Strengths, limitations, and practical considerations of using eDNA techniques to detect amphibian pathogens in wild populations

Panel discussion on the use of eDNA

Dr. Laura A Brannelly
Research Fellow
The University of Melbourne, Victoria, Australia

Presentation Aims

- Overview eDNA and its uses
- Benefits of using eDNA techniques for wildlife pathogens
- Discuss the variety of methods and techniques
- Limitations of eDNA use in the field
- Example from my research of uses and limitations

- Panel discussion

Environmental DNA?

- Extracted DNA from environmental samples to identify a target species
  - Example: Water samples to look for an invasive fish species
- Picks up the DNA traces an organism leaves behind
- Allows detection of species without sampling individuals

How is eDNA used?

- Find rare or threatened species
  - Long Finned Pilot Whale in the Baltic Sea
  - 6 species of shark in New Caledonia
- Invasive species
  - Asian Grass Carp in Great Lakes
  - Cane Toads in Australia
- Pathogen detection
  - Wildlife (Bd, Bsal, RV), Human (Cholera) and Agricultural (bovine TB)
- Search for the Loch Ness Monster
  - No Jurassic-aged reptile found
  - Plenty of eel DNA

Amphibian pathogens in environmental samples

Batrachochytrium dendrobatids (*Bd*), *Batrachochytrium salamandrivorans* (*Bsal*), Ranavirus

- Detected in ponds, streams, and even water inside bromeliads (Kirshtein et al. 2007; Walker et al. 2007; Cossel Jr. and Lindquist 2009; Hyman and Collins 2012)
- Presence can be detected outside of die-off events and at low concentrations (Miaud et al 2019; Spitzen-van der Sluijs et al 2020)
- Large quantities of *Bd* DNA detected just prior to die-off (Kamoroff and Goldberg 2017)
- Quantity of Ranavirus DNA in environmental samples correlated with amphibian load (Hall et al 2015)

Benefits eDNA for amphibian pathogens

- eDNA is non-invasive
  - Cryptic, threatened, low density species
  - Remote/restrictive environments
- Might be used to predict declines
- Particularly important for biosecurity
  - Bsal is not currently worldwide
- For modeling transmission/disease dynamics
  - Possibly used as a proxy for force of infection in the environment
  - Can be used for occupational modeling

eDNA extraction methods

- Methods vary widely (Rees et al. 2014)
- Sporadic PCR inhibition is common (Hyman and Collins 2012; Kolby et al. 2015)
- Most common for *Bd* eDNA is a spin column, sometimes with a homogenization step followed by an inhibitor removal step (Kirshtein et al. 2007; Kolby et al. 2015; Kamoroff and Goldberg 2017; Mosher et al. 2018)

Extraction method comparison

- Published comparisons of methods of eDNA extraction are rare
  - Assumed that no one extraction method is more robust (Rees et al. 2014).
- Systematically tested five commonly used extraction methods (Brannelly et al 2020)
- Powersoil/Powerfecal were far superior for my frog pond water samples (Brannelly et al 2020)
  - 2-4x higher detection than other kits tested
  - Minimum detection level ~100 zoospores/filter in this study
  - Notes:
    - For this trial, inhibitor removal step decreased inhibition but did not eliminate it, with no change in DNA quantity detected
    - Piloted an environmental master mix – which improved quantity detected for some extraction methods, but still not equal to high quality extraction methods

Sampling techniques to consider

- Contamination
  - Major consideration for eDNA work in field and lab
  - Clean room/hygiene protocols
  - 50% bleach, freshly made each day
  - Use disposables (filter funnels, etc)
- Sample collection
  - Minimize contamination (don't step in before the sample is collected)
  - Maximize volume that can be filtered
    - Try for the clearest water possible
  - Negative controls!
  - Sample replication: multiple per site
- Sample processing
  - Keep on ice/in fridge once collected
  - Filter the water as soon as possible (<24hrs)
  - Try for >500ml per filter
- Sample storage
  - Dry the filter as best you can before storage
  - Multiple methods: frozen or dry at room temp
- Extraction and qPCR
  - Troubleshoot!!
  - IPCs are critical (internal pos control)
  - Positive and Negative controls!
  - Triplicate (or more) qPCR

