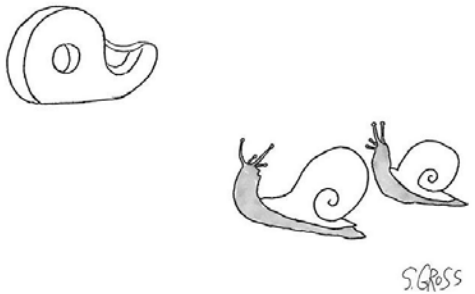
ABCs of apple snails and eDNA
WEBINAR FOR TPWD

Dr. Romi Burks
&
research with MANY Southwestern undergraduates



"I don't care if she is a tape dispenser. I love her."

Notes regarding presentation – this talk contains preliminary, unpublished research with speculation regarding its importance and interpretation to date. Please do not hesitate to contact me with questions.

Our on-going eDNA program continues to work on apple snails and the ecology of eDNA. Major collaborators of this work include Dr. Matt Barnes (eDNA), Dr. Ken Hayes (apple snails) & Dr. Russ Minton (mysterysnails). All mistakes – slides and verbally - are my own.

About me: Dr. Romi Burks aka ProfRomi

- Undergraduate – Loyola University Chicago – '95
  - Biology & English
  - **First research on non-native species (zebs!)**
- Graduate work – Ph.D. – University of Notre Dame '00 – Aquatic Ecology
  - **Cultivated interest in community ecology**
- Postdoctoral Fellow – '01 – The Ohio State University – Wetlands
  - **Gained appreciate for applied ecology**
- Faculty Fellow –'01-'03 Rhodes College –
  - **Committed to undergraduate research**
- Faculty at Southwestern University since 2003

My first paper with undergraduates – Published Fall 2008

J. N. Am. Benthol. Soc., 2008, 27(3):738–745
© 2008 by The North American Benthological Society
DOI: 10.1899/08-013.1
Published online: 8 July 2008

Fecundity of the exotic applesnail, *Pomacea insularum*

Matthew A. Barnes[1], Rebecca K. Fordham[2], AND Romi L. Burks[3]
Department of Biology, Southwestern University, 1001 E. University Avenue, Georgetown, Texas 78626 USA

Jennifer J. Hand[4]
School of Science and Computer Engineering, University of Houston-Clear Lake, Houston, Texas 77058 USA

Becca

Now Dr. Matthew Barnes

Graduation Dates: 2005 2006

Now faculty & collaborator at Texas Tech

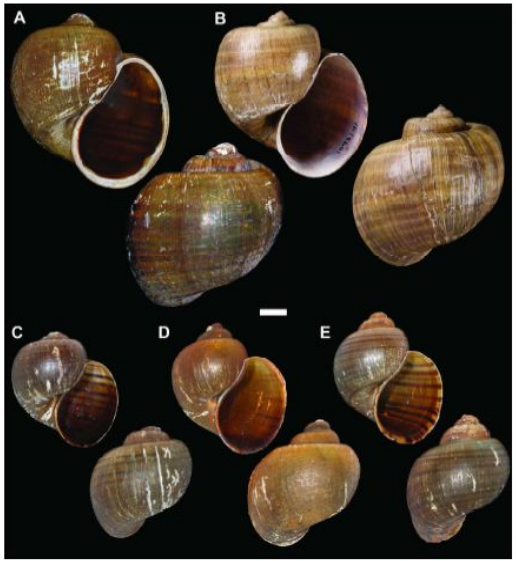
A is for Assumptions we have about snails…

… the RESEARCH goes FAST!!

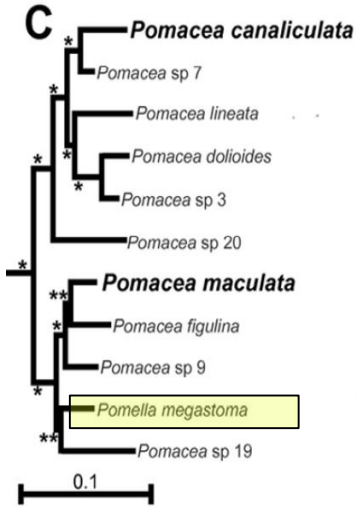
Why Study Apple Snails? (A = Ampullariidae)

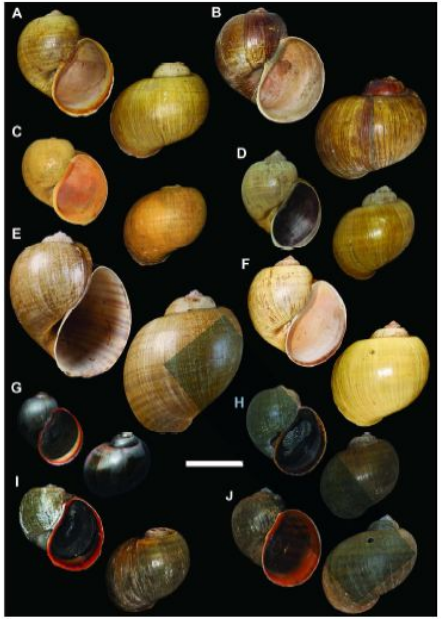
- Models of evolutionary history (ancient and diverse group)
    - Apple snails global – 160 mya; mollusks even older
- Interesting biological adaptations
- Pose threats to wildlife, habitats and humans
- Always keep you questioning everything!

“Channeled” (many more than just one) Apple Snails Exhibit Considerable Morphological Variation Too



P. canaliculata





P. gigas
P. insularum
P. haustrum
P. amazonicas

All images from Hayes et al. 2012

History of work with TPWD

About 125 results (0.26 seconds)

Invasive Apple Snails near Houston - Texas parks and wildlife [official ...

www.allreadable.com/b5306vmk

Jun 11, 2009 - Uploaded by Texas Parks and Wildlife

Invasive Apple Snails near Houston - **Texas parks** and **wildlife** [official] ... has led to years of research ...

https://youtu.be/Pi2wcFPPJsI

2008/9…10 years later…What's changed?

