Revealing the illegal harvesting of Manila clams (*Ruditapes philippinarum*) using fatty acid profiles of the adductor muscle

Renato Mamede, Fernando Ricardo, Andreia Santos, Seila Díaz, Sónia A.O. Santos, Regina Bispo, M. Rosário M. Domingues, Ricardo Calado

PII: S0956-7135(20)30284-X

DOI: https://doi.org/10.1016/j.foodcont.2020.107368

Reference: JFCO 107368

To appear in: *Food Control*

Received Date: 14 February 2020

Revised Date: 11 May 2020

Accepted Date: 17 May 2020

Please cite this article as: Mamede R., Ricardo F., Santos A., Díaz S., Santos Só.A.O., Bispo R., Domingues M.Rosá.M. & Calado R., Revealing the illegal harvesting of Manila clams (*Ruditapes philippinarum*) using fatty acid profiles of the adductor muscle, *Food Control* (2020), doi: https://doi.org/10.1016/j.foodcont.2020.107368.

Author's contribution:

Conceived and designed the experiments: R.M., F.R. and R.C. Performed the experiments: R.M., F.R and A.S. Analyzed the data: R.M., F.R., R.B., M.R.M.D. and R.C. Contributed reagents/materials/analysis tools: S.D., S.A.O.S., M.R.M.D. and R.C. All authors wrote and reviewed the manuscript.

Revealing the illegal harvesting of Manila clams (*Ruditapes philippinarum*) using fatty acid profiles of the adductor muscle

Renato Mamede[1*], Fernando Ricardo[1], Andreia Santos[1], Seila Díaz[2,3], Sónia A. O. Santos[4], Regina Bispo[5], M. Rosário M. Domingues[6,7], Ricardo Calado[1*]

[1] ECOMARE, CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal

[2] Department of Zoology, Genetics and Physical Anthropology, Genomes and Disease Group, CIMUS, Universidade de Santiago de Compostela, Campus of Santiago de Compostela, 15706 Santiago de Compostela, Spain

[3] Centro de Investigaciones Marinas, Universidade de Vigo, Illa de Toralla, 36331, Coruxo, Vigo, Spain

[4] CICECO - Instituto de Materiais de Aveiro, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

[5] Departamento de Matemática & Centro de Matemática e Aplicações (CMA), Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal

[6] Departamento de Química & CESAM - Centre for Environmental and Marine Studies & ECOMARE, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

[7] Centro de Espetrometria de Massa, Departamento de Química & QOPNA & ECOMARE, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

* Corresponding author.

Email address: renatomamederes@gmail.com (R. Mamede); rjcalado@hua.pt (R. Calado)

Abstract

The Manila clam (*Ruditapes philippinarum*) is one of the most traded bivalves in the world. Knowing its harvesting location is therefore paramount to guarantee the safety of consumers. The present study employs fatty acid (FA) profiles of the adductor muscle (AM) to reveal the most likely harvesting location of four batches of Manila clams suspected of having been illegally sourced from the Tagus estuary. In this ecosystem, where the collection of Manila

clams is currently prohibited for food safety reasons, illegal, unreported and unregulated (IUU) capture is known to occur. In order to trace the geographic origin of these four batches of Manila clams, a reference model based on the FA profiles of the AM was developed with specimens originating from the two most representative ecosystems supplying the trade-chain of this species in mainland Portugal (the Tagus estuary and Ria de Aveiro), as well as Ría de Vigo, a production area outside Portugal and that is also an important supplier. The ability of this model to allocate clams to its origin ecosystem was evaluated using independent datasets, with an allocation success of 100% (all samples were correctly assigned to its origin ecosystem, thus validating the model). Based on the reference model established, the harvesting location of the four batches suspected of originating from ongoing IUU in the Tagus estuary was investigated. Specimens from 3 of the 4 batches screened were classified, as most likely originating from the Tagus estuary (with a likelihood ranging from 90% up to 100%). These results highlight the potential of this approach to fight the IUU capture of Manila clams, as this practice endangers important habitats and threatens public health.

Keywords: traceability, mislabeling, bivalves, food safety, lipid markers

1. Introduction

Marine bivalves, such as oysters, mussels, cockles and clams are among the most consumed seafood products worldwide. The Manila clam (*Ruditapes philippinarum*) is one of the most representative of such bivalves, with a production in 2017 of over 35.000 tons in Europe alone (FAO, 2019). Native from South-east Asia (Indo-Pacific), *R. philippinarum* is an invasive species in European coasts (Humphreys et al., 2015), where it was introduced in the early 1970's. In Iberian Peninsula, this species has been reported since late 1980's (Campos

& Cachola, 2005; Chiesa et al., 2017), presenting currently well-stablished populations which turned it an important economic resource in this area (FAO, 2019).

Despite their high nutritional value, bivalves can at times threaten human health. Due to their suspension feeding nature, bivalves can accumulate pathogenic bacteria, being this especially dangerous for human health if consumed raw or lightly cooked (Cook, 1991; Wright, Fan, & Baker, 2018), as well as metals and metalloids present in the water (Karouna-Renier, Snyder, Allison, Wagner, & Ranga Rao, 2007; Velez, Figueira, Soares, & Freitas, 2015). These issues are related with water and sediment quality of the harvesting location (Li, Yu, Song, & Mu, 2006; Stabili, Terlizzi, & Cavallo, 2013). In order to safeguard public health, the European Union (EU) already produced several pieces of legislation (Regulation (EC) No 853/2004, No 854/2004, No 2073/2005 and No 1021/2008) classifying bivalves harvesting areas according to the levels of *Escherichia coli* they display per g of bivalves flesh and intravalvular liquid (EC, 2004a, 2004b, 2005, 2008). Moreover, to ensure the traceability of each batch of seafood traded in the European Union, it also stablished several labelling regulations (Regulation (European Commission (EC)) No 104/2000 and No 1224/2009; Regulation (EU) No 404/2011 and No 1379/2013) (EC, 2000, 2009; EU, 2011, 2013). The more recent and demanding of these labeling regulations (Regulation (EU) No 1379/2013) (EU, 2013) stipulates, among other specifications, that marketed seafood products need to display the catch area, production method and fishing gear used. In this way, the development of traceability tools for origin certification is paramount to avoid seafood mislabeling, being key to ensure safety for human consumption (Leal, Pimentel, Ricardo, Rosa, & Calado, 2015; Moretti, Turchini, Bellagamba, & Caprino, 2003).