Know your limitations

- Can your question be addressed through eDNA techniques? Or is there a better technique for your question?
- There is a detection limit for DNA extraction methods and qPCR methods
  - Detection correlated both with abundance of frogs, and infection dynamics in the frogs
  - If environment has a lot of inhibitors/dissolved particles this could reduce precision
- Cost-benefit analysis of utilizing eDNA
  - eDNA is much more expensive and time intensive per sample, but requires fewer samples per site
  - Adjust methods based on your conditions for maximum benefit at lowest cost
- Troubleshoot extraction and qPCR to ensure its working with your samples

Example using eDNA methods for *Bd* detection in the field

Aims

1) Understand whether environmental *Bd* correlates with pathogen load and prevalence on amphibian hosts across sites and seasons

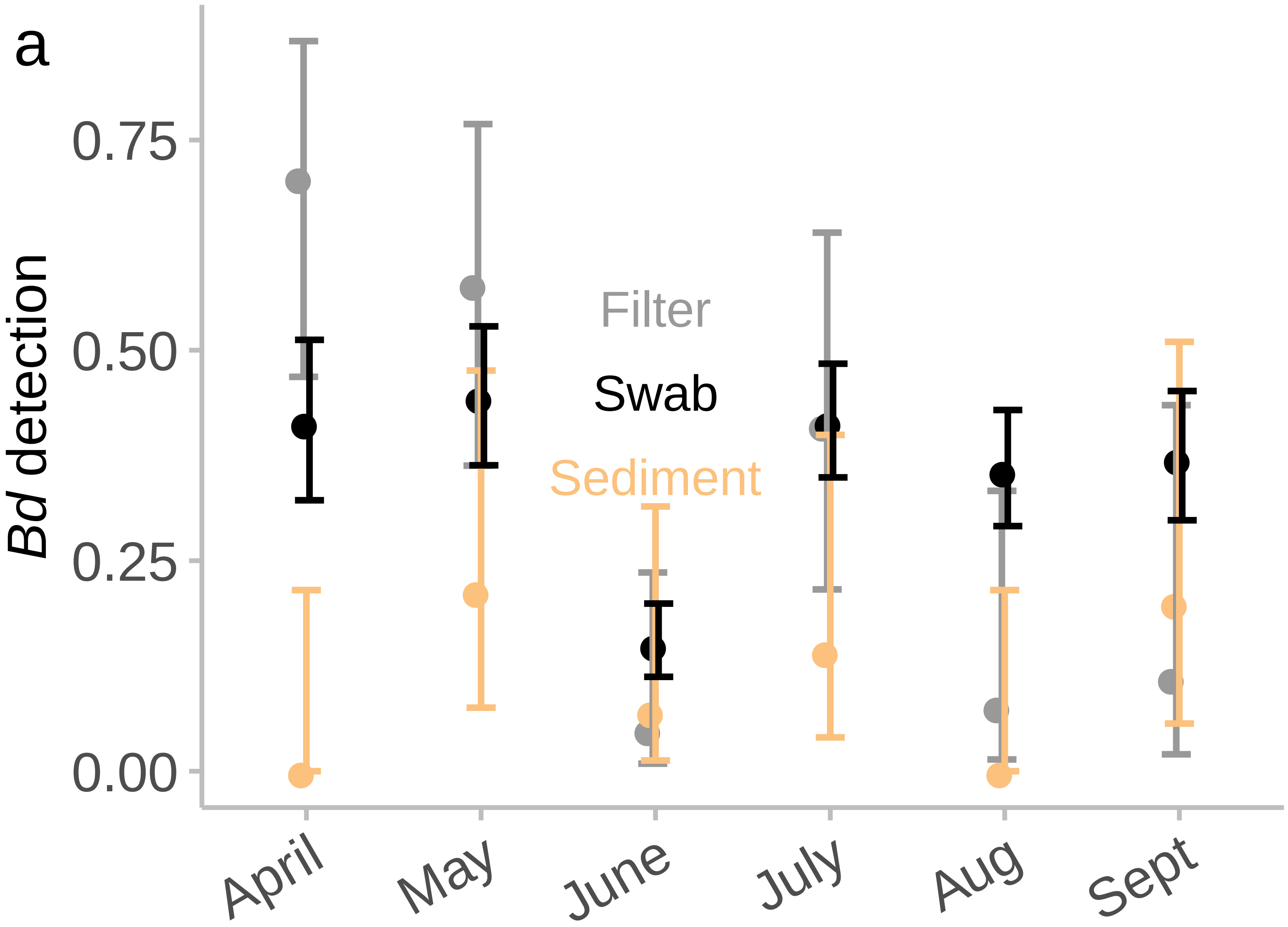
2) To identify whether eDNA can be used as a proxy for force of infection in transmission models

