Environmental DNA (eDNA)protocols for the early detection of rusty crayfish in lotic systems

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Rusty Crayfish

- Native to Ohio river basin
- Invasive in 20 states, including North Carolina
- Introduced through bait buckets, educational use, intentional release
- Inhabits wide range of aquatic habitats, including both pools and riffles

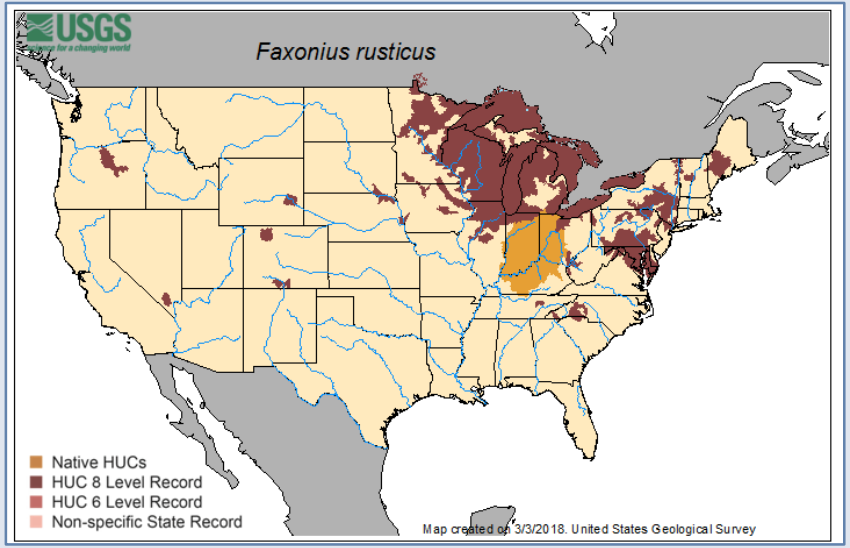


Photo credit: USGS

Rusty Crayfish

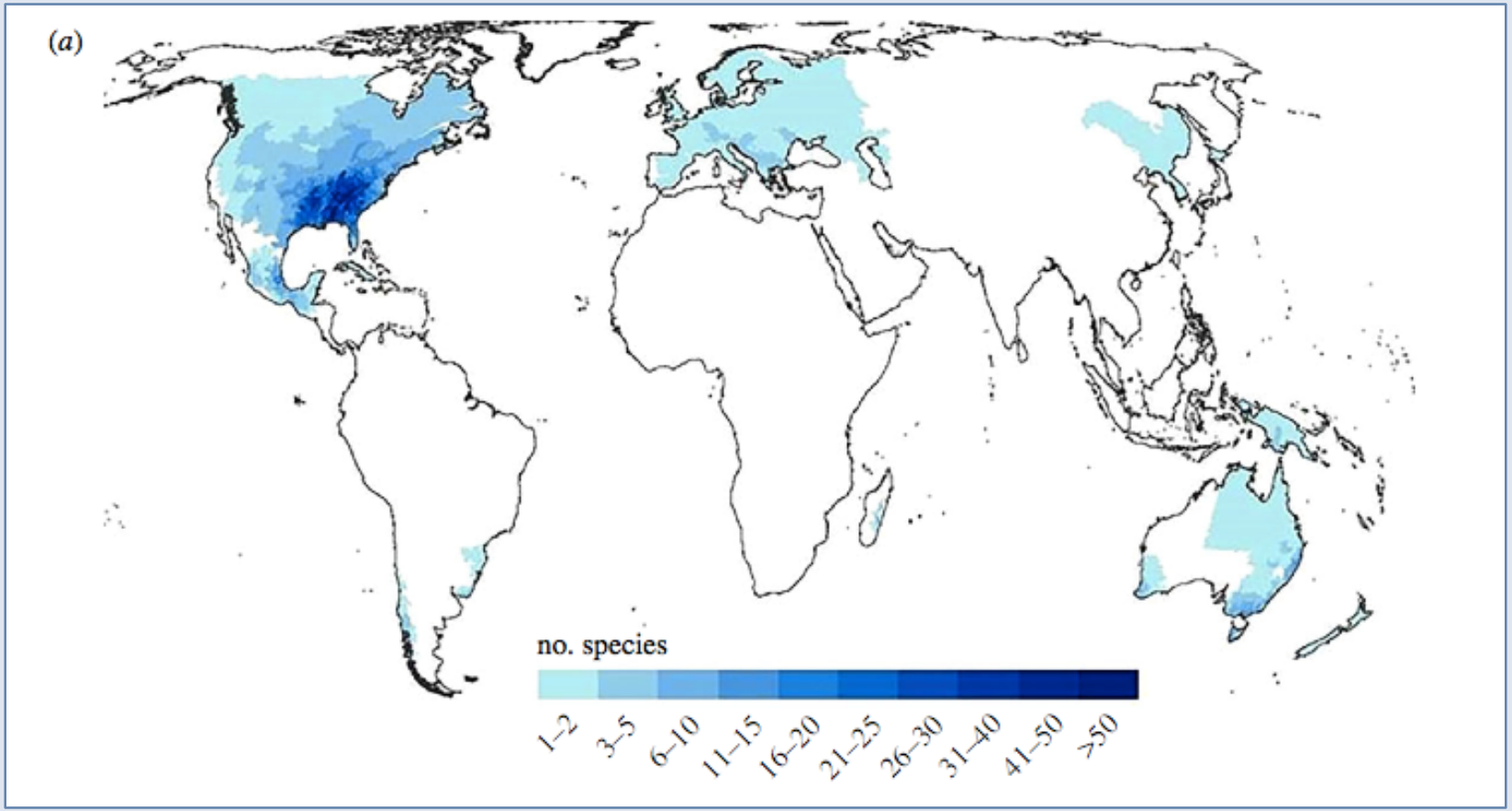
Impacts native species through:

