Case study: Improved fish survey outcomes using eDNA in aquatic ecological monitoring, contrasted to traditional techniques

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Key points

- eDNA was effective in completing a more comprehensive appraisal of fish assemblages over a larger area than traditional netting or observer techniques utilised in the original baseline assessment.
- Adopting eDNA resulted in significantly improved safety outcomes, which were a critical consideration in the hazardous terrain present across the study area, and represented a more cost-effective approach.
- Standardising survey effort, and the detection of additional terrestrial and semi-aquatic species provided additional benefits.
- A combination of traditional and eDNA sampling techniques may yield optimal detections.

Abstract

Niche (a specialist biodiversity and heritage consultancy) undertook an updated baseline aquatic ecological survey in autumn and spring 2024 on behalf of the Marulan South Limestone Mine, in accordance with approval requirements. This established an updated assessment of conditions and captured temporal variation in stream health compared to the original baseline assessment completed in spring 2014 and autumn 2015. Survey occurred within Bungonia and Morton National Parks across highly rugged terrain. This terrain presented significant challenges to completing the fish survey during the original baseline assessment and therefore novel approaches were considered during the updated assessment.

Niche completed the fish survey component using eDNA sampling as an alternative to traditional techniques to improve survey effectiveness, safety during collection and reduce cost. Utilising the original dataset enabled comparison of the eDNA approach with traditional survey methods to assess whether these aims were achieved.

The study demonstrated the efficacy of eDNA sampling in achieving the monitoring program objectives. Fish species detections were improved in comparison to traditional techniques, with survey effort increased and standardised across sites. Adoption of this approach also reduced field safety risks and overall program costs.

This case study provided evidence that eDNA can be a cost-effective tool in aquatic ecological monitoring, particularly for fish surveys in rugged or hazardous environments, where it improved survey effectiveness, safety and value.

Keywords

Aquatic ecology, ecological monitoring, fish, eDNA, traditional survey

Introduction

Niche undertook a baseline aquatic ecological assessment in autumn and spring 2024 on behalf of the Marulan South Limestone Mine (Niche, 2024), in accordance with approval requirements (Marulan South Limestone Mine, 2022). The purpose of the assessment was to establish an updated understanding of baseline aquatic ecology conditions and capture any temporal variation in stream health condition (water quality, macroinvertebrates and fish assemblages). This is compared to the

original baseline assessment which was completed in spring 2014 and autumn 2015 (Niche, 2018). The appraisal of the fish assemblages present is the focus of this study.

Survey occurred within Bungonia and Morton National Parks across highly rugged and hazardous terrain, including very steep grades in remote areas, around major rivers and tributaries (Figure 1). Hazardous conditions were exacerbated by extreme rainfall events prior to the 2024 surveys. Consequently, the terrain presented significant challenges to completing fish survey using traditional sampling techniques in an effective and safe manner.

These challenges promoted the adoption of using eDNA (environmental Deoxyribonucleic Acid) as an emerging technique to assess fish assemblages that was well suited to the study environment and project aims. This also presented a unique opportunity to contrast the efficacy of eDNA sampling to the traditional survey techniques applied in the original baseline (spring 2014 and autumn 2015) surveys. Studies have shown that eDNA metabarcoding can be more effective than traditional techniques in some settings (McColl-Gausden et al., 2020). As part of the uptake of emerging techniques such as eDNA sampling, there is a need to evaluate its performance against traditional techniques, including an assessment of its cost-effectiveness (Lahoz-Monfort & Tingley, 2018). Accordingly, this study aimed to evaluate the performance of eDNA sampling relative to traditional survey methods, including its effectiveness, safety, and cost-efficiency in the study environment.

Figure 1 Examples of hazardous terrain

Methods

A total of 18 monitoring sites were established as part of the baseline studies (Figure 2) using a Before-After, Control-Impact design (Underwood, 1997). All sites addressed in the original baseline were assessed in the updated baseline. Fish survey was not completed at the three Main Gully sites due to habitat unsuitability.

