



Subterranean Fauna and eDNA Survey Outcomes

Subterranean Fauna Research Program

June, 2024

Report prepared by: The Western Australian Biodiversity Science Institute

Acknowledgements

WABSI acknowledges the Department of Water and Environmental Regulation for providing input into creating the eDNA survey.

We are particularly grateful to the survey respondents and thank the eDNA workshop participants for their time, expertise and for sharing information in an open, respectful and constructive way.

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Contents

Acknowledgements	1
Contents	2
1. Background	3
Subterranean Fauna.....	3
The Subterranean Fauna Research Program	3
The Western Australia Biodiversity Science Institute	3
2. Subterranean Fauna and eDNA workshop	4
3. Survey Outcomes	5
Overview of Survey	5
Question 1 - What is your role?	5
Question 2 - How long have you been involved with subterranean fauna in your work?	6
Question 3 - Do you provide subterranean fauna survey services?	6
Question 4 - Do you provide eDNA analysis services?.....	7
Question 5 - How familiar were you with eDNA before the workshop?	7
Question 6 - Are you currently using eDNA?	8
Question 7 - If you are not using eDNA, why not? Other concerns, please specify.	8
Question 8 - Based on the presentation at the workshop, do you think eDNA is a useful tool for: ..	9
Question 9 - When do you plan to use eDNA?	10
Question 10 - Would you be willing to share eDNA and subterranean fauna specimen data by contributing to open access databases (i.e. BOLD, GenBank or similar)?	10
Question 11 - What phase of the assessment process do you think is reasonable to make data publicly available?	11
Question 12 - Do you have any comments on making data publicly available?	11
Question 13 - Would your organisation be willing to contribute to further research to advance eDNA?	12
Question 14 - Do you have specific research questions you would like to see addressed? E.g. degradation time of DNA was discussed as part of the eDNA workshop.....	13

1. Background

Subterranean Fauna

Subterranean environments contain a unique and diverse fauna: either aquatic, living in the groundwater (stygofauna), or air-breathing, living in rock voids above the water table (troglofauna).

The decision by the Western Australian Environmental Protection Agency (EPA) in the mid-1990s to recognise subterranean fauna as a potential factor to be considered in environmental impact assessments highlighted the dearth of information available to make informed decisions.

Since then, research on this group of mainly invertebrates has grown exponentially. However, much of this research has focused on taxonomy, diversity and evolutionary history, and recent reviews have indicated that large knowledge gaps still exist in relation to their basic biology and ecology.

The Subterranean Fauna Research Program

In 2018 WABSI published the Subterranean Fauna Research Program. The specific objective of the program is to address research gaps that were identified by end-users, and to encourage complementarity and collaboration, rather than duplication of research effort.

The research program is designed to be a collaborative effort with expertise from multiple disciplines contributing. A strong governance structure has been established, including a steering committee to oversee the program and to ensure end-user expectations are being met.

Overarching objectives of project clusters in this program are to:

- Develop a standardised best practice approach for recognising species boundaries based on multiple criteria
- Refine and test new sampling and survey protocols to ensure contemporary approaches are efficient, repeatable and effective
- Develop, test and apply a generic approach for advanced subterranean fauna assessment based on fine-resolution dynamic three-dimensional habitat characterisation
- Characterise ecosystem function and food webs of subterranean environments
- Determine the response, resilience to and persistence after change in habitat conditions for stygofauna and troglofauna
- Establish laboratory-based subterranean fauna breeding programs for selected species
- Consolidate existing subterranean fauna records and associated habitat attributes in a publicly accessible information system

The Western Australia Biodiversity Science Institute

WABSI is an independent, collaboration mechanism facilitating relevant, end user driven biodiversity science research. It is a joint venture partnership with leading Western Australian science research organisations, with multi-disciplinary research expertise.

WABSI research programs bring together a diversity of stakeholders to achieve consensus on the most important factors limiting progress against challenges of great importance for biodiversity conservation.

