DETECTION AND MONITORING OF NON-INDIGENOUS INVERTEBRATE SPECIES IN RECREATIONAL MARINAS THROUGH DNA METABARCODING OF ZOOPLANKTON COMMUNITIES IN THE NORTH OF PORTUGAL

Ana S. Lavrador[1,2], Fábio G. Amaral[1,2], Jorge Moutinho[1,2], Pedro E. Vieira[1,2], Filipe O. Costa[1,2], Sofia Duarte[1,2]

[1] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Braga, Portugal

[2] Institute of Science and Innovation for Sustainability (IB-S), University of Minho, Braga, Portugal

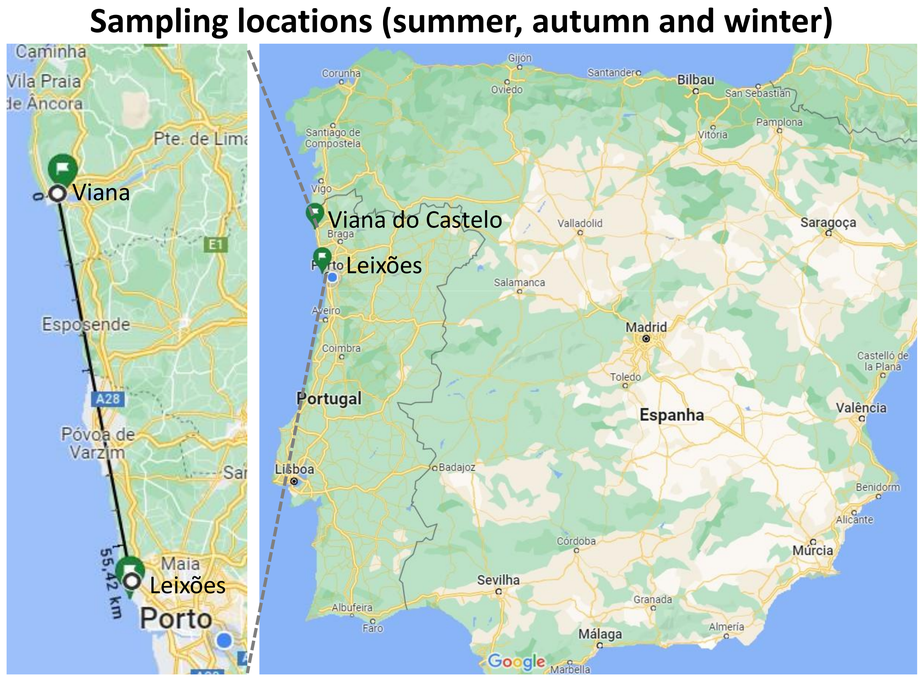
INTRODUCTION

- When introduced to new locations, non-indigenous species (NIS) can establish and become invasive, causing severe alterations in native ecosystems.
- Most introductions in coastal ecosystems occur in ports and marinas, and, thus, these are priority hubs for NIS detection.
- Zooplankton monitoring can allow the detection of earlier life stages, facilitating a quicker response to possible biological invasions.
- DNA metabarcoding constitutes a fit approach to monitor taxonomically-challenging zooplankton communities, as it can detect NIS regardless of life stage or density, providing accurate species identifications.

OUR GOAL WAS TO EMPLOY DNA METABARCODING TO MONITOR NIS IN THE ZOOPLANKTON OF 2 MARINAS FROM THE NORTH OF PORTUGAL, ON THREE SEASONS OF THE YEAR, AND USING 2 MOLECULAR MARKERS.

