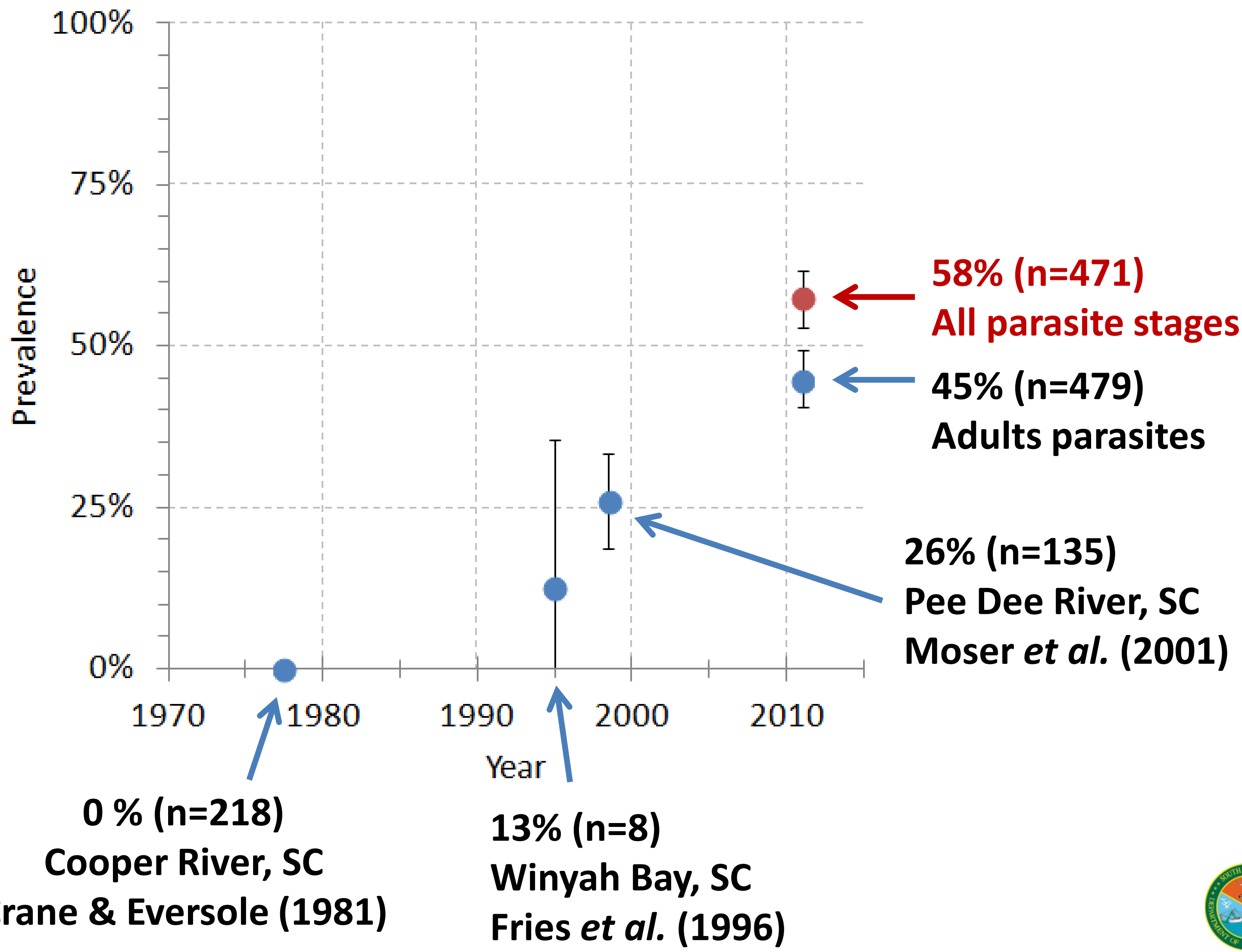
Damaged

Swim bladder damage in yellow/silver eels – SCDNR electrofishing surveys

89% - higher than prevalence of parasite; damage from previous infections (parasite cleared from lumen once eggs released).



