Environmental DNA (eDNA) as a monitoring tool for zebra mussels in Lake Winnipeg

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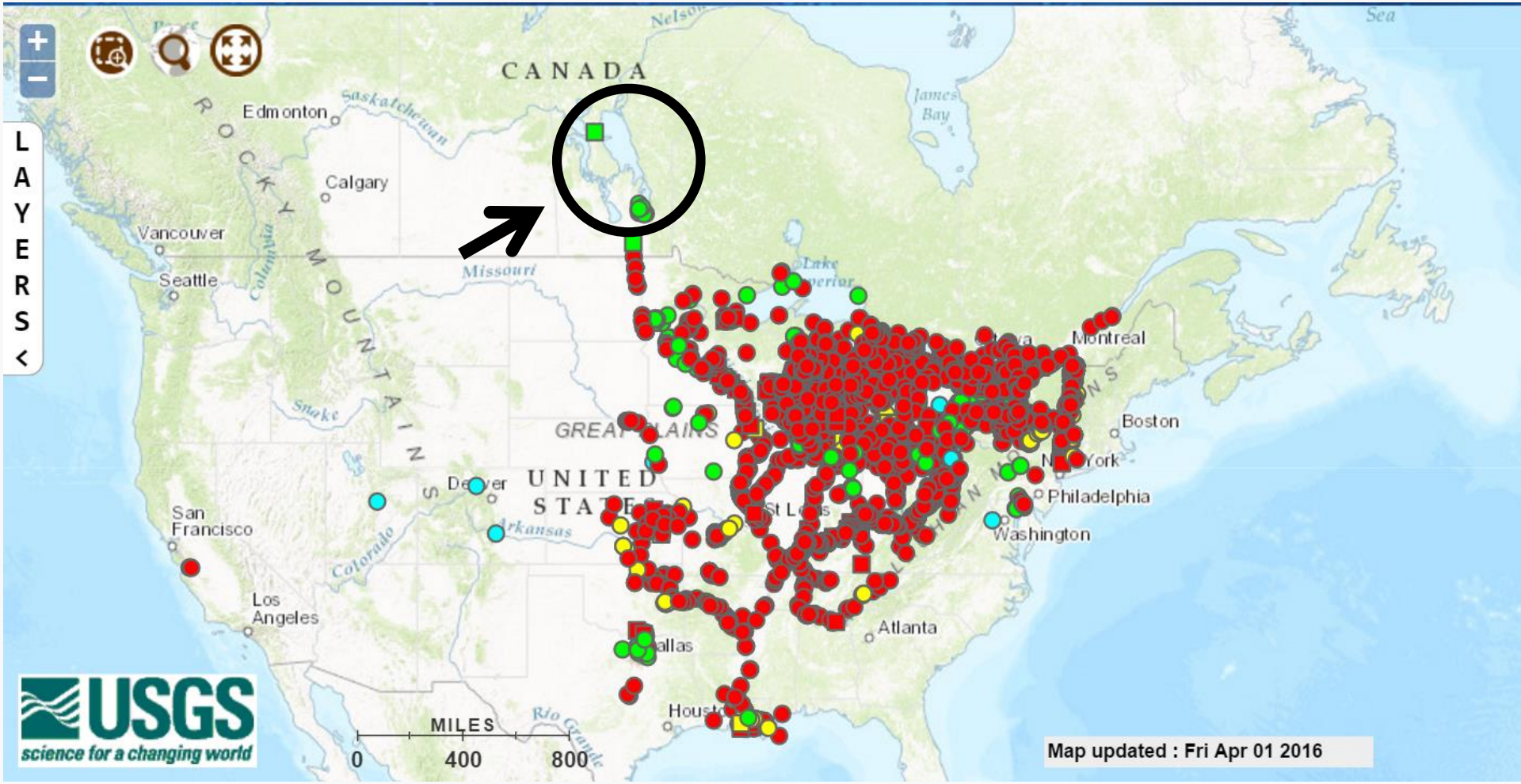
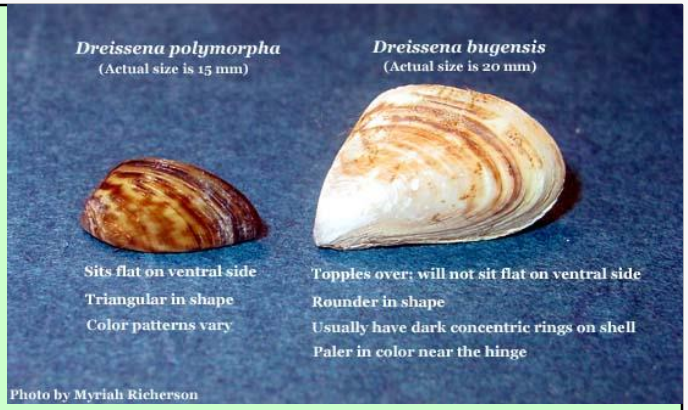