Original baseline assessment (Niche, 2018)

Fish survey was conducted in Marulan Creek, Bungonia Creek, Barbers Creek and Shoalhaven River during both spring 2014 and autumn 2015 monitoring. Survey was conducted at nine of the monitoring sites, opportunistically located within proximity of macroinvertebrate sampling sites, representing upstream and downstream fish assemblages. Surveys were undertaken using fyke netting, bait traps, seine nets and visual observation. Where possible, effort targeted Macquarie Perch *Macquaria australasica*. including targeted nocturnal spotlighting for this species. Fish surveys were described as 'limited', because not all techniques were used at all sites due to difficult/remote access habitat constraints, and time required to set traps. As such, the survey was opportunistic in nature and provides for presence/absence consideration of fauna rather than repeatable measures of abundance per unit effort.

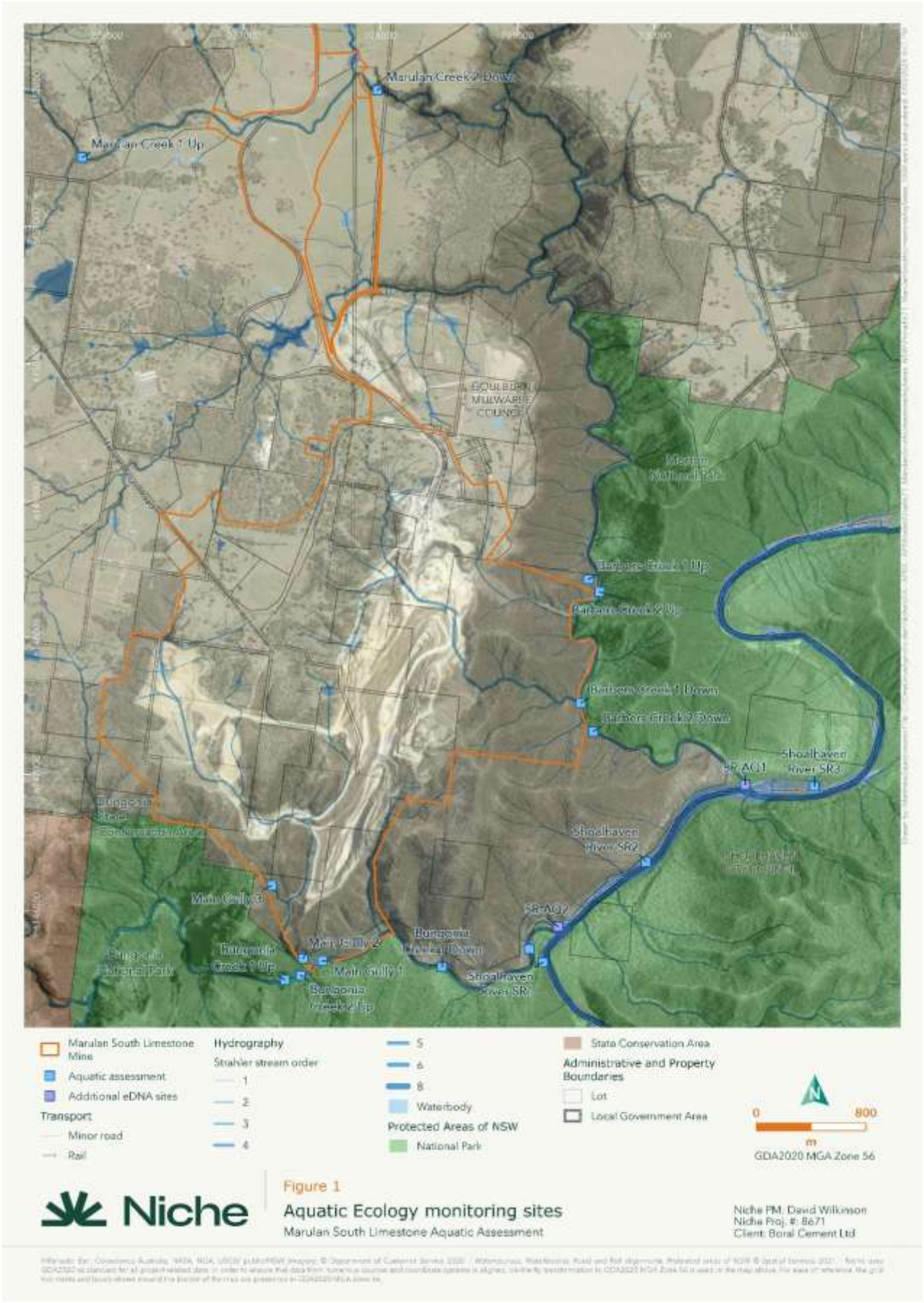

Figure 2 Aquatic ecology monitoring sites

Updated baseline assessment (Niche, 2024)

Sampling of fish assemblages was completed during the spring 2024 survey only, using eDNA sampling. A single survey was considered sufficient to provide a suitable appraisal of the fish assemblage present, commensurate to the project aims. Survey was completed in spring as the threatened species that could conceivably occur were more likely to occur in this season. The timing also avoided the effects of significant flooding that occurred prior to the autumn survey, driven by an extreme rainfall event. A total of 30 samples were collected from 15 sites (Figure 2), including two additional fish sampling sites (SR-AQ1, SR-AQ2) added to augment assessment within the larger Shoalhaven River. Two samples (replicates) were collected at each site, by passing up to 1000 millilitres of sampled water through a 1.2 µm disc filter. Filtration was conducted on site to reduce potential DNA degradation or contamination during transport of water samples, and a preservative added. The samples were then transferred to a laboratory for analysis. Extracted DNA from the filters was analysed through the use of two rounds of Polymerase Chain Reaction (PCR) and comparison to a metabarcoding library of species DNA. This library included all fish species previously detected during the original baseline (Niche 2018) and two threatened fish species of relevance to the program, being the Australian Grayling *Prototroctes maraena* and the Macquarie Perch.