Environmental factors, such as temperature, salinity and sediment type, influence the spatial distribution of bivalves (Gosling, 2003) modulating their fatty acids (FA) profile (Calado & Leal, 2015). For instance, high salinity fluctuations and low temperatures influence the

structure and fluidity of cell membranes. This results in a lower saturated FA (SFA) content, that stabilize bilayer cellular membranes, with these biomolecules being replaced by polyunsaturated FA (PUFA), which allow higher membrane fluidity (Fokina, Ruokolainen, Bakhmet, & Nemova, 2015; Nemova, Fokina, Nefedova, Ruokolainen, & Bakhmet, 2013). Other driving factor of the FA composition in bivalve tissues is trophic history, with the predominance of certain FA revealing their dietary regimes (Calado & Leal, 2015; Prato, Danieli, Maffia, & Biandolino, 2010). The FAs 16:0, 16:1*n*-7 and 20:5*n*-3 (eicosapentaenoic acid, EPA) in bivalves tissues reveal the consumption of diatoms, while PUFA 18:3*n*-3 and 18:2*n*-6 of green microalgae, 22:6*n*-3 (docosahexaenoic acid, DHA) and 18:4*n*-3 of dinoflagellates, and odd chain FA (15:0 and 17:0) and 18:1*n*-7 of detritus/bacteria (among others, Calado & Leal, 2015; Dalsgaard, John, Kattner, Müller-Navarra, & Hagen, 2003; Ezgeta-Balić, Najdek, Peharda, & Blažina, 2012; Fujibayashi, Nishimura, & Tanaka, 2016; Nerot et al., 2015). These indicative features allow to apply the profiling of FA signatures of tissues from different marine species for multiple scopes, such as identifying their feeding habitats (e.g. Coelho et al., 2011; Xu, Xu, Zhang, Peng, & Yang, 2016), diet composition (e.g. Bosley, Copeman, Dumbauld, & Bosley, 2017; White et al., 2017), seasonal variations in dietary habits (e.g. Soler-Membrives, Rossi, & Munilla, 2011) or trace their geographic origin (Ricardo, Maciel, Domingues, & Calado, 2017; Ricardo et al., 2015; Zhang, Liu, Li, & Zhao, 2017).

The FA profile of the adductor muscle (AM) proved to be suitable in geographic origin traceability studies targeting diverse bivalve species, such as cockles (*Cerastoderme edule*; Ricardo et al., 20157, 2015), scallops (*Pecten maximus*; Grahl-Nielsen, Jacobsen, Christophersen, & Magnesen, 2010) and clams (*Astarte sulcata*; Olsen, Grahl-Nielsen, & Schander, 2009). The AM is of particular interest in traceability studies, mainly due to its

high content in polar lipids, which prevents short-term turnover in the FA profile related to dietary shifts (Grahl-Nielsen et al., 2010; Leal et al., 2015; Olsen et al., 2009).

To avoid the fraudulent mislabeling of seafood geographic origin, it is important to develop and refine traceability tools. Therefore, the present study aimed to develop a model based on the FA profile of the AM that could indicate the most likely harvesting location of four batches of *R. philippinarum* suspected of having been illegally harvested from the Tagus estuary (where the harvesting of Manila clams is forbidden due to food safety issues). A two-step approach was employed to develop this model: i) Manila clam samples harvested from three ecosystems (the Tagus estuary and Ria de Aveiro, two Portuguese ecosystems that supply ~95% of the whole Manila clam traded in Portugal, and Ría de Vigo, a Spanish ecosystem that is also an important supplier of this species to the Portuguese market) were used to validate a predictive model to trace their geographic origin; and following the validation of the predictive model ii) the FA profile of the AM of clams suspected of originating from the Tagus estuary were screened to verify if these clams had indeed been harvested in this area where their capture is illegal.

2. Material and methods

2.1 Study areas and clam collection

Thirty specimens of *R. philippinarum* were collected in May 2018 in the Tagus estuary (Te, Portugal), Ria de Aveiro (RAv, Portugal) and Ría de Vigo (RV, Spain) (3 ecosystems X 30 replicates = 90 samples; Figure 1). These ecosystems play an important role in the Portuguese trade of *R. philippinarum*, with these clams being intensively harvested in these locations. However, it must be highlighted, that regardless of the Tagus estuary being the main source of Manila clams supplying the Portuguese trade, and most likely the Spanish trade as well, the harvesting of Manila clams is currently illegal in this ecosystem due to food safety issues.

Recurrent apprehensions of several tons of this bivalve originating from the illegal, unreported and unregulated (IUU) capture of this bivalve are commonly reported on Portuguese and Spanish media, with authorities from both countries continuously pursuing inspection actions to fight this practice. The specimens collected from these three ecosystems were used to assemble a predictive reference model to assign the most likely place of origin to sampled Manila clams (see below). Stakeholders provided 4 batches of Manila clams whom they strongly suspected had been illegally harvested from the Tagus estuary, with the 1st batch of 12 clams being obtained from a retailer (Rt), the 2nd batch of 7 clams from a wholesaler (Ws) and the 3rd and 4th batches of 30 clams each originating from two separated tanks from a depuration center (DC1 and DC2).