Who we are



2. Subterranean Fauna and eDNA workshop

WABSI hosted a workshop on Wednesday 28th February 2024 at the Kieran McNamara Conservation Science Centre in Kensington, Western Australia.

Aim of the workshop was to find out more about the latest developments around the use of eDNA in relation to subterranean fauna monitoring in Western Australia.

The ARC Linkage project: *Taking eDNA underground: transforming assessment of subterranean ecosystems* has wrapped up. The aim of the project was to develop a novel environmental DNA (eDNA) approach using new assays and a DNA barcode reference library for comprehensive, accurate, cost effective and reproducible monitoring of the Pilbara's unique groundwater communities. These eDNA approaches will also be applicable to groundwater monitoring across Australia and elsewhere.

During the workshop researchers presented the project's research outcomes and took part in an extended discussion with industry and regulators on integrating these outcomes into bioassessment and monitoring of subterranean fauna in Western Australia.

An outcome of the workshop was the need for a survey, to review the current use of eDNA and how stakeholders would like to use it in the future. Outcomes of the survey are described in this report.

3. Survey Outcomes

Overview of Survey

An online survey was conducted to collect representative data on the current and future use of eDNA in subterranean fauna surveys. The survey was developed using an online platform, Qualtrics (QualtricsXM, Provo, UT, USA). The survey could be completed by either a single person or multiple people from the same organisation.

An email with a link to the survey was sent directly to participants of the eDNA workshop, and the survey was also promoted through the Environmental Consultants association e-newsletter and the Environment Institute of Australia & New Zealand (WA Division) social media pages.

The survey collected data on the role of respondents, involvement in subterranean fauna work, involvement in eDNA work, familiarity and opinion on eDNA use, willingness to contribute to an open access database, and comments on publicly available data. The survey also asked respondents to detail specific research questions they would like to see addressed.

The survey was open from the 15th of April to 14th of May 2024. A total of 33 recorded responses to the survey was recorded. However, only 24 of the 33 responses were completed in full. There were six responses partially completed, and three responses that were blank entries. The blank entries were not counted towards the results of the survey, while the questions completed in the partial responses were included in the results. Questions requiring written responses were commonly unanswered indicating that respondents did not have time to answer or did not have anything to add to the response.

Question 1 - What is your role?

All 30 participants provided responses to the question, giving a solid overview of industry influence over the survey results. The largest sector is the Consultant role with 11 people, followed by Research and Proponent with an equal seven. Additional roles identified includes a Strategic policy/Government role and a student.

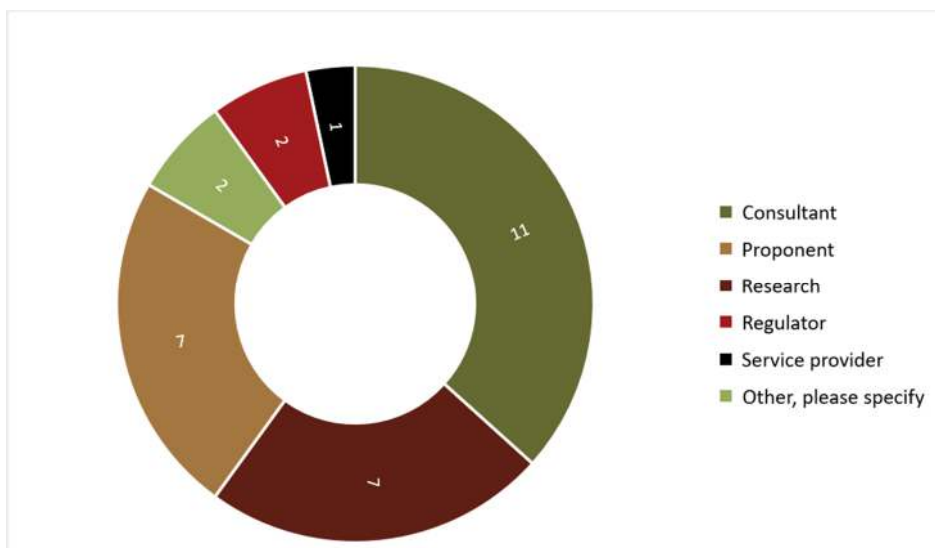


Figure 1: Overview of the role of respondents.