Limitations

There is a temporal element to the comparison that is acknowledged as the study did not assess methods concurrently against the same fish assemblage. Comparative assessments of detection however, been addressed elsewhere in the literature (e.g. Piggot et al. 2020). Nevertheless, this comparison provides a useful case study for evaluating eDNA beyond detection success alone, including its practical application, utility and cost-effectiveness. Furthermore, given the remote area and National Park status, no external pressures are anticipated to have significantly changed the residing fish assemblages. There is an imbalance in number of sites addressed between the baseline assessments, as the eDNA approach enabled a greater number of sampling points to be established, however this is considered as a benefit of the method (eDNA vs traditional sampling approach) rather than a limitation to this comparison.

The eDNA library utilised in this assessment included vertebrates, specifically fish. It did not include freshwater crustacea. It is not always possible to resolve all taxa to the species level in eDNA analysis. This may be due to inadequate genetic sequence data available in the reference library for the region. In metabarcoding, often only a small sequence of a species genome is interrogated, and there is not always enough genetic variation in the marker sequence utilised to differentiate different species. For example, there is difficulty in accurately distinguishing between closely related Galaxias species. In this study, 75% of all taxa were resolved to species level.

Results

Survey design, safety and costs

Fish sampling was completed at 15 monitoring sites during the updated baseline surveys (six more than the original). Estimates of distances at which eDNA sampling can detect biota are variable in literature (McColl-Gausden et al., 2020). General guidance from the commercial laboratory indicated that eDNA data can be expected to provide information on the presence of species up to 1 kilometre upstream of the sampling point, depending on factors such as connectivity and flow (which were high in this study). Therefore, the updated baseline effectively assessed a larger extent of the stream network than the original assessment.