All samples were collected fresh, stored in aseptic bags and kept refrigerated until arrival to the laboratory. All specimens were taxonomically confirmed as *R. philippinarum* and the AM was dissected, freeze-dried and stored at -80 °C until the FA analysis was performed.

2.2. Fatty acids analysis

Methyl esters of fatty acids (FAME) were obtained through transmethylation, using a modified method from Aued-Pimentel, Lago, Chaves, & Kumagai (2004). In brief, 5-10 mg of the adductor muscle was suspended in 1 mL n-hexane, 0.2 mL of methanolic solution KOH (2 M) and 2 mL saturated NaCl solution, followed by intense vortexing. Posteriorly, the samples were centrifuged at 2000 rpm for 5 minutes, with the organic phase then being collected. The FAME obtained were injected and analyzed by gas chromatography-mass spectrometry (GC-MS - QP2010 Ultra, Shimadzu, Kyoto, Japan), equipped with an auto-sampler, a DB-FFAP column with 30 m length, 0.32 mm internal diameter and 0.25 µm film thickness (J&W Scientific, Folsom, CA). The column was initially programed to 80 °C, increasing 25 °C min^{-1} until 160 °C, 2 °C min^{-1} from 160 to 220 °C and 30 °C min^{-1} from 220

153 to 250 °C, using helium as the carrier gas, at a flow of 1.8 mL min^{-1}. All FAME were
154 identified using the equipment built-in software by comparing retention times, the mass
155 spectrum of each relative to mixed FAME standards (C4-C24, Supelco 37 Component Fame
156 Mix) and standard spectra from the library "AOCS Lipid Library"
157 (http://lipidlibrary.aocs.org/).
158

159 **2.3. Data and statistical analysis**

160 The relative FA composition was obtained for each sample, being calculated as the mean and
161 standard deviation for each FA per sampling group. All FA were classified either as saturated
162 FA (SFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA) or highly unsaturated
163 FA (HUFA). Usually, FA with ≥ 2 double bonds are only classified as PUFA, however, for a
164 better characterization of the FA profile, these biomolecules were separated in the present
165 study as PUFA (FAs displaying 2 or 3 double bonds) and HUFA (FAs with ≥ 4 double
166 bonds).
167 The relative FA composition of each sample was submitted to a log (x + 1) transformation
168 and a dissimilitary matrix between samples was obtained using the Bray-Curtis coefficient.
169 The existence of significant differences ($p < 0.05$) between the FA profiles of the AM of
170 clams from different ecosystems was investigated trough a one-way analysis of similarity
171 (ANOSIM). Additionally, a similarity percentage analysis (SIMPER) was performed to find
172 which FAs contributed the most to the separation recorded between pairs of ecosystems.
173 A reference model was built using a canonical analysis of principal coordinates (CAP) with
174 the groups corresponding to the ecosystems of origin of the Manila clams surveyed, namely
175 Te, RAv and RV. First, to evaluate the accuracy of the reference model, an independent
176 training and test datasets was produced. The matrix with all samples was randomly split with
177 a ratio of 0.67 to 0.33, resulting in training and test matrices with 60 samples (20 replicates

per ecosystem) and 30 samples (10 replicates per ecosystem), respectively. Following this procedure, a CAP was performed under the training dataset. The generated model was then evaluated introducing samples of the test dataset, one group at a time, verifying in which ecosystem the samples were allocated. Finally, the reference model with all sites per ecosystem (30 replicates per ecosystem = 90 samples) was built. This model was used to verify the most likely harvesting location of the samples suspected of originating from the Tagus estuary provided by the stakeholders, namely Rt, Ws, DC1 and DC2, through their allocation to one of the following locations: Te, RAv or RV.

To justify the allocation of the samples suspected of originating from the Tagus estuary to the ecosystems, under each FA, nonparametric Nemenyi tests were performed to investigate the existence of significant differences ($p < 0.05$) between pairs of sampling groups.

Multivariate analysis (CAP, ANOSIM and SIMPER) were performed using PRIMER v7 with the add-on PERMANOVA+ (Anderson, Gorley, & Clarke, 2008; Clarke & Gorley, 2015), while the Nemenyi tests were performed using R environment v3.2.5 (R Core Team, 2016).

3. Results

The mean relative abundance of each FA per sampling group is presented in Table 1. A total of 26 FAs were identified, being HUFA the most dominant class (50-53%), with eicosapentaenoic (20:5*n*-3; EPA) and docosahexaenoic (22:6*n*-3; DHA) acids being the most relevant ones, followed by SFA (22-26%), with the predominance of palmitic (16:0; PA) and stearic (18:0) acids. The least represented classes were MUFA (13-18%), mostly present due to oleic (18:1*n*-9) and eicosenoic (20:1*n*-9/11) acids, and PUFA (7-12%), with docosadienoic (22:2*n*-6) acid being the most predominant.

The results of global and pairwise ANOSIM tests performed under the ecosystems surveyed (Te, RAv and RV) revealed significant differences ($p < 0.001$), with the higher value of R

obtained for the RAv vs. RV comparison (R = 1), followed by Te vs. RV (R = 0.999) and Te vs. RAv (0.877). Results from the SIMPER analysis are presented in Table 2 and revealed that DHA and EPA were, for the three comparisons, the FAs that contributed the most for the differences recorded between ecosystems. The lowest mean DHA content was registered for RV (18.80%) and the highest for RAv (30.17%), while the lowest mean EPA content was presented by RAv (9.22%) and the highest by RV (17.63%, see Table 1).