Question 2 - How long have you been involved with subterranean fauna in your work?

All 30 participants provided responses to the question. A majority of respondents have been involved with subterranean fauna for more than five years. While the rest of the respondents are consistently distributed between three to five years, one to three years, and less than a year. Suggesting that respondents possess considerable expertise in this field and are likely to provide responses grounded in substantial experience.

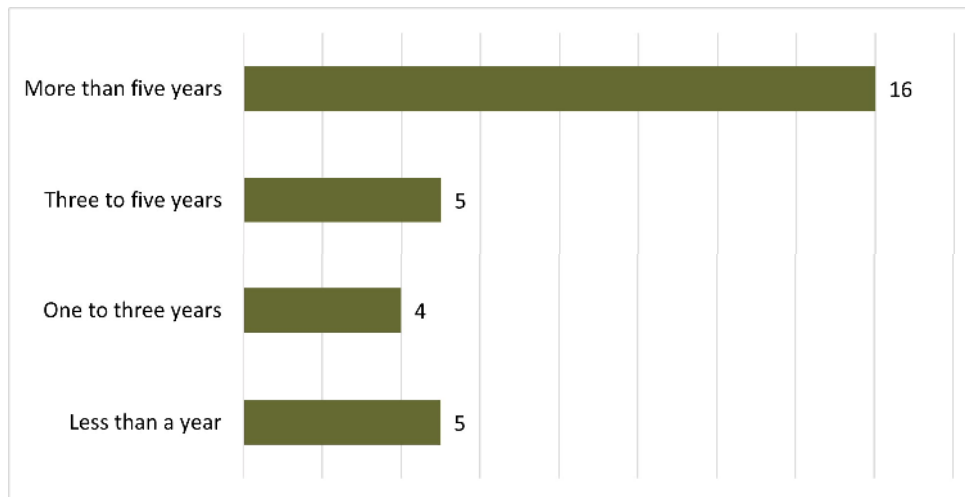


Figure 2: Overview of involvement with subterranean fauna.

Question 3 - Do you provide subterranean fauna survey services?

All 30 participants provided responses to the question. The majority of respondents indicated that they do not offer subterranean fauna survey services. This contrasts with the findings of the previous question, wherein most respondents demonstrated significant experience in subterranean fauna-related work. However, despite their expertise in this field, the majority do not possess experience specifically in providing survey services.

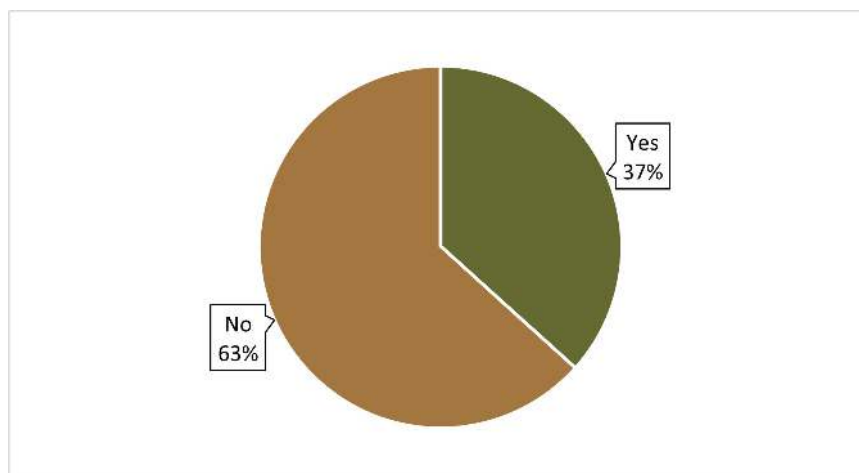


Figure 3: Analysis of the use of subterranean fauna survey services.

Question 4 - Do you provide eDNA analysis services?