Figure 1: Distribution of ZM in the US and Canada

History of zebra mussels in Lake Winnipeg

- First detected in summer of 2013
- Found in four South Basin harbours
  - Balsam Bay Harbour
  - Gimli Harbour
  - Silver Harbour
  - Winnipeg Beach Harbour



- 425 adults removed in October 2013 from these harbours
- All harbours treated with KCL to kill ZM in May-June 2014
  - Initially successful but ZM re-established in late fall
- Shift in strategy from eradication to prevention to limit spread in Manitoba

History of zebra mussels in Lake Winnipeg

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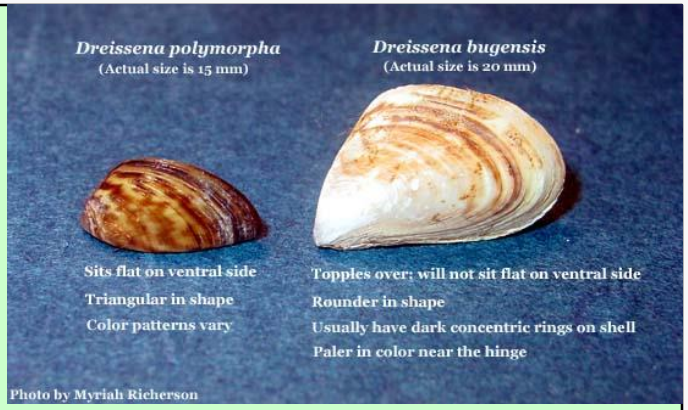
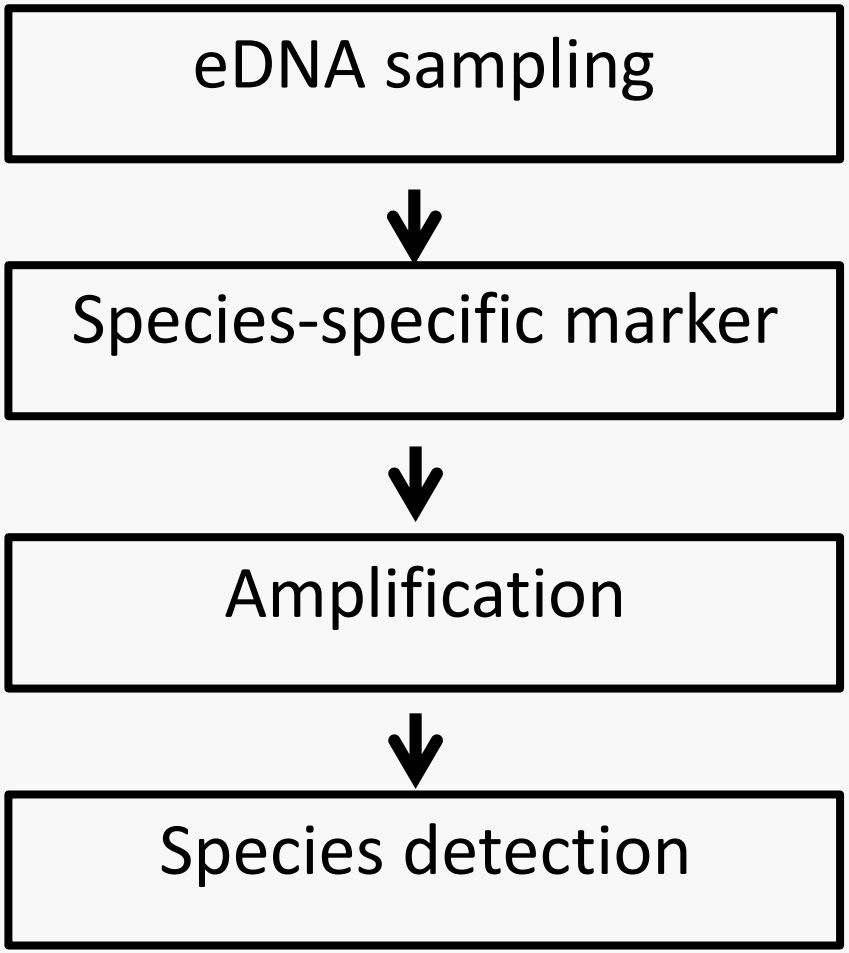


