#	Question	Answer
1	How is eDNA being used in identifying regions of ecological significance, and how does it link to other monitoring aspects?	eDNA allows us to detect a wide range of organisms, including rare, cryptic, or threatened species, all from one method of sampling. This wide breadth of data can help to identify biodiversity hotspots, ecological corridors, and areas of high conservation value, you may also use this data for natural capital accounting. Like any biodievrsity/species detection data, the results can be correlated with environmental and habitat data (e.g remote sensing, land use, water quality etc etc).
2	Hi Josh, Is eDNA robust enough to be use in determining marine benthic infauna communities, to determine macro-invert populations for monitoring? In addition to Shona's question I wondered if you can determine abundance of marine invertebrates through eDNA?	Yes, although resolution to species level will be unlikley for most taxa (typically Family/Genus). Water smpling at depth would be required to better tagert benthic communitiies. Sediment sampling is also possible. Abundance estimates are not possible but other population metrics like site occupancy can be used.
3	How can/can eDNA be used in assessing soil health and/or plant health?	Soil microbiome can be assessed using eDNA. Translating that data into "health" requires existing baselines of health for that soil type or habitat. Increasingly, reference sites are being used to baseline data.
4	Where there is little existing environmental information on downstream environments, what is the feasibility of using eDNA to make preliminary assessments of the potential impact of the construction of or potential failure of a water storage or dam?	Absolutley, eDNA can be used to determine presence of threatened species, or whole community analyses to provide greater power to assess impacts. Any impact assessment should have before and after monitoring to quantify impacts.

5	I would like to know about the database of eDNA system.	I assume this is refering to reference libraries? The vast majority of reefernce sequences for specioes is derived from public repositories (e.g. NCBI). EnviroDNA does internal sequencing to help fill gaps for particular species as required if tisue samples can be sourced.
6	in what extent that you you use eDNA as a tool to analize a river either polluted or not, disturbed, failed or not?	eDNA can be very useful for assessing river health. By analysing changes over time in biological communities such as fish, macroinvertebrates, and microbes, eDNA can help indicate whether a river is healthy, disturbed, or impacted by pollution. You may also use this data to compare the biodiversity levels across different rivers to assess health or changes in environments.
7	Have any new species been detected through eDNA?	eDNA relies on comparison against existing reference databases, so it cannot directly identify a completely new species that has never been genetically characterised.
8	What's the timeframe for detecting species? Eg sampling a creek, will the eDNA results have data from just the last 24hrs, or longer timescale?	eDNA persistence varies depending on the environment and conditions, but generally in surface water, eDNA typically persists from 1-7 days. DNA can persist for longer in sediment or cave environments. Studies have used eDNA on sediment cores to determine historical fauna compositions.
15	how long does the dna last in the system?	
9	What could be a potential reason that sediment eDNA from certain lakes will show hardly any positives, even though prior surveys have shown a species' presence? Could this be organic material? Acidity? Rapid digestion? Any ideas?	Generally, only very small volumes of solid substrates like sediment can be processed (<1g) so large sample numbers may be required to detect lake species. Sediments can also contain PCR inhibitors which should be minimised durign extraction and assessed during processing.

10	In interpretation how do we account for the differentials in rates of DNA shedding, and in different carriers (eg turbid v clean water)	Knowledge of species biology (e.g. breeding activity) should be used for interpretation of any survey data. Results can also be correlated with any environmental or sampling data (i.e. sample volumes, river flow, turbidity etc)
11	how are probes created for endangered (rare) species? how is that validated to ensure specimens from different populations have the same markers? (to ensure there are not false negatives)?	qPCR assays are derived from existing sequence data and tissue samples from target species to determine a unique geen region for the species. This needs to be carefully validated against known co-occurring species to ensure specificity. Sequences/tissues from across the target species range helps understand genetic variability (gene regions taregeted are usually conserved) and determine regional specificity.
12	if it is accurate what is your graph showing a possible detection? Shouldn't it just show present or not present?	Trace amounts of DNA or faint positive detectipons can arise from reasons other than species presence. Therefore threshold criteria should always be applied before concludign species presence depending on level of confidence required.
13	How much sample (water and soil) do you need to do Biodiversity assessment?	This will verey much depend on the system, spatial scope of project, project objectives etc. We can get some information from a small amount of water, better information on more water. Replicate samples at each site and replicate sites across a study area are important and need to be considered when designing the sampling program to meet project objectives.
14	Is there public access to any information collected to date?	Most fo the data that EnviroDNA produces is the Intellectual Property of our clients. Some orgnaisations make this data available or upload to public databases (e.g. ALA).

16	So this requires a set of library of DNA to match the samples. How would you use this process for different regions/ countries that have species' DNA not available in the library? Where could we possibly get additional library?	The databases currently used are global but some countries hjave better coverage than others. Targeted sequencing to fill gaps is always possible if tissue smples can be obtained. Even without speceis resolution, useful data can obtained from Genus/Family data and biodiversity assessment can be done at a sequence level only (ASV's). Reference libraries are rapidly growing globally.
17	What role (if any) does machine learning or AI play in recognising detection?	As with most data intensive applications, AI will be increasingly used in this space. Most of the analysis done now is using code/algorithms and AI will be natural progression from this. Currently, we ensure all outputs have a manual/human sense check and groundtruthing to improve confidence.
19	Is there a set of environmental data that you can collect which can be predictive of the duration that eDNA markers will persist in the environment	Not reliably. DNA source, UV, oxygen, temperature, bacterial communites can all impact DNA degeradation rates.