All 30 participants provided responses to this question. Similar to the findings of question 3, the majority of respondents indicated that they do not offer eDNA analysis services. This reaffirms the reliance of current groundwater survey techniques on traditional taxonomy and DNA sequencing of individual specimens. Consequently, the establishment of eDNA analysis services would likely enhance these survey methods.

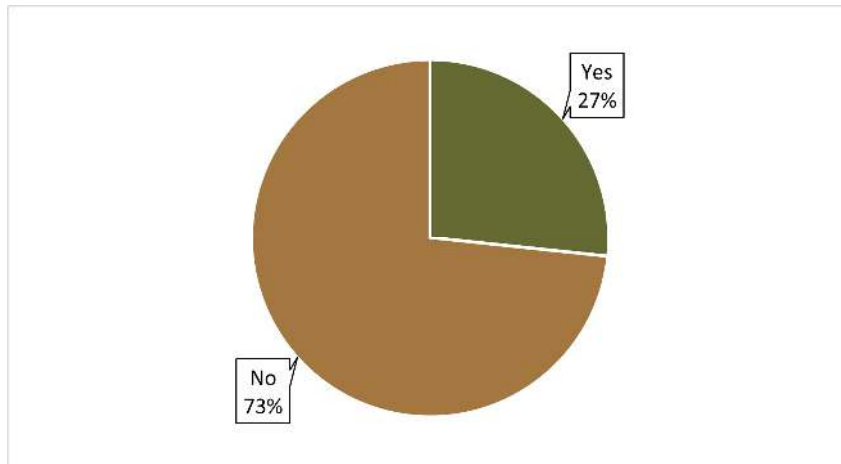


Figure 4: Analysis of the use of eDNA analysis services.

Question 5 - How familiar were you with eDNA before the workshop?

Of the 27 participants who responded to this question, all possessed at least a basic level of familiarity with eDNA prior to the workshop. The majority indicated being either very familiar or extremely familiar with eDNA. This suggests a high level of pre-existing knowledge and understanding among the participants regarding eDNA technology.

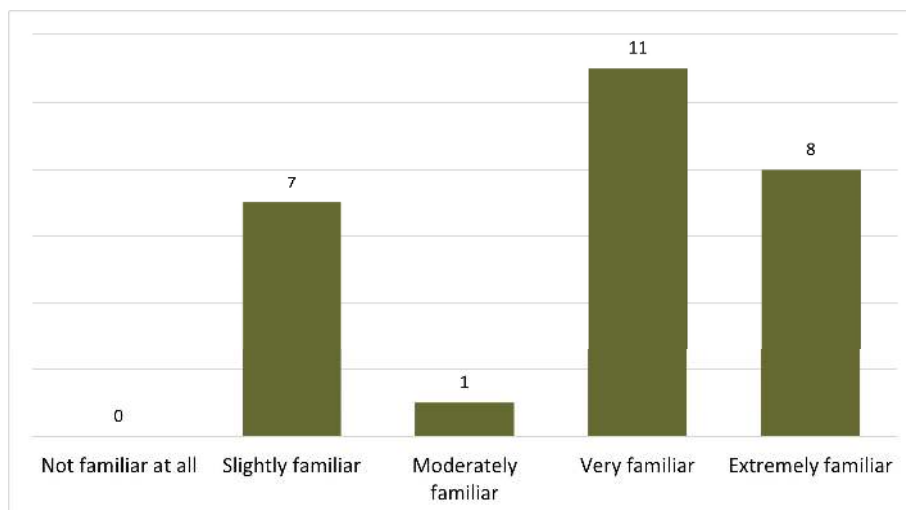


Figure 5: Familiarity with eDNA before the workshop.

Question 6 - Are you currently using eDNA?

From the 27 participants who responded to this question, it was found that the majority are presently utilising eDNA. The level of familiarity demonstrated in question 5 can thus be attributed to this current usage of eDNA. However, as observed in Question 4, most respondents are not offering eDNA analysis services. Therefore, the current use of eDNA likely pertains to other applications or purposes beyond the provision of analysis services.