- Know a lot more about the ecology of the snails
- Name change
  - *Pomacea insularum* to *Pomacea maculata*
- Expanded distribution
- **Techniques used to study snails**
  - **Molecular ecology**


Fisher Scientific
Cat. No. 09-016

Apple Snails

Ampullariidae

-Lay eggs

-Globus (spherical) Shell

-Found more in southern U.S.

-Consume vascular plants

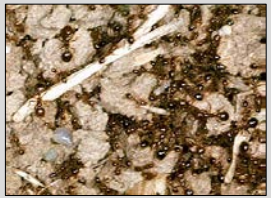
Why Care about Apple Snails

P. paludosa
~40-80 eggs
80% hatch

P. maculata
~2000 eggs
70% hatch

- Large native distributions
- Can obtain large sizes
- Freshwater
- ***Subject to parasites Causing zoonotic diseases***
- Opercula
- ***Aquarium trade***
- ***Environmental threats***

Less documented predators



Well documented predators

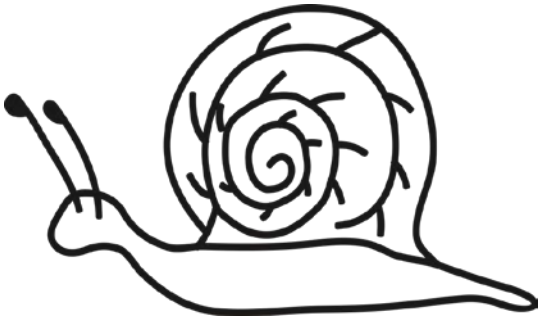
Effects move up the food web

B is for Biological Invasions

Invasive Freshwater Snails

Common name in aquarium trade

Apple Snails ≠ **Mysterysnails**



Nicole Kelly & Shannon Odell

MYSTERYSNAILS?

Cipangopaludina chinensis

AT LEAST TWO SPECIES

VISUALLY SIMILAR, GENETICALLY DIFFERENT

NOT WELL STUDIED

Cipangopaludina japonica

What's in a name?

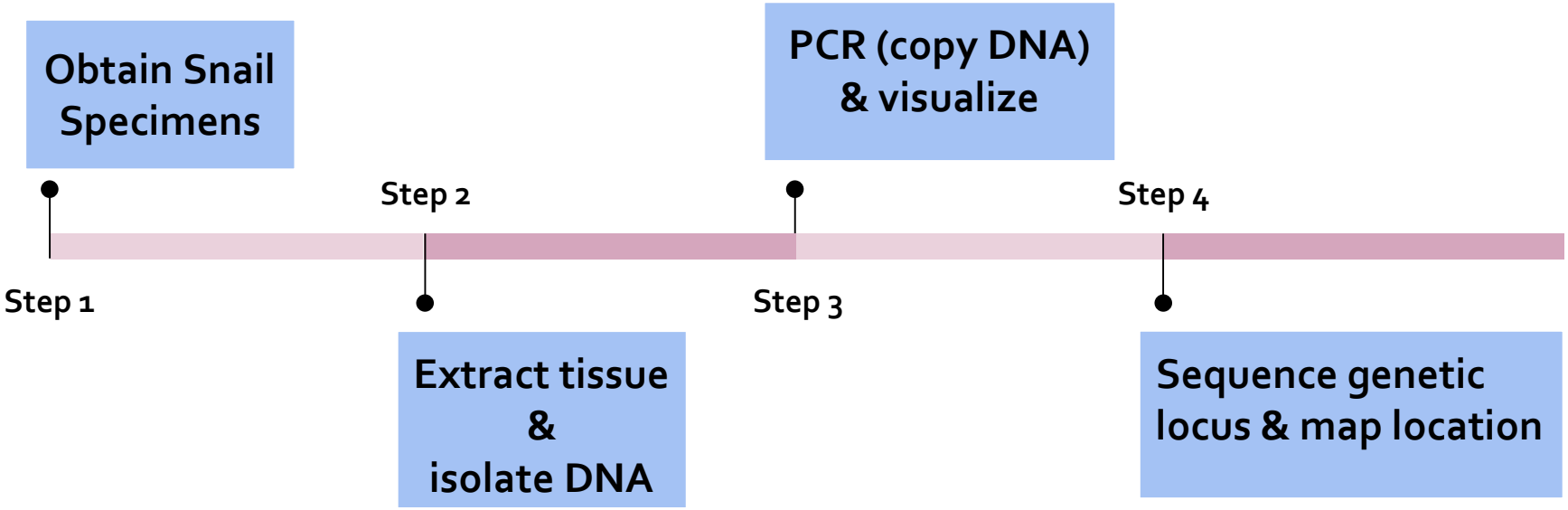
- Common language
- Regulatory power
- Biological insight
- Scientific advancement

Avoid: channeled, giant, island, golden

C is for Confirmation

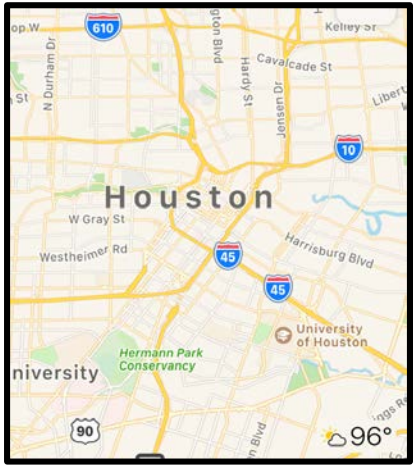
- Often morphologically difficult
- Now rely on molecular means