A. crassus in South Carolina



DNR

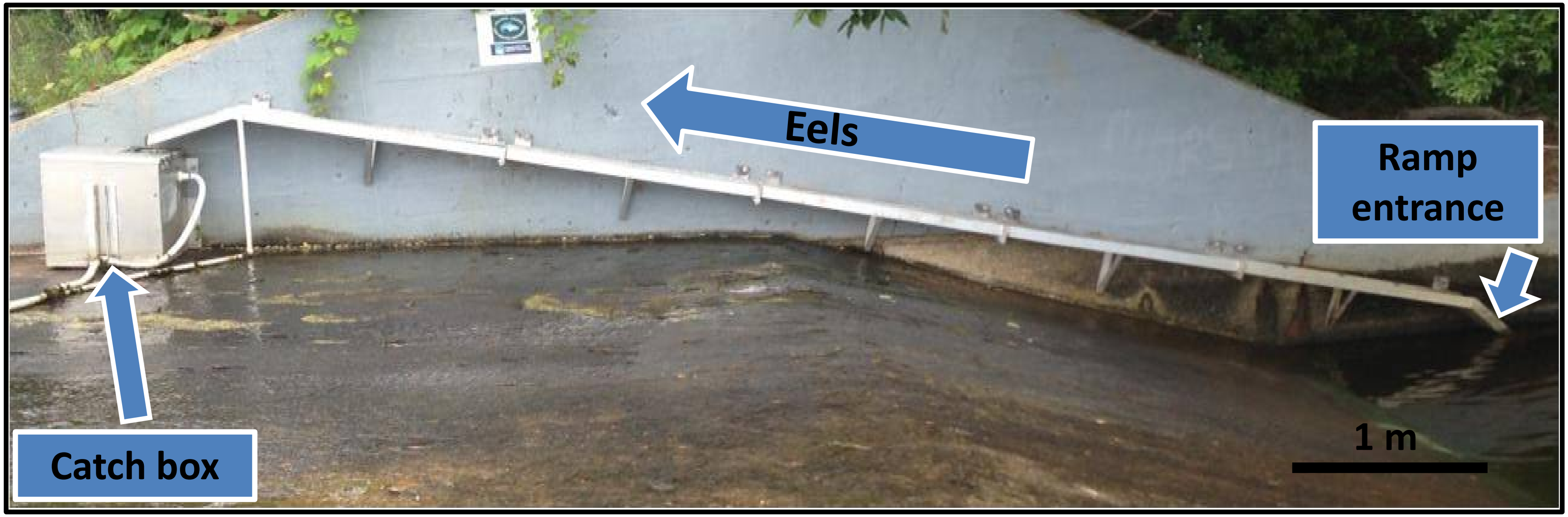
A. crassus infections since 2012

- Gravid female *A. crassus* have been observed year-round, such that the life cycle may be maintained all year; for more northern populations, occurrence of gravid female *A. crassus* is seasonal.
- 71% infection compared with 58%
- 6 parasites/infected eel compared with 3 parasites/infected eel in 2011
- 58% infection in silver eels (n=118) - implications for migration success

[State Wildlife Grant-funded research by J. Hein post-MS]

Switching focus to glass eels and elvers:

- Little is known about the effects of *A. crassus* on glass eels and elvers; how susceptible are these early stages to infection?
- Long-term monitoring of glass eels by the SCDNR Diadromous Finfish Research Section provides access to glass eels.
- Dam at Goose Creek Reservoir; eel passages installed in 2012.





Project Objectives:

- **Determine whether wild-caught glass eels and elvers are infected.**
- **Identify the earliest eel life stage infected.**
- **Determine the factors influencing infection.**

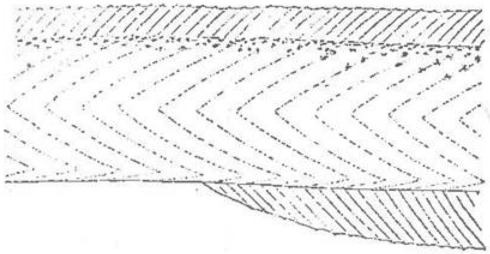
Sampling:

- **Subsamples of eels collected on a weekly or bi-weekly basis between March and December 2013; returned to laboratory.**
- **Eel length, eel pigment stage and the numbers of *A. crassus* larvae (L3 and L4) and adults in dissected swimbladders were recorded.**

Glass Eel Pigment Stages

From Haro *et al.* (1988)

1



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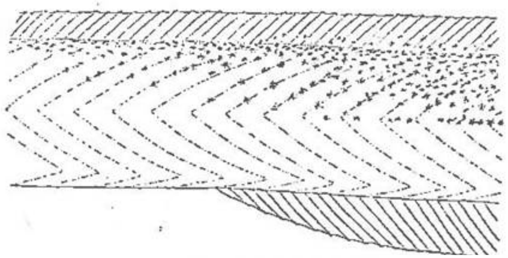

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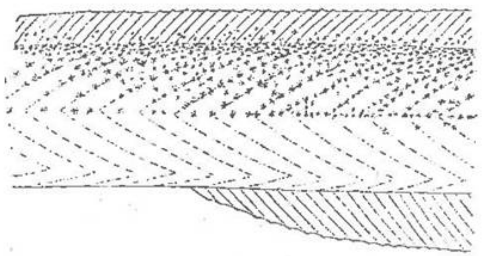

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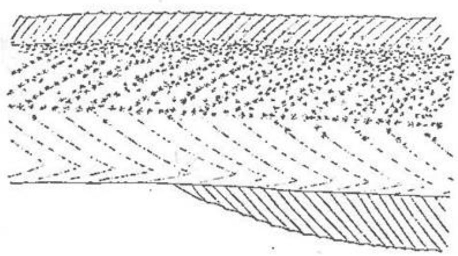

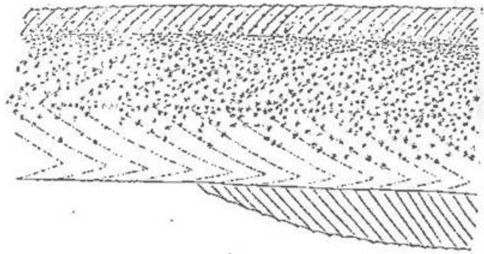

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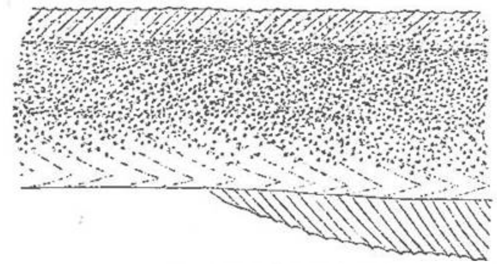