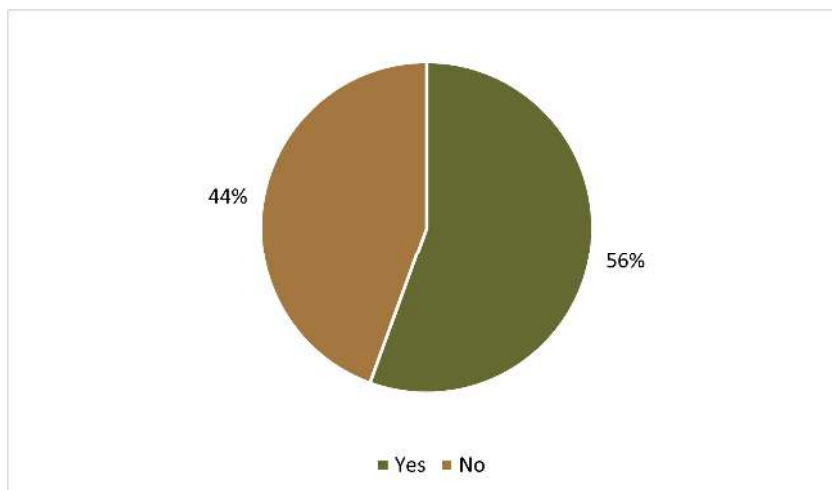


Figure 6: Overview of the current use of eDNA.

Question 7 - If you are not using eDNA, why not? Other concerns, please specify.

We had 18 participants who responded to this question, it is notable that the response rate is lower, possibly due to the question's relevance primarily to those not currently utilising eDNA. As established in the previous question, the majority of respondents are indeed using eDNA, which could have led to uncertainty or lack of applicability for some participants, resulting in a lower response rate.

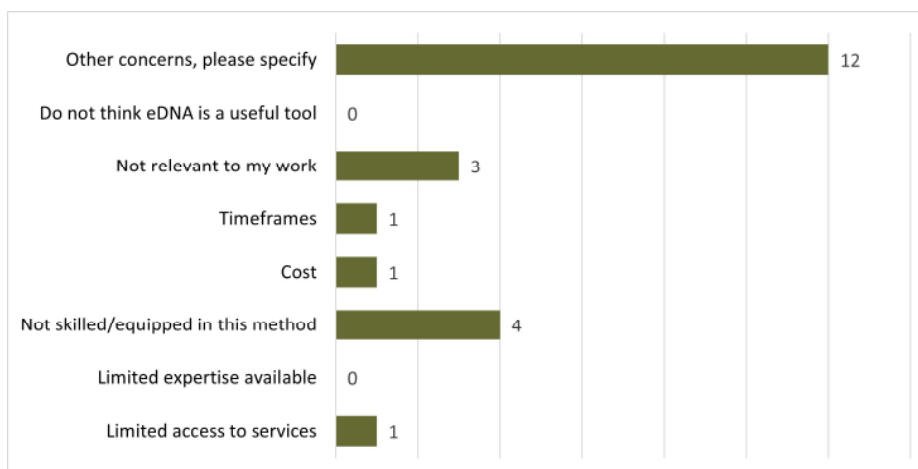


Figure 7: Why are people not using eDNA an overview.

Figure 7.5 outlines additional concerns gathered from the responses to question 7. Despite receiving 12 votes for the 'Other concerns' option, only seven specific responses were provided. The remaining responses consisted of blank or N/A entries, or explicitly stated the use of eDNA, rendering them irrelevant to this inquiry. Among the specific concerns received were inquiries regarding the efficacy of eDNA for small animals and its scalability for large-scale applications. Furthermore, uncertainty surrounding the current utilisation of eDNA was also noted among the responses.

