eDNA Notes

B. Wright on 4/29/19, 2:10pm –

I wanted to follow up on our promise to initiate a data request, but first a quick question: Under Action Item #1, are the dates you have listed for meetings a through d proposed options that you would like everyone to weigh in on?

As for the data request, I would like to initiate the request on this group's behalf, but first I wanted to make sure we are on the same page with regard to the data we are requesting. Here is what I'm thinking, let me know your thoughts if you have them:

query all substantiated northern leopard frog occurrences front range wide (Larimer, Boulder, Denver, Jefferson, Douglas, Arapahoe, Adams)

fields to include in the query: UTM's, date, county, associated water body (if available), observer, data source (i.e. SciColl Permit, Citizen Science, CPW), life stages present (if available), other amphibians present (if available). Is there anything I am missing here that we should be asking for? ...recognizing that there will be a wide range of additional information available for each data point

Do we want to do a separate query for American Bullfrog or is the data that Lauren Livo compiled (assume someone has a shape file for that?) sufficient?

I would envision this as an opportunity incorporate your information for known breeding sites into our database if they are not already captured by CPW's data. To that end, I was wondering if each of you would be willing to compile a table of your known Northern Leopard Frog breeding sites. Such a table would include fields for geographic coordinates (UTM's preferred), year breeding was first documented, years breeding site was active, qualitative assessment of breeding site robustness (maybe scale of 1 to 5, or something like that), bullfrogs present (Y/N), bullfrogs actively managed (Y/N and method)

Finally, on the eDNA front, we have a paper that should be published very soon in the Journal of Herpetology titled, "Determining Presence of Rare Amphibian Species: Testing and Combining Novel Survey Methods." I will share it once it hits the press, as it has summarized results and methods from our eDNA work with boreal toads that would be of use if considering eDNA for leopard frogs. I'm also happy to demonstrate the protocol to anyone who thinks they might be interested in filtering water soon. In the meantime, we would need to figure out if there is already a published primer for Norther Leopard Frog that we could use, or if we want to develop our own primer. If you want me to, I can talk to John Woods at Pisces Molecular in Boulder, who has done all of our toad eDNA work. A couple of years ago when I was considering eDNA for leopard frogs there was not a primer available, but that may have changed since then. Again, let me know your thoughts.

Thanks,

Boyd

F. Boyd Wright III

Native Aquatic Species Biologist

Platte River Basin Aquatics

M. Kobza on 4/29/19, 5:24pm –

Just FYI, I had contacted several researchers about eDNA for frogs and chytrid over the past few years.

The Tangled Bank folks (JJ Apodaca) came up as a very reputable lab for eDNA.

USGS Fort Collins Science Center, Aquatic Systems Branch, has a frog expert, **Dr. Erin Muths**. Erin said this in an email from 10/2018: "We can assess water samples for leopard frog and the amphibian chytrid fungus (Batrachochytrium dendrobatidis, Bd) but would need some lead time to have assays running and tested. Cost would be approximately $50-60 per sample for DNA extraction and to run qPCR. Once the process is up and running, samples should be processed in approximately 4-6 weeks. It sounded like you would be collecting the samples. We have expertise in sample design, including eDNA sampling designs, to maximize detection that we could share with you. It is likely that we could do the sampling if needed, but again that would require lead time to schedule the trips as well as some cost. Please let us know exactly what questions you are working on answering and how we might be of help." 970-226-9474, muthse@usgs.gov. (Quan Dong is the branch chief of the Aquatic Systems branch of USGS and an old colleague from Florida who I trust very much).

Dr. Michael Young, Research Fisheries Biologist, Rocky Mountain Field Station USFS, said this: "**There is a northern leopard frog assay that was published by a group in Ontario (Beauclerc et al. 2018)**. We would have to perform optimization work to ensure that it would work in our lab, and we cannot vouch for how well it would work for this species in Colorado without additional testing of specimens in your area. But that group had access to

the tissue library that was part of the Barcode of Life database, and this is likely to be a reliable assay." **p: 406-542-3254, mkyoung@fs.fed.us. Paper attached.** [Beauclerc et al 2018]