- increased interspecific competition and displacement of native crayfishes
- increased rates of predation by fishes
- hybridization with native crayfishes

Photo credit: Animal Diversity Web

Impacts other species through:

- destruction of aquatic plant beds and trophic shifts in predator-prey/grazer-vegetation relationships
- shifts in macroinvertebrate/fish assemblages



Distribution of global crayfish populations. Image: Richman et al (2015) Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). Phil. Trans. R. Soc. B

Environmental DNA (eDNA) detection of invasive species

- Potential advantages: provides a highly sensitive method for detecting invasive species at low densities without the need for invasive sampling
- Potential issues for eDNA detection of crayfish: presence of an exoskeleton, benthic habitats
- eDNA sampling for crayfish has had varying degrees of success (Dougherty et al. 2016; Rice et al., 2018; Tréguier et al 2014)

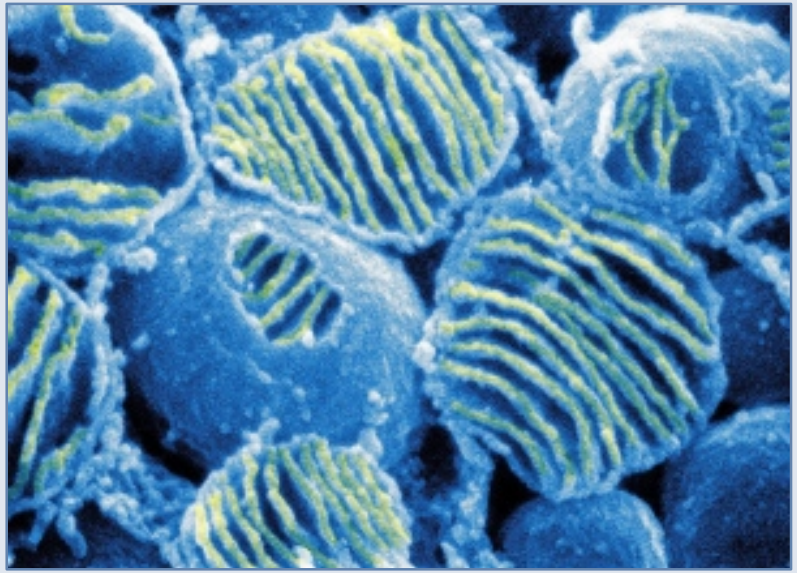


Photo credit: Pierce *Genetics Essentials*

Questions

- What factors affect eDNA detection rates for rusty crayfish?
- Is eDNA sampling an effective method for detecting rusty crayfish in lotic systems?