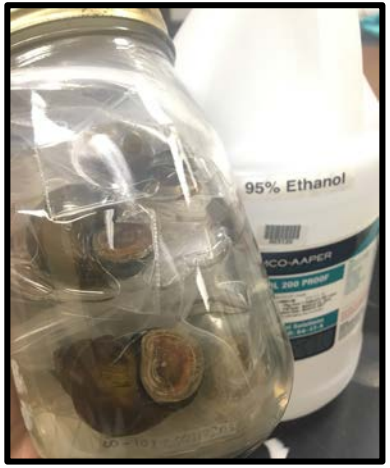
Molecular Approach



Snail mail

- Acquire samples
- TPWD Exotic Species Permit
- USDA APHIS – Mail (if live)





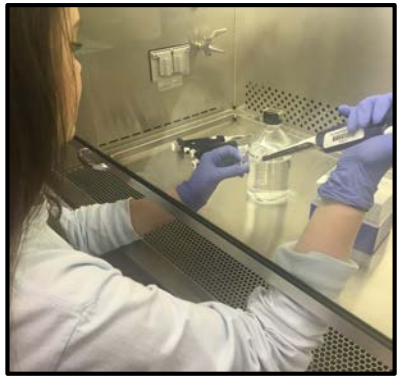
Dissect & Extract

- Isolating total genomic DNA from tissue
- Spin Kit or Chloroform extractions

Polymerase Chain Reaction

- Amplifies target gene using specific primers, master mix & Total Genomic DNA

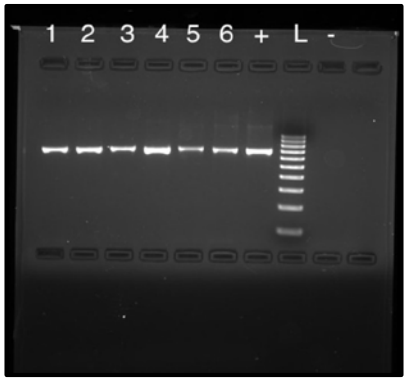
- Thermocycler induces multiple rounds of DNA replication



Gel Electrophoresis

- Separates DNA fragments according to size & compares results to a positive

- Porous agarose gel, Ethidium Bromide, & electric current



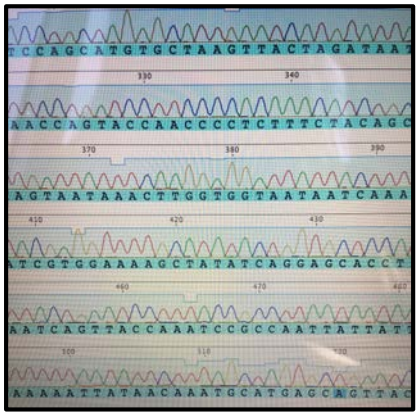
Sequencing & Mapping Locations

- Edit in Geneious Computer Program

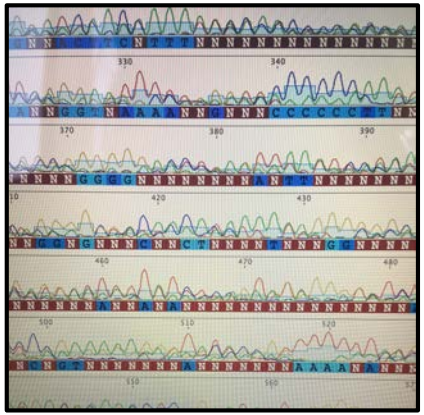
- GenBank BLAST (Identifying sequences)

-Record the species identification

Good Sequence

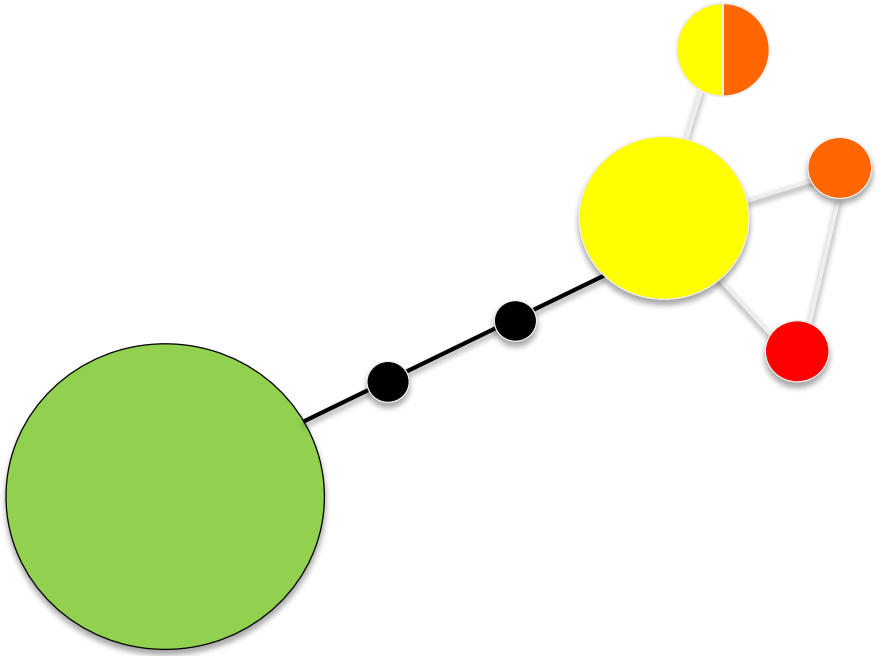


Bad Sequence



- J model Test
- Phylogenetic Modeling (ML)

Haplotype Mapping (single species)

