**FY 2021 SCTF Funding Request**

**Project Title:** Investigation of Novel Sampling Methods to Assess Distribution of Northern Leopard Frogs, American Bullfrogs, and Chytrid Fungus in Northeastern Colorado

**Project Manager:** F. Boyd Wright, Native Aquatic Species Biologist, CPW

**Project # if on-going SCTF:**

**Project Start Date:**

**Project End Date:**

**Research or Management Project:**

**Description of the Proposal** (one-two sentences)**:**

This study will investigate novel survey methods, including environmental DNA (eDNA) and automated (call) recording devices (ARDs), with the goal of informing a consistent survey protocol. Such a protocol would optimize Northern Leopard Frog, American Bullfrog and *Bd* detection probabilities in an occupancy framework, and increase understanding of species status trends.

**Management Implications/ Project Benefits** (explain how information gathered or the actions taken to conserve, improve conditions for, or reduce threats to qualified species of concern)**:**

This project would enhance conservation efforts of Northern Leopard Frogs, a Tier 1 Species of Greatest Conservation Need (SGCN; CPW 2015) in Colorado by:

1. Improving our understanding of the distribution of Northern Leopard Frogs in northeastern Colorado, as well as the distribution of two invasive threats identified in Colorado’s State Wildlife Action Plan (SWAP; CPW 2015), American Bullfrogs and the Chytrid Fungus (*Batrochytrium dentrabititis; Bd*).
2. Developing a survey protocol that optimizes species detection based on multiple methods to increase consistency across various monitoring efforts. Such a protocol could be applied statewide and potentially for other amphibian species.
3. Testing novel methods that could considerably increase efficiency in amphibian survey efforts.
4. Fostering collaboration among CPW and multiple municipal open space programs, to benefit conservation status of the species.

Such benefits would represent marked progress toward information needs identified for the species in the SWAP, including ecology of *Bd* and susceptibility of the species, *Bd* status of populations in Colorado, and inventory to identify occupied habitats of Northern Leopard Frogs and American Bullfrogs.

**Introduction** (briefly summarize background and need)**:**

The Northern Leopard Frog (*Lithobates pipiens*) is broadly distributed across the United States, where it was once considered common (Smith and Keinath 2006). However, localized declines have been observed across the native range, particularly in the west, including Colorado (Johnson et al. 2011; CPW 2015). Colorado’s State Wildlife Action Plan (SWAP) identifies Northern Leopard Frogs as a Tier 1 Species of Greatest Conservation Need (SGCN). The decline of the species in Colorado has been notably prevalent east of the Continental divide and along the Front Range. Johnson et al. (2011) found that Northern Leopard Frogs occurred at only 1 of 65 historical sites (1.7%) they studied in northeastern Colorado, as compared to approximately 50% in western Colorado.

Colorado’s SWAP identifies three primary threats to Northern Leopard Frogs: (1) residential and commercial development, (2) problematic invasive species and pathogens, and (3) climate change and severe weather (CPW 2015). Habitat loss to development and invasive species and pathogens appear to be particularly problematic in northeastern Colorado. Of 65 sites where the species once occurred in northeastern Colorado, 21 no longer existed either due to destruction or drying (Johnson et al. 2011). The introduced American Bullfrog (*Lithobates catesbeianus)* is known to compete with leopard frogs, both through predation and competition for resources, and was found at 24.1% of historical Northern Leopard Frog sites and 31.9 % of randomly selected wetlands in northeastern Colorado (Johnson et al. 2011). The chyrtid fungus *(Batrochytrium dendratbatitis; Bd)* is known to be a driving factor in decline of certain amphibian species (Muths et al. 2003), but less is understood about its effect on Northern Leopard Frog populations (Johnson et al. 2011). However, it is difficult to detect *Bd* in the absence of amphibians, yet the absence of amphibians could be the result of a prior deleterious *Bd* outbreak (Johnson et al. 2011; Mosher et al. 2017)

Fortunately, there is a burgeoning grassroots movement supporting Northern Leopard Frog conservation in northeastern Colorado, involving multiple city and county open space programs. A Front Range Northern Leopard Frog Working Group has formed that involves participation from Boulder Open Space and Mountain Parks (BOSMP), Boulder County Parks and Open Space (BCPOS), City of Longmont Natural Areas, City of Fort Collins Natural Areas (FCNA), and Jefferson County Open Space (JCOS). A few of these agencies are deploying comprehensive bullfrog control and habitat restoration, while all of the agencies are either engaged in surveying habitat and monitoring populations, or developing such plans.

With CPW relying on local municipalities to monitor Northern Leopard Frogs on their lands, there is a need for a more standardized monitoring and surveillance approach that may utilize a range of available methods to optimize species detection. For example, JCOS is primarily relying on a combination of automated recording devices (ARDs) and nighttime auditory surveys for amphibian call detections, while BOSMP is conducting two visual estimate surveys (VES) at up to 111 wetlands per year. Other programs, such as BCPOS and FCNA are looking to CPW and other counties and cities for guidance as they increase monitoring and surveillance. None of these organizations are currently surveying for *Bd* in wetland habitat on their properties. Developing a standardized approach to monitoring leopard frogs and Bd could also be expanded to monitor the species across all of Colorado, and be potentially applied to other amphibian species.