The CAP results are summarized in Tables 3 and 4 and graphically presented in Figures 2 and 3. The evaluation of the CAP model built with the training dataset revealed a high performance (100% of correct allocations, see Table 3). For the reference model built with all the ecosystem samples collected, the percentage of the one-leave-out cross-validation was also 100% of correct allocation (see Table 4), being this illustrated by the perfect separation of the group samples shown in the respective CAP diagram (Figure 2). Concerning the most likely harvesting location of the four batches of Manila clams suspected of originating from the Tagus estuary, these showed high allocation percentages (≥ 90%) for samples of Rt, Ws and DC2 to TE (see Table 4 and Figure 3 A, B and D, respectively). The sole exception was batch DC1, with samples being mostly allocated to RAv (83.3% of allocation) (see Figure 3C and Table 4).

The results of the Nemenyi tests are summarized in Table 5, where comparisons between the FAs of the AM of Manila clams from the sampled ecosystems and between clams from the batches suspected of originating from the Tagus estuary are presented. Regarding the comparisons between ecosystems, Te vs. RAv, Te vs. RV and RAv vs. RV, significant differences were recorded for most of the FAs surveyed (see Table 5). The batches of Manila clams being investigated with most samples classified as Te in the CAP analysis (namely Rt, Ws and DC2), displayed only 4 FAs (or less) with significant differences between these groups and Te. It is worth highlighting that these significant differences were always higher

when the comparison was performed with the two other ecosystems surveyed (RAv and RV) (see Table 5). Concerning DC1, a higher number of FAs presented significant differences in the comparison Te vs. DC1 than in the comparison RAv vs. DC1. This result is in line with the allocation of most samples from DC1 to RAv as their most likely harvesting location. Overall, the results of the Nemenyi tests support the results of the CAP.

4. Discussion

The fraudulent mislabeling of geographic origin is a well-known problem in the seafood trade (Leal et al., 2015). The significant differences recorded in the FA profiles of the AM of Manila clams originating from the three ecosystems surveyed in the present study, confirmed the potential of this biochemical tool to trace the geographic origin of bivalves, as already highlighted by previous studies (Grahl-Nielsen et al., 2010; Olsen et al., 2009; Ricardo et al., 2017, 2015).

The FA profile of the AM of *R. philippinarum* presented general features similar to other bivalves, such as cockles (*Cerastoderme edule*; Ricardo et al., 2017, 2015), scallops *(Pecten maximus*; Grahl-Nielsen et al., 2010) or other clams (*Astarte sulcata*; Olsen et al., 2009), with the most dominant FAs being 16:0 (PA), 18:0, 20:5*n*-3 (EPA) and 22:6*n*-3 (DHA), as well as by the FA classes with PUFA plus HUFA presenting the highest relative abundance, followed by SFA and MUFA. The high contents of *n*-3 HUFA, namely EPA and DHA in the AM of *R. philippinarum* in all sampling groups screened, is likely related to its phytoplankton diet, as previously suggested for other bivalve species (Ackman, Epstein, & Kelleher, 1974; Nemova et al., 2013). The highest contents on DHA, as in the case of Manila clams originating from RV, suggests the prevalence of a dinoflagellate based diet, whereas specimens from other ecosystems displayed in their AM higher levels of EPA, likely related to a predominant consumption of diatoms (e.g., Dalsgaard et al., 2003; Fujibayashi et al., 2016; Napolitano,

1999). The areas surveyed in the present work display a latitudinal gradient, being expected that the northernmost ecosystems would likely host specimens with higher levels of unsaturated FA, as these contribute to maintain membrane fluidity in colder waters (Fokina et al., 2015; Nemova et al., 2013). Nevertheless, this pattern was not found in the present study, likely because the latitudinal cline was not sufficient to promote such contrasting water temperatures and, as such, to be reflected in the level of unsaturated FA on the AM of *R. philippinarium*. The FA profile of the AM is influenced by long term dietary tendencies and environmental conditions (Dalsgaard et al., 2003; Napolitano, Pollero, Gayoso, Macdonald, & Thompson, 1997; Nerot et al., 2015), thus, the results here obtained reflect the prevalent abiotic and trophic conditions on the ecosystems surveyed.

The reference model assembled displayed accuracy values of 100%, as previously obtained by Ricardo, *et al.* (2017) using the FA profile of the AM of cockles originating from different ecosystems along the Portuguese coast. The high percentage of samples allocation ($\geq$ 90%) to the Tagus estuary from three of the four batches of Manila clams suspected from being illegally collected in that ecosystem is in agreement with the suspicions of Portuguese law enforcement agencies that a significant part of *R. philippinarum* traded in mainland Portugal originate from IUU captures. The findings from this study are certainly of concern as: i) the Tagus estuary holds a production/capture area classification of C that impairs the trade of live Manila clams, even if depurated (IPMA, 2019); ii) the damaging nature of the harvesting gears employed in this estuary to pursue this IUU fishery, which endangers multiple habitats of the largest wetland zone in Portugal, and one of the biggest in Europe (Ramajal et al., 2016); and iii) the study by Chiesa *et al.* (2018) that refers the high loads of metals and arsenic (As) recorded in the edible tissues of *R. philippinarum* from the Tagus estuary. Overall, the IUU capture of Manila clams in this ecosystem is certainly of concern if live specimens are traded for human consumption, as they pose a serious threat to public health.

The present study built upon the findings reported by Ricardo, *et al.* (2017, 2015) that used the FA profile of the AM to trace the geographic origin of the bivalve *C. edule*. It advanced the state of the art by applying this approach to another commercially relevant bivalve species (*R. philippinarum*) targeted by IUU. The independent training and test datasets employed in the present study to evaluate the reference model, allowed a more reliable and accurate analysis, when compared with one-leave-out cross-validation (Franklin, 2010). The framework presented in this study will help to strengthen food safety measures aiming to fight the fraudulent mislabeling of the geographic origin of seafood, namely for bivalves. Future studies should enhance the robustness of the reference model by including more origin ecosystems (even if these only repent a small fraction of the supply chain supporting the trade of Manila clams) and investigate how seasonal and/or interannual variations on the FA profile of the AM may challenge the accuracy of predictive models.