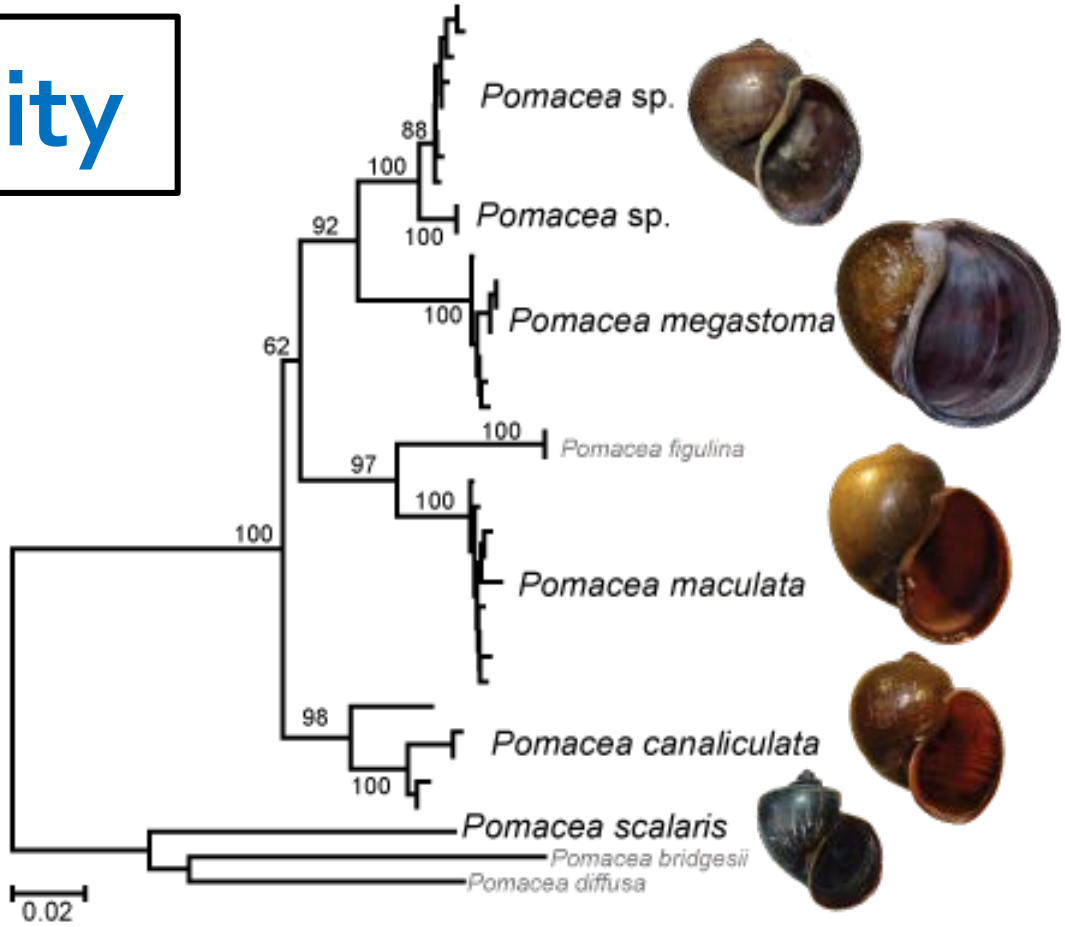


D is for Diversity

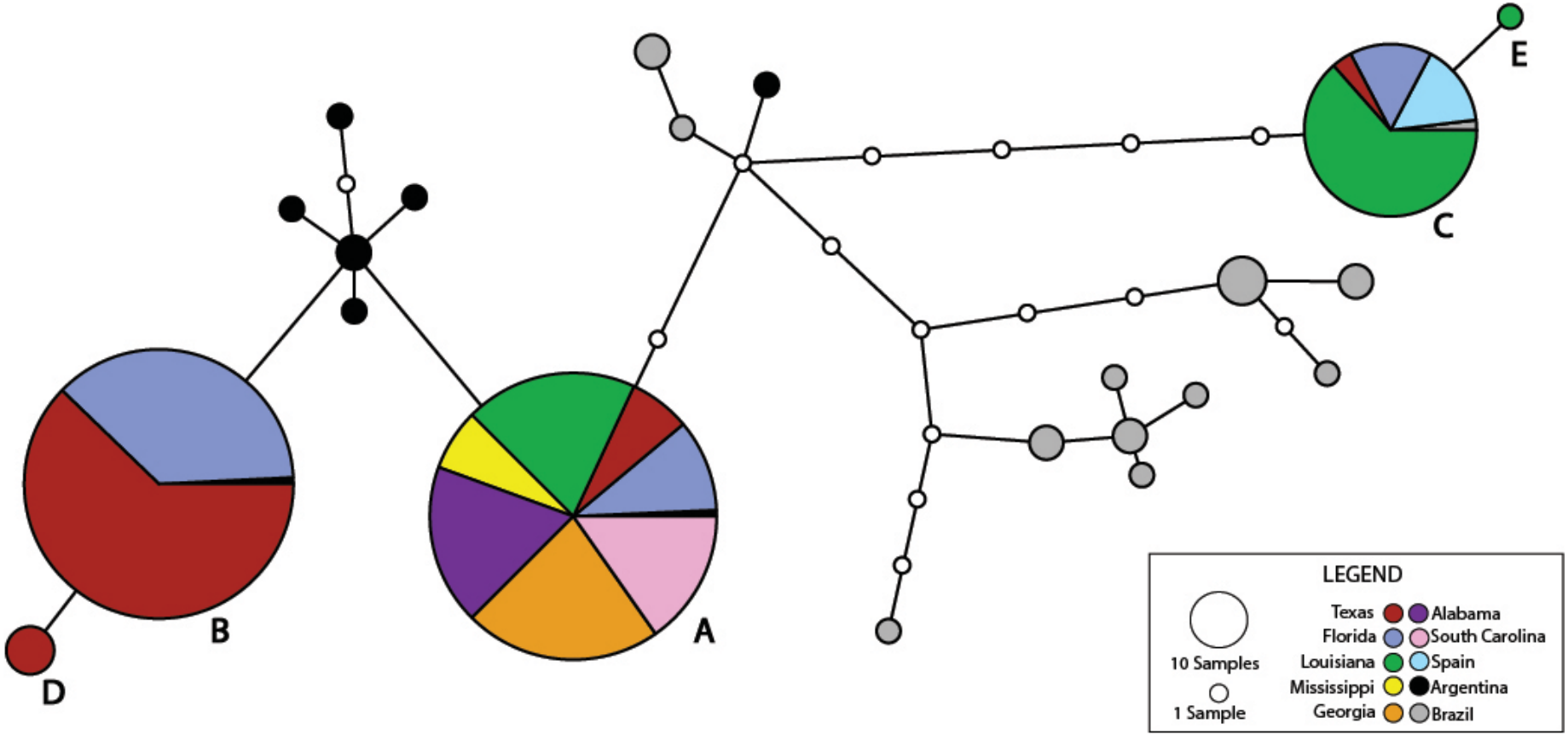
Pomacea most diverse genus
(***Marisa*, *Felipponea*, *Asolene*)**

