Environmental DNA (eDNA) and Environmental RNA (eRNA) Markers for Detection of Grass Carp (*Ctenopharyngodon idella*)

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Grass Carp: Introduction and Spread



Figure 2. Native distribution of grass carp (modified from Opuszynski and Shireman 1995).

Grass Carp Risk Assessment 2017

Now:

- Arrival in lakes Michigan, Erie
- Survival throughout Great Lakes high with high certainty
- Reproducing populations in tributaries connected to Lake Erie

Future:

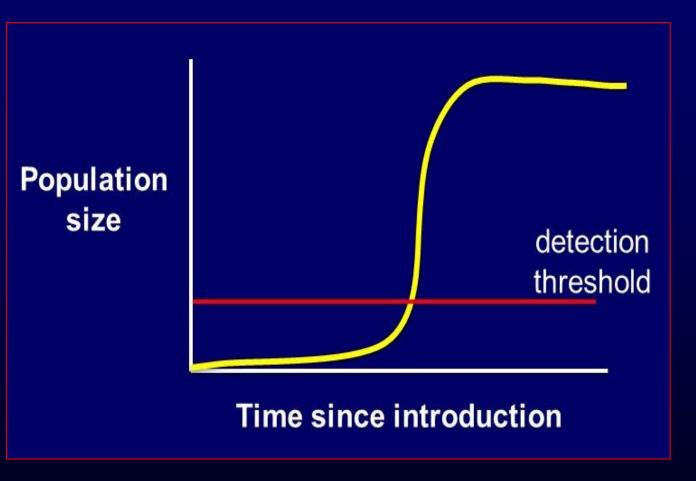
- Over the next 5-10 years: Spread to Lake Huron, low consequences
- Over next 50 years: large consequences expected for lakes Michigan and Erie

Early Detection and Mitigation

 Often difficult to detect low abundance (newly introduced) species

Prevention less costly

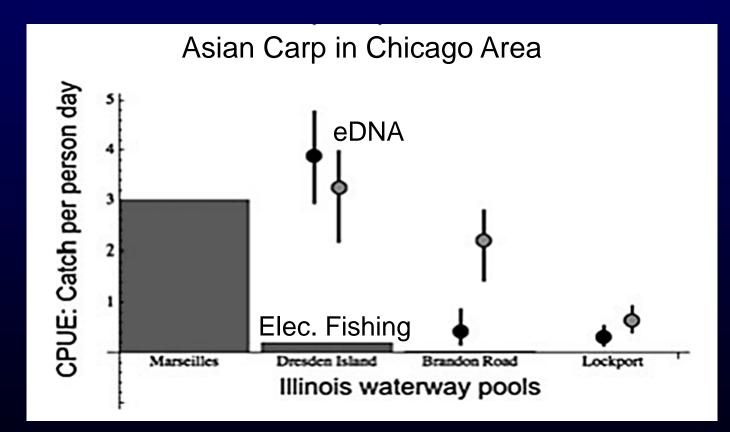
 Abundance inversely proportional to chance of eradication success



