

ISOTOPIC TISSUE FRACTIONATION AT BIVALVE *PINNA NOBILIS*, A NON-INVASIVE APPROACH

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Abstract

Carbon and nitrogen isotopic values of muscle and mantle in Mediterranean endemic bivalve *Pinna nobilis* individuals have been analyzed to study tissue fractionation. Muscle tissue is enriched in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ compared to mantle on average 1.11 ‰ and 0.71 ‰, respectively. Analyses of mantle tissue do not involve sacrifice of individuals and are therefore proposed as a conservation tool in the study of *P. nobilis*.

Keywords: *Bio-indicators, Bivalves, Conservation, Balearic Islands*

Introduction- *Pinna nobilis* is one of the largest bivalves in the world [1] and endemic to the Mediterranean. Populations have been reduced due to direct and indirect impacts [2]. As a consequence, it has been listed as a Mediterranean endangered species (92/43/EEC). Previous studies have suggested muscle as the most appropriate for stable isotopes analyses [3]. The aim of this study was to analyze mantle and muscle tissues of *P. nobilis* at three different localities to i) study fractionation between tissues, ii) study isotopic differences among localities and iii) propose sampling of mantle instead of muscle to study the trophic ecology of the fan mussel. Results will allow comparison of muscle and mantle tissue in order to provide a non-invasive technique for sampling vulnerable fan mussel.

Materials and Methods- The study was carried out at 3 sampling sites: Cabrera, Es Freus and Andratx, Balearic Islands (Western Mediterranean). Individuals of *P. nobilis* were collected under licence from the Government for research purposes by experienced scuba divers in February 2013. For each *P. nobilis* individual, muscle and mantle tissues were obtained. Stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were analyzed following standard procedures [4]. A multivariate analyses of variance PERMANOVA tested significant spatial differences among sites and tissues.

Results- For all localities, muscle tissue were enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ compared to mantle tissue (Fig. 1). Values of $\delta^{13}\text{C}$ were highest at Andratx and lowest at Es Freus. Values of $\delta^{15}\text{N}$ were highest at Andratx and lowest at Cabrera (Fig. 1). Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of *P. nobilis* showed significant differences for locality (Lo) (PERMANOVA, Lo, $\delta^{13}\text{C}$, $p < 0.001$ and Lo, $\delta^{15}\text{N}$, $p < 0.001$, Fig 2.) and tissue (Ti) (PERMANOVA, Ti, $\delta^{13}\text{C}$, $p < 0.001$ and Ti, $\delta^{15}\text{N}$, $p < 0.001$, Fig 2). Only the interaction between tissue and locality for $\delta^{15}\text{N}$ showed significant differences (PERMANOVA, Lo x Ti, $\delta^{15}\text{N}$, $p < 0.05$, Fig 2).

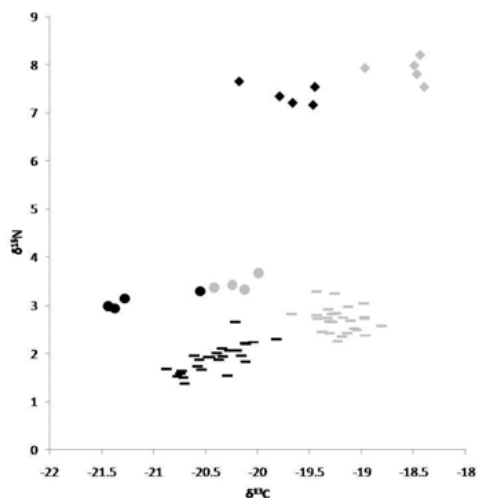


Fig. 1. Individual values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for *P. nobilis* at Andratx ♦, Cabrera – and Es Freu •. Mantle is represented in black and muscle in grey.

Discussion- The present study shows significant differences between tissues and localities. Muscle is enriched compared to mantle of the same individual. Both tissues are nitrogen enriched in localities which receive higher anthropogenic pressure, Andratx. Mantle has shown to follow the same isotopic pattern as muscle. Studying mantle in place of muscle does not involve sacrificing individuals and minimizes the sampling impact on the existing populations contributing to the conservation of this endangered species.

Tab. 1. Results of multivariate analyses of variance PERMANOVA for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in *Pinna nobilis* and posterior pair wise test for factors locality and tissue. ** $p < 0.001$ * $p < 0.05$

Source of variation	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$					
	df	SS	MS	Pseudo-F	df	SS	Pseudo-F		
Locality (Lo)	2	10.602		5.3012	87.446**	2	239.25	119.63	1737**
Tissue (Ti)	1	11.283		11.283	186.12**	1	2.8349	2.8349	41.16**
Locality x Tissue	2	0.091		0.046	0.75293	2	0.4587	0.22935	3.33*
Residual	64	3.8799		0.06		64	4.4075	0.069	
Total	69	38.274				69	252.88		

Pair wise within level of factor		
Locality	Andratx ≠ Cabrera ≠ Es freus	Andratx ≠ Cabrera ≠ Es freus
Pair wise within level of factor		
Tissue	Muscle ≠ Mantle	Muscle ≠ Mantle
Pair wise test		
Lo x Ti		Lo x Ti
		Andratx: muscle > mantle
		Cabrera: muscle > mantle
		Es freus: muscle > mantle

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