Project costs are commercial in confidence. However, key differences in survey approach achieved a relative cost reduction for the updated baseline surveys. The original baseline surveys required three team members to carry additional equipment required and completed fish survey during two (autumn and spring) survey seasons. By contrast, the updated baseline surveys required only two team members and fish survey was completed during spring only. During the original baseline surveys, team

members were required to camp on-site due to the effort in transporting equipment into the sites, time required to set nets and nocturnal survey. The small size of the eDNA sampling equipment and reduced time required for sampling, together with the removal of nocturnal survey requirements, eliminated the need for on-site camping, thereby reducing exposure to associated safety risks. While eDNA sampling incurs higher direct costs associated with equipment and laboratory analysis, the overall program cost is reduced due to lower field effort requirements, including fewer personnel, reduced survey duration, and simplified logistics.

Fish assemblage data

A high level of filtered water was achieved during sampling, with the average across samples being 913 millilitres (a maximum of 1000 millilitres may be filtered per sample). A total of eight fish species were recorded across seven monitoring sites during the original baseline surveys, over two seasons (Table 1). Two species were recorded in autumn only; Flathead Gudgeon *Philypnodon grandiceps*, and Australian Bass *Macquaria novemaculeata*. A total of 10 species were recorded across 13 monitoring sites, in the updated baseline (Table 2). Additional species detected were; Short-finned *Eel Anguilla australis*, Dwarf Flathead Gudgeon *Philypnodon macrostomus* and Eel-tailed Catfish *Tandanus tandanus*. In addition, there were detections from four taxa that were not resolved to species level. Of these, no detections of species from the Carp Gudgeon or the Salmon and Trout genus were recorded during the original baseline surveys, and are considered to be additional taxa recorded in the updated baseline. It is likely that the genus of Galaxias detections are the Mountain Galaxias *Galaxias olidus* recorded during the original baseline. The genus of Goby detections may represent sequence reads for Cox's Gudgeon that were not resolved to the species level. Both Flathead Gudgeon and Australian Bass were detected in spring (previously only recorded in autumn). When site data were aggregated by watercourse (e.g. across Shoalhaven River sites), there were 11 instances in which eDNA detected a species not recorded by traditional techniques. This is compared to two instances where traditional techniques identified species that were not detected by eDNA sampling. These instances occurred most commonly within the Shoalhaven River.

Table 1 Fish survey data collected during the original baseline surveys, adapted from Niche (2018)

Species	Season	Sites and number of individuals caught (observed)								
		Bungonia Creek upstream	Bungonia Creek downstr	Barbers Creek upstrea	Barbers Creek downstr	Shoalhaven River 1	Shoalhaven River 2	Shoalhaven River 3	Marulan Creek upstrea	Marulan Creek downstr
Australian Smelt *Retropinna semoni*	**Autumn**	**60**	**41**	**0**	**0**	**100**	**163**	**100**	**0**	**0**
	Spring	**1**	**18**	**0**	**0**	**300**	**Obs.**	**0**	**0**	**0**
Cox's Gudgeon *Gobiomorphus coxii*	**Autumn**	**9**	**11**	**0**	**2 (11)**	**0**	**0**	**(2)**	**0**	**0**
	Spring	**25**	**11**	**10**	**0**	**0**	**0**	**0**	**0**	**0**
Mountain Galaxias *Galaxias olidus*	**Autumn**	**(5)**	**0**	**0**	**1 (7)**	**0**	**0**	**0**	**0**	**0**
	Spring	**0**	**0**	**10**	**0**	**0**	**0**	**0**	**0**	**0**
Flathead Gudgeon *Philypnodon grandiceps*	**Autumn**	**22**	**6**	**0**	**0**	**3**	**16**	**0**	**0**	**0**
	Spring	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Long-finned Eel *Anguilla reinhardtii*.	**Autumn**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Spring	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Australian Bass *Macquaria novemaculeata*	**Autumn**	**0**	**0**	**0**	**0**	**(1)**	**0**	**0**	**0**	**0**
	Spring	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Eastern Gambusia* *Gambusia holbrooki*	**Autumn**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**14**	**50+**
	Spring	**0**	**0**	**0**	**0**	**51**	**Obs.**	**0**	**346**	**1 (50+)**
Common Carp* *Cyprinus carpio*	**Autumn**	**0**	**(5+)**	**0**	**0**	**1**	**0**	**(4)**	**0**	**0**
	Spring	**0**	**0**	**0**	**0**	**0**	**Obs.**	**0**	**0**	**0**