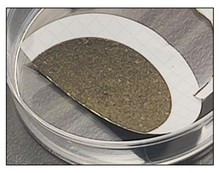
METHODOLOGY

Sampling locations (summer, autumn and winter)



55 µm mesh zooplankton net with 40 cm opening diameter and 100 cm of length

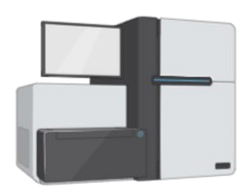
DNA metabarcoding workflow



Filtered zooplankton samples

DNA Extraction (DNeasy® Power Soil® Kit)

PCR
COI: mlCOIintF/LoboR1
18S: TAReuk454FWD1/TAReukREV3



High throughput sequencing (MiSeq)

Data analyses (97% OTU threshold)

RESULTS

- The highest number of species was detected with 18S (413/ 8 NIS), compared to COI (158/6 NIS), but very few were detected by both markers (33/1 NIS only)
- Annelida was the most well represented phylum in the COI dataset, while Nematoda (Viana) and Arthropoda (Leixões), in the 18S dataset (Fig. 2).
- Twelve NIS were recovered in all the seasons, but only 2 were detected in both marinas, and only one NIS was simultaneously detected with both markers (Table 1, Fig. 1B).
- Maximum NIS were found in autumn in Leixões (6) and in winter in Viana (4). In both marinas no NIS were detected in all seasons (Fig. 1B).

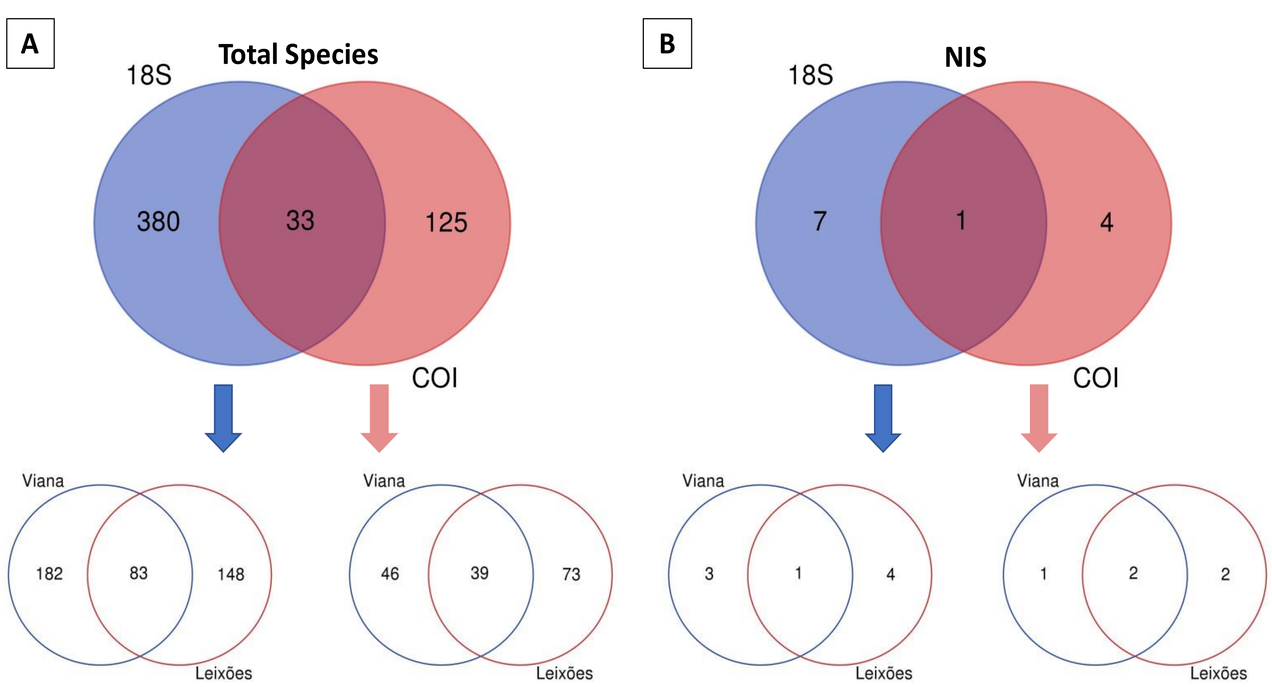


Figure 1. Venn diagrams of the total species (A) and NIS (B) recovered on the three seasons in both marinas.