Acknowledgements

This work was financially supported by project TraSeafood (Tracing the geographic origin of seafood as a pathway towards the smart valorization of endogenous marine resources) (PTDC/BIA-BMA/29491/2017), funded by FEDER, through PT2020 Partnership Agreement and Compete 2020 and by national funds (OE), through FCT/MCTES. This work was also funded by the Integrated Programme SR&TD ‘Smart Valorization of Endogenous Marine Biological Resources Under a Changing Climate’ (Centro-01-0145-FEDER-000018), co-funded by the Centro 2020 program, Portugal 2020, European Union, through the European Regional Development Fund. We also acknowledge FCT/MEC for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020) and CICECO – Aveiro Institute of Materials (UIDB/50011/2020 & UIDP/5011/2020) through national funds and co-funding by

FEDER, within the PT2020 Partnership Agreement and Compete 2020. This is a contribution of Marine Lipidomic Laboratory, University of Aveiro.

Declaration of interest

The authors declare that they have no conflict of interest.

5. References

Ackman, R. G., Epstein, S., & Kelleher, M. (1974). A comparison of lipids and fatty acids of the Ocean Quahaug, *Arctica islandica*, from Nova Scotia and New Brunswick. *Journal of the Fisheries Board of Canada*, *31*(11), 1803–1811.

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). PERMANOVA+ for PRIMER: Guide to software and statistical methods. *PRIMER-E: Plymouth*.

Aued-Pimentel, S., Lago, J. H. G., Chaves, M. H., & Kumagai, E. E. (2004). Evaluation of a methylation procedure to determine cyclopropenoids fatty acids from *Sterculia striata* St. Hil. Et Nauds seed oil. *Journal of Chromatography A*, *1054*(1–2), 235–239.

Bosley, K. M., Copeman, L. A., Dumbauld, B. R., & Bosley, K. L. (2017). Identification of Burrowing Shrimp Food Sources Along an Estuarine Gradient Using Fatty Acid Analysis and Stable Isotope Ratios. *Estuaries and Coasts*, *40*(4), 1113–1130.

Calado, R., & Leal, M. C. (2015). Trophic ecology of benthic marine invertebrates with bi-phasic life cycles: what are we still missing? In *Advances in marine biology*, *71* (pp. 1–70). Elsevier Ltd.

Campos, C. J. A., & Cachola, R. A. (2005). The introduction of the Japanese Carpet Shell in coastal lagoon systems of the Algarve (south Portugal): a food safety concern. *Internet Journal of Food Safety*, *8*, 1–2.

Chiesa, S., Chainho, P., Almeida, Â., Figueira, E., Soares, A. M. V. M., & Freitas, R. (2018).

Metals and As content in sediments and Manila clam *Ruditapes philippinarum* in the Tagus estuary (Portugal): Impacts and risk for human consumption. *Marine Pollution Bulletin*, *126*, 281–292.

Chiesa, S., Lucentini, L., Freitas, R., Marzano, F. N., Breda, S., Figueira, E., Caill-Milly, N., Herbert, R. J. H., Soares, A. M. V. M., & Argese, E. (2017). A history of invasion: COI phylogeny of Manila clam *Ruditapes philippinarum* in Europe. *Fisheries Research*, *186*, 25–35.

Clarke, K. R., & Gorley, R. N. (2015). PRIMER v7: User Manual/Tutorial. *PRIMER-E: Plymouth*.

Coelho, H., Lopes da Silva, T., Reis, A., Queiroga, H., Serôdio, J., & Calado, R. (2011). Fatty acid profiles indicate the habitat of mud snails *Hydrobia ulvae* within the same estuary: Mudflats vs. seagrass meadows. *Estuarine, Coastal and Shelf Science*, *92*(1), 181–187.

Cook, D. W. (1991). Microbiology of bivalve molluscan shellfish. In *Microbiology of marine food products* (pp. 19–39). Boston, MA: Springer.

Dalsgaard, J., John, M. S., Kattner, G., Müller-Navarra, D., & Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. In *Advances in marine biology, 46* (pp. 225–340). Elsevier Science Ltd.

EC. (2000). Council Regulation (EC) No 104/2000 of 17 December 1999 on the commomn organisation of the markets in fishery and aquaculture products. *Official Journal of the European Communities L17*, pp. 22–52.

EC. (2004a). Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for food of animal origin. *Official Journal of the European Union L226*, pp. 22–82.

EC. (2004b). Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for food of animal origin intended for human

consumption. *Official Journal of the European Union L226*, pp. 83–127.

EC. (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union L338*, pp. 1–26.

EC. (2008). Commission Regulation (EC) No 1021/2008 of 17 October 2008 amending Annexes I, II and III to Regulation (EC) No 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption and Regulation (EC) No 2076/2005 as regards live bivalve molluscs, certain fishery products and staff assisting with official controls in slaughterhouse. *Official Journal of the European Union L277*, pp. 15-17.

EC. (2009). Council Regulation (EC) No 1224/2009 of 20 November 2009 establishing a Community control system for ensuring compliance with the rules of the common fisheries policy, amending Regulations (EC) No 847/96, (EC) No 2371/2002, (EC) No 811/2004, (EC) No 768/2005, (EC) No 2115/2005, (EC) No 2166/2005, (EC) No 388/2006, (EC) No 509/2007, (EC) No 676/2007, (EC) No 1098/2007, (EC) No 1300/2008, (EC) No 1342/2008 and repealing Regulations (EEC) No 2847/93, (EC) No 1627/94 and (EC) No 1966/2006. *Official Journal of the European Union L343*, pp. 1–50.

EU. (2011). Comission implementing Regulation (EU) No 404/2011 of 8 April 2011 laying down detailed rules for the implementation of Council Regulation (EC) No 1224/2009 establishing a Community control system for ensuring compliance with the rules of the Common Fisheries Policy. *Official Journal of the European Union L112*, pp. 1–153.