Table 2 Fish survey data collected during the updated baseline surveys (autumn and spring), adapted from Niche (2024)

Species	Sites and sequence reads (total of 2 replicate samples)														
	Bungonia Creek 1 Up	Bungonia Creek 2 Up	Bungonia Creek 1 Down	Bungonia Creek 2 Down	Barbers Creek 1 Up	Barbers Creek 2 Up	Barbers Creek 1 Down	Barbers Creek 2 Down	Shoalhaven River SR1	Shoalhaven River SR2	Shoalhaven River SR3	Shoalhaven River SR-AQ1	Shoalhaven River SR-AQ2	Main Gully 1	Main Gully 2
Australian Smelt *Retropinna semoni*	**0**	**0**	**89895**	**67736**	**329**	**4223**	**0**	**14318**	**9659**	**24547**	**109578**	**18767**	**543**	**0**	**0**
Cox's Gudgeon *Gobiomorphus coxii*	**101900**	**78989**	**6231**	**2107**	**155**	**0**	**1**	**2912**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Genus of Galaxias *Galaxias* sp.	**12950**	**2644**	**3906**	**0**	**17686**	**71757**	**29249**	**988**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Flathead Gudgeon *Philypnodon grandiceps*	**0**	**0**	**143**	**1894**	**0**	**0**	**0**	**0**	**13188**	**197**	**0**	**0**	**187**	**7166**	**6968**
Dwarf Flathead Gudgeon *Philypnodon macrostomus*	**0**	**0**	**0**	**6437**	**0**	**0**	**0**	**0**	**27199**	**826**	**1523**	**1446**	**580**	**0**	**0**
Long-finned Eel *Anguilla reinhardtii*.	**1708**	**3848**	**130**	**279**	**700**	**0**	**1218**	**0**	**174**	**200**	**19429**	**0**	**52**	**0**	**0**
Short-finned Eel *Anguilla australis*	**3823**	**13898**	**0**	**0**	**262**	**0**	**0**	**0**	**0**	**49**	**0**	**0**	**0**	**0**	**0**
Australian Bass *Macquaria novemaculeata*	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**54**	**0**	**0**	**1139**	**0**	**0**	**0**
Freshwater Catfish *Tandanus tandanus*	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**256**	**9139**	**437**	**0**	**0**	**0**	**0**
Genus of Goby *Gobiidae* sp.	**657**	**575**	**52**	**151**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Genus of Carp Gudgeons *Hypseleotris* sp.	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1226**	**161**	**0**	**0**	**5740**	**0**	**0**
Salmon and Trout	**281**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Species	Sites and sequence reads (total of 2 replicate samples)														
	Bungonia Creek 1 Up	Bungonia Creek 2 Up	Bungonia Creek 1 Down	Bungonia Creek 2 Down	Barbers Creek 1 Up	Barbers Creek 2 Up	Barbers Creek 1 Down	Barbers Creek 2 Down	Shoalhaven River SR1	Shoalhaven River SR2	Shoalhaven River SR3	Shoalhaven River SR-AQ1	Shoalhaven River SR-AQ2	Main Gully 1	Main Gully 2
***Salmo* sp.**															
Eastern Gambusia* *Gambusia holbrooki*	**0**	**0**	**0**	**17161**	**0**	**0**	**0**	**0**	**193607**	**157752**	**126514**	**59646**	**27359**	**219703**	**0**
Common Carp* *Cyprinus carpio*	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**153**	**0**	**0**	**0**	**0**	**0**