In a follow-up to Michael, I asked about "optimizing" the kit, and using Smith-Roots eDNA field kit: "We can do it, with a little up-front investment. To design an assay from scratch is ~$10K. To optimize a published assay, sequence a handful of local tissues, and run a handful of samples to verify that the assay is working can be done for ~$2K plus 8% overhead (assuming you can bill for that total on a credit card, and assuming that this is a decent assay that doesn't require too much additional work). After that, individual samples are $85/site (for the first species) and $35/site (for each additional species, if desired), plus overhead. My understanding is that Smith-Root optimizes existing assays for their device. I think some of the assays they offer are ones we initially designed, but I may be in error. I am uncertain whether their device addresses inhibition, which we (see Jane et al. 2016, available on our eDNAtlas webpage) found long ago was fundamental to correct interpretation of eDNA sampling and has been a common issue when sampling wetlands for amphibians. But this is speculation on my part because we don't work directly with Smith-Root and are unfamiliar with their equipment. If you are dedicated to the Smith-Root platform, you should contact them directly about optimizing the existing assay for use on their equipment." [Smith-Root does address inhibition in their equipment].

Dr. Melanie Murphy, Univ. Wyoming, gave a talk at ESA and mentioned she developed a PCR test for NLF, and she wrote back in 6/2012 and could be contacted again: "I would be happy to talk about leopard frog water samples. We are refining the methods this summer. After optimization, I would estimate $30/sample to run the test." (307) 766-5295, melanie.murphy@uwyo.edu, https://sites.google.com/site/murphylabuwyo/

5. Finally, the Aquatic eDNAtlas Project has been running a while now with a neat eMap, and I guess Boyd is involved with this large program on native fish and toads. Leopard Frog is not in this system yet it appears.

-Mac

B. Wright on 4/30/19, 10:18am –

Hey Mac (everyone else please let me know if you want off this email string),

Thanks for doing all that leg work to get cost estimates for eDNA sampling. A quick point of clarification, none of my toad eDNA data is represented in the USFS eDNA atlas. The sampling you see in the Rockies of northern CO (still being processed) look like they are from RMNP. The eDNA Atlas is super cool. It's run through Mike Young's lab and I was fortunate enough to see Mike give a presentation on it a few years back at AFS. I am working with Matt Fairchild (USFS) to develop assays for plains topminnow and plains killifish and those are being run through Young's lab and I expect those should ultimately appear on the atlas.

For the most part, it looks like the "quotes" you revived are competitive if not better than our sole source contract with Pisces Molecular. For a new species, we would be looking at $5,300 for assay/optimization and then $93.50 per sample- it is a little unclear to me what the cost would be if we added a second species (i.e. Bd)- but I could talk to John Woods at Pisces about that. I know the work that Mike Young and the USFS are doing is top notch and they quickly established themselves as leaders in the aquatic eDNA world. That said, I know there is a considerable lag time for processing samples- just look at how many samples are are being processed according to the atlas. The costs and turnaround time that Erin provided for USGS's lab seem too good to be true! I contacted Melanie a few years ago about using their assay for NLF, and with their data not yet published at that time she was understandably reticent about sharing the assay with Pisces- looks like that has changed for the better. I think I will get to meet JJ at the upcoming PARCA workshop- will have to pick his brain. I will say that if we wanted to go after funding available through CPW (such as Species Conservation Trust Fund) to pay for eDNA samples (target year: 2020), it would probably be easiest to go through Pisces Molecular since we already have the sole source contract with them.

As Erin suggested, however, I think study design should be worked through first. Some considerations from the burgeoning body of literature on this topic (eDNA for amphibians and Bd) are that the number of samples at a given site is more important than the volume sampled, and that spatiatemporal scale of sampling is also critical. In our boreal toad paper, we recommend at least 10 samples during the breeding season spread across a given sample site. This doesn't necessarily equate to more cost, as different labs have different strategies for pooling samples- which is another good question to ask when you get quotes from the labs- can they reliably pool samples, how many samples in a pool, and cost per pool? Finally, pairing investigation of eDNA utility alongside of other more conventional sample methods will enable comparison of detection probabilities between methods, and would also add power to species occupancy models. As far as study design goes, I think the first step is the data request that I would like to work on alongside of compiling a list of known breeding sites. From there, perhaps we could look at a Dual Frame type study design where we use multiple survey methods to survey historic and

stratified random (spatially balanced) sites (e.g. NWI layer within specified counties and specified elevation range).

I'm attaching a good paper [Mosher et al 2018] on detecting Bd using eDNA sampling.

Thanks,

Boyd