Report for the Rakali River Conservation Society

Introduction

Monitoring rakali (*Hydromys chryogaster*), also known as water rats, is vital because this species act as a critical indicator for the health of freshwater ecosystems. Rakali are nocturnal, semi-aquatic, solitary and shelter in burrows along banks of permanent water bodies such as rivers or lakes (Bytheway et al., 2025; Crook & Crook, 2025). Their survival is currently threatened by habitat alteration, decline in water quality, predation, and livestock grazing. They are regarded as of least concern by the IUCN RedList (2016), but are a priority 4 classification in Australia, meaning they are considered near threatened and in need of regular monitoring.

The nature of water rats being hard to monitor means there is limited knowledge about their distribution and behaviour. A three-year study conducted by the River Conservation Society in York between 2022 and 2025 aimed to improve the knowledge of Rakali distribution along the Avon River (Crook & Crook, 2025). The Avon River in York is an ephemeral river that dries in summer to form a chain of permanent pools. They used both camera traps and kayak sitings for 12 permanent pools to determine if there are breeding populations, and if they are under threat of extinction in this ecosystem.

Our study aims to support the findings of the River Conservation Society. Our aim is to investigate the detection and distribution of rakali using eDNA across 10 monitoring sites along the Avon River, and to explore how detections relate to water quality and vegetation characteristics. Environmental DNA (eDNA) metabarcoding is a non-invasive method for identifying rare and elusive species (Sahu et al., 2023). Traditional monitoring methods, such as visual surveys and camera traps can be labour-intensive and prone to observer bias, often missing small or cryptic species. eDNA uses samples from the environment, such as water, that include DNA shed by organisms.

Methods

Two sample types were collected from 10 of the 12 sampling points previously monitored by the River Conservation Society, including eDNA filters and floating puffs left over night at each site. At each sampling point we also measured water temperature, pH, conductivity, salinity and dissolved oxygen using a Hanna multiparameter probe and conducted a simple vegetation assessment. Four 1-liter water samples were collected separately for eDNA analysis at each sampling point and passed through individual eDNA filters using a drill-powered filtration system. Filters were preserved in buffer, stored in a sky with ice and transported to the laboratory within 48 hours of collection.

DNA was extracted from the preserved filters using the Qiagen PowerFecal DNA extraction kit following the steps on the manufacturer's protocol. A species-specific quantitative PCR (qPCR) assay targeting the mitochondrial cytochrome *b*(*cyt*b*) region was used for rakali species detection. Each sample was analysed in duplicate using both neat and diluted DNA templates to assess amplification and improve detection reliability in case of chemical inhibitors in water.

Results and Discussion

No rakali eDNA was detected in any of the environmental samples collected across the 10 Avon River sites. This was the case for both neat and diluted samples, suggesting inhibition was not a factor influencing this result. However, the positive controls containing known rakali DNA amplified successfully, showing that the qPCR assay was working correctly.

Importantly, the absence of an eDNA detection should not be interpreted as confirmation that rakali is absent from these sites. Rakali are notoriously difficult to detect using conventional survey methods, and detection rates can vary considerably depending on the monitoring approach used (Sanders et al., 2024). Environmental DNA detection also depends on several factors beyond whether the target species is present.

Rakali are mobile, semi aquatic mammals and may only spend a limited amount of time in the water at any one location potentially resulting in low concentrations of DNA being released. eDNA can also be unevenly distributed within a waterbody and influenced by water flow, distance from source, degradation, among other factors, meaning detections can vary between samples even when a species is present.

The timing of sampling may also have influenced the results. Sampling was conducted in mid-August, during winter when the Avon River was flowing more continuously rather than being divided into the distinct pools that form during drier months. In flowing systems, eDNA can be transported away from its source and dispersed through the river, affecting the concentration available at a particular sampling location (Curtis et al., 2021). This may have reduced the likelihood of capturing rakali DNA at detectable concentrations.

As field sampling was completed only over 2 days the survey represents only a very short snapshot in time and rakali activity and eDNA may vary across different days or seasons.

Overall, the non-detections may reflect low or uneven eDNA concentrations, winter flow conditions and the short sampling period rather than a confirmed absence of rakali from this part of the Avon River.

Recommendations

There are various elements of the sampling design that we could modify for future attempts. Sampling during lower-flow periods may improve detection rates due to less diluted eDNA. Sampling can be repeated in summer/different seasons as well as in multiple locations/depths within the pools, rather than only near riverbanks. Increasing the number of water samples/replicates will also improve the ability to detect eDNA. Additional data regarding the size of permanent populations in the pools may be useful to gather before future sampling attempts, due to the solitary nature of rakali and to account for transient individuals. The absence of breeding populations in pools may result in insufficient eDNA in water for detection, therefore breeding populations should be further investigated before additional sampling.

References

Bytheway, J. P., Goumas, M., & Banks, P. B. (2025). First evidence of aggressive interactions by native rakali towards invasive Black rats. *Australian Mammalogy*, *47*(2). https://doi.org/10.1071/am25009

Crook, J., & Crook, M. (2025). *RAKALI, RIPARIAN AND RIVER HEALTH PROJECT*. River Conservation Society Incorporated York, Western Australia. https://www.riverconservationsociety.org/_files/ugd/11bdad_6623efeb84c446a2becc74ec7a179215.pdf.

Curtis, A. N., Tiemann, J. S., Douglass, S. A., Davis, M. A., & Larson, E. R. (2021). High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. Diversity and Distributions, 27, 1918–1931. https://doi.org/10.1111/ddi.13196

Sanders, E., Nimmo, D. G., Turner, J. M., Wassens, S., & Michael, D. R. (2024). Putting rakali in the spotlight: Innovative methods for detecting an elusive semi aquatic mammal. Wildlife Research, 51(6), WR24002. https://doi.org/10.1071/WR24002

Sahu, A., Kumar, N., Pal Singh, C., & Singh, M. (2023). Environmental DNA (Edna): Powerful technique for biodiversity conservation. *Journal for Nature Conservation*, *71*, 126325. https://doi.org/10.1016/j.jnc.2022.126325