Three species of freshwater crustacean were recorded during the original baseline fish surveys; Common Yabby *Cherax destructor*, Long-armed Shrimp *Macrobrachium australiens*, Freshwater Shrimp *Parataya australiensis*. Crustacea were not addressed via the eDNA sampling in the updated baseline fish surveys, although Freshwater Shrimp were detected as part of macroinvertebrate sampling. In addition to the fish species present, the eDNA sampling detected a number of bird, mammal, amphibian and reptile species. Of most relevance to the aquatic ecological assessment this included Platypus *Ornithorhynchus anatinus*, Eastern Long-necked Turtle *Chelodina longicollis,* Eastern Water Skink *Eulamprus quoyii*, Lesueur's frog *Litoria lesueurii* and Common Eastern froglet *Crinia signifera*. The eDNA results also included detections of Spotted-tailed Quoll *Dasyurus maculatus*, listed as a Vulnerable species in NSW. As well as feral species such as Pig *Sus scrofa*.

Discussion

The eDNA results were successful in providing an effective appraisal of the fish assemblage present. Overall, all species previously detected during the original baseline assessment were detected in the updated baseline. Five additional species were recorded by eDNA sampling demonstrating a higher degree of efficacy than traditional techniques. It is acknowledged that no abundance, sex, size or condition data is gathered by eDNA sampling. However, these factors are not important to the scope of the assessment set by the approval requirements under which the studies have been completed. Collection of two samples (replicates) per site are considered to provide a reasonable level of confidence in detection. This recognises the strong level of coverage of monitoring sites across the stream network, additional sites included for the updated baseline, the relatively close position of some sites together and high level of filtered water achieved.

When the site detections are considered at the watercourse level, eDNA was more effective than traditional techniques, identifying more species in 11 instances. The majority of the additional detections occurred within the Shoalhaven River, which is the largest waterway considered and likely reflects the better alignment of eDNA sampling to this size of waterway, and also the ability to deploy this technique at greater number of sites across the updated baseline. However, there were two instances in which traditional techniques detected a species within a watercourse that was not identified via eDNA. This suggests a combination of approaches would be optimal. Combining different traditional survey methods is often considered (e.g. Lintermans, 2016), as a combination of techniques may yield better results or overcome bias associated with individual techniques (Mueller et al., 2017). A combination approach may also help address species that there are difficulties resolving to the species level (such as Galaxiids). Further support for this was the detections of freshwater crustacea that were recorded in the original baseline (not addressed via eDNA sampling), balanced against the additional terrestrial or semi-aquatic biota detected via eDNA (e.g. Platypus) that are of interest, but not a key concern of the studies.

Importantly, the deployment of eDNA sampling allowed for a greater number of sites to be considered, allowing a more thorough representation of the study reaches. This advantage is further strengthened by the stream length over which eDNA can provide information (up to one kilometre). Additionally, the updated baseline standardised survey effort, establishing a more reliable and useful basis for any between-site comparison and follow-up assessment (Radinger et al., 2019). This would be particularly valuable if ongoing monitoring was triggered.

A key consideration in the adoption of eDNA sampling in this study was the opportunity to reduce risk to field teams due to hazardous terrain and weather conditions. Key safety outcomes achieved included removing the need for camping and carrying additional sampling gear, as well as reducing exposure to in-water work. Cost is a key constraint in ecological monitoring (Radinger et al., 2019) and the use of eDNA is not always the most cost-effective technique (Smart et al., 2016). However, in this study, the updated baseline achieved cost savings relative to the original baseline, by reducing the field team required from three to two ecologists and reducing the fish survey rounds from two to one.