Limiting spread is where environmental DNA comes in

eDNA sampling: species detection

- Most studies use species-specific quantitative polymerase chain reaction (qPCR) TaqMan assays
- Assays use short species-specific genetic markers which target short DNA fragments of target species

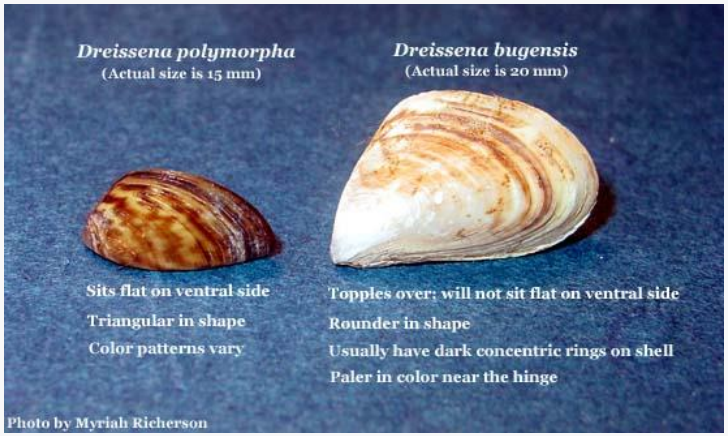
Species detection with eDNA



- False negative = no detection but target present
- False positive = detection but target absent
- Negative controls are added at every step to indicate false positives
- Multiple assays and replicates reduce likelihood of false negatives

3 Independent Qpcr Assays for "Triplechecking" of Results

- Target fragments of COI, cyt *b*, 16s rRNA genes
  - One genus-specific (*Dreissena* – 16s rRNA)
  - Two species-specific (*Dreissena polymorpha* – COI and cyt *b*)
- Enables indirect detection of quagga mussel (*Dreissena bugensis*)



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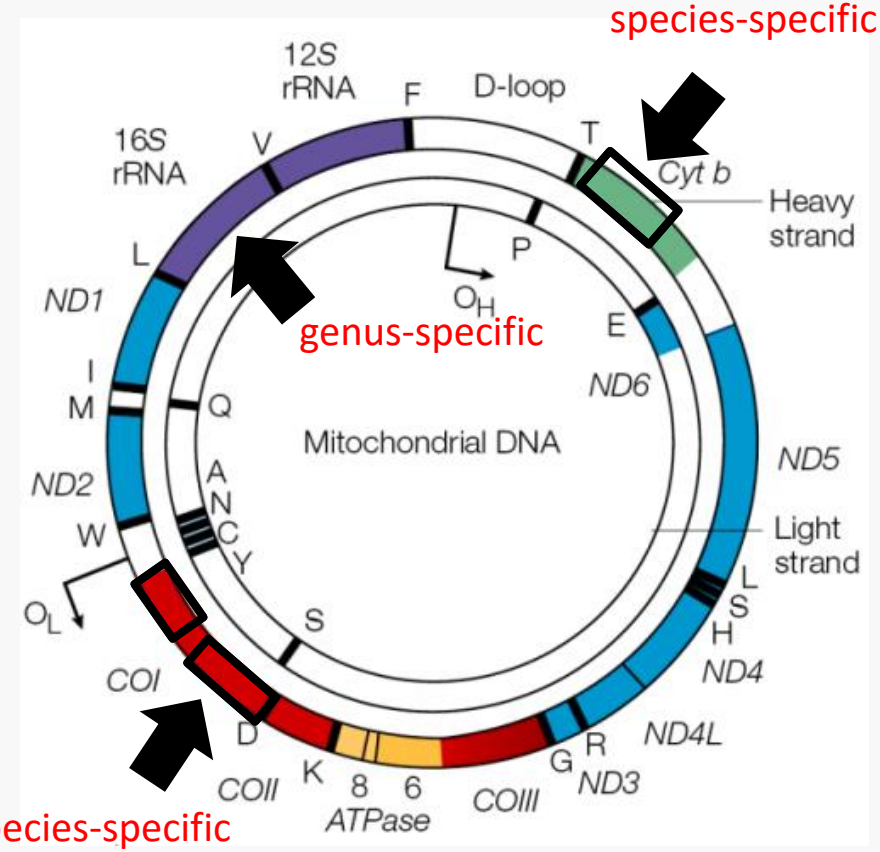