EU. (2013). Regulation (EU) No 1379/2013 of the European Parliament and of the Council of 11 December 2013 on the common organisation of the markets in fishery and

aquaculture products, amending Council Regulations (EC) No 1184/2006 and (EC) No 1224/2009 and repealing Council Regulation (EC) No 104/2000. *Official Journal of the European Union L354*, pp. 1–21.

Ezgeta-Balić, D., Najdek, M., Peharda, M., & Blažina, M. (2012). Seasonal fatty acid profile analysis to trace origin of food sources of four commercially important bivalves. *Aquaculture*, *334–337*, 89–100.

FAO. (2019). Fisheries and aquaculture software. FishStatJ-software for fishery statistical time series. In *FAO Fisheries and Aquaculture Department, Rome*. Available at: http://www.fao.org/fishery/statistics/software/fishstatj/en/ Accessed 26 July 2019.

Fokina, N. N., Ruokolainen, T. R., Bakhmet, I. N., & Nemova, N. N. (2015). Lipid composition in response to temperature changes in blue mussels *Mytilus edulis* L. from the White Sea. *Journal of the Marine Biological Association of the United Kingdom*, *95*(8), 1629–1634.

Franklin, J. (2010). *Mapping species distributions: spatial inference and prediction*. Cambridge, UK: Cambridge University Press.

Fujibayashi, M., Nishimura, O., & Tanaka, H. (2016). Evaluation of food sources assimilated by unionid mussels using fatty acid trophic markers in Japanese freshwater ecosystems. *Journal of Shellfish Research*, *35*(1), 231–235.

Gosling, E. (2003). *Bivalve Molluscs: Biology, Ecology and Culture.* Oxford, England: Blackwell Publishing Ltd.

Grahl-Nielsen, O., Jacobsen, A., Christophersen, G., & Magnesen, T. (2010). Fatty acid composition in adductor muscle of juvenile scallops (*Pecten maximus*) from five Norwegian populations reared in the same environment. *Biochemical Systematics and Ecology*, *38*(4), 478–488.

Humphreys, J., Harris, M. R. C., Herbert, R. J. H., Farrell, P., Jensen, A., & Cragg, S. M.

(2015). Introduction, dispersal and naturalization of the Manila clam *Ruditapes philippinarum* in British estuaries, 1980-2010. *Journal of the Marine Biological Association of the United Kingdom*, *95*(6), 1163–1172.

IPMA. (2019). Despacho n.º 2102/2019. *In Diário da República 2ª série, 43*.

Karouna-Renier, N. K., Snyder, R. A., Allison, J. G., Wagner, M. G., & Ranga Rao, K. (2007). Accumulation of organic and inorganic contaminants in shellfish collected in estuarine waters near Pensacola, Florida: Contamination profiles and risks to human consumers. *Environmental Pollution*, *145*(2), 474–488.

Leal, M. C., Pimentel, T., Ricardo, F., Rosa, R., & Calado, R. (2015). Seafood traceability: current needs, available tools, and biotechnological challenges for origin certification. *Trends in Biotechnology*, *33*(6), 331–336.

Li, Y., Yu, Z., Song, X., & Mu, Q. (2006). Trace metal concentrations in suspended particles, sediments and clams (*Ruditapes philippinarum*) from Jiaozhou Bay of China. *Environmental Monitoring and Assessment*, *121*(1–3), 489–499.

Moretti, V. M., Turchini, G. M., Bellagamba, F., & Caprino, F. (2003). Traceability issues in fishery and aquaculture products. *Veterinary Research Communications*, *27*(1), 497–505.

Napolitano, G. E. (1999). Fatty acids as trophic and chemical markers in freshwater ecosystems. In *Lipids in freshwater ecosystems* (pp. 21–44). Springer, New York, U.S.A.

Napolitano, G. E., Pollero, R. J., Gayoso, A. M., Macdonald, B. A., & Thompson, R. J. (1997). Fatty acids as trophic markers of phytoplankton blooms in the Bahia Blanca estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). *Biochemical Systematics and Ecology*, *25*(8), 739–755.

Nemova, N. N., Fokina, N. N., Nefedova, Z. A., Ruokolainen, T. R., & Bakhmet, I. N.

(2013). Modifications of gill lipid composition in littoral and cultured blue mussels *Mytilus edulis* L. under the influence of ambient salinity. *Polar Record*, *49*(3), 272–277.

Nerot, C., Meziane, T., Schaal, G., Grall, J., Lorrain, A., Paulet, Y. M., & Kraffe, E. (2015). Spatial changes in fatty acids signatures of the great scallop Pecten maximus across the Bay of Biscay continental shelf. *Continental Shelf Research*, *109*, 1–9.

Olsen, B. R., Grahl-Nielsen, O., & Schander, C. (2009). Population study of *Astarte sulcata*, da Costa, 1778, (Mollusca, Bivalvia) from two Norwegian fjords based on the fatty acid composition of the adductor muscle. *Biochemical Systematics and Ecology*, *37*(5), 662–669.

Prato, E., Danieli, A., Maffia, M., & Biandolino, F. (2010). Lipid and fatty acid compositions of *Mytilus galloprovincialis* cultured in the Mar Grande of Taranto (Southern Italy): Feeding strategies and trophic relationships. *Zoological Studies*, *49*(2), 211–219.

R Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria. URL https://www.R-project.org/: R Foundation for Statistical Computing.

Ramajal, J., Picard, D., Costa, J. L., Carvalho, F. B., Gaspar, M. B., & Chainho, P.(2016). Amêijoa-japonesa, uma nova realidade no estuário do Rio Tejo: pesca e pressão social e impacto socio-económico. In *Entre Rios e Mares: Um Património de Ambientes, História e Saberes* (pp. 17–30). UERJ.

