Poster 51

# eDNA metabarcoding as a way to evaluate myxozoan presence and diversity in the sediment of a transboundary estuary 

G. Ferreira ${ }^{1, *}$, A. Machado ${ }^{1,2}$ and S. Rocha ${ }^{1,3}$<br>${ }^{1}$ School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira n ${ }^{\circ}$ 288, Porto, Portugal<br>${ }^{2}$ Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Novo Edifício do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal<br>${ }^{3}$ Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Rua Alfredo Allen n no 208, Porto, Portugal<br>* Correspondence: aqb.gabriel.ferreira15@gmail.com


#### Abstract

Background: Myxozoans are a diverse group of cnidarian endoparasites cosmopolitan in the aquatic environment, responsible for causing serious diseases in fish [1]. Traditional methods of detection and characterization of these parasites are very cumbersome and complex by nature [1-3]. Therefore, it is essential to implement simpler, non-destructive approaches to assess myxozoan presence and diversity, such as eDNA analysis [3]. Most eDNA-based parasitological assessments focus on water samples to indicate potential disease risk. However, Turner et al. [4] demonstrated that fish DNA is more concentrated in sediment than in water, which could also be true for myxozoan DNA. Objective: This study aimed to compare traditional methods of myxozoan detection versus a novel eDNA approach from sediment samples. Methods: Sediment was collected monthly from the three distinct stretches of the Minho River estuary, near Caminha (lower estuary), Boega (middle estuary), and Morraceira (upper estuary). Collected annelids were identified and microscopically surveyed for myxozoan infection. eDNA was extracted from the sediment samples and a nested PCR protocol targeting a variable region of the 18 S rDNA ( $450-490 \mathrm{bp}$ ) was performed using metabarcoding primers [3]. Results: A total of 1,746 oligochaetes and 327 polychaetes were isolated, among which only one oligochaete collected in September from the upper estuary displayed microscopic evidence of myxozoan infection. Actinospores were identified as belonging to the sphaeractinomyxon collective group, based on morphology and 18 S rDNA sequence. Conversely, eDNA metabarcoding from sediment samples revealed positive amplification throughout the sampling period, and in all three locations. Preliminary results identified amplicons as having the highest genetic similarity with myxozoan 18 S rDNA sequences. Conclusion: This work highlights the utility of eDNA metabarcoding of sediment samples for evaluating myxozoan presence and diversity in estuarine environments, allowing the acquisition of high-fidelity results at a faster rate and superior sensitivity than those obtained via traditional methods.


Keywords: Cnidaria; 18S rDNA; Minho River; annelids; sphaeractinomyxon

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