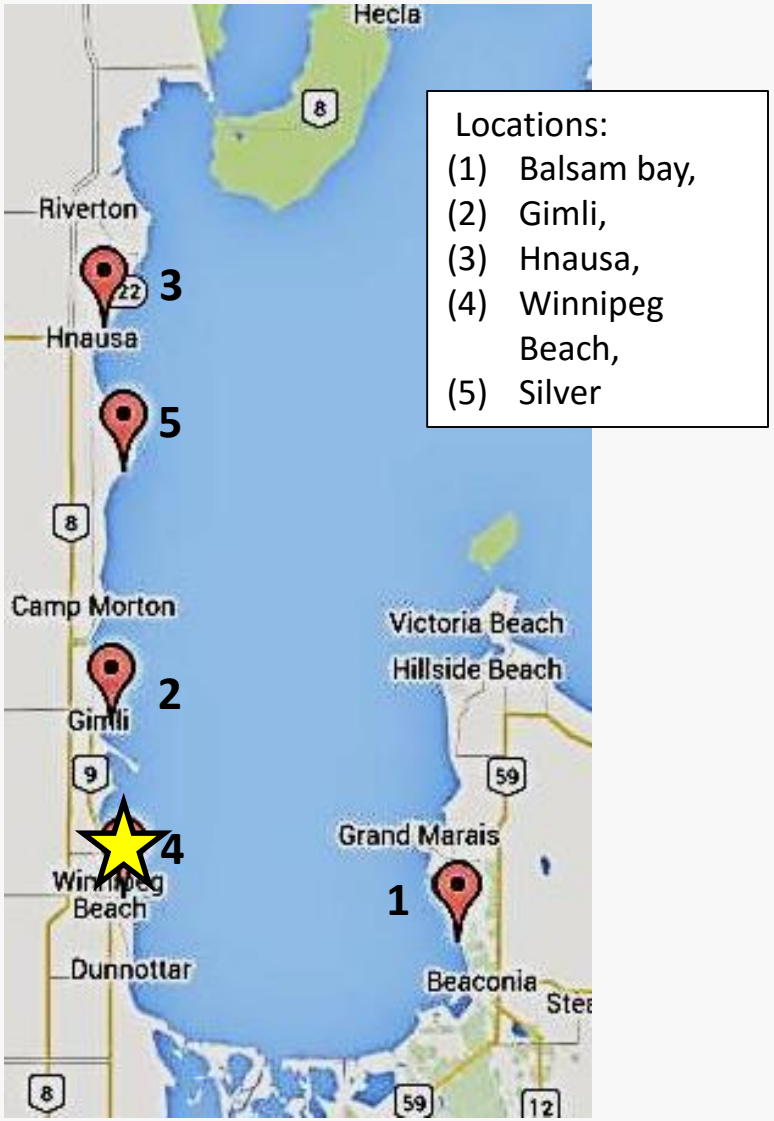
Redundancy is king!

eDNA techniques for detecting ZM

- Step 1: develop assays and validate them to ensure that species other than ZM are not detected
- Step 2: sample sites within Lake Wpg (May, October 2014)
  - Areas that should be positive for ZM:
    - Balsam bay Harbour
    - Gimli Harbour
    - Silver Harbour
    - Winnipeg Beach Harbour
    - Hnausa Harbour
  - Areas should be negative for ZM in late fall:
    - Grindstone
    - Gull Harbour
    - Hecla
    - Red River
- Step 3: comparison of larvae netting and eDNA
  - Namao 2015 fall survey

