

Questions & Answers for CESU 18-08 Gopher Frog.

1. As the NFE understands the SOW, there will be a minimum of 60 samples (3 samples from 20 wetlands) taken each month during the breeding season. We have two questions about the number of samples to be taken:

a. How many months are you assuming constitute the breeding season? Should the NFE assume four months: January – April. **If the frogs are thought to breed within a certain timeframe, that is when they should be sampling and it should be in their proposal. The proposal is then evaluated for accuracy and completeness.**

b. NFE assumes that the SOW is requesting that ONCE each month, within two weeks of a significant rain event, these samples be taken. Please CONFIRM this is the intended sampling frequency, as opposed to that samples should be taken after any “significant rain event” during the breeding season, even if that means more than one sampling effort per month. If the latter, please define “significant rain event”. **Sentence 2 of SOW 2.0 states “on a monthly basis” which = once each month so the NFE is correct. It also says “Water samples within two weeks following significant rain events from selected wetlands on a monthly basis (weather permitting) during the possible breeding season.” So the monthly sampling should occur within two weeks of a significant rain event.**

c. Thus, the NFE assumes a minimum of four sampling efforts, with a minimum total of 240 water samples to be taken and tested, during the entirety of the project. **Yes, 240 minimum, but there are larger, more complex wetlands that may need more. Hence 2.0 states: “Three or more water samples of at least 250 mL will be collected at each wetland, determined by size and complexity of the wetland. “**

2. The SOW states: Established sequences/markers for gopher frogs should be utilized if possible, but new ones may need to be determined since species present is not assured. As we understand it, a marker has been developed/applied for eDNA testing for *Lithobates capito*. Can you clarify why a new marker might need to be developed? Are you concerned that the existing marker would not be sufficient and would need to be improved for a specific sub-population in the proximity of Arnold AFB?

And if so, please clarify that you would then expect improvements/validation to be conducted as possible using the two tissue samples, stored in formaldehyde, obtained in 1993 and 1997. Or are you requesting that the two tissue samples be tested for eDNA to confirm that they are *Lithobates capito*, as opposed to *L. aesopus* or *L. sevosus*; however, this would still not require new marker development given these tissues (if DNA was extractable) could be tested against the existing *L. capito* marker. Please CONFIRM if the NFE is misunderstanding the task. **Last sentence of 1.0 states “ due to nomenclature changes, we are unsure which species occurs on AAFB and it is possible it is the species protected under**

the ESA.” Second to last sentence of 2.0 states “, but new shall be determined since species present is not assured.” So if the species on AAFB is not L. capito, a new marker will need to be developed if it doesn’t already exist to fulfill the SOW requirements.

3. Is this work considered to be non-severable?

This work is non-severable.