Study design

- Collect environmental samples (100g soil, 1L of water) and amphibian skin swab samples
- 2 locations
  - Pennsylvania: 7 sites, 6 sampling points April-September
  - Louisiana: 5 sites, 3 sampling points (spring, summer and fall)

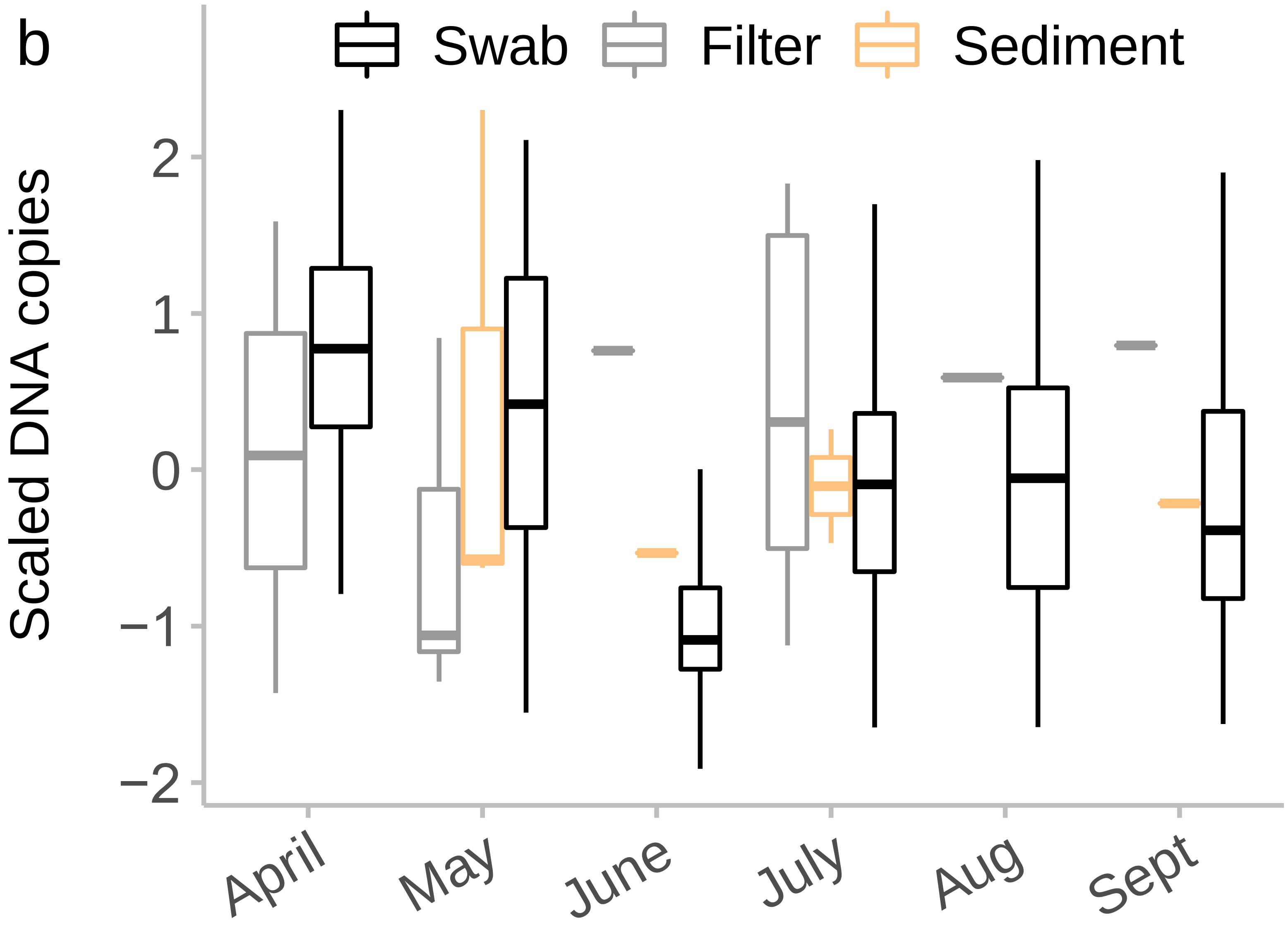
Field results: Pennsylvania

- *Proportion* of positive samples was consistent between water and swab samples
  - Sediment detection was more than 4.5x lower
- Prevalence varied seasonally