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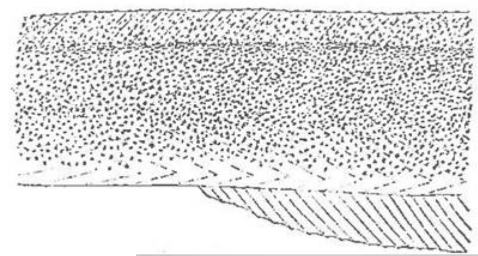
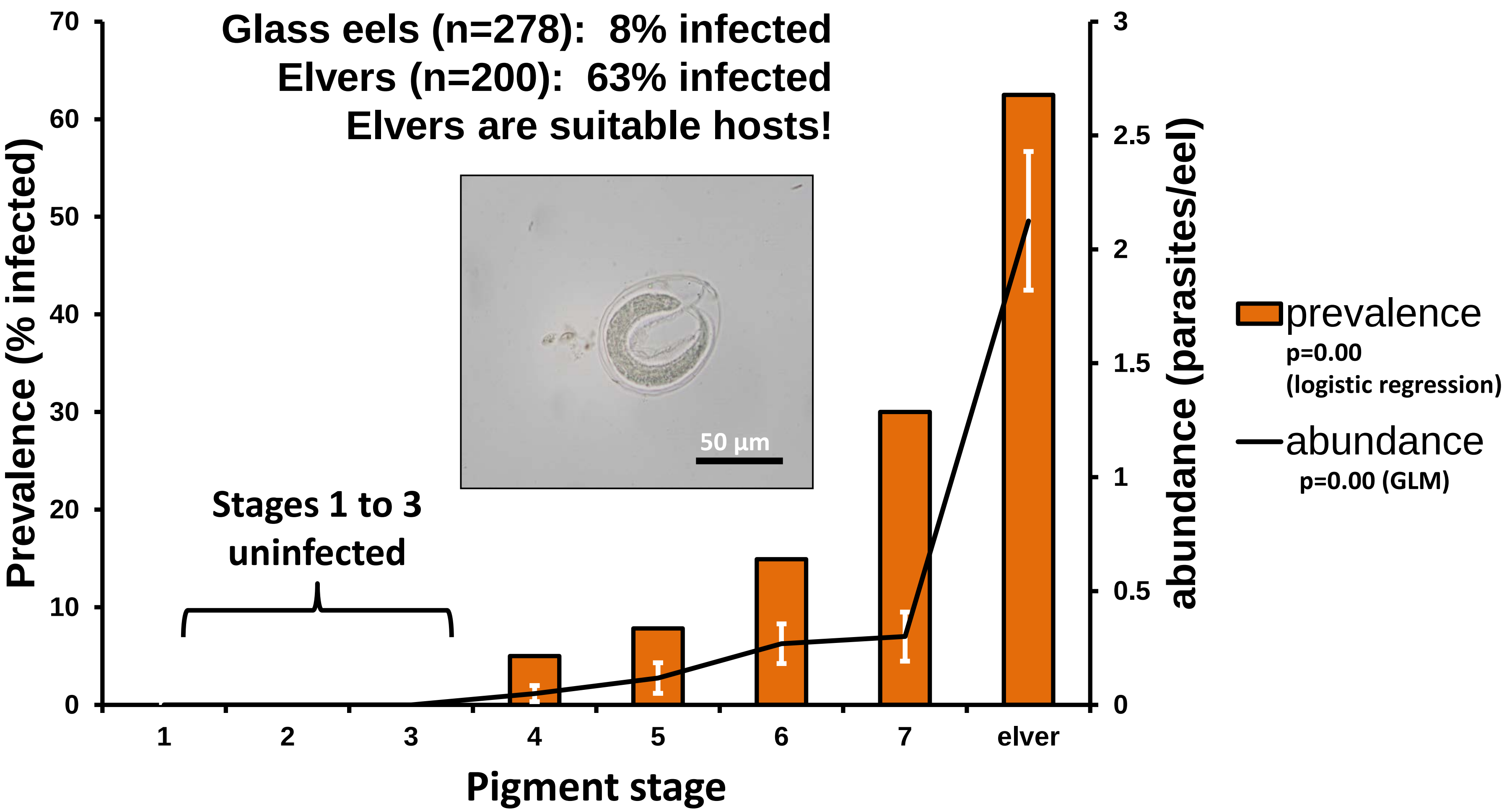
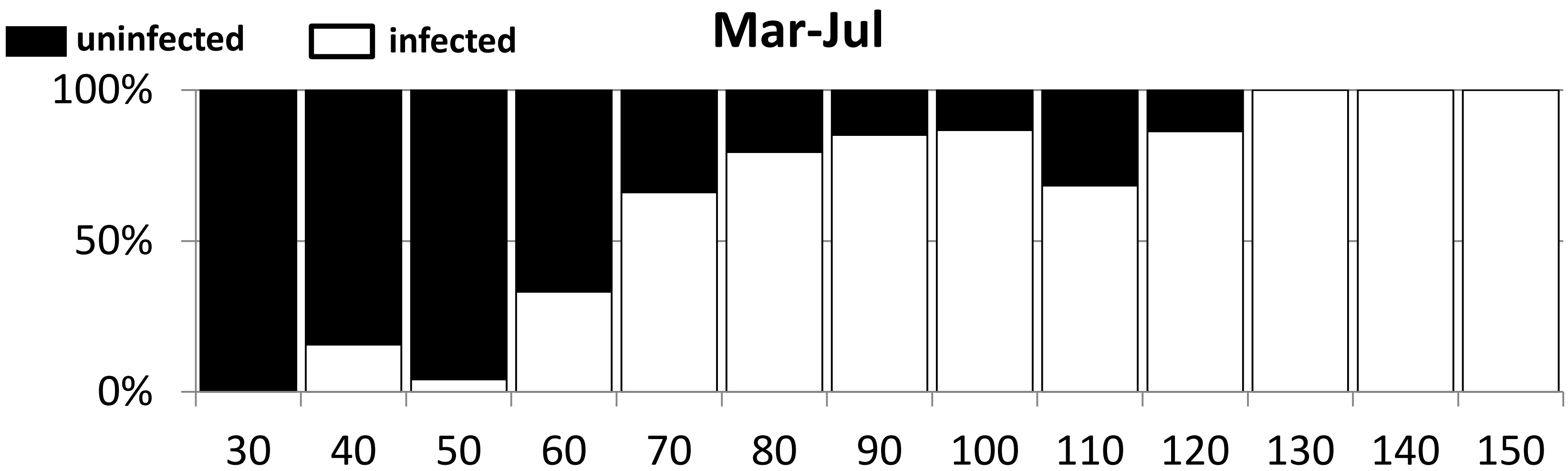


