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25 March 2025

Review of eDNA report for *Heleioporus australiacus* at Broadwater State Forest

Thank you for the opportunity for me to provide a review and feedback on the report produced by EnviroDNA. The report was produced in response to a review of the Management Plan for *Heleioporus australiacus* (Giant Burrowing Frog) in far southern NSW, which identified that the Plan was proving to be ineffective in meeting its aim to monitor the status of known populations in the region. This was because the species had not been located at four of five previously known locations and this prevented any assessment of the broader impacts of forestry activities on the species and what the effect might be on the status of the species in the region. The review led to a meeting to understand options moving forward. The focus then became finding alternative ways to determine if populations are present at the monitoring sites and, if they are no longer present, locate new monitoring sites. A clear objective was to understand if the previously identified populations still existed, indicating the proposed methods were inappropriate for monitoring, or if these populations were no longer present and the methods may still be relevant for any populations that could be found.

This led to a discussion for the potential for the sampling of environmental DNA (eDNA) as a means to establish if Giant Burrowing Frogs are present at a site, to be completed through collection of water samples. The discussion focused on how easily this approach could be employed for sampling and how effective it would be in detecting the frog if it was present. Of specific interest was the distance downstream an extant population of the Giant Burrowing Frog could be detected. It was hoped that eDNA could locate frogs hundreds to thousands of metres away and so be used to provide a potential catchment level of detection for the species, thereby allowing faster detection over broad areas. Hence an important aim for understanding the use of eDNA was to determine the distances and relative dilutions of eDNA that could be effectively detected with eDNA water samples.

The scope of work for this review then is to consider the EnviroDNA report and metadata submitted to the Commission in August 2024 (attached) and other available literature on eDNA to comment on:

- Is the pilot sufficient to make decisions on eDNA as a survey method for GBF?
- Noting the results of the eDNA surveys and the aims of the Species Management Plan, is there potential for eDNA to locate new or existing GBF populations and show if they are stable or declining?
- If no, what are the issues or concerns with the eDNA method? Discuss flow rates, population density, life cycle, detectability etc
- Is further work required to better understand if this method is suitable for use for the GBF? If yes, what is required?

The report itself is easy to read and the approach used follows recommended standard approaches to eDNA sampling (e.g. Rees et al. 2014). It provides a logical run through of the process and explains how the results were assessed and conclusions reached.

Is the pilot sufficient to make decisions on the use of eDNA as a survey method? Yes, but with some caveats. The pilot has been able to clearly identify that the eDNA approach will confidently detect the presence of Giant Burrowing Frogs, at least where there are tadpoles present. As there has not been sampling at known sites at times when tadpoles are not present (I think that there is typically continuous recruitment) it is not possible to determine how well it detects adult frogs alone. As the adult frogs typically only enter the water for very short periods when calling or laying eggs, the period to detect the presence through adult frogs alone is likely very limited and uncertain right now if they provide enough DNA to be detectable. eDNA material typically lasts for not more than 5 days and likely at most 10 days, depending on conditions (Harrison et al. 2019). It is maybe as easy to just sample for tadpoles as they are often relatively visible and so be as detectable compared to using eDNA, but the advantage is that people with no skill in tadpole survey ID skills and minimal time can still easily do the eDNA sampling whereas tadpole surveys take some skill and experience. So, in summary, the tests show that detecting eDNA where tadpoles are present is a reliable method to confirm species presence. It is unlikely to detect the frog where tadpoles are not present, at least with any confidence. This could be applied to any area where there is water by taking three samples at any point of interest and expect to detect presence if there are tadpoles present.

It was discussed at the initial eDNA meeting that, if possible, testing be included that could provide information on how far at least downstream eDNA could be detected in order to understand at what scale this approach could be used to assess the presence of Giant Burrowing Frogs. That is, could information be obtained that would indicate how regularly along a stream sampling needed to talk place to be confident that the frog was present or absent on that stream and/or adjacent feeder streams. The eDNA report does supply some limited evidence on this from the sampling with the second period of sampling where tadpoles were and a positive ID of eDNA was obtained at Site 4-8, no tadpoles were seen but there was still a positive ID for Site 4-12 (less than 200 m downstream) but no tadpoles and no eDNA was detected a Site 4-16, which was another 250 m or so further away downstream. This is not inconsistent in with the findings of Harrison et al. (2019) where DNA dispersion is highly variable and dependent on flow rates and chemical conditions and could be hundreds of metres, as indicated here, to potentially greater than 100 km. The data available here is limited, but does indicate eDNA cannot reliably be detected in this environment (albeit at just the one time) more than 300-400 m downstream, providing a minimum sampling distance to work on. This was when water flows were high and pools connected and so likely represent a reasonable starting point for minimal distance between samples.