Cryptic species

Hybridization*



Multi-locus tree; K. Hayes


E
C
B
A
D
LEGEND
10 Samples
1 Sample
Texas
Alabama
Florida
South Carolina
Louisiana
Spain
Mississippi
Argentina
Georgia
Brazil

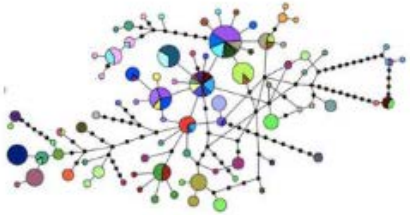
Molecular Ecology Projects

Hybridization

Hybridization as a means for invasion success

Phylogeography & Phylogenetics

Diversity and distribution of native and non-native apple snails



NEWS: PRESS RELEASE ON APPLE SNAILS IN S. TEXAS

eDNA

'Ecology of eDNA' investigations using apple snails as a model for detection of invertebrate invasive species

Other Invasive Snails

Discovery of a Japanese Mystery Snail in TX prompts questions of identity and distribution of this other group of invasive snails

Invasion Process

Introduction

Transport

Spread

Native elsewhere

Impact

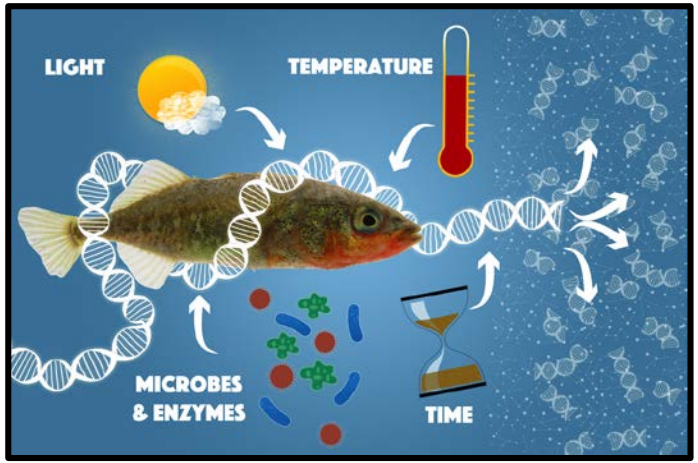
Shellsea Miller & Lauren Muskara

eDNA's detectives

E is for Environmental DNA (eDNA)

What is it?

"Environmental DNA (eDNA) refers to the extracellular, residual DNA shed from an organism found in various abiotic environments (Barnes et al. 2014)."



HOW IS IT USED?

MOLECULAR ECOLOGY

PERSPECTIVE | Open Access

Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA

DAVID M. LODGE, CAMERON R. TURNER, CHRISTOPHER L. JERDE, MATTHEW A. BARNES, LINDSAY CHADDERTON, SCOTT P. EGAN, JEFFREY L. FEDER, ANDREW R. MAHON,