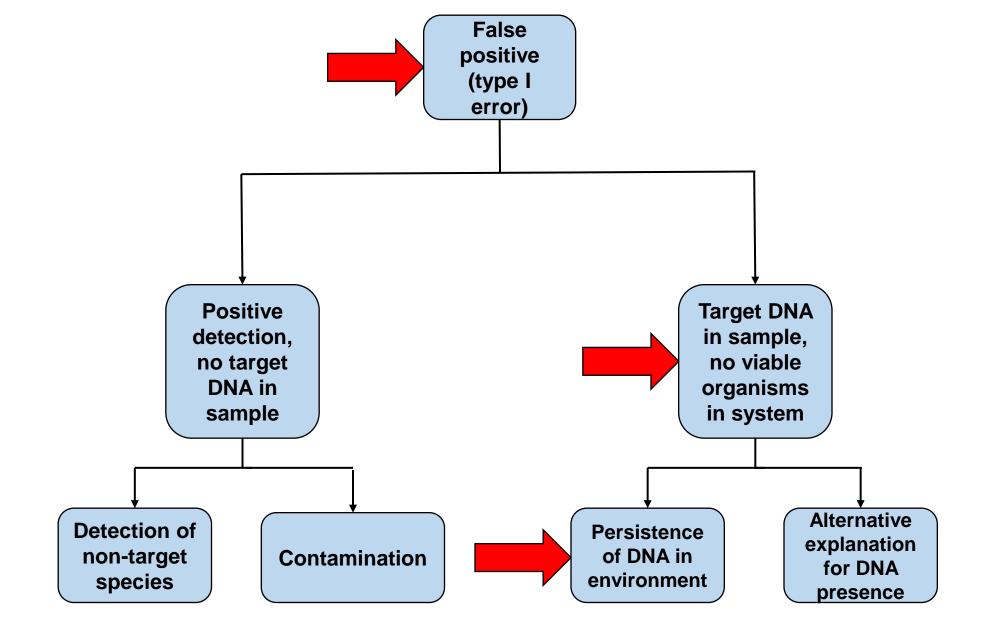
Lockwood et al. (2013)

Surveillance Using eDNA

One way to detect presence early is through the use of environmental DNA (eDNA), DNA shed or excreted from organisms present in the environment

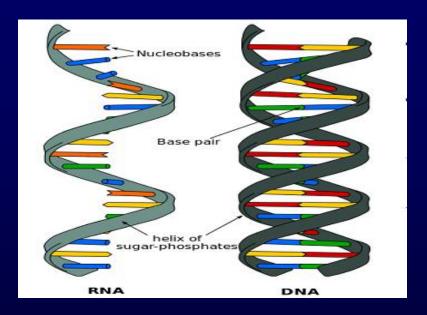


Jerde et al. (2011)



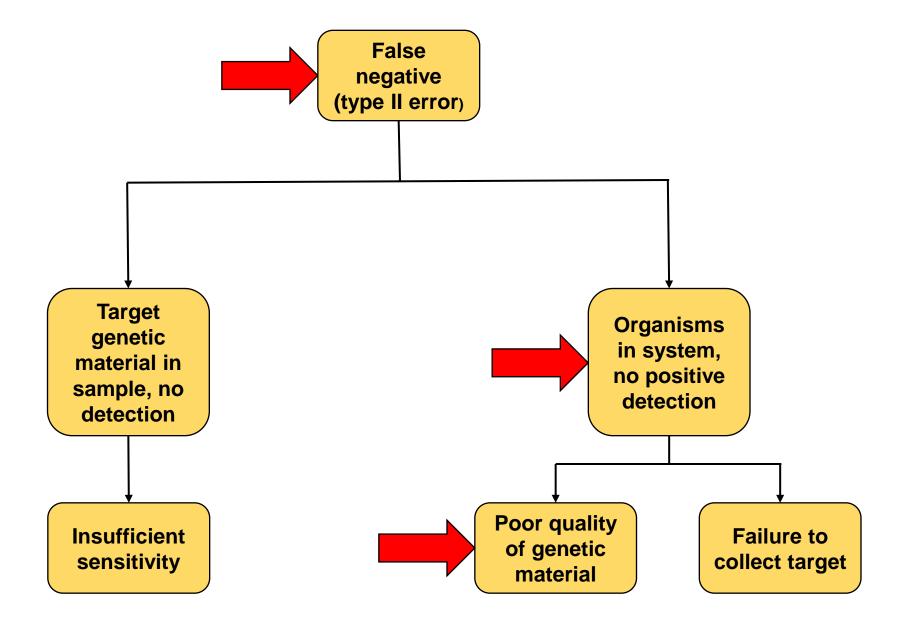
Modified from Darling and Mahon (2011)

Environmental RNA (eRNA)



 Used reliably for detection of microbes; novel for vertebrate presence detection

- Less persistence and increased selection compared to eDNA
- Measure of recent occupancy and reduction of false positives