Strategy

- Collection of water samples under varying laboratory conditions
- Preservation of water samples
  - Filtration
  - Preservation in ethanol and 3M sodium acetate
- Field test protocol

Laboratory model stream system

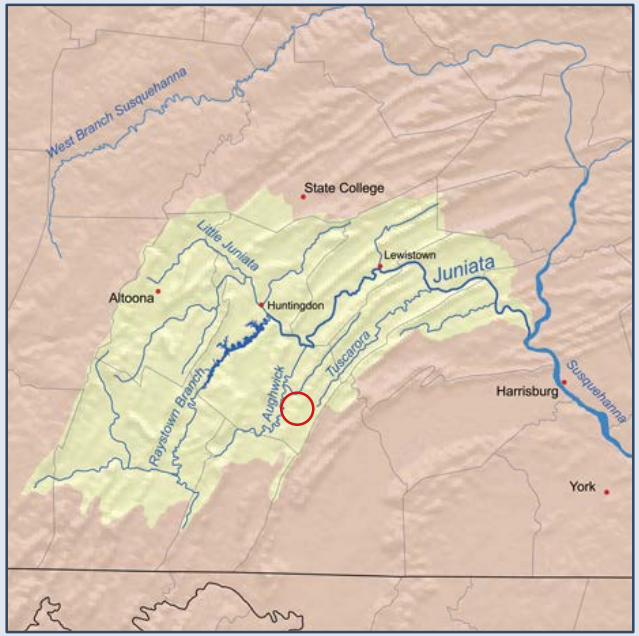
- Stream with a catchment pool
- Flow rates from 0.167 l/s to 2 l/s

Laboratory model stream system

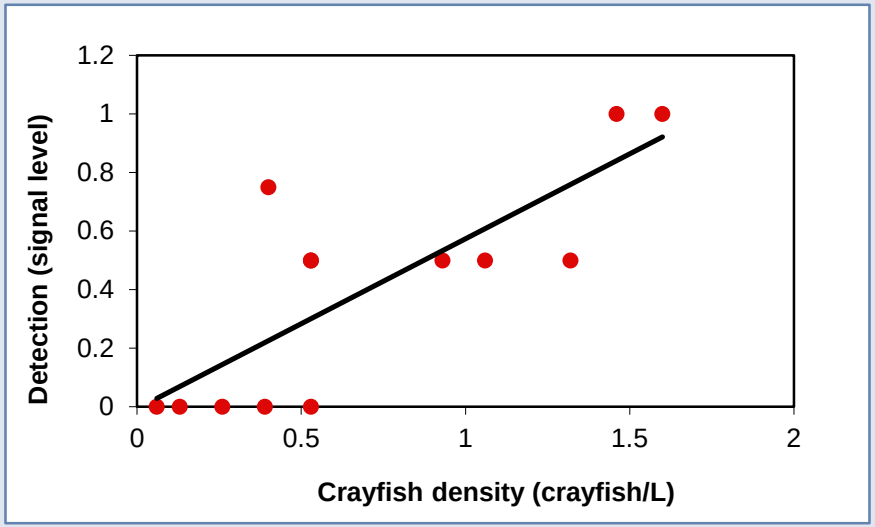
- Stream with a catchment pool
- Flow rates from 0.167 l/s to 2 l/s
- Crayfish introduced to stream in tethered cages

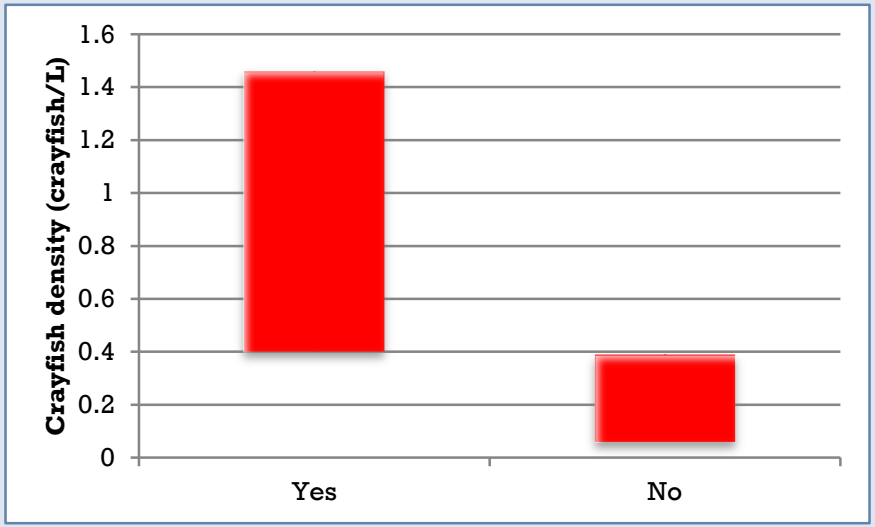
Initial protocol development

- Crayfish collected from Blacklog creek
  - ***Faxonius rusticus***
  - ***Faxonius obscurus***
- Maintained in aquaria
- Used tissue samples for initial protocol testing
- PCR primers (Dougherty et al., 2016) amplify cytochrome c oxidase subunit 1 (COI) gene