South basin sampling – May 2014

- 2 to 3 samples taken from each harbour (and 2 to 4 replicates per sample)
- All harbours tested negative for ZM except for Winnipeg Beach ★
- The 2013 eradication and winter freeze/die-off in shallow water likely resulted in ZM density below detection limits



South basin sampling – October 2014

- 2 to 8 samples taken from each harbour (and 2 to 4 replicates per sample)
- All harbours tested positive for ZM

- Zebra mussels recovered after 2013 potash treatment
- Numbers were high enough to be detected consistently with eDNA

Locations:
(1) Balsam Bay,
(2) Gimli,
(3) Hnausa,
(4) Silver
(5) Winnipeg Beach.

South basin – October 2014

- Between 2 and 8 samples taken from each harbour (2 to four replicates per sample)
- All harbours tested positive for ZM
- After reproductive and growth season, eDNA becomes more detectable
- Necessary to take multiple samples

Sample number	Balsam bay	Gimli	Hnausa	Silver	Winnipeg Beach
1	0/3	1/4	1/4	4/4	1/4
2	0/3	1/4	3/4	0/4	1/4
3	0/3	0/4		1/3	2/4
4	1/3	0/4		0/4	0/4
5	3/3	0/4			1/4
6	0/3	1/4			0/4
7	2/3	0/4			2/4
8	0/2				
Total replicates	6/23	3/28	4/8	5/15	7/28
Total samples	2/8	3/7	2/2	2/4	5/7

Narrows sampling – November 2014

- eDNA samples from Grindstone, Hecla, and Gull harbour
- No samples tested positive for ZM DNA
- ZM were not likely present based on veliger count data

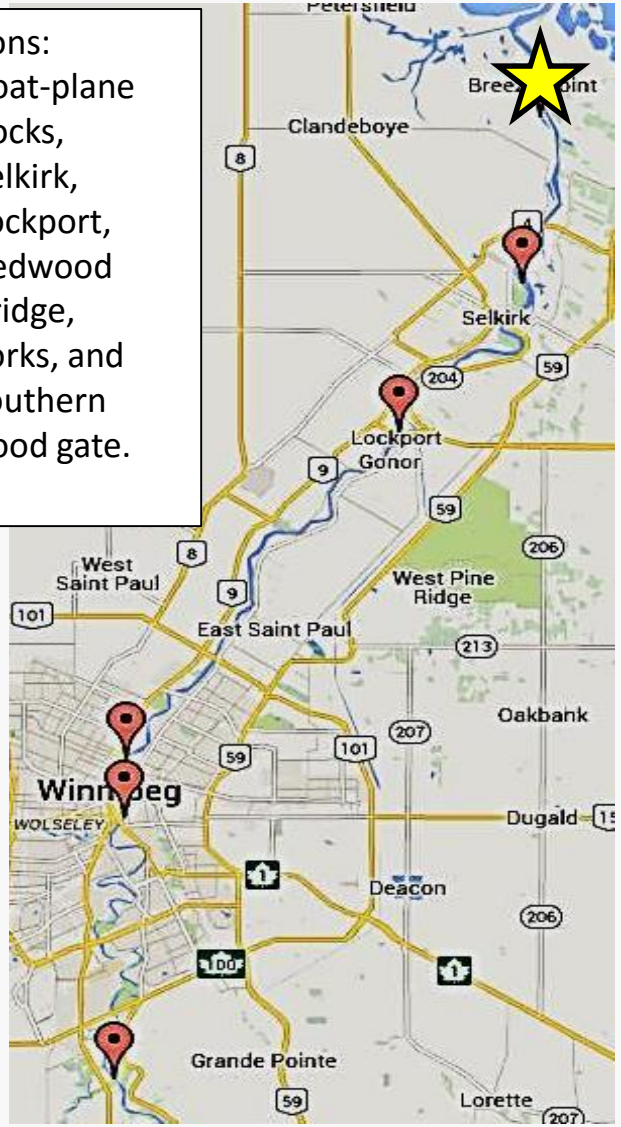
- ZM have since expanded their range into the Narrows (veliger and eDNA data)

Locations: (1) Grindstone, (2) Gull Harbour, and (3) Hecla Village Harbour.