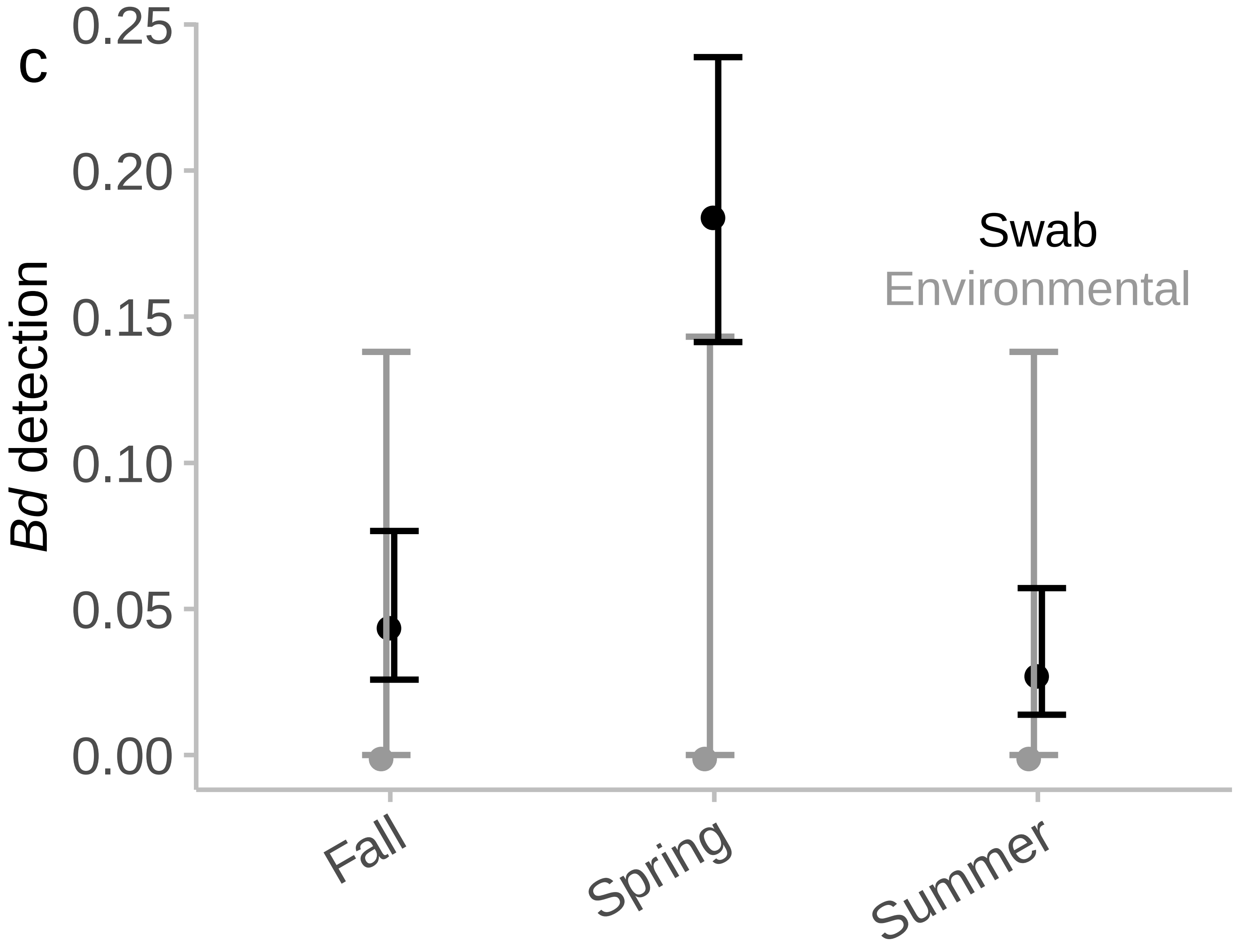
Field results: Pennsylvania

- Environmental DNA *quantity* did not vary over the sampling period
- Swab DNA quantity did vary over the sampling period
  - Highest load in April, 2x higher than the lowest month, June



Field results: Louisiana

- Overall swab *Bd* presence was low – 8.5% average infection prevalence
- No positive sediment samples or water samples
- No statistical difference between swab and environmental samples, or through time



Detecting *Bd* DNA in the field

- Water filter and swabs yielded similar results detection (prevalence of the pathogen) overall
- *Bd* eDNA quantity was not correlated with swab load
- Pathogen presence in swab samples but not in eDNA

eDNA for presence but not absence of *Bd*

(i.e. swab samples are more sensitive)

Summary of eDNA for pathogen detection

- eDNA is an exciting new technology!
- But there are limitations
  - Detection limit
  - Not useful for pathogen absence
  - Volume of water per filter
  - Pathogen DNA quantity is not always proportional infection in the frogs
- Need to troubleshoot before embarking on a project, especially if new to eDNA research
  - Utility is likely site and species dependent
  - Extraction methods matters
- Can be used for detecting pathogen presence as a preliminary step before animal surveys

eDNA should always complement traditional tools, not replace.