Conclusions

eDNA provided a more comprehensive appraisal of fish assemblages over a larger area than the netting and observational techniques used in the original baseline, as reflected by the greater number of species detections. Additional benefits included standardising survey effort, and the detection of additional terrestrial and semi-aquatic species. Adopting eDNA resulted in significantly improved safety outcomes, which were a critical consideration in the hazardous terrain present across the study area, and represented a more cost-effective approach. Balancing the trade-offs in survey techniques deployed is a constant challenge faced by applied scientists and eDNA is no exception. The results suggest that although a combination of traditional and eDNA sampling techniques may be the optimal approach, eDNA alone provided an assessment that was overall superior in this context sufficient to meet aims of the study. Applied scientists must balance the need to undertake scientifically robust assessments with value for money and safety requirements. As these pressures increase, eDNA presents a useful tool in assessments such as this, improving both efficiency and survey rigour.

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References

Lahoz-Monfort, J. J., & Tingley, R. (2018). The technology revolution: *Improving species detection and monitoring using new tools and statistical methods*. In S. Legge, D. B. Lindenmayer, N. Robinson, B. C. Scheele, D. Southwell, & B. A. Wintle (Eds.), Monitoring threatened species and ecological communities, CSIRO Publishing, 303–313.

Lintermans, M. (2016). Finding the needle in the haystack: comparing sampling methods for detecting an endangered freshwater fish. *Marine & Freshwater Research* 67(11), 1740–1749.

McColl-Gausden, E.F., Weeks, A., & Tingley, R. (2020) A field ecologist's guide to environmental DNA sampling in freshwater environments. *Australian Zoologist* 40(4), 641 – 651.

Marulan South Limestone Mine (2022). Biodiversity Management Plan. SSD 7009. Prepared by Cambium Group for Boral Cement Ltd. Report dated 25 October 2022.

McColl-Gausden, E.F., Weeks, A.R., Coleman, R.A, Robinson, K.L., Song, S., Raadik, T.A., & Tingley, R. (2021). Multispecies models reveal that eDNA metabarcoding is more sensitive than backpack electrofishing for conducting fish surveys in freshwater streams. *Molecular Ecology*. 30(13), 3111-3126.

Mueller, M., Pander, J., Knott, J. & Geist, J. (2017). Comparison of nine different methods to assess fish communities in lentic flood-plain habitats. *Journal of Fish Biology* 91: 144-174.

Niche Environment and Heritage (2018). *Marulan South Limestone Mine Continued Operations: Aquatic Assessment*. Prepared for Boral Cement Ltd. Report dated: November 2018. Project number 2155.

Niche (2024). *Marulan South Baseline Aquatic Biodiversity Monitoring – Autumn 2024*. Prepared for Boral Cement Ltd. Report dated 05/03/2025. Project number: 8671.

Piggot, M.P., Banks, S.C., Broadhurst, B.T., Fulton, C.J., & Lintermans, M. (2020). Comparison of traditional and environmental DNA survey methods for detecting rare and abundant freshwater fish. *Aquatic Conservation* 31(1), 173-184.

Radinger J., Britton J.R., Carlson S.M.,

et al. (2019). Effective monitoring of freshwater fish. *Fish and Fisheries* 20, 729–747.

Radinger, J., Britton, J. R., Carlson, S. M., Magurran, A. E., Alcaraz-Hernández, J. D., ... & García-Berthou, E. (2019). Effective monitoring of freshwater fish. *Fish and Fisheries* 20(4), 729–747.

Smart, A.S., Weeks, A.R., van Rooyen, A.R., Moore, A., McCarthy, M.A. and Tingley, R. (2016). Assessing the cost-efficiency of environmental DNA sampling. *Methods in Ecology and Evolution*, 7, 1291-1298.

Underwood, A. J. (1997). *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge: Cambridge University Press.