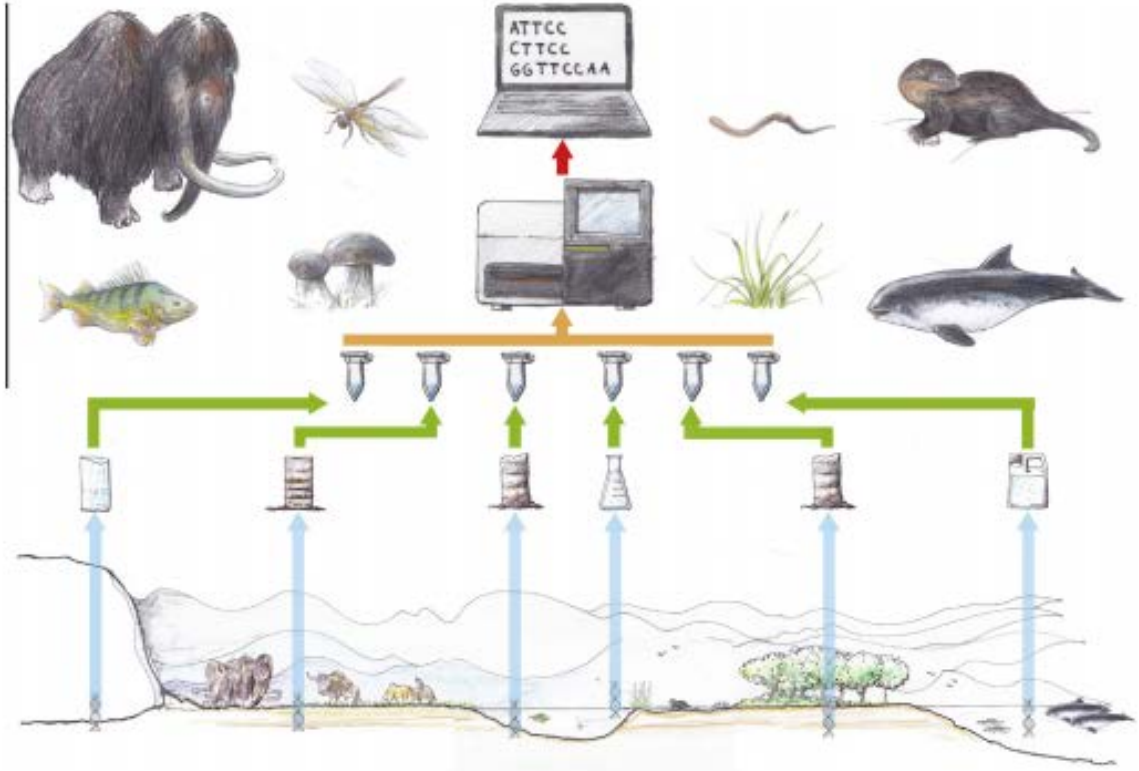
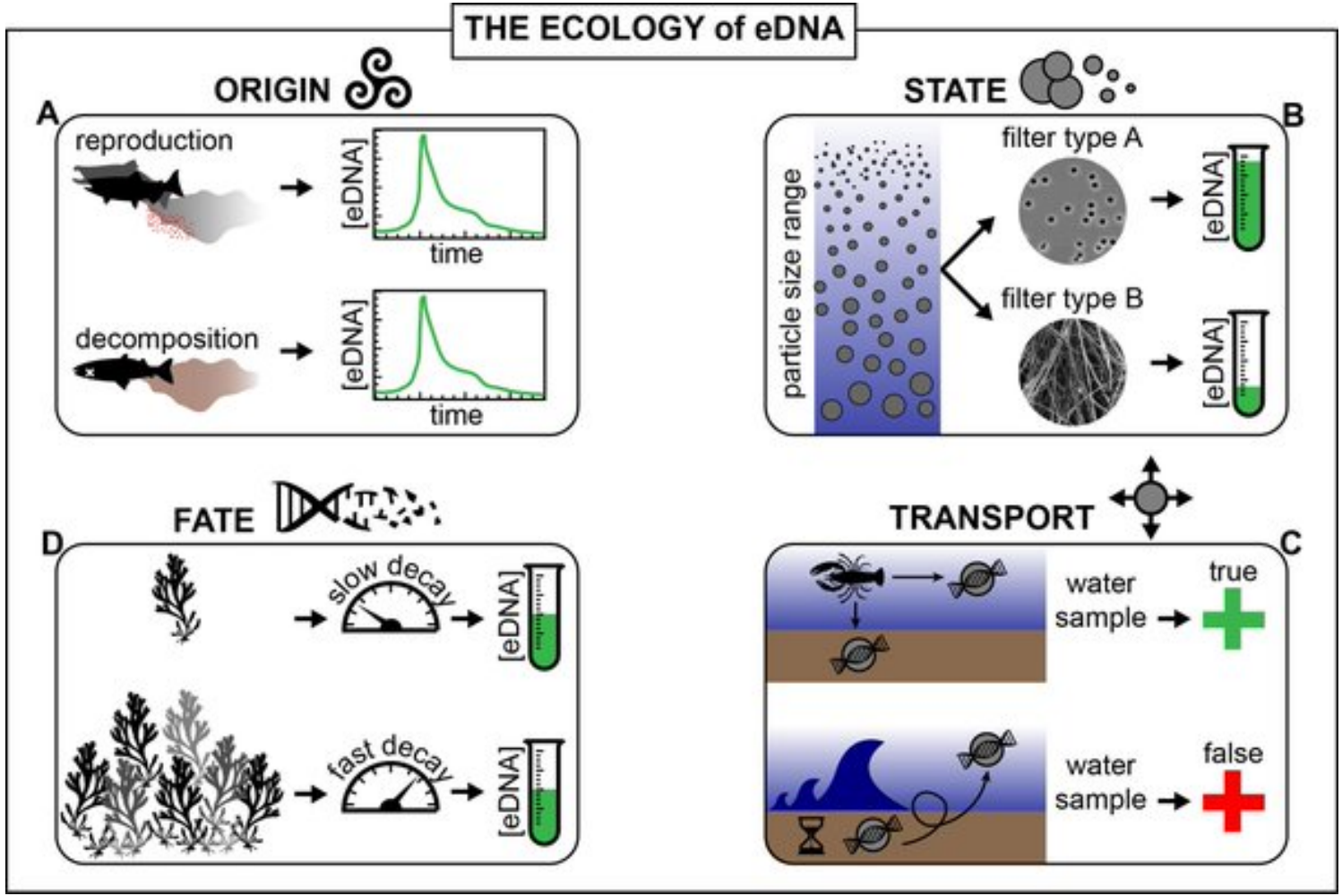
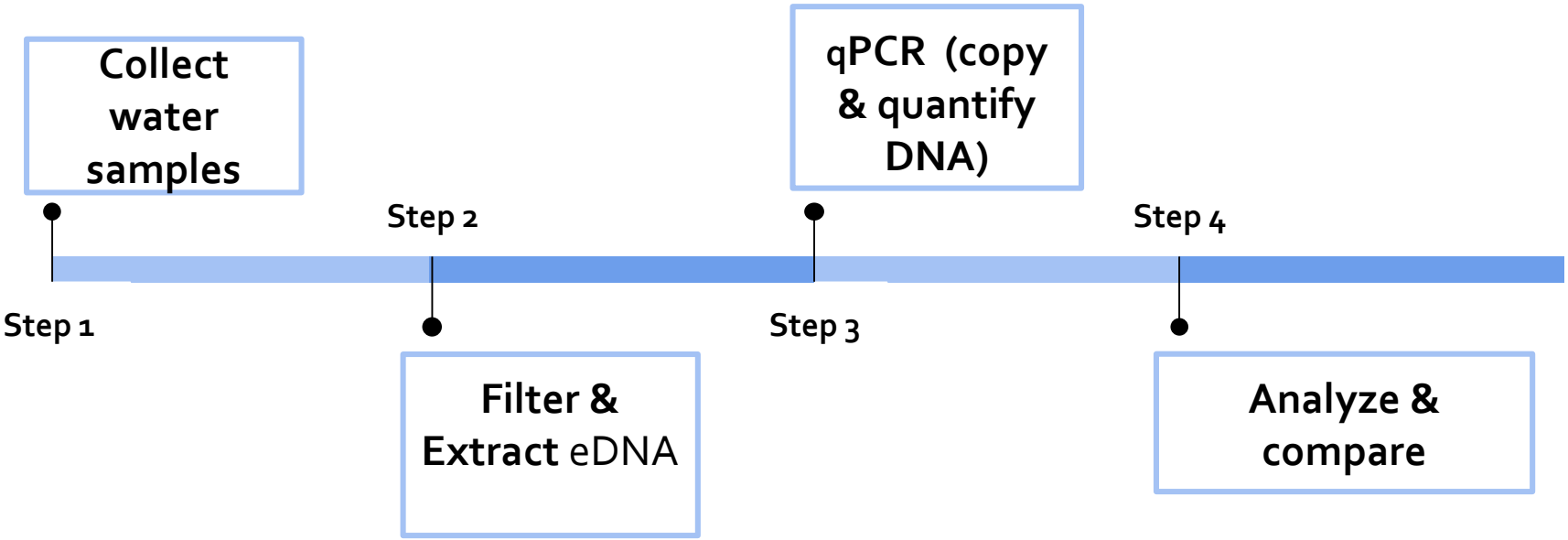


