	Webinar Q&A | Thursday 3rd October 2024	
	Webinar: Beyond tracks and scats: The power of eDNA for aquatic monitoring	
Question Details #	Question	Answer
1	Is the data submitted to EnviroDNA publicly available ?	We currently don't publish your data publicly after completion of your project with us (it's your IP). However, you are able to upload your data after you receive it to a public database such as ALA.
2	Would appreciate if you can comment on the sampling techniques. How many samples are required and what is the depth of sampling in water?	This is very much dependent on type of sytstem, target organisms and objectives of your survey.
3	Can eDNA be used to determine HOW MUCH of a particular species is present	Great question: eDNA analysis can be both quantitative and qualitative (depending on the analysis undertaken), we usually can't get down to specific numbers of individuals in a population - but relative abundance of species is very typical. Survey design is very important here as a number of factors can influence DNA concenrations at a site level.
		how do you determine relative abundance?
4	Can this technology be used for plants too? also interested in this question and the detection of other diseases that can impact aquatic species, eg frogs	Hi Daniel, yes it can be! We have multiple plant metabarcoding assays that we can employ depending on your interest. We also have an assay for Chytrid in frogs - occassionally detected in water but more commonly from swabbing frogs directly.
5	What can you tell us about using eDNA in the event of a HPAI outbreak, to identify areas of water contamination?	eDNA is used to monitor for viruses commonly (although, at enviroDNA we don't do much with human pathogens - typically requires quite strict lab certifications). Pathogen detection works largely the same as anything else with the probe based technology often being employed. A known concentration is usually run alongside samples to figure out viral load / concentration. To add on, Brett - we can use this technology for diseases that impact other species as well, and often use it for Chytrid fungus in frogs!
6	Hi. Great topic! I know CSIRO has a National bioDiversity DNA Library - but is comparison with this library dependent on the DNA test procedure?	Most of our sequences come from GenBank as a global repository (although we do some internal sequencing to bolster this). I'm not sure if CSIRO library links to GenBank.
7	Can you associate the type of found eDNA to the abundance of a species in the water body or could that really be just one animal/bacteria/fungus/protist?	A number of studies have linked abundnace to DNA concentrations but there are a number of factors that can influence this. Sampling design needs to be carefully considered but we have used this technique to quantify effective ness of e.g. carp control measures.
8	How long will eDNA be present and detectable for after a species left the waterway?	good question!! Depends on a number of factors but typically 1-7 days.
		This is a great question! DNA tends to degrade pretty quickly in water (from as little as 1 day to a week, depending on weather / flow / temperature). Therefore, eDNA is a good measure of what is in a waterway at any given time, and is not very accumulative.
9	Yes - as a more specific clarification I have on Dominek's question.....is eDNA just present/ absense or can it also give an indication of numbers also?	Hi Cherie, thank you for your question! Haylo outlined this a little in Peter Cook's question but yes, eDNA data does give presence/ absence data so for any metabarcoding analyses you get a good understanding of species richness. Typically, we can't get down to specific numbers of individuals in a population, however, relative abundance of species is typical. Site occupancy can also be used a different metric for a population size/health (i prefer this for platypus these days).
10	With detecting different life stages, can they be differentiated?	We don't typically differentiate life stages using eDNA - traditional methods may be better suited for this. There may be scope for this kind of work in the future, but a significant amount of R&D would need to be done before assessing whether it is doable!
10a	Thank! Since gametes have only half the chromosomes, can they be identified? It would be useful to know if we are finding egg/sperm or organisms when targeting broadcast spawners.	eDNA techniques almost always target mDNA as there are more copies than nuclear DNA. We certainly can see massive spikes in DNA during fish spawning which is presumably from gametes.
11	With Water Quality it needs a chain of custody form & tested in a NATA lab to be defendable in court.....is there an equivalent process/use limit for eDNA?	TO clarify this query, what QA/QC apply to ensure results are defendable in court? Cheers
		There are no national/inetrnational standards for eDNA processes yet. Each lab will (should!) have there own QA/QC and can vary deepnding on techniques used. Whether a court will accept this is a lawyer question!
12	Can you separate stocked v natural / naturalised fish species?	eDNA itself can't tell us which species are stocked / naturalised. We tap into other databases to help inform us on this!
13	What is the recommended decontamination solution? I have seen bleach used, as well as Decon90 in industry. Particularly when collecting eDNA samples from groundwater in water bores.	We use bleach and also an enzyme based decontamination spray (LookOut is the brand name), for when bleach is too corrosive for what we are trying to decontaminate (machines / computers / anything metal).
14	Could this be used to detect contamination and what type of contamination? Would this work in shallow groundwater sources potentially such as river/creek alluvial units? How does pH and salinity affect testing results?	In theory, any type of biological contamination can be detected if appropriate genetic information is available and an assay can be properly developed and validated. Yes, can absolutley work in groundwater - DNA tends to be pretty sparse just like the stygofauna. Salinity doesn't seem to be a problem (used in marine situations), pH likely contributes to faster DNA degradation.