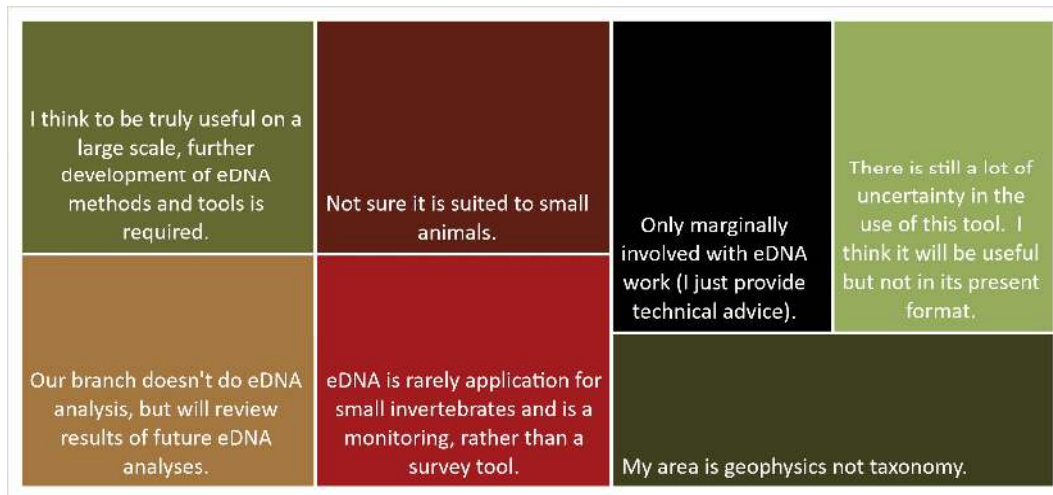


Figure 7.5: Other concerns why people are not using eDNA.

Question 8 - Based on the presentation at the workshop, do you think eDNA is a useful tool for:

Out of the 26 participants who responded to this question, the majority selected 'Targeted survey', accounting for 81 per cent of the responses. Following closely behind, 'Monitoring' was chosen by 69 per cent of respondents. In contrast, 'Detailed survey' was the least favoured option, selected by only 27 per cent of participants. This distribution suggests a clear preference among respondents for 'Targeted survey' and 'Monitoring' methodologies over 'Detailed survey' approaches.

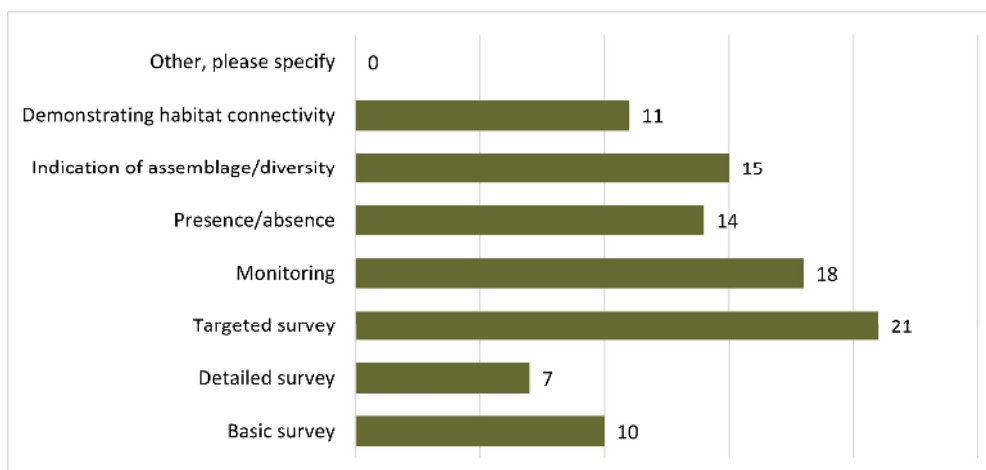


Figure 8: Overview of the use of eDNA, based on the presentation at the workshop.

Question 9 - When do you plan to use eDNA?

From the 26 participants who responded to this question, the majority of respondents are indeed currently utilising eDNA confirming the previous findings. The remaining respondents are evenly distributed across various timeframes, including in the immediate future, in two years, and in five years.