Another discussion point at the initial eDNA discussion meeting was to try to collect information on the influence of density of tadpoles, understanding that low densities of tadpoles would likely impact eDNA detection rates. It would be reasonably assumed that high densities of tadpoles would lead to more available eDNA and so better detections, but numbers/densities of tadpoles were not recorded to compare with detection rates. The data may be too coarse to make this a viable option anyway and it appears that moderate numbers of tadpoles were consistently present, thus preventing a useful assessment to be made with the available sampling.

eDNA sampling at this time cannot be used to undertake monitoring as proposed in the Giant Burrowing Frog Management Plan. This proposed the monitoring of adult frogs and tadpoles to provide data to understand population trends and changes over time to assess if mitigations were maintaining local populations. This is best done with counts of individuals to compere numbers of frogs/tadpoles present over time. The use of eDNA was proposed as a means to determine if a population was present, but was not expected to be a sensitive method (at least at this time) for assessing local population status and changes. It can determine if the frog is present, but cannot say anything about the size of that population – so one frog is the same as 100 frogs or 10 tadpoles the same as 10,000 tadpoles. It just says that there is DNA present and so the species is present. It cannot tell if the population is in clear decline or is increasing. It cannot tell if the DNA came from an adult frog or a tadpole. A declining population would not be clearly evident from eDNA sampling. It is possible that declining populations could be identified by monitoring multiple points through time and, as detection rates dropped, this would indicate a declining population. Where tadpoles are present only two to three samples are needed for 95% confidence in detecting the frog. Hence a declining detection rate may be usable for detecting declining populations, but it would be a coarse approach and declines may not be identifiable until after the decline can no longer be reversed. The potential to use this approach needs sensitivity testing for statistical power. This is something that should be explored with a biometrician.

Given the above considerations, there are some areas that could be considered for further investigation in regards to eDNA. The most important one is to understand the effect of tadpole density on detection. The density of tadpoles at the Broadwater sites have apparently always been high given that they are constantly seen, whereas they have not been observed in the other locations. This indicates that, if present, tadpole densities in the other monitoring sites are low and it is not clear how easily eDNA sampling can detect the presence of tadpoles in such situations. If there were only one tenth of the tadpole numbers would three samples still be adequate for 95% confidence in detection of would it need to be double or triple the number or would each sample need to include double or triple the number of subsamples. This is important as the greatest value that eDNA sampling may have is to at least determine that a population of the Giant Burrowing Frog is present somewhere within the catchment so that decisions to allocate resources to conservation can be made. But we do not have any understanding of how well the process detects the presence of tadpoles when they do occur, but at very low densities. It may be possible to return to the current sampling locations and take counts of tadpoles to match up to eDNA samples, but I don't know if low number of tadpoles can be located or that if the counts would be accurate enough to provide confidence in the assessment. Another option would be to take a more experimental approach and place set densities of tadpoles into holding areas and then sample those to provide a more direct measure of detections and graph the changes to see if there are thresholds or patterns in detections and what is a suitable level of sampling to provide confidence in detecting eDNA when tadpole densities are very low.

The question of how far downstream the eDNA can be detected downstream from where they occur still relatively uncertain. Although the sampling I think provided some useful information, it was only one location where tadpoles were absent and the detection was a positive and only one absence location with a negative detection. Given that eDNA detection is affected by a whole range of biotic and abiotic factors (Shogren et al. 2017; Harrison et al. 2019), the results may be quite different even within different locations on the same stream. Completing a few more

similar tests in other areas would help to determine how variable are the results in the distance of detection using eDNA for downstream populations that could greatly assist in determining suitable sampling regimes to have confidence in detecting the presence of the Giant Burrowing Frog in a catchment.

Flow rates of water could also be considered for further assessment, but my experience with this species including at the Broadwater Road site is that mostly flow rates are very low to nil. It would be simpler to just ensure that sampling was completed consistently in similar circumstances as it is likely that higher flow rates will disperse DNA rapidly and make detections much less likely. It could be argued that higher flow rates may increase opportunities to record eDNA as it provides a large "catchment" from which material may be sampled. This is possible, but eDNA typically disperses quickly from the source point and it would seem probable that it would become almost undetectable under a high flow rate where the larger volumes of water would dilute the presence of eDNA (but see above point that might provide some evidence on what is detectable).

In summary, the use of eDNA appears to be a viable means of detecting the presence of the Giant Burrowing Frog at a given point of a stream or in a specific water body where tadpoles are present. It is not clear if it able to detect the presence of adult frogs alone and likely is not. As it only provides for a presence/absence answer it cannot provide detailed data to assess/monitor population status and declines in a given location, just extinction. It may be possible to provide a coarse estimate of decline through the number of negative vs positive samples obtained through a series of repeated samplings. The main use of eDNA at this time would appear to be as a means to determine if a population of the species still exists within the catchments of the populations being monitored under the Management Plan or to survey for new populations. If a detection is obtained through eDNA, other monitoring methods would be required to achieve more sensitive population monitoring. This could be the currently recommended traditional methods of tadpole capture and spotlighting for adults. These appear to work well at the Broadwater Road site where the population remains large and is easily detected. If populations are small at other locations, as indicated by the lack of success in monitoring them, then this approach may not be practical. More detailed monitoring using genetic structuring of populations would be a worthwhile consideration and talking to an expert in this area should be considered to assess its viability. But presence of the other populations needs to be established first before it is worth taking this path, at least as far as progressing the current Management Plan is concerned. In the 1990s and 2000s individuals in the Yambulla State Forest population was relatively easy to locate after rainfall and this might be the best option for targeting if more work is to be considered.

Regards,

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References

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