15	Further to Ross' question can you comment on extensiveness of eDNA reference libraries with respect to different areas of Australia (eg regional SA vs Vic) and different species groups (eg stygofauna?)	Not easily in this forum as that's pretty complex! Stygofauna in general is pretty poorly characterised (although I believe there is heaps of data sitting with various consultants). However, even without identification, we can use OTU's/ASV's as metrics of diversity and similarties between aquifers or to assess temporal changes.
16	Can this approach be used to identify plant species in scats? How fresh would the scats need to be?	Great question! We have completed projects previously for plant species idenitification in scats. For any scat analysis, as fresh as possible is always best. It is possible but results can vary depending on on the plant species you are trying to idetify due to how closely related they can be and hybridisation as well. Another important part is how well researched the plant species may be and if they are present in reference databases. Having tissue samples from the plants in the region/ species of interest can also be helpful so we can sequence these and ensure it is in our reference database.
		Thanks Emma, I am looking for a method to identify species that are consuming seedlings of river red gum, coolibah and black box. This is likely to included domestic sheep and cows, feral goats, kangaroos and wallabies. I know the red gum genome has been sequenced. Not sure about the other trees.
17	Is there a way to determine how close/far the target species is from where the sample is taken from?	Generally, not. But you can design your sampling to take sequential samples throughout a waterway, and areas with higher concentration of your species DNA would be indicative of source. There is also scope in bioinformatics / datascience to produce some simulations around water flow to try and figure out where the DNA may have been sourced, but they're estimations at most.
18	How long after the species has been in an area can the analysis be able to detect it? For migratory species that go over long distances?	Depends on a number of factors but typically 1-7 days in aquatic systems.
19	What degree of detection differentiation is possible when seeking eDNA matches for closely-related, yet potentially more threatened, species/subspecies?	Great question, and unfortunately the answer is it depends! It depends on the specific species, and what their genomes look like (how much differentiation are we working with here, how much genetic data is out there for us to work with). With probe based (qPCR) detection, we can usually design a probe around small areas of variation - so if the variation is there in the genomes, we can try and design a probe to differentiate the species / subspecies.
20	Why are the assays different "north" and south" ..is their dna slightly different?	Not entirely clear on this question, but you can have genetic variation across a species range that may requrie different assays. Generally, the regions we use for assays are reasonably well conserved within species. Best practise is always to get a local tissue sample to verify the assay (if it hasn't been used in that area before).
21	ie. one species is threatened perhaps critically so and the other is not	
22	Have you looked into the application of eDNA for determining the impacts of contaminants on the biodiversity? or using eDNA to define extent of contamination in the nevironment by assessing the biodiversity data from eDNA?	We often don't know what the ultimate use of the daat we produce gets used for. However, we have had a couple of contamination events where in areas where we were doing previous monitoring and were able to go back afterwrads. The comparisons were pretty stark! Getting pre-event data is always problematic.
23	How long does DNA last in the environment?	Great question. The answer is somewhat dull - it depends on the environment. Temperature, humidity, how much sun, water turbidity all play into how quickly DNA degrades. In freshwater DNA can last for a couple days to a week.
24	What if a bird eats a specific fish species (say A) from a watershed, and flies to another watershed, drops the excrement in a new place where it is not observed. Will it not give a wrong assessment of biodiversity? How does eDNA address this issue?	Great question, and this is definitely something we are always working on. We generally double check all of our eDNA detections against preexisting databases built from years of traditional surveying, so if anything seems out of place - we can pick up on it and try and investigate further.
		Okay, that makes sense! So it needs to be backed by other physical surveys to back eDNA identification. Thank you!
		Not always but groundtruthing with other data sources helps to flag unusual detections. The analysis can say whether DNA is present but not where it has come from. 99% of the time, presence of DNA will indicate presence of speices. There are indicators in the data of what may be contamination vs actual presence (e.g. number of reads, detection across multiple replicates). If it is critical, resampling always recommended to confirm.
25	If you analyse a sample, can the DNA breakdown be used at a later date to identify a different species than currently detectable?	Great question Naomi. We hold onto the DNA produced from samples for up to a few years, and generally have quite a large volume of DNA from every sample. We can always go back and reanalyse the DNA for newer assays/ new discoveries.
		Thank you.
26	Given that eDNA degrades over time, how does EnviroLab assure that sample integrity is maintaned from creek - storage - lab so we can be confident that what we are sampling is what is being tested? And have there been any case studies of regulator acceptance / uptake of eDNA results in approval determinations?	Hey Dan, great question. We use a DNA preservative in our samples, which is placed onto the filter as soon as the sample is taken. We also can use (more expensive) self-preserving filters that can preserve DNA at room temperature for up to a couple months. We give directions to clients about how to keep their samples, and maximum timelines for sample shipping/storage to make sure the DNA is in the best shape it can be in. eDNA is still making its way into policy and how to best be accepted / used in these cases - watch this space!
27	Were they ground parrots being fed on by foxes?	Re-introduced Plains Wanderer was the example given predated on by fox, given along with the Great White shark predated on by the Orca.
		cheers Allie
28	Does the positive indication indicate that the species is present in the environment or that the specied has come into contact with the water sampled. As a random example the presence of frogs in shallow groundwater samples?	Isnt that the same thing? If a species has come in contact with the water, then it is present in the surrounding environment.