eDNA detection *is* affected by crayfish density





$R^2 = 0.627$

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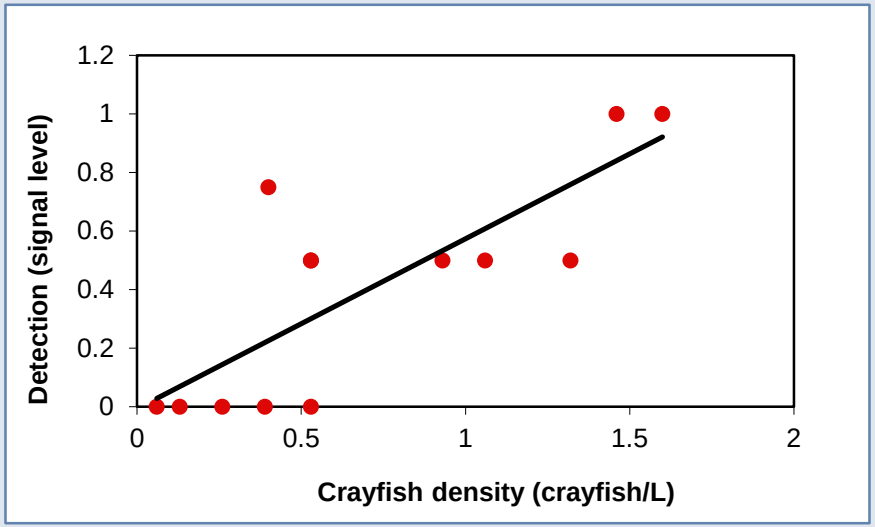
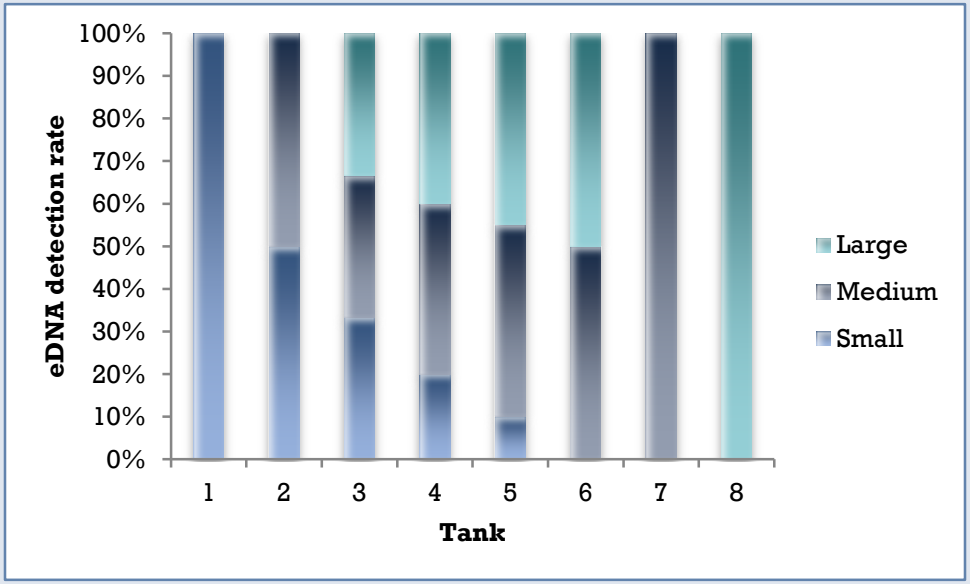