Key eDNA articles

- Goldberg et al. (2016) Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolution 7: 1299–1307. http://doi.org/10.1111/2041-210X.12595
- McColl-Gausden et al. (2020) A field ecologist's guide to environmental DNA sampling in freshwater environments. Australian Zoologist: 2020, Vol. 40, No. 4, pp. 641-651
- Rees et al. (2014) The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. Journal of Applied Ecology 51(5): 1450–1459. http://doi.org/10.1111/1365-2664.12306
- Roussel et al. (2015) The downside of eDNA as a survey tool in water bodies. Journal of Applied Ecology 52: 823–826. doi: 10.1111/1365-2664.12428
- Smith and Goldberg (2019) Occupancy in dynamic systems: accounting for multiple scales and false positives using environmental DNA to inform monitoring. Ecography 43: 376–386. doi: 10.1111/ecog.04743

Contact information for webinar panelists

Laura A Brannelly, Research Fellow – Melbourne Veterinary School, University of Melbourne, laura.brannelly@unimelb.edu.au

Maria Forzan, Wildlife Pathologist, Long Island University, maria.forzan@liu.edu

Alyssa Wetterau Kaganer, PhD Candidate - Zoology and Wildlife Conservation, Cornell-Smithsonian Joint Graduate Training Program, amw268@cornell.edu

Emily H Le Sage, Postdoctoral Researcher – Integrative Biologist, Vanderbilt University Medical Center, emily.m.hall@vanderbilt.edu

Danna Schock, Wildlife Biologist, Palustris Environmental, danna.schock@palustrisenvironmental.ca

Travis Seaborn, Postdoctoral Research Associate – Spatial Ecologist, University of Idaho, tseaborn@uidaho.edu

John Wood, Molecular Biologist, Pisces Molecular, jwood@pisces-molecular.com

Articles cited here

- Brannelly, L.A., Wetzel, D.P., Ohmer, M.E.B., Zimmerman, L., Saenz, V., Zawacki, C.L.R., 2020. Evaluating environmental DNA as a tool for detecting an amphibian pathogen using an optimized extraction method. Oecologia 194, 267–281. doi:10.1007/s00442-020-04743-4
- Cossel Jr., J.O., Lindquist, E.D., 2009. Batrachochytrium dendrobatidis in arboreal and lotic water sources in Panama. Herpetol. Rev. 40, 45–47.
- Hall, E.M., Crespi, E.J., Goldberg, C.S., 2015. Evaluating environmental DNA-based quantification of ranavirus infection in wood frog populations. Mol. Ecol. Resour. 16, 423–433. doi:10.1111/1755-0998.12461
- Hyman, O.J., Collins, J.P., 2012. Evaluation of a filtration-based method for detecting Batrachochytrium dendrobatidis in natural bodies of water. Dis. Aquat. Organ. 97, 185–195. doi:10.3354/dao02423
- Kamoroff, C., Goldberg, C., 2017. Using environmental DNA for early detection of amphibian chytrid fungus Batrachochytrium dendrobatidis prior to a ranid die-off. Dis. Aquat. Organ. 127, 75–79. doi:10.3354/dao03183
- Kirshtein, J.D., Anderson, C.W., Wood, J.S., Longcore, J.E., Voytek, M.A., 2007. Quantitative PCR detection of Batrachochytrium dendrobatidis DNA from sediments and water. Dis. Aquat. Organ. 77, 11–15. doi:10.3354/dao01831
- Kolby, J.E., Smith, K.M., Ramirez, S.D., Rabemananjara, F., Pessier, A.P., Brunner, J.L., Goldberg, C.S., Berger, L., Skerratt, L.F., 2015. Rapid response to evaluate the presence of amphibian chytrid fungus (Batrachochytrium dendrobatidis) and ranavirus in wild amphibian populations in Madagascar. PLoS One 10, 1–21. doi:10.1371/journal.pone.0125330
- Miaud, C., Arnal, V., Poulain, M., Valentini, A., Dejean, T., 2019. eDNA increases the detectability of ranavirus infection in an alpine amphibian population. Viruses 11, 1–15. doi:10.3390/v11060526
- Mosher, B.A., Huyvaert, K.P., Bailey, L.L., 2018. Beyond the swab: ecosystem sampling to understand the persistence of an amphibian pathogen. Oecologia 188, 319–330. doi:10.1007/s00442-018-4167-6
- Rees, H.C., Maddison, B.C., Middleditch, D.J., Patmore, J.R.M., Gough, K.C., 2014. Review: The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. J. Appl. Ecol. 51, 1450–1459. doi:10.1111/1365-2664.12306
- Spitzen-van der Sluijs, A., Stark, T., DeJean, T., Verbrugghe, E., Herder, J., Gilbert, M., Janse, J., Martel, A., Pasmans, F., Valentini, A., 2020. Using environmental DNA for detection of Batrachochytrium salamandrivorans in natural water. Environ. DNA 1–7. doi:10.1002/edn3.86
- Walker, S.F., Salas, M.B., Jenkins, D., Garner, T.W.J., Cunningham, A.A., Hyatt, A.D., Bosch, J., Fisher, M.C., 2007. Environmental detection of Batrachochytrium dendrobatidis in a temperate climate. Dis. Aquat. Organ. 77, 105–112. doi:10.3354/dao01850

