TRACING THE GEOGRAPHICAL ORIGIN OF THE MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*) IN FOOD AUTHENTICATION USING STABLE ISOTOPE AND TRACE ELEMENT ANALYSIS

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Introduction

The global production of molluscs, mainly bivalves, reached 17.7 million tonnes (USD 34.6 billion) in 2018 with consistent growth in production (FAO, 2020). Among them, mussels are an important product, which is globally traded and with strict requirements and standards, particularly when entering the European Union (EU) market. On a global scale, Europe is a major producer of mussels, supplying over a third of the total production, with *Mytilus edulis* and *M. galloprovincialis* being the two main species harvested (FAO, 2019).

Considering the economic relevance of this extensively farmed species and its importance in international trade, the verification of the geographic origin is necessary for labelling, traceability and food safety purposes (Luque and Donlan, 2019). Among the exiting methods for seafood traceability, stable isotope and elemental fingerprinting have been recognized as useful origin discriminants (Li et al., 2016). In the case of mussels, stable isotope ratio analysis (SIRA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) has been used to trace their origin (Deudero et al., 2009; Zhao et al., 2019). Additionally, trace element fingerprinting (TEF) has also been successfully applied to trace the geographical origin of bivalves (Dunphy et al., 2015; Ricardo et al., 2015; Bennion et al., 2019; Morrison et al., 2019).

Moreover, the combination of both methodological approaches, organic stable isotope and inorganic trace element analysis, is an innovation in seafood authentication. In this sense, we present a combined study of stable isotope ratio and trace element analysis for the authentication of the geographical origin of *M. galloprovincialis* samples from the Mediterranean Sea, the European Atlantic coast and the Chilean Pacific coast.

Material and Methods

Mytilus galloprovincialis samples for SIRA and TEF were collected between autumn 2018 and autumn 2019. Samples were obtained from 11 different locations in 7 different countries namely Spain, Portugal, France, Italy, Ireland, Tunisia, and Chile. The sample set for SIRA comprised 183 individual mussels, whose tissue (without digestive gland) was dried and mortared before stable isotope analysis. Simultaneous $\delta^{13}C$ and $\delta^{15}N$ measurements were performed using a Flash EA 1112 elemental analyser coupled to a Delta Plus XL isotope-ratio mass spectrometer (Thermo Scientific, USA) according to Molkentin 2018. For TEF analysis, a total of 106 mussel shells were processed following a methodology similar to previous studies (Ricardo et al., 2015; Bennion et al., 2019). After organic tissue removal, microwave-assisted digestion procedure was carried out. The quantitative analysis of trace metals (B, Al, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, As, Cd, Ba, Pb) was performed by using ICP-MS (7700x, Agilent Technologies, USA). All statistical analyses were performed using R software. PERMANOVA was used to test the significance of differences in isotopes and trace elements. Differences in sampling points were examined using non-metric multidimensional scaling (nMDS). To test whether isotope and element fingerprinting could be used to successfully assign samples to their harvesting locations, random forest (RF) classification method was used.

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Results

Significant differences were recorded among locations for both methodological approaches. The RF analysis revealed that the combination of stable isotope ratio and trace element analysis was able to assign significantly the samples to each respective farming location, with successful classification in 97% of samples. Most relevant elements for provenance discrimination were $\delta^{15}N$, Pb, $\delta^{13}C$, Ba, Mn, Zn and Al.

Conclusions

In conclusion, this study reveals that the combiantion of stable isotope ratio and trace element analysis is an effective technique for the authentication of the geographical origin of *M. galloprovincialis* mussels farmed in Mediterranean Sea, the European Atlantic coast and the Chilean Pacific coast. The technique described here provides a reliable traceability tool applicable for labelling and seafood safety purposes.

Acknowledgments

Our study was carried out thanks to the financial support of the SEA-TRACES (EAPA_87/2016) INTERREG Atlantic Area EU funded project. The authors thank for the ICPMS MW service provided by SGIker (UPV/EHU/ ERDF, EU). Ane del Rio is the recipient of a PhD grant from the Education Department of the Basque Government. Samples were kindly provided by Consello Regulador Mexillón de Galicia, IIM-CSIC, Fepromodel, Universidad de Chile, Muséum National D' Histoire Naturelle and Indigo Rock Marine Research Station.

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