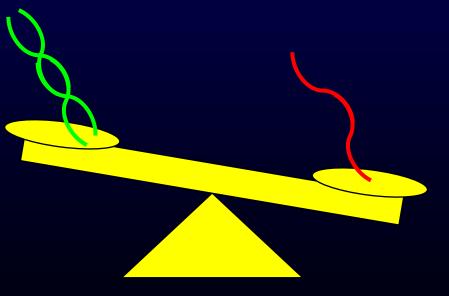
Modified from Darling and Mahon (2011)

DNA

RNA

- Persists longer in the environment
- Source could be alive or dead
- Contamination more likely
- Higher likelihood
 of false positives

- Doesn't last long outside living cell
- Low quality genetic material more likely
- Higher likelihood
 of false negatives

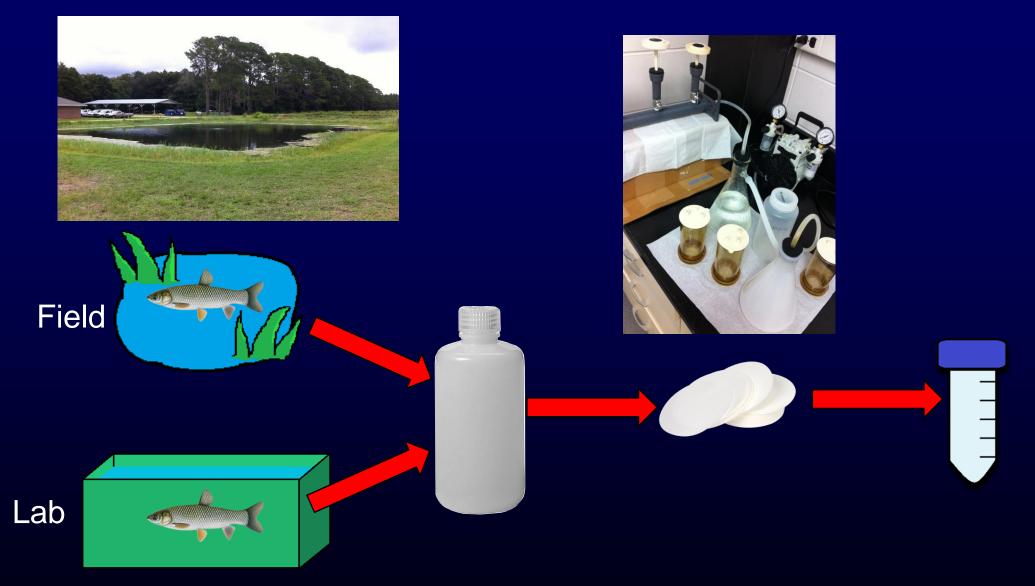


Objectives

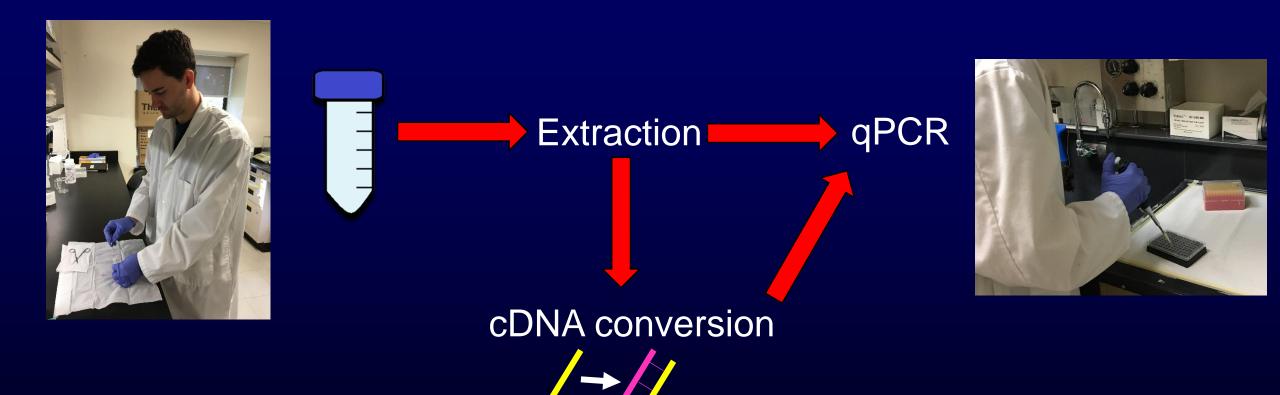
Can eRNA be adequately detected and used in conjunction with eDNA to limit false positives?