Red River sampling – November 2014

- 1 to 3 samples taken from five sites along the Red River (with 2-4 replicates per site)
- One eDNA sample tested positive: Float-plane dock
- ZM were later discovered in Selkirk (June 2015)

Locations:
(1) float-plane docks,
(2) Selkirk,
(3) Lockport,
(4) Redwood bridge,
(5) Forks, and
(6) southern flood gate.



Red River – November 2014

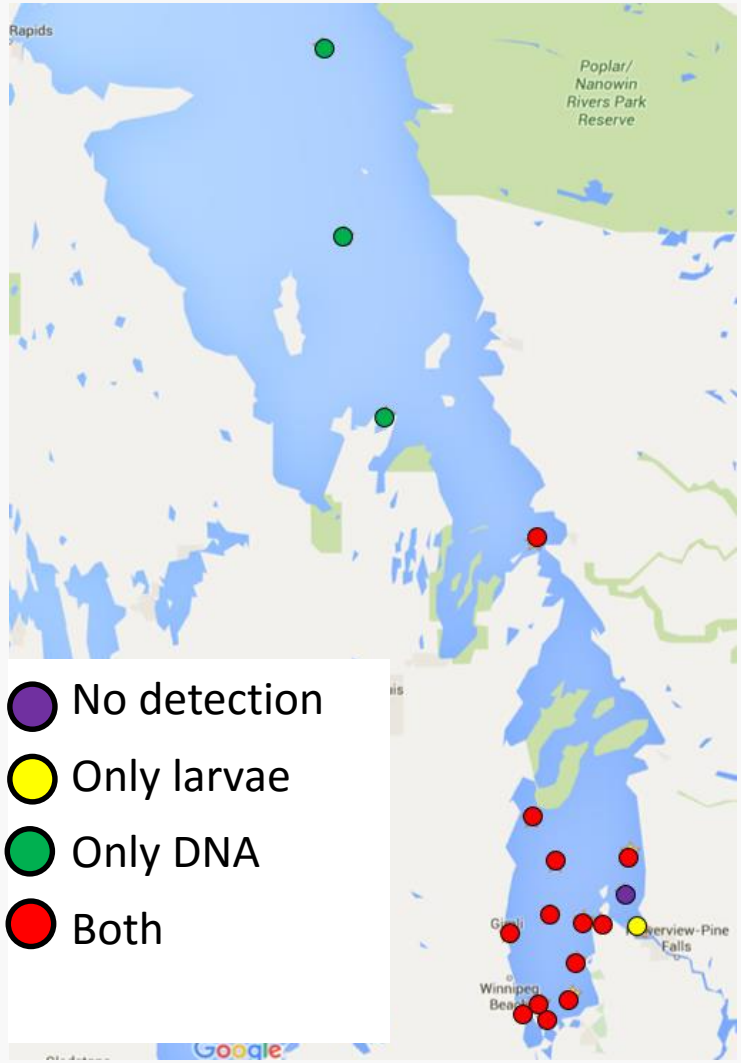
- One eDNA sample tested positive: Float-plane dock
- ZM were later discovered in Selkirk (June 2015)

Sample number	Float-plane dock	Selkirk	Lockport	Redwood bridge	Forks	Southern flood gates
1	1/4	0/4	0/4	0/4	0/4	0/4
2	0/4	0/4	0/4		0/4	0/4
Total replicates	1/8	0	0	0	0	0
Total samples	1/2	0	0	0	0	0

Veliger comparison – September and October 2015

- 3 replicate samples collected from 17 sites from Lake Wpg.
- Parallel larval netting samples

- 1 site where no larvae or DNA were detected
- 1 site where larvae were detected but DNA was not
- 12 sites where larvae and DNA were detected
- 3 sites where no larvae were detected but DNA was



Conclusions

1. eDNA detects ZM
2. Detection appears to be dependent upon amount of eDNA in the water (i.e., late-season samples show more positives)
3. eDNA techniques responsible for the first detection of ZM in upstream areas of the Red River
4. ZM distribution may extend further north than previously thought, but…
5. False negatives are a constant threat!

Thank you