29	Are there some species you have had trouble developing probes for? Do you mean the same thing as a primer when you use the term probe.	Hey Patrick, we generally use a primer pair (foward + reverse) as well as fluorescence tagged probe (that will bind to a region in between the primers in the amplified DNA) in our qPCR assays. Species that are not very differentiated from close relatives, or subspecies, can be a big challenge designing probes for (e.g. pygmy perch!).
		My basic ecological brain often interchanges probe/primer/assay during talks (sorry!).
30	Is there anything we can do to help build the reference database?? (i.e. if there are endemic species in our area we are interested in etc)	Hi Cherie, thanks for this question! For any projects where you may have species of interest that aren't currently in our reference databases and you are able to obtain a tissue sample for this species - ethically of course! Then you can send this to us so we can sequence and include it in our reference database so it can be identified if the DNA is present.
		Fantastic - thanks Emma. I'll contact Enviro DNA to find out more details on the correct process if others in our team want to pursue this :)
31	thanks Haylo - plenty of different scenarios to consider there, across suites of different attributes. What about instances where there are larger areas of variation, can probe design be tweaked (accurately) enough or does it need to be used in conjunction with perhaps other tools?	If there's a large area of variation, we can usually design a reliable qPCR probe for it. It's all about what is there for us to work with!
32	Is there a global biodiversity DNA library that one can refer to when analyzing eDNA? If yes, is it accessible to researchers?	Yes - GenBank.
33	Is there any published (or upcoming) literature on using eDNA to detect sewage contamination?	I did a search - alot

34	Do you a database for identifying stygofuana?	The vast majority of sequences we use come from a public global database - GenBank. Stygofauna are pretty poorly charaacterised (although I recenetly discovered there is actually a heap of gentic data sitting with various consultants). We can use OTU/ASV's to characterise diversity without identifications tho.
35	Could eDNA be used to map connectivity of underground karst systems?	think its a really inetrsting application. Not aware of it being done to date though. In theory, you could use to test for simarities between genetic sequences between aquifers to infer connectivity.
36	I'm interested in soil sampling of for ecohealth. Are there sufficient assays for macro/micro organisms to allow development of a project now, or is it a future direction?	I'm also interested in this question, as it relates to the invertebrates and bacteria and associated with hypoxia and eutrophication
		Presumanly E. coli is often detectable, and you say there is some quantitive level of assessment, so that could be one level of ecohealth assessment
		I'm not a presenter, but I have done microbial analysis in marine sediments and it works really really well.
		Absolutely can be done now recognising that not everyhting going to be able to identified. Typically a combination of baceria/fungi used to assess soil biome/health. Would love to see this start to happen in aquatic systems as well.
37	I believe this is a great technology for disease detection in aquaculture. What do you believe is the minimum concentration of a disease vector that you can detect. For example, out of a pond containing 500,000 prawns, how many prawns need to be sick before you can detect the virus, causing the disease?	Several factors will impact detection rates, primarily sensitivity/specificty of assay and then how much virus DNA is actually shed into water. Most of our assays are typically in the detection range <1 DNA copy per uL.
38	Josh this looks like a good tool for use in applications to EPA for using high quality recycled water for beneficial environmental flow to assist meeting shortfall targets for waterways. Evidence based risk assessment to the health of the waterways is required for these applications.	Absolutely, get in touch to chat about what this may look like.
39	Hi - I'm really curious hew definite the results are and the level of uncertainty for false positivesb or false absenses is known ???	Depends on tye of analysis but there's a number of studies that have repeatedly shown false negatives are much lower than traditional techniques and false positive rate is very low. What is the false negative and false positive rates of your current methods?
40	What is the ball park cost of sample analysis?	Varies quite a bit depending on type of analysis, number of assays, total samples etc.
41	How robust is the sampling and analyses methods with regards to verifiable and repeatable results, with reference to been tested in legal juristriction?	Cant comment of legal aspects, but methods of analysis are pretty well developed and robust. Many of the methods are what has been used in genetics for decades just applied in a different setting.
42	can you give us a rough idea on the cost of analysis?	Varies quite a bit depending on type of analysis, number of assays, total samples etc.
44	How much would it cost to set up a group in an organisation with the device and tools to do sampling independently?	Sampling can be as simple as manual syringe and filters (~$10/sample) to automated pumps ($3000-$15,000).
45	Curious if this technology has ever been used to prove the existence of a previously unknown species/declared extinct species?	Not that I'm aware of but we've certainly been involved in projects rhat have expended known ranges (particularly crytpic species like mussels, burrowing crayfish etc).
46	Does that mean we are missing benthc data by doing water samples in top ten cm at high tide? i.e should we be sampling at low tide, close to shore	Interested in the benthic questions - and could be broken down further into epibenthic, hyporheic - should get different results above and within sediments. Would be interested in that
		Potentially, depending on depth. Close to shore there is likely to be good mixing from wave action, but less so further out. Impacts of depth and currents need to be considered for marine sampling, not as simple as laminar river flows.