7

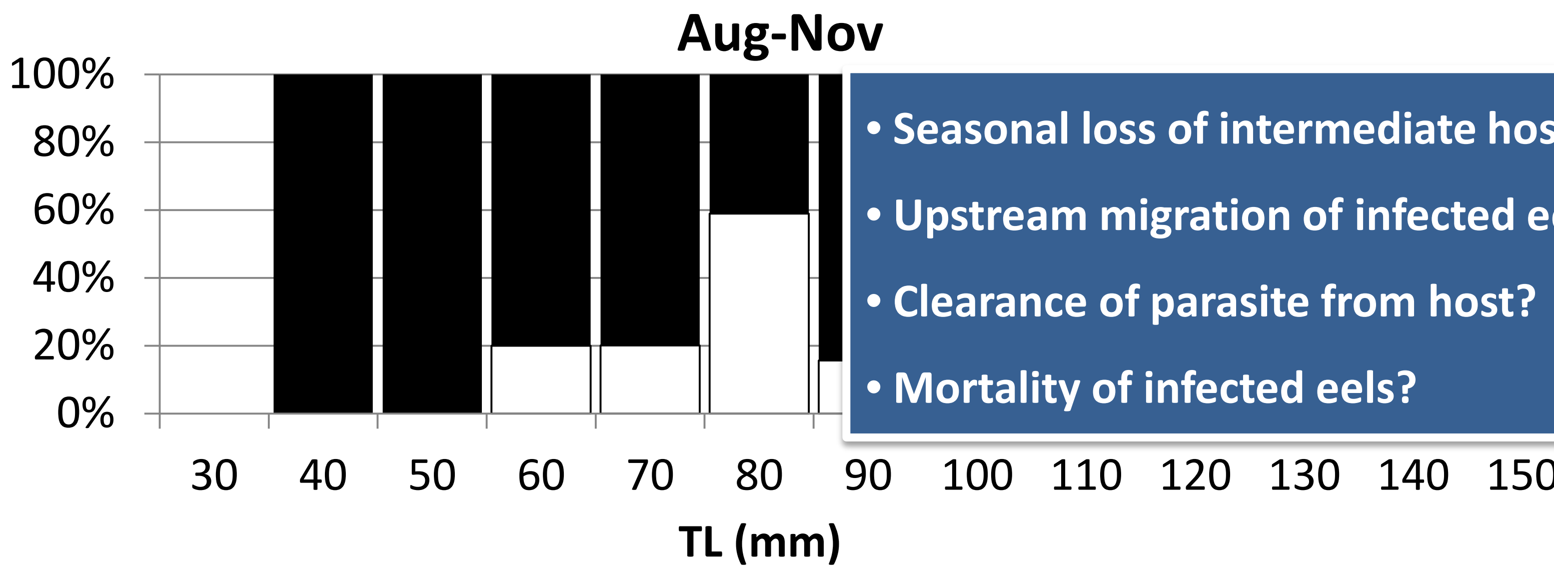
elver



- **Late stage glass eels and elvers were infected; infection occurs within months of eel recruitment. *A. crassus* eggs observed in elver swimbladder.**
- **Recent eel recruits to estuary may not be feeding on intermediate parasite hosts which are unknown in North America, but likely include various species of copepod, based on European studies and Hubbard *et al.* study.**