Fig. 1. The overall workflow for environmental DNA (eDNA) studies with examples of organisms that have been identified from environmental samples. Environments and their respective samplings from left to right: (i) glaciers; (ii) permafrost/tundra; (iii) aquatic sediments; (iv) lakes and streams; (v) terrestrial habitats; (vi) oceans. The first three are ancient environments while the latter three are modern. Color codes of arrows represent the different steps in the analyses: (blue) environmental sampling (ice cores, soil/sediment core samples, freshwater/seawater samples); (green) DNA extraction using procedures specific for the individual type of sample; (orange) PCR amplification of extracted DNA using generic or species-specific primers targeting biodiversity and subsequent sequencing of amplicons (here shown as Illumina MiSeq technology); (red) bioinformatic data-processing including error trimming, sequence sorting, and identification pipelines leading to various taxonomic level or MOTUs and the final interpretation and publication of results. Drawing by Lars Holm.


THE ECOLOGY of eDNA
ORIGIN
A
reproduction
[eDNA]
time
decomposition
[eDNA]
time
STATE
B
particle size range
filter type A
[eDNA]
filter type B
[eDNA]
FATE
D
slow decay
[eDNA]
fast decay
[eDNA]
TRANSPORT
C
water sample
true
water sample
false

Approach


Collect water samples
Step 1
Step 2
Filter & Extract eDNA
qPCR (copy & quantify DNA)
Step 3
Step 4
Analyze & compare

eDNA **primers**

Primers amplify a specific Section of the CO1 Region

AS1- 80 bp AS2-101 bp AS3- 123 bp AS4- 150 bp

Each primer Varies in length and location

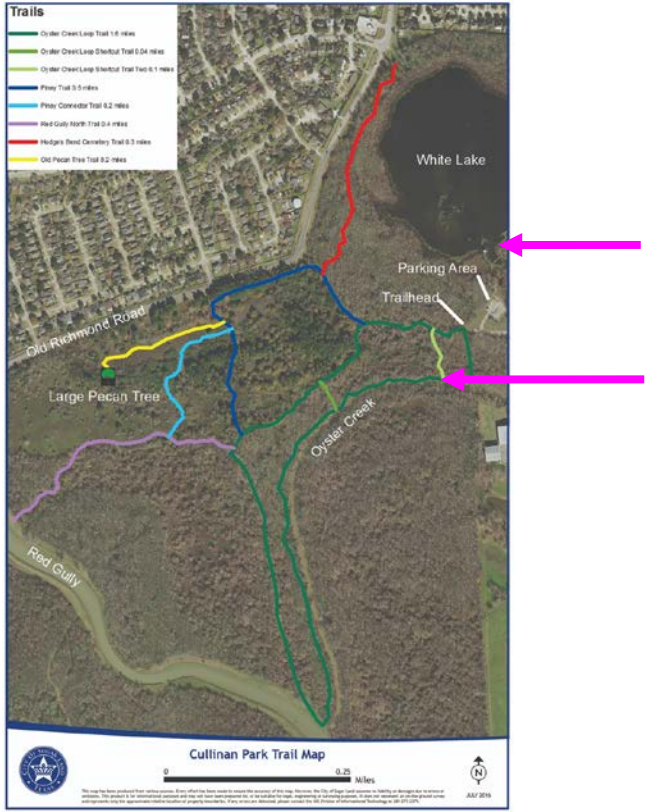
Does performance vary among the primers?

What is the best primer to use for field sampling?

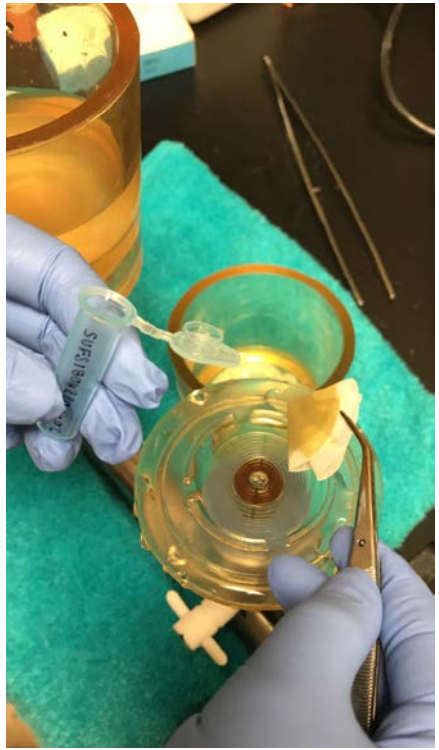
eDNA **Field Research**

Are there snails present in all 3 field sites in Cullinan Park?