Additional articles and resources

The Goldberg lab's resources:

- https://labs.wsu.edu/goldberglab/
- https://ednaresources.science/

This is a Mendeley reference collection where you can find so many good references:

- https://www.mendeley.com/community/usgs-cdi-edna-cop/

USGS run webinars are sometimes on here:

- https://www.pnamp.org/project/emerging-technology-information-sessions

listserv: https://listserv.usgs.gov/mailman/listinfo/cdi-edna

- Huver, J. R., et al. "Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems." Ecological Applications 25.4 (2015): 991-1002.
- Chestnut, T., C. Anderson, R. Popa, A. R. Blaustein, M. Voytek, D. H. Olson, and J. Kirshtein. 2014. Heterogeneous Occupancy and Density Estimates of the Pathogenic Fungus *Batrachochytrium dendrobatidis* in Waters of North America. PLoS ONE 9(9):e106790. doi:10.1371/journal.pone.0106790
- Schmidt, B. R., M. Kéry, S. Ursenbacher, O. J. Hyman, and J. P. Collins. 2013. Site occupancy models in the analysis of environmental DNA presence/absence surveys: a case study of an emerging amphibian pathogen. Methods in Ecology and Evolution 4:646-653.

Important considerations for piloting an eDNA study:

- Stewart, Kathryn A. "Understanding the effects of biotic and abiotic factors on sources of aquatic environmental DNA." *Biodiversity and Conservation* 28.5 (2019): 983-1001.

How to report Limit of Detection:

- Klymus, KE, Merkes, CM, Allison, MJ, et al. Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*. 2020; 2: 271–282. https://doi.org/10.1002/edn3.29

The utility of eDNA in the pet trade:

- Brunner, J.L. Pooled samples and eDNA-based detection can facilitate the "clean trade" of aquatic animals. *Sci Rep* **10,** 10280 (2020). https://doi.org/10.1038/s41598-020-66280-7

Particle-bound eDNA:

- Turner, C.R., Barnes, M.A., Xu, C.C.Y., Jones, S.E., Jerde, C.L. and Lodge, D.M. (2014), Particle size distribution and optimal capture of aqueous macrobial eDNA. Methods Ecol Evol, 5: 676-684. https://doi.org/10.1111/2041-210X.12206

Additional Notes

Field hygiene practices

EVERYONE should be practicing field hygiene

- Whether or not you study disease
- In all habitats (fishing, animal collection, spelunking)
- To reduce the SPREAD of disease
- To protect the integrity of your samples (reduce contamination)

Cleaning equipment

- Scrub and remove mud
- Soak in disinfectant (lots of options!)
- Rinse and allow to completely dry

Use gloves

- New pair for EVERY ANIMAL
- Change often – when in doubt, change!

Interpreting your results

FALSE NEGATIVES

- NOT detecting the target DNA when it is present
- Possible causes
  - Sample collection
  - Low DNA quantity
  - Poor DNA quantity
  - Inhibition
  - Mutation at marker site

FALSE POSITIVES

- Detecting the target DNA when it is NOT present
- Possible causes
  - Sample contamination
  - Amplification of another species DNA
  - Mutation at marker site for either target or non-target species