

A magnifying glass on cetaceans and their environmental DNA

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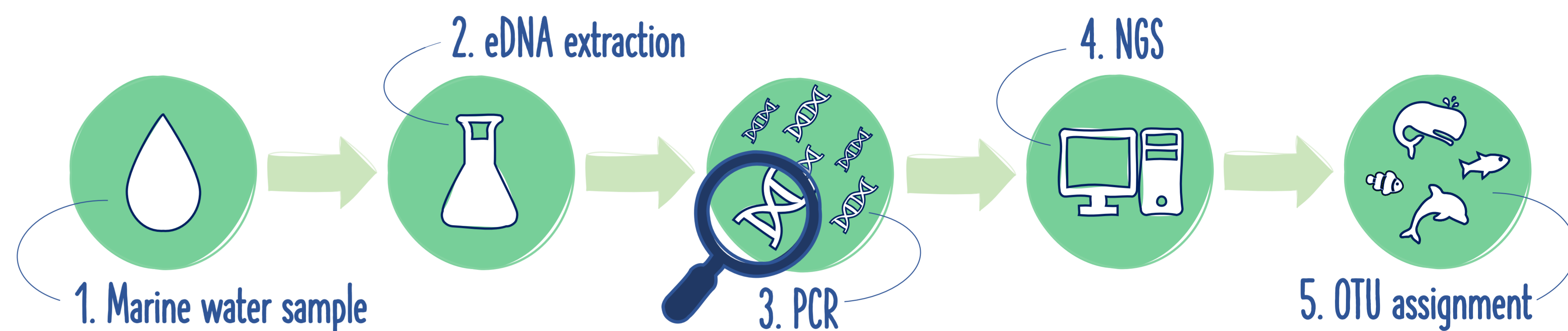
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INTRODUCTION

Marine mammal species are the most vulnerable in the marine environment. Yet, information about their conservation status are usually lacking. In this context, newly developed approaches, such as **environmental DNA (eDNA) sequencing**, are considered a good option to achieve biodiversity monitoring, being fast, non-invasive and sensitive. However, their application on high dispersed and broadly distributed species, such as the cetofauna, is still scarcely explored. The first pivotal step in implementing the use of an eDNA approach is the design of an efficient and replicable protocol that addresses technical issues, such as the species detection bias towards more abundant taxa, like bony fishes. To do so, we made use of water samples from Maldives, a well-known biodiversity hotspot, to identify suitable mtDNA regions for cetacean detection and to optimize **the metabarcoding protocol**.

METHODS

- Seawater samples were taken between 2020 and 2021 from different types of marine habitats in Maldives
- Targeting 2 hypervariable regions: the 12S rDNA and 16S rDNA genes
- 2 set of primers for each target: **vertebrate-specific MARVER** and **ceto-specific MARMAM/CETO**
- NGS **multiplex approach**: two libraries in one run