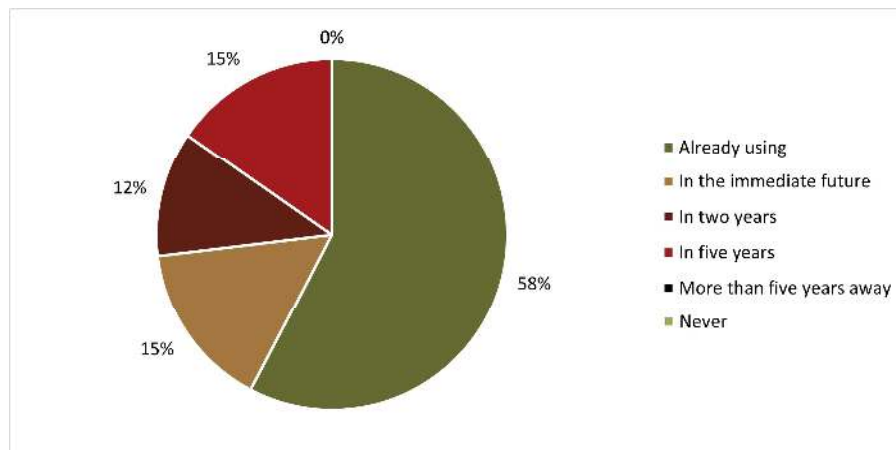


Figure 9: Overview of future plans to use eDNA.

Question 10 - Would you be willing to share eDNA and subterranean fauna specimen data by contributing to open access databases (i.e. BOLD, GenBank or similar)?

Out of the 26 participants who responded to this question, the majority expressed high levels of optimism, with 'Extremely likely' being the most selected option, followed by 'Somewhat likely'. This indicates a promising outlook for the future of open access databases in sharing subterranean fauna data. However, it is important to note that there were still some respondents who remained neutral on the subject, and one participant who expressed a 'Somewhat unlikely' probability to contribute.

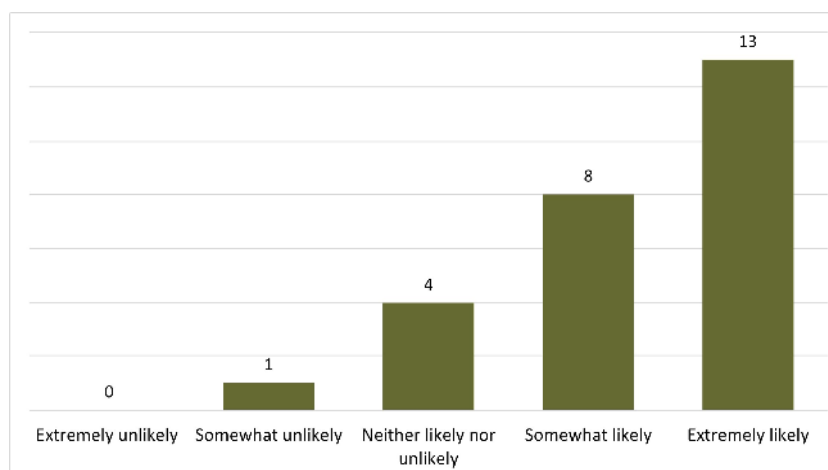


Figure 10: Overview of eDNA and subterranean fauna contribution to open access databases.

Question 11 - What phase of the assessment process do you think is reasonable to make data publicly available?

Out of the 26 participants who responded to this question, the results are diverse, lacking a clear majority. Slightly leading the responses is 'Post assessment' with 10 votes, while 'During assessment' and 'On completion' are tied with eight votes each. This indicates that while individuals are inclined to adopt publicly available data, there is uncertainty regarding the optimal timing for its release to the public.