Ricardo, F., Maciel, E., Domingues, M. R., & Calado, R. (2017). Spatio-temporal variability in the fatty acid profile of the adductor muscle of the common cockle *Cerastoderma edule* and its relevance for tracing geographic origin. *Food Control*, *81*, 173–180.

Ricardo, F., Pimentel, T., Moreira, A. S. P., Rey, F., Coimbra, M. A., Domingues, M. R., Domingues, P., Leal, M. C., & Calado, R. (2015). Potential use of fatty acid profiles of the adductor muscle of cockles (*Cerastoderma edule*) for traceability of collection site. In *Scientific report*, *5* (11125).

Soler-Membrives, A., Rossi, S., & Munilla, T. (2011). Feeding ecology of *Ammothella longipes* (Arthropoda: Pycnogonida) in the Mediterranean Sea: A fatty acid biomarker approach. *Estuarine, Coastal and Shelf Science*, *92*(4), 588–597.

Stabili, L., Terlizzi, A., & Cavallo, R. A. (2013). Sewage-exposed marine invertebrates: Survival rates and microbiological accumulation. *Environmental Science and Pollution Research*, *20*(3), 1606–1616

Velez, C., Figueira, E., Soares, A., & Freitas, R. (2015). Spatial distribution and bioaccumulation patterns in three clam populations from a low contaminated ecosystem. *Estuarine, Coastal and Shelf Science*, *155*, 114–125.

White, C. A., Bannister, R. J., Dworjanyn, S. A., Husa, V., Nichols, P. D., Kutti, T., & Dempster, T. (2017). Consumption of aquaculture waste affects the fatty acid metabolism of a benthic invertebrate. *Science of the Total Environment*, *586*, 1170–1181.

Wright, A. C., Fan, Y., & Baker, G. L. (2018). Nutritional Value and Food Safety of Bivalve Molluscan Shellfish. *Journal of Shellfish Research*, *37*(4), 695–708.

Xu, Q., Xu, Q., Zhang, X., Peng, Q., & Yang, H. (2016). Fatty acid component in sea cucumber *Apostichopus japonicus* from different tissues and habitats. *Journal of the Marine Biological Association of the United Kingdom*, *96*(1), 197–204.

Zhang, X., Liu, Y., Li, Y., & Zhao, X. (2017). Identification of the geographical origins of sea cucumber (*Apostichopus japonicus*) in northern China by using stable isotope ratios and fatty acid profiles. *Food Chemistry*, *218*, 269–276.

Figure captions:

Figure 1. Sampling locations of Manila clams *Ruditapes philippinarum* in mainland Portugal and Spain: Ría de Vigo (8° 43' 9.59"W, 42° 15' 38.44"N), Ria de Aveiro (8° 41' 18.93"W, 40° 46' 6.95"N) and Tagus estuary (9° 0' 58.66"W, 38° 45' 16.55"N).

Figure 2. Reference model produced by a canonical analysis of principal coordinates (CAP) based on the fatty acid composition of the adductor muscle of Manila clams *Ruditapes philippinarum* originating from the Tagus estuary (Te), Ria de Aveiro (RAv) and Ría de Vigo (RV).

Figure 3. Pinpoint of the harvesting location of the samples suspected of originating from the Tagus estuary produced by a canonical analysis of principal coordinates (CAP) based on the fatty acid composition of the adductor muscle of Manila clams *Ruditapes philippinarum*. The reference model was validated using samples from the Tagus estuary (Te), Ria de Aveiro (RAv) and Ría de Vigo (RV), on which were introduced, one group at a time, samples from the batches originating from Retailer (Rt, A), Wholesaler (Ws, B), Depuration Center tank1 (DC1, C) and Depuration Center tank 2 (DC2, D).

Table 1. Fatty acid profile (presented as % of relative abundance of the total pool of fatty acids) of the adductor muscle of Manila clams *Ruditapes philippinarum* (values are means of replicates ± SD) collected from the Tagus estuary (Te), Ria de Aveiro (RAv) and Ría de Vigo (RV) and from the four batches of clams with unknown geographic origin, namely Retail (Rt), Wholesaler (Ws) and two different tanks from a depuration center (DC1 and DC2). SFA - Saturated fatty acids; MUFA - Monounsaturated fatty acids; PUFA - Polyunsaturated fatty acids; HUFA – Highly unsaturated fatty acids.