Conventional methods to monitor amphibians include VES and nighttime call surveys for species with males that exhibit breeding calls, such as leopard frogs (Corn et al. 2000). Dosch et al. (2008) provided a VES protocol to survey wetlands in Colorado for Northern Leopard Frogs, though it is uncertain if the protocol is being followed by practitioners. Nighttime call surveys are utilized to detect breeding male amphibians that exhibit auditory calls to attract mates (Corn et al. 2000). These surveys are widely used because they can be accomplished by citizen scientist volunteers, but may be biased because timing of surveys do not always align with phenology of the target taxa (Corn et al. 2000).

A few novel methods are becoming increasingly utilized to detect amphibian species and model their occupancy. This SCTF project previously investigated the utility of environmental DNA (eDNA) to detect Boreal Toads and culminated in a peer reviewed publication (Bailey et al. 2019). That study suggested that practitioners employ multiple methods, including eDNA, to optimize Boreal Toad detections, but that eDNA samples should be supplementary to visual occupancy surveys which featured significantly higher detection probabilities. However, several studies investigating the use of eDNA to survey for “more aquatic” amphibians, such as American Bullfrogs and Eastern Hellbenders, suggest that the method could hold more promise for Northern Leopard Frogs (DeJean et al. 2012; Wineland et al 2018). A CPW pilot study conducted on BOSMP lands detected Northern Leopard Frog eDNA in 49 of 50 samples collected in waters where the species was known to occur. Environmental DNA has been shown to be less sensitive to detecting *Bd*, but this is an area that warrants additional research (Mosher et al. 2017). While automated recording devices (ARD) were once deemed less advantageous for monitoring calling amphibians, due to overburdening maintenance and staff time (Corn et al. 2000), recent technological advances, including terabytes of digital data storage and advanced frog call recognition models have increased their popularity (Crump and Houlahan 2016). Though costly, these devices have the distinct advantage of logging months of continuous call data, spanning the phenology of the target organism.

This study will investigate these novel methods and compare them with the other more conventional methods, with the goal of informing a survey protocol. Such a protocol would seek to optimize Northern Leopard Frog, American Bullfrog, and *Bd* detection probabilities in an occupancy framework. This would promote consistency in monitoring across the range of Northern Leopard Frogs in Colorado, thus aiding in better understanding of species status trends and enhancing the conservations status of the species.

**If project addresses a Tier II species (Statewide Wildlife Action Plan ranking), explain why/how species prioritized over Tier I species:**

**Progress to date** (if on-going SCTF work)**:**

This study represents a repurposing of remaining funds, while maintaining a similar scope to the original project. Previous work investigated the utility of eDNA to detect Boreal Toads in Colorado. This work was conducted in the SW region in 2014, the NW and SE Region in 2015, and in the NE Region in 2016, culminating in a peer reviewed publication (Bailey et al. 2019). We are requesting that the funds be slightly repurposed to follow a similar approach investigating the utility of eDNA and another novel method for detection of Northern Leopard Frog, American Bullfrog, and *Bd* in northeastern Colorado.

**Approach** (briefly describe procedures used to complete the project)**:**

Working in collaboration with municipal open space program biologists, this study will investigate 3 different survey methods for detection of Northern Leopard Frogs and American Bullfrogs, and two different survey methods for the detection of *Bd*. For the detection of leopard frogs and bullfrogs, we will test a combination of eDNA, ARDs, and VES. For the detection of Bd we will combine eDNA tests and qPCR from swabbed amphibians to detect *Bd* where amphibians are detected, which will inform detection probabilities of eDNA samples in the absence of amphibian detections. The general concept will be to complement existing monitoring programs where they already exist and pilot different survey protocols where monitoring programs are in the early stages of being developed.

Combining and analyzing multiple survey techniques across a range of taxa at a landscape scale is inherently complicated, but has the potential to be statistically powerful if strategically planned. Thus, the first phase of this project (FY 21) will include study design, which we would like to contract out to Dr. Larissa Bailey at Colorado State University (CSU). Dr. Bailey is a renowned statistician and herpetologist in the field of occupancy modeling, and played a critical role in the Boreal Toad study previously funded by this SCTF project (Bailey et al. 2019).

The second phase of the project will begin in April 2021 and conclude in August 2022 (FY 21-23) and will consist of all of the field work and data collection. Two technicians with strong herpetological backgrounds will be trained in using CPW’s eDNA protocol (CPW 2020) and deploying and maintaining ARDs. Depending on the study design developed in the first phase, they would utilize eDNA and ARDs to compliment other agency survey efforts on their properties. On lands where little such work is currently planned, they will utilize eDNA, ARDs, and VES. The majority of the work will be conducted in close coordination with the local land management agencies to include: BCPOS, BOSMP, JCOS, FCNA, and City of Longmont Natural Areas. Additionally, Douglas County is of interest due to several anecdotal accounts of robust Northern Leopard Frog populations lacking technical documentation. All laboratory work, including qPCR on eDNA and skin swab samples (for *Bd*) will be contracted to Pisces Molecular, Boulder, CO.