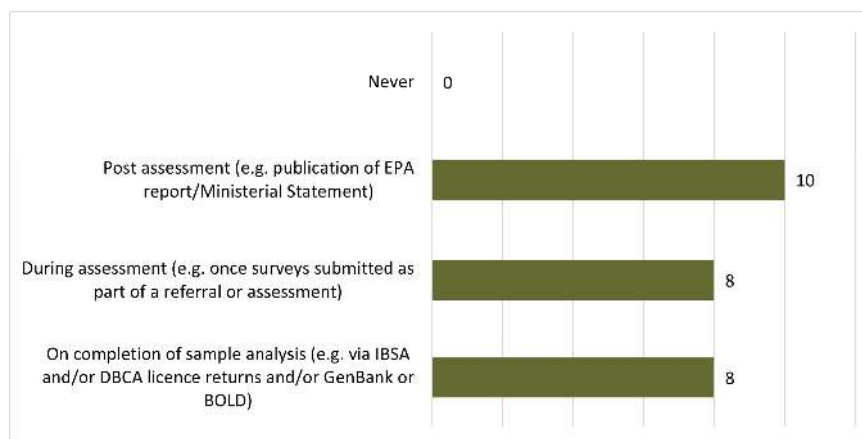


Figure 11: Overview of which phase of the assessment process data should be publicly available.

Question 12 - Do you have any comments on making data publicly available?

Following on from the previous question, it is notable that only 11 responses were received for Question 12, indicating that not all respondents had specific comments to contribute on making data publicly available. Most of the comments are reinforcing the idea that data should be made publicly available, while acknowledging some difficulties in doing so. All responses are listed below (Figure 12), but highlighting some key remarks:

“It is in everyone's best interest to make data sharable among relevant users ASAP after being generated. Making it public (e.g., GenBank) is a different category of access, and I think we need to be careful not to conflate them. A centralised database of seq data needs to be maintained somewhere, that can be accessed while protecting clients' proprietary concerns, but ensuring we don't continue to generate OTU synonyms and incomplete OTU distributions. We have one of the most powerful tools to understand species distribution across the state, and we are instead using it to create more biodiversity confusion, rather than less.”

“As a service provider we have contractual obligations to the Client, most notably around the ability to share data. Further we can only invest a small amount of time finding everything in there and assessing it if it's okay to release. We don't specifically screen for potentially problematic content so would need acknowledgement of the risks in doing that.”



Figure 12: Specific comments on making data publicly available.

Question 13 - Would your organisation be willing to contribute to further research to advance eDNA?

From the 23 participants who responded to this question, the responses were largely divided between 'Yes' and 'Maybe', with only one respondent selecting 'No'. Given the majority indicating a positive response to further research on eDNA, it can be inferred that there is support from various sectors to advance this area of study.

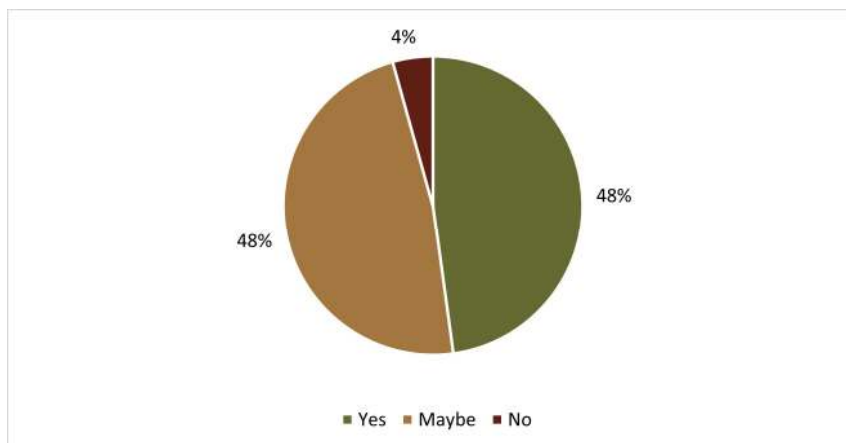


Figure 13: Overview of the willingness to contribute to further research to advance eDNA.

Question 14 - Do you have specific research questions you would like to see addressed? E.g. degradation time of DNA was discussed as part of the eDNA workshop.

From the eight participants who responded to this question, specific research questions were provided, offering a diverse range of areas for further investigation and presenting potential avenues for advancing the adoption of eDNA as a tool for subterranean fauna surveying.