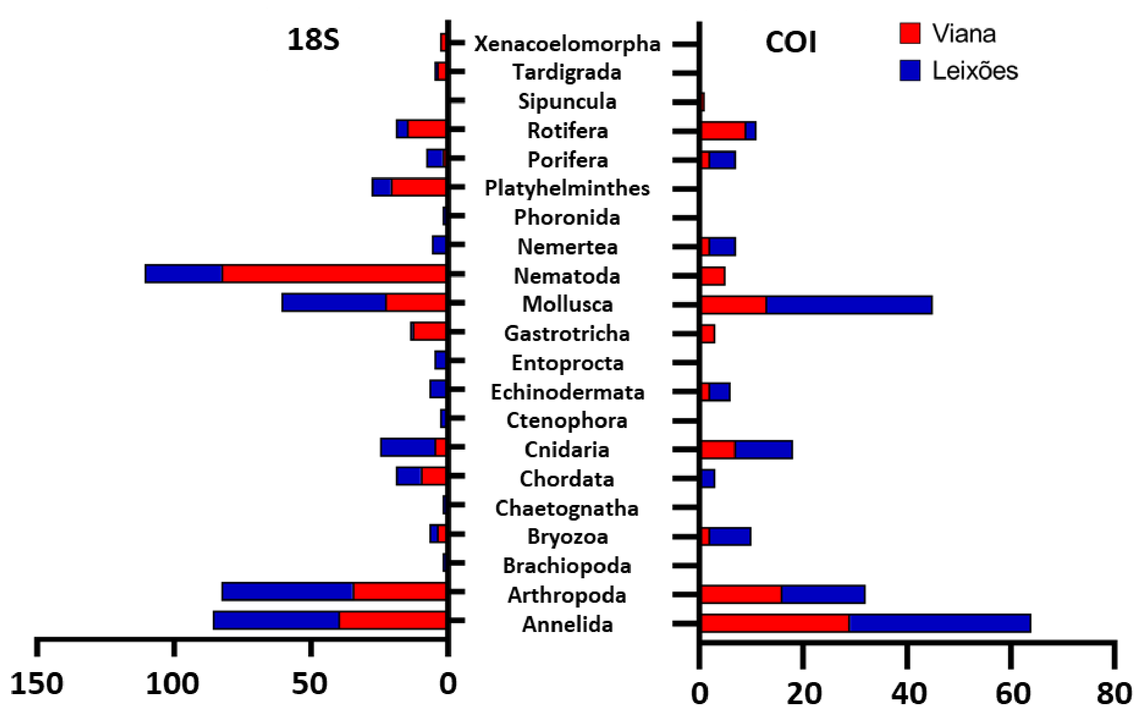


Figure 2. Taxonomic profiling of the total species detected in all seasons in both marinas with each marker.

Table 1. List of the NIS detected in this study

Species	Leixões			Viana		
	Summer	Autumn	Winter	Summer	Autumn	Winter
Amphibalanus amphitrite				X	X	
Balanus trigonus	X	X			X	
Botrylloides violaceus			X			
Bugula neritina		X				
Ciona intestinalis		X				
Corbicula fluminea						X
Cordylophora caspia						X
Mya arenaria				X		
Potamopyrgus antipodarum						X
Pseudodiaptomus marinus		X	X		X	X
Pseudopolydora paucibranchiata		X	X			
Styela plicata		X	X			

CONCLUSION

These results show the efficiency of DNA metabarcoding for early detection of NIS in zooplankton, but also **reveal the need to employ different molecular markers and sampling different seasons** to guarantee a more thorough detection of NIS in these environments. This work is a part of the NIS-DNA project (https://sites.google.com/view/nis-dna/home) that comprises sampling of up to seven marinas and using five different sampling schemes.

Acknowledgments: This work was funded by the Portuguese foundation of Science and Technology (FCT, I.P.) under the project PTDC/BIA-BMA/29754/2017. Ana S. Lavrador work was also supported by the Collaboration Protocol for Financing the Multiannual Research Grants Plan for Doctoral Students with financial support from the Foundation for Science and Technology (FCT) and the European Social Fund under the Northern Regional Operational Program - Norte2020 (UI/BD/150871/2021).