- **% Infection increases with age, likely with pigment stage (p=0.001).**
- **% Infection decreases significantly after July (p = 0.001).**



- **Seasonal loss of intermediate hosts?**
- **Upstream migration of infected eels?**
- **Clearance of parasite from host?**
- **Mortality of infected eels?**

- **Molnár (1993): infected eels died at < 3 mg/L DO and > 33°C; this temperature threshold was exceeded in July in our studies, after which time infection and eel numbers declined.**

Gulf States Marine Fisheries Commission (GSMFC) Subcontract Award:

Detection of an invasive parasite of American eels using qPCR.

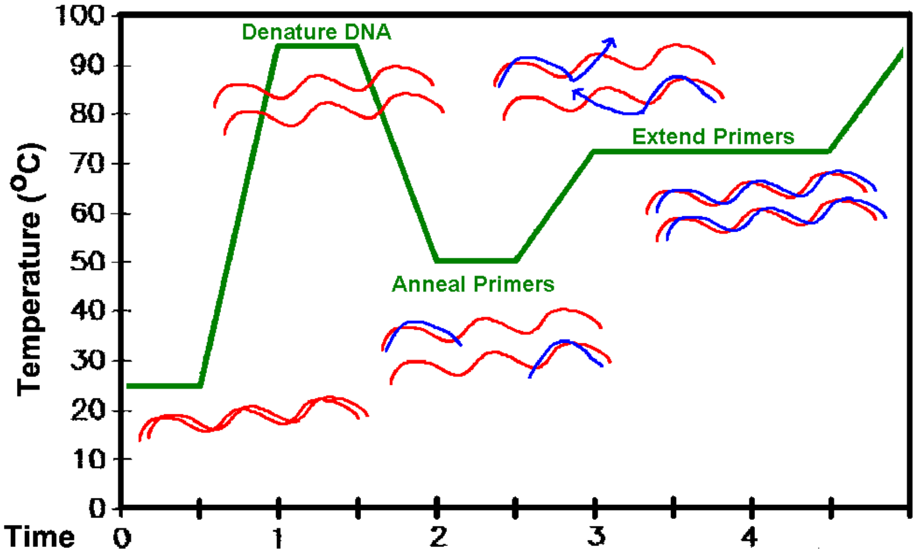
Stephen A. Arnott (PI)[1], Jennifer L. Hein[1], Isaure de Buron[2], Aaron M. Watson[1] & Peter R. Kingsley-Smith[1]

[1] Marine Resources Research Institute, SCDNR, PO Box 12559, Charleston, SC, 29422.
[2] Department of Biology, College of Charleston, Charleston, SC, 29401.