The third and final phase (FY 23) of the study will be to contract data analysis again to Dr. Bailey. Working with Dr. Bailey, we will prepare a final report and make recommendations for developing a monitoring and survey protocol.

**Deliverables or Expected Outcomes** (tie back to Management Implications/Project Benefits)**:**

Our goal is to report findings from this study in a technical report and/or a peer reviewed journal. Such a report would provide:

1. A description of the current distribution of northern leopard frogs, as well as invasive American Bullfrogs and *Bd*, in northeastern Colorado for comparison with past studies (Johnson et al. 2011). Our results would be bolstered by advanced techniques and investigation of geographic areas previously overlooked (e.g. Douglas County).
2. Recommendations for a standard survey protocol that would optimize species detection probability and could be applied to survey for the Northern Leopard frogs, American Bullfrogs, and *Bd* in all suitable habitats across Colorado. This will ultimately enhance our understanding of the species’ distribution and conservation status and make survey efforts more cost effective.

**Project Budget** (please round to the nearest five hundred dollars; rows can be inserted/deleted as needed)**:**

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| --- | --- | --- | --- | --- |
| **Object Group** | **FY 21** | **FY 22** | **FY 23** | **Total** |
| Study Design and Data Analysis | $ 9,000 |  | $ 9,000 | $ 18,000 |
| Technician time | $ 18,000 | $ 42,000 | $ 24,000 | $ 84,000 |
| eDNA filter Unit (500 total) | $ 10,500 |  |  | $ 10,500 |
| Leopard Frog eDNA test (n=500) |  | $ 10,000 | $ 10,000 | $ 20,000 |
| Bd eDNA test (n=500) |  | $ 9,000 | $ 9,000 | $ 18,000 |
| Bullfrog eDNA test (n=500) | $ 1,000 | $ 14,000 | $ 14,000 | $ 29,000 |
| Bd swab individual samples (n=120) |  | $ 2,500 | $ 2,500 | $ 5,000 |
| Song Meter SM4 Acoustic Recorder (12) | $ 10,000 |  |  | $ 10,000 |
|  |  |  |  |  |
|  |  |  |  |  |
| **Total Annual Cost** | **$ 58,500** | **$ 77,500** | **$ 68,500** |  |
| **Total Project Cost** |  |  |  | **$ 194,000** |

**Sources of funding that will support project:**

**Confirmed** (list individually)**:**

Aside from leveraged staff time, including that of CPW, BCPOS, BOSMP, JCOS, FCNA, and City of Longmont, no additional funds have been secured for this project.

**Pending** (list individually)**:**

Although no figures have been discussed, the City of Longmont has mentioned they have funding they would be willing to contribute to this project. Additionally, small grants of up to $10,000 are available through BCPOS and BOSM. We may seek funding though these programs if deemed necessary. Any additional funding would likely be used to increase the number of eDNA and swab samples.

**If pending funding not approved, what is your “plan B” to fund project?**

There are no alternate plans to fund this project.

**On-going project expenses after initial project work ends** (identify annual costs; length of on-going funding need; source of approved funding for on-going expenses)**:**

None.

**Project Personnel** (identify internal and external; ***indicate the extent to which internal collaboration has taken place with researchers; biologists; the appropriate senior biologists and AWM’s within the Regions (at a minimum)*** ; identify all internal units that have agreed to participate, if participation/coordination with multiple units is proposed).

*I have notified/coordinated/ clearly discussed the details of this proposal with all appropriate CPW staff from other Sections/Regions. The specific Sections/Regions and individuals identified below (including any comments and/or concerns expressed):*

*I have coordinated with CWCB staff on projects that may involve their funding and/or approval and have determined who the project lead is. The project lead will be responsible for implementing and monitoring any contracts, purchase orders, additional participant approvals and/or funding etc. The project lead is responsible for ensuring compliance with state fiscal and procurement rules (funding and/or approval details, additional participants/collaborators listed here):*

*If this project has a construction component, coordination with appropriate Capital Development (Engineering) staff is required. Determine: 1) if capital development staff time and/or resources needed and to what extent; 2) project is included in the large capital construction process (over $100,000) or the Wildlife Small Capital Process and have submitted the request. Details of staff time and/or resources needed and if large cap or small cap noted here as well as any comments or concerns expressed by Capital Development staff.*

Literature Cited

Bailey, L.L., P. Jones, K.G. Thompson, H.P. Foutz, J.M. Logan, F.B. Wright, and H.J. Crockett. 2019. Determining presence of a rare amphibian species: testing and combining novel survey methods. Journal of Herpetology 53(2): 115-124.

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