- 1. Determine temporal limit of eDNA and eRNA persistence after carp are removed from a system
- 2. Determine the effects of different densities on detection as genetic material accumulates over time.

Method Overview



Method Overview



Degradation

	96hr		 Hours After GC Removal	RNA	DNA
P. Y			Before	\checkmark	\checkmark
			0	\checkmark	\checkmark
and the second			1/2	\checkmark	
			1	\checkmark	
			4	\checkmark	\checkmark
-			8	\checkmark	\checkmark
- T			12	\checkmark	
			24	\checkmark	
			48	\checkmark	\checkmark
			72	\checkmark	\checkmark
Water		Water	192		\checkmark
Only		Only	384		\checkmark
, , , , , , , , , , , , , , , , , , ,			576		\checkmark
			768		\checkmark

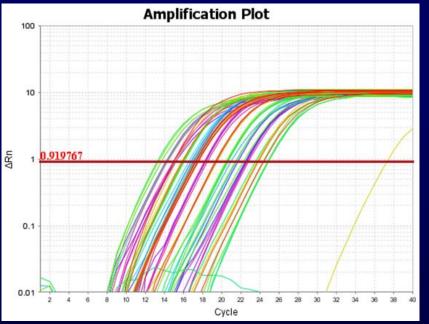
Density and Accumulation

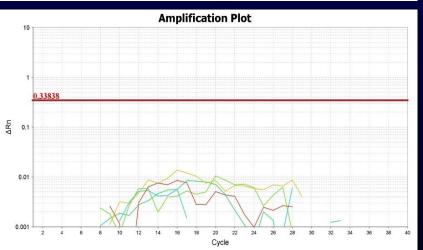
	72hr	-			
			Hours		
			After GC	RNA	DNA
			Addition		
-		-	Before	\checkmark	\checkmark
			0	\checkmark	\checkmark
			1	\checkmark	\checkmark
			6	\checkmark	\checkmark
			12	\checkmark	\checkmark
			24	\checkmark	\checkmark
			48	\checkmark	\checkmark
			72	\checkmark	\checkmark
Water Only		Water Only			

Density and Accumulation

	72hr		 Hours After GC Addition	RNA	DNA
			Before	\checkmark	\checkmark
			0	\checkmark	\checkmark
			1	\checkmark	\checkmark
			6	\checkmark	\checkmark
			12	\checkmark	\checkmark
			24	\checkmark	\checkmark
			48	\checkmark	\checkmark
			72	\checkmark	\checkmark
Water Only		Water Only			

Preliminary qPCR Results





DNA detection:

Density (1 Fish):

Hours after carp added to tank: 12, 24, 48, 72

Density (5 Fish):

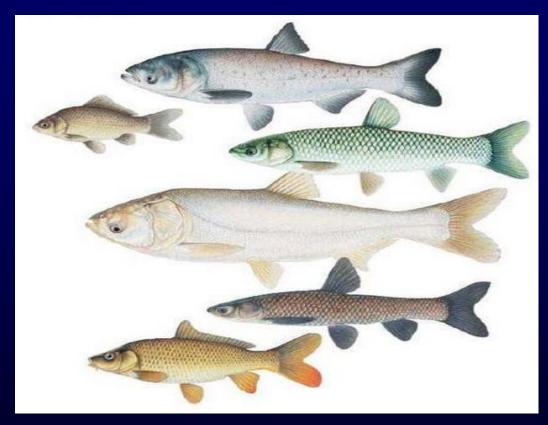
• Hours after carp added to tank: 12, 24, 48

Degradation experiment:

• Hours after carp removed: 0, 4, 8

Future work

- Further sampling and modified testing
- Testing of eRNA using universal primers
- Potential adaption of eRNA detection for other species if successful



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Thank you.

Questions?