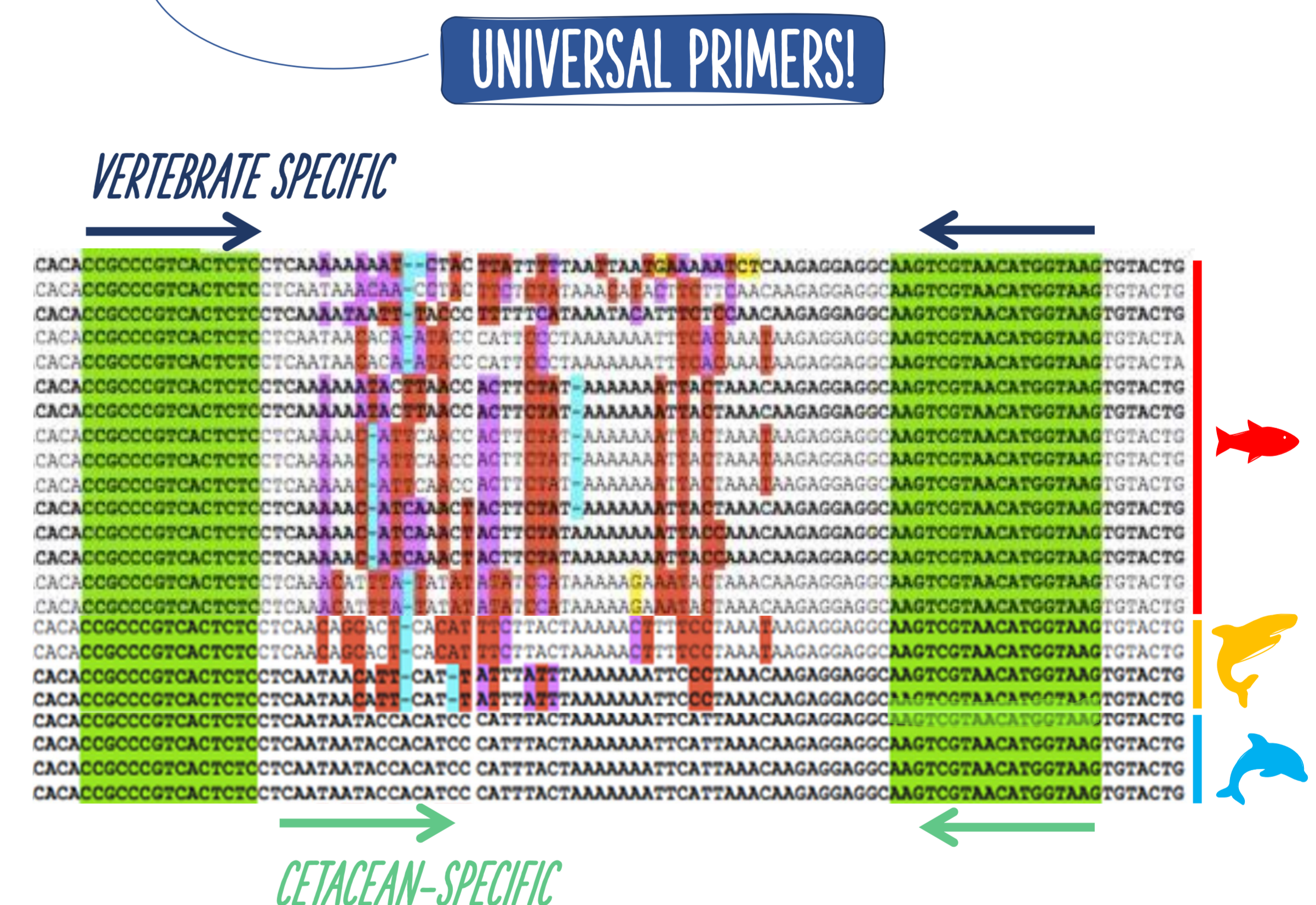


RESULTS

Taxonomic group	Cetacean taxa	12S-rDNA		16S-rDNA	
		MarVer1	MarMam1	MarVer3	Ceto3
Species	<i>Balaenoptera physalus</i>	11	24	1	30
	<i>Kogia sima</i>			1	1
	<i>Ziphius cavirostris</i>	2	34	9	31
	<i>Peponocephala electra</i>				2
	<i>Tursiops aduncus</i>		1		
	<i>Tursiops truncatus</i>	7			
	<i>Stenella coeruleoalba</i>				17
	<i>Stenella longirostris</i>		9	9	12
	<i>Lagenodelphis hosei</i>		1		1
	<i>Grampus griseus</i>				1
Genus	<i>Sotalia</i>		4		
Families	Balaenopteridae fam.	11	3		
	Delphinidae fam.	20	22	3	7

- Ceto-specific primers performed better**: higher cetacean detection and in a larger number of samples compared to vertebrate-specific primer set
- The **most abundant species** match the **most frequently observed** in visual surveys
- Compatibility of the cetacean and vertebrate primer sets to be run in the same NGS run

- Many sequences not resolved to the genus/species level: higher resolution needed
- Detection of the genus *Sotalia* and of the fin whale, which are not found in this geographic range: contamination or lack of reference sequences?



CONCLUSIONS

The study revealed the efficiency of ceto-specific primer in enhancing the resolution of marine mammals detection, especially for gene16S, underlying the advantage of targeting cetofauna when adopting a metabarcoding approach. The multiplex NGS maximised data output, while reducing the costs. However, there is still room for improvement: a higher taxonomic resolution is needed for all those sequences resolved only at family level. Once optimized, the eDNA protocol will then be applied to a larger and more comprehensive survey in the Mediterranean basin in the context of the project LIFE- CONCEPTU Maris:

“CONservation of CETaceans and Pelagic sea TURtles in Med: Managing Actions for their Recovery In Sustainability”

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- Valsecchi, Elena, Antonella Arcangeli, Roberto Lombardi, Elizabeth Boyse, Ian M. Carr, Paolo Galli, and Simon J. Goodman. 'Ferries and Environmental DNA: Underway Sampling From Commercial Vessels Provides New Opportunities for Systematic Genetic Surveys of Marine Biodiversity'. *Frontiers in Marine Science* 8 (August 2021):704786.

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