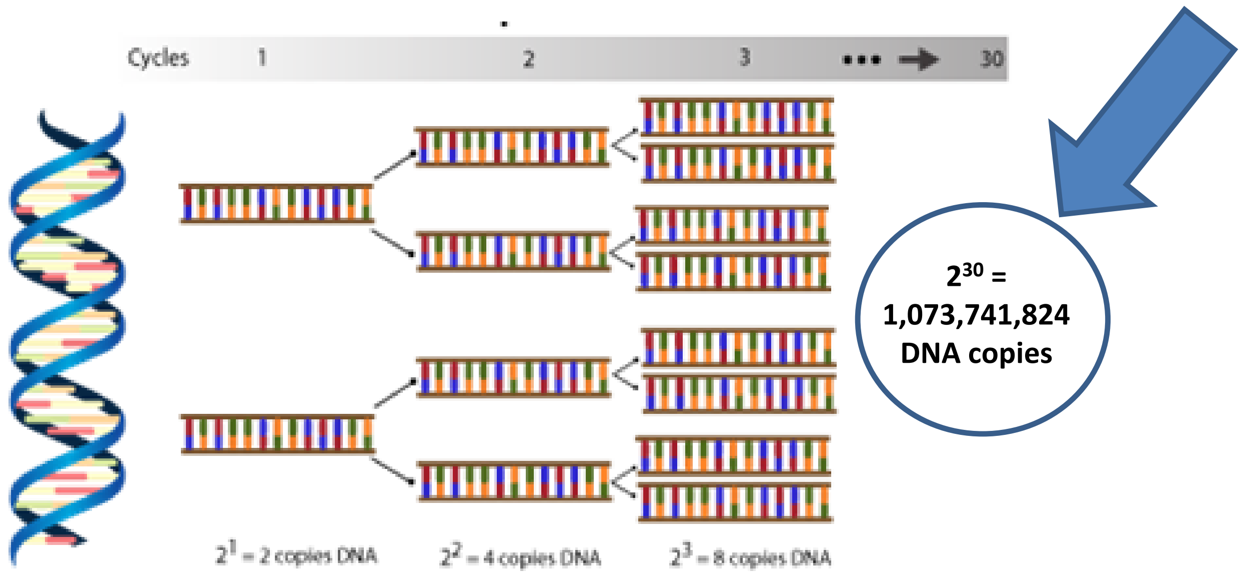
PROJECT GOALS:

- **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.**

Polymerase Chain Reaction (PCR)

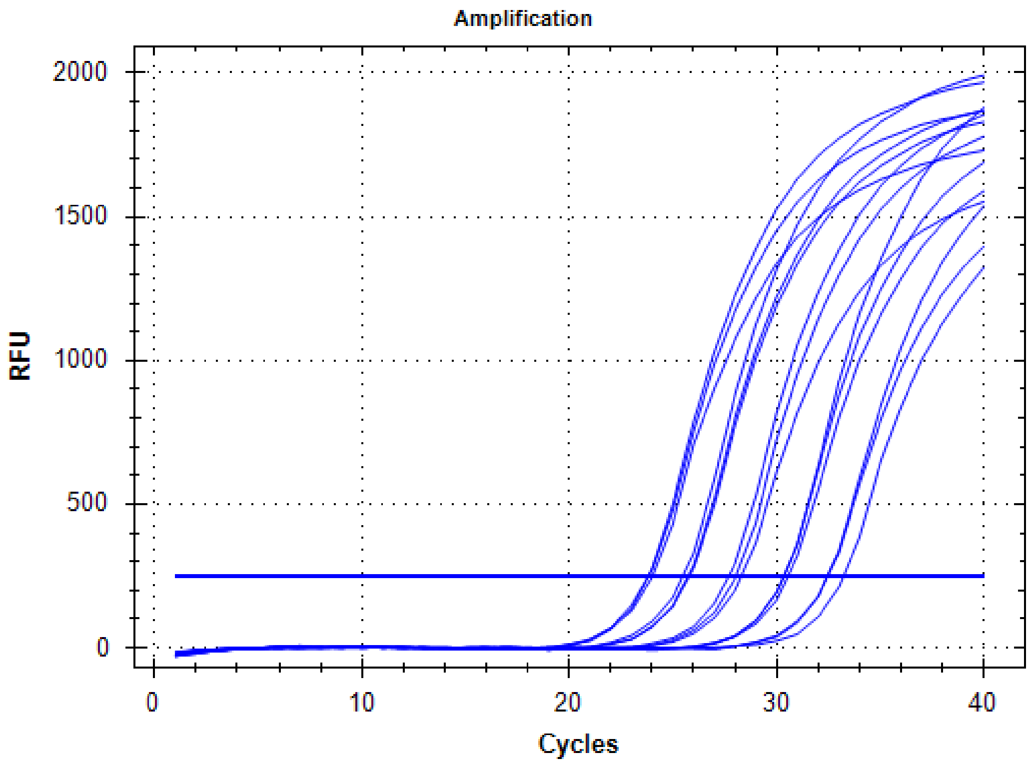


- Simulates natural cell DNA replication
- Allows for DNA detection/visualization
- Key is the precise thermal cycling
- DNA quantity doubles with each thermal cycle



Quantitative PCR (q-PCR)

- S-shaped curves initially, but 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.)

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