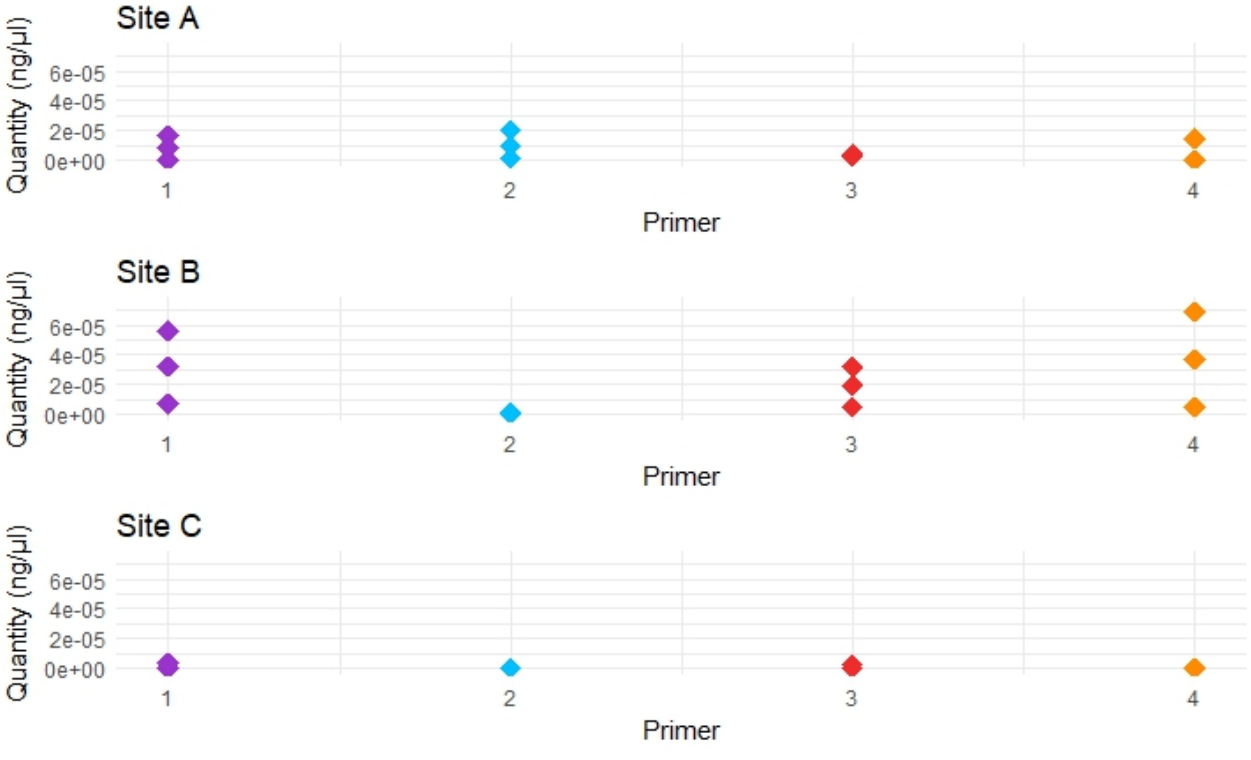
- White Lake (visible apple snails) - Offshore (site A) vs nearshore (site B)
- Oyster Creek (no visible apple snails) - nearshore (site C)



eDNA **Field Testing - Using** eDNA **to Detect Snails (*P. maculata*) in Cullinan Park**



Primer Performance Results



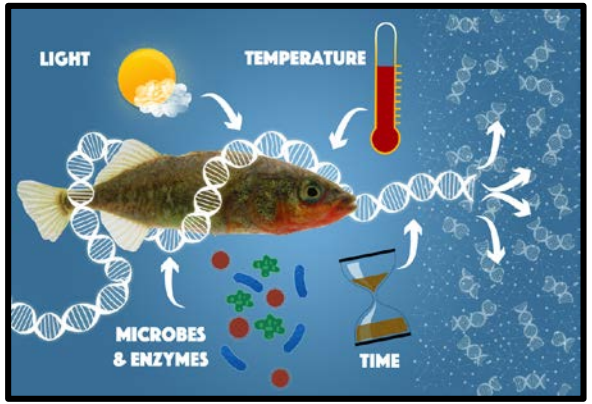
Quantity Of Target?

AS1, AS2, AS3, AS4

Specificity?

What Does This Mean?

- Evidence of Apple Snails at all 3 Sites in Cullinan Park
- Determining the optimal Primer for Field Sampling
- Application for species detection and monitoring



Future eDNA work

- eDNA **Accumulation in the presence of antibiotics**

F is for Future Work

Introduction (detection & ID)

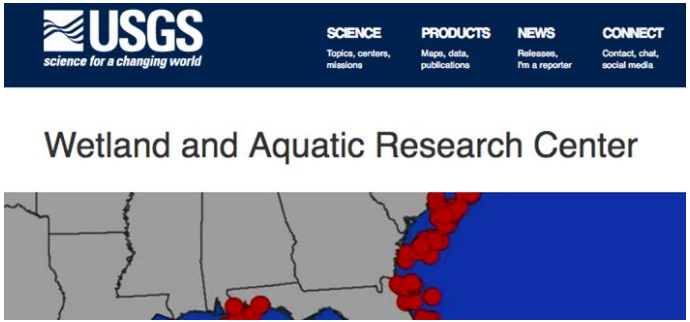
Transport (investigate aquarium trade)

Spread (distributions across habitats)

Native elsewhere (compare to Genbank)

Impact (reduce before they are a problem)

What can you do?



- Contact professionals
- Report sightings to USGS
- texasinvasives.org
- Sacrifice *correctly identified* snails (in the name of science!)

G is for the Gist

- **A**pple snails = Family **A**mpullariidae
- **B**iological invasions = challenging
- **C**ommon names = problematic
- **D**iversity = possible source of good invaders
- **E**nvironmental DNA = molecular detectives
- **F**uture research = ecology of eDNA

H is for the Hand-off

Keep invasive species from running WILD

Questions?

Please feel free to contact
burksr@southwestern.edu