

Evaluation of eDNA primers for coastal UK marine mammal species



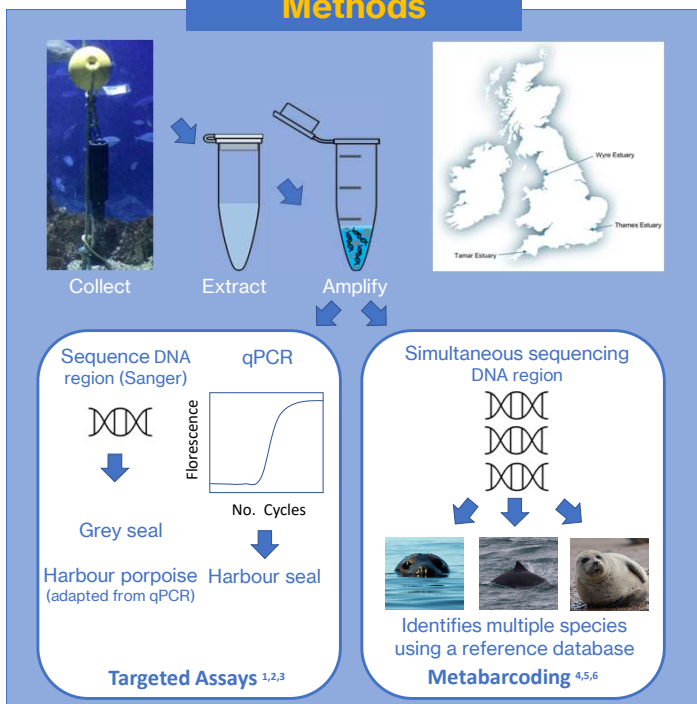
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Aims

- Evaluate the most effective eDNA analysis method (metabarcoding versus single species assays)
 - Understand the effectiveness of open-source metabarcoding primer sets
- Understand which of the primers is most effective for conducting eDNA studies of UK coastal marine mammal species

Methods



Discussion

The metataxonomic assays showed more promising results than the targeted assays for detecting marine mammals in eDNA samples.

Targeted assays: Based on the poor performance we consider that none of the targeted assays^{1,2,3} used in this study would currently be suitable for use in eDNA studies for marine mammal monitoring.

Metataxonomic assays: Ceto2, MiMammal and MiFish did not detect the target species. The MarVer1 assay⁶ identified the greatest number of amplicon sequence variants of species (ASV*), however MarVer3 outperformed MarVer1 for marine mammals. MarVer3 returned more unique mammal ASVs (N=19, incl. 6 ASVs for seals identified to Family and 4 ASVs for harbour porpoise identified to Species), compared to MarVer1 (ASV N=9, incl. 2 ASVs for seals identified to Family).

*ASVs (amplicon sequence variants): the unique DNA sequences from each genetic locus. These matched to a reference database in order to identify which species they came from. ASVs also give a detailed picture of DNA sequence diversity within the sample.



Main Findings

All targeted assays^{1,2,3} performed as expected against tissue / faecal samples, but poorly against eDNA samples. Metabarcoding had varying results.

The **MarVer1⁶** and **MarVer3⁶** assays are considered the most promising assays for use in the monitoring of UK coastal marine mammals using eDNA techniques, with MarVer3 outperforming MarVer1.

More extensive validation studies are needed to confirm the performance of these metataxonomic assays in a range of marine environments and against a wider range of marine mammal species.

Results

All three targeted assays performed as expected against true positive tissue and faecal samples, but performed poorly against eDNA samples. qPCR for harbour porpoise amplified all target species, so a Sanger method was used.

Targeted Assay Performance

	Harbour porpoise ¹	Harbour seal ²	Grey seal ³
Control sample	Y tissue N/A faecal	Y tissue Y faecal	Y tissue Y faecal
eDNA sample	3/9 Thames & Tamar	0/9	0/9

The metataxonomic assays had a wide range of performance, ranging from Ceto2 detecting no species, to MarVer3 detecting 19 unique mammal species, including all three target species.

Metataxonomic Assay Performance

	Ceto2 ⁶	MiFish ⁴	MiMammal ⁵	MarVer1 ⁶	MarVer3 ⁶
Total ASVs*	254 ± 49	132 ± 67	86 ± 13	275 ± 118	124 ± 42
Mammal ASV to species	0	10	48	64	54
Unique mammal sp.	0	3	11	9	19
Grey seal	0/9	0/9	0/9	1/9 Tamar	5/9 Tamar, Thames & Wyre
Harbour seal	0/9	0/9	0/9	1/9 Wyre	1/9 Thames
Harbour porp.	0/9	0/9	0/9	0	2/9 Wyre

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REFERENCES

- Footo, A. D., Thomsen, P. F., Sveegaard, S., Wahlberg, M., Kielgast, J., Kyhn, L. A., ... & Gilbert, M. T. P. (2012). Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals.
- Matejusková, I., Bland, F., Hall, A. J., Harris, R. N., Snow, M., Douglas, A., & Middlemas, S. J. (2013). Real-time PCR assays for the identification of harbor and gray seal species and sex: A molecular tool for ecology and management. *Marine Mammal Science*, 29(1), 186-194.
- van Bleijswijk, J. D., Begeman, L., Witte, H. J., IJsseldijk, L. L., Brasseur, S. M., Gröne, A., & Leopold, M. F. (2014). Detection of grey seal *Halichoerus grypus* DNA in attack wounds on stranded harbour porpoises *Phocoena phocoena*. *Marine Ecology Progress Series*, 513, 277-281.
- Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., et al. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *R. Soc Open Sci*, 2, 1-33.
- Ushio, M., Fukuda, H., Inoue, T., Makoto, K., Kishida, O., Sato, K., ... & Miya, M. (2017). Environmental DNA enables detection of terrestrial mammals from forest pond water. *Molecular Ecology Resources*, 17(6), e63-e75.
- Valsecchi, E., Bylemans, J., Goodman, S. J., Lombardi, R., Carr, I., Castellano, L., ... & Galli, P. (2020). Novel universal primers for metabarcoding environmental DNA surveys of marine mammals and other marine vertebrates. *Environmental DNA*, 2(4), 460-476.