$R^2 = 0.627$

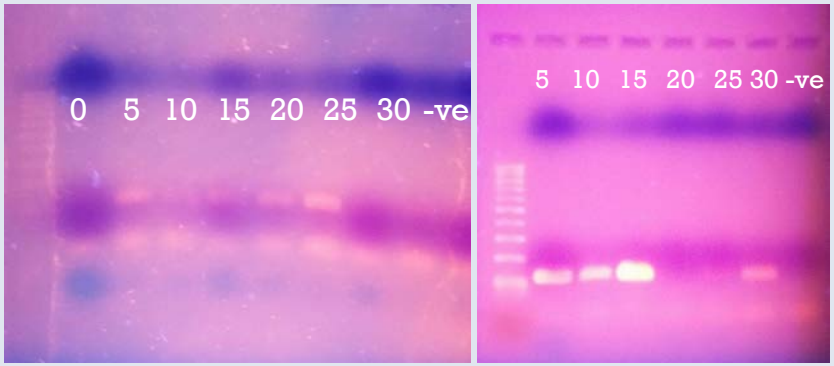
- Sex ratio affects detection strength

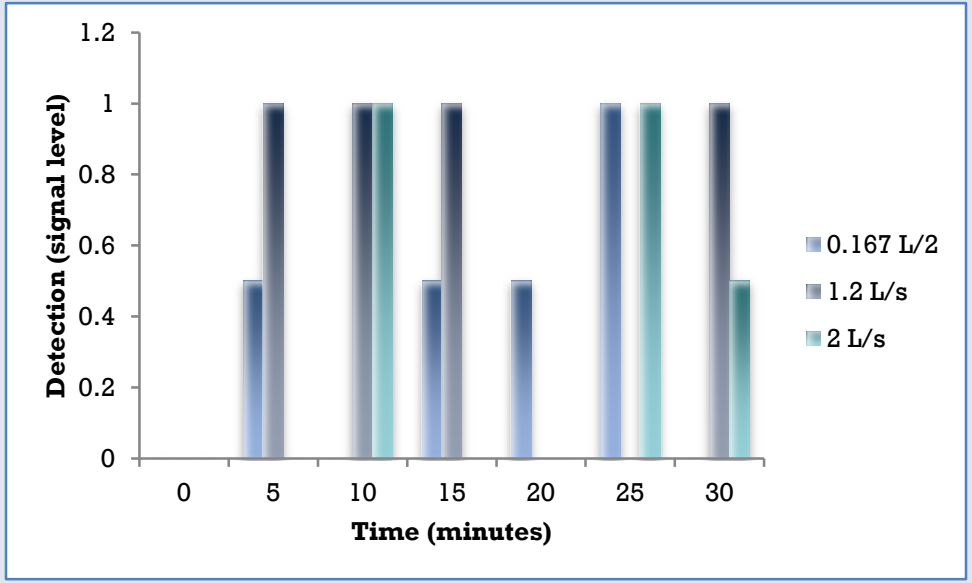
eDNA detection success is not affected by crayfish size



eDNA detection is more variable in lotic conditions

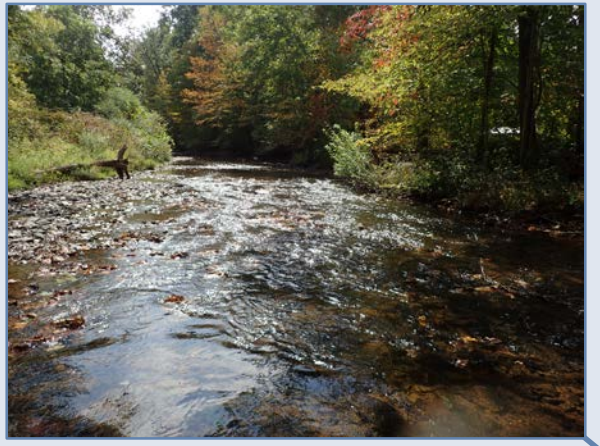
- Water samples collected at 5-minute intervals
- Flow rates: 0.167 L/s, 1.2 L/s, 2 L/s





- Detection reliability increased when appendages/moulted exoskeletons were present

Stream locations



Sideling Hill

N. branch Little Aughwick

Wooden Bridge

Aughwick Creek

Photo credit: Weather.gov

- eDNA collected by filtration and by preservation in ethanol and 3M sodium acetate

Stream	GPS Location	Comparative speed	Rusty Crayfish Observed	eDNA Detection
Aughwich Creek	40.17306 - 77.92086	Slow	No	No
Ninemile Run	40.07020 - 77.93018	Medium	No	No
Wooden Bridge Creek	40.08860 - 78.02242	Medium	No	No
Ft. Littlelton (Little Aughwich Creek)	40.06740 - 77.96388	Slow	Yes	No
N. Branch Little Aughwich Creek	40.09184 - 77.90920	Fast	Yes	No
Sideling Hill	40.12207 - 78.02421	Slow	Yes	No

Conclusions

- Density and sex ratio affects eDNA detection rates
- Likelihood of detection may vary seasonally. Field sampling be more successful conducted in the spring and summer, during the breeding and molting season
- Crayfish exoskeleton may inhibit the release of cells and extracellular DNA into the environment, making detection more difficult for this species
- Unclear whether eDNA may be an effective early detection method in lotic systems

Future directions

- Re-sample sites in Spring/Summer, when crayfish likely to be moulting
- Modify detection method (nested PCR)
- Identify water quality parameters that influence detection rates

Acknowledgements

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