Fatty acids (%)	Sampling groups						
	Te (n=30)	RAv (n=30)	RV (n=30)	Rt (n=12)	Ws (n=7)	DC1 (n=30)	DC2 (n=30)
14:0	0.36±0.08	0.52±0.13	0.80±0.18	0.53±0.09	0.33±0.06	0.41±0.14	0.33±0.08
15:0	0.50±0.08	0.57±0.10	0.43±0.06	0.49±0.06	0.47±0.03	0.40±0.07	0.44±0.06
16:0	12.75±0.93	13.71±1.66	14.04±0.66	13.47±0.82	12.04±0.56	11.34±1.84	11.91±0.94
17:0	1.29±0.17	1.33±0.14	1.01±0.10	1.26±0.13	1.36±0.16	1.45±0.15	1.42±0.09
18:0	7.91±0.70	9.81±1.22	8.07±0.67	8.00±0.94	8.19±0.71	8.86±0.77	8.60±0.78
∑SFA	**22.81±1.96**	**25.94±3.24**	**24.36±1.67**	**23.75±2.04**	**22.39±1.53**	**22.45±2.98**	**22.71±1.94**
16:1*n*-9	0.18±0.04	0.14±0.03	0.13±0.03	0.15±0.02	0.20±0.03	0.15±0.04	0.17±0.03
16:1*n*-7	2.33±0.39	1.92±0.36	3.88±0.79	2.55±0.40	2.21±0.61	1.78±0.73	2.26±0.32
18:1*n*-9	4.79±0.67	4.79±0.81	3.80±0.49	4.33±0.62	4.80±0.66	4.88±1.29	4.66±0.56
18:1*n*-7	1.92±0.24	1.63±0.17	2.67±0.29	2.17±0.21	1.84±0.14	1.29±0.39	1.72±0.20
20:1*n*-9/11	4.78±0.41	4.84±0.56	4.08±0.42	5.07±0.66	4.40±0.37	2.93±0.38	4.57±0.36
20:1*n*-7	2.74±0.25	2.41±0.24	3.25±0.25	3.17±0.23	2.75±0.21	2.43±0.29	2.84±0.26
∑MUFA	**16.74±1.99**	**15.72±2.17**	**17.81±2.25**	**17.43±2.14**	**16.20±2.01**	**13.47±3.11**	**16.23±1.72**
18:2*n*-6	0.30±0.05	0.23±0.05	0.19±0.05	0.24±0.05	0.29±0.08	0.26±0.29	0.25±0.05
18:3*n*-3	0.84±0.14	0.38±0.06	0.52±0.10	0.63±0.11	0.82±0.13	0.38±0.20	0.75±0.08
20:2*n*-6	1.80±0.28	1.61±0.32	1.69±0.15	1.85±0.22	1.75±0.17	1.08±0.20	1.82±0.22
20:3*n*-6	0.22±0.06	0.11±0.04	0.22±0.05	0.19±0.04	0.24±0.04	0.17±0.05	0.15±0.03
22:2*n*-9	1.18±0.15	1.31±0.19	0.87±0.13	0.92±0.14	1.10±0.11	2.14±0.44	1.09±0.17
22:2*n*-6	3.23±0.41	2.71±0.41	2.91±0.41	2.84±0.64	3.09±0.40	6.90±1.20	3.33±0.41
22:3*n*-6	1.22±0.12	0.76±0.09	1.47±0.16	1.17±0.17	1.21±0.15	1.00±0.35	1.16±0.11
∑PUFA	**8.79±1.21**	**7.10±1.15**	**7.87±1.05**	**7.83±1.37**	**8.50±1.08**	**11.93±2.72**	**8.55±1.06**
18:4*n*-3	1.48±0.25	1.06±0.16	0.91±0.17	1.24±0.11	1.45±0.18	1.13±0.33	1.22±0.18
20:4*n*-6	3.70±0.34	3.50±0.39	3.03±0.32	3.66±0.40	3.69±0.17	4.31±0.46	3.75±0.26
20:4*n*-3	0.72±0.17	0.47±0.10	0.92±0.15	0.74±0.12	0.72±0.10	0.30±0.07	0.69±0.08
20:5*n*-3	11.55±0.77	9.22±0.67	17.63±1.20	13.00±1.58	11.16±0.93	7.30±1.28	10.80±0.77
22:4*n*-6	1.58±0.23	1.37±0.19	1.92±0.22	1.59±0.24	1.64±0.32	1.75±0.44	1.90±0.23
22:5*n*-6	2.21±0.62	1.44±0.20	0.95±0.16	1.57±0.12	1.96±0.25	2.01±0.29	1.85±0.17
22:5*n*-3	4.45±0.46	4.00±0.50	5.79±0.56	4.85±0.51	4.25±0.47	5.81±1.02	4.57±0.70
22:6*n*-3	25.98±1.99	30.17±1.77	18.80±1.04	24.34±1.46	28.05±1.38	29.54±3.98	27.73±1.58
∑HUFA	**51.66±4.83**	**51.24±3.99**	**49.96±3.83**	**50.99±4.54**	**52.92±3.79**	**52.15±7.86**	**52.51±3.97**

Table 2. Similarity percentage analysis (SIMPER) identifying which fatty acids of the adductor muscle of Manila clams *Ruditapes philippinarum* contributed to the differences recorded between ecosystems. Te: Tagus estuary; RAv: Ria de Aveiro; RV: Ría de Vigo.

Te vs. RAv			Te vs. RV			RAv vs. RV		
Fatty Acids	**Ind. (%)**	**Cum. (%)**	**Fatty Acids**	**Ind. (%)**	**Cum. (%)**	**Fatty Acids**	**Ind. (%)**	**Cum. (%)**
22:6*n*-3	20.28	20.28	22:6*n*-3	23.60	23.60	22:6*n*-3	28.44	28.44
20:5*n*-3	12.34	32.61	20:5*n*-3	21.35	44.95	20:5*n*-3	23.05	51.49
18:0	10.57	43.19	16:1*n*-7	6.13	51.09	16:1*n*-7	5.95	57.44
16:0	9.04	52.22	16:0	5.25	56.33	22:5*n*-3	5.33	62.77
18:1*n*-9	4.56	56.78	22:5*n*-3	5.20	61.53	18:0	5.25	68.02
22:5*n*-6	4.53	61.31	22:5*n*-6	5.00	66.53	16:0	3.98	72.00
22:5*n*-3	3.72	65.04	18:1*n*-9	4.13	70.66	18:1*n*-9	3.35	75.36
22:2*n*-6	3.66	68.70	18:1*n*-7	2.99	73.65	18:1*n*-7	3.17	78.53
16:1*n*-7	3.12	71.81	20:1*n*-9/11	2.94	76.59	20:1*n*-7	2.57	81.10
20:1*n*-9/11	3.02	74.83	18:0	2.84	79.43			
22:3*n*-6	2.67	77.50	20:4*n*-6	2.74	82.17			
18:3*n*-3	2.64	80.14						

Table 3. Allocation success (by sampling group) of the canonical analysis of principal coordinates (CAP) based on fatty acid profiles of the adductor muscle of Manila clams *Ruditapes philippinarum*. Te: Tagus estuary; RAv: Ria de Aveiro; RV: Ría de Vigo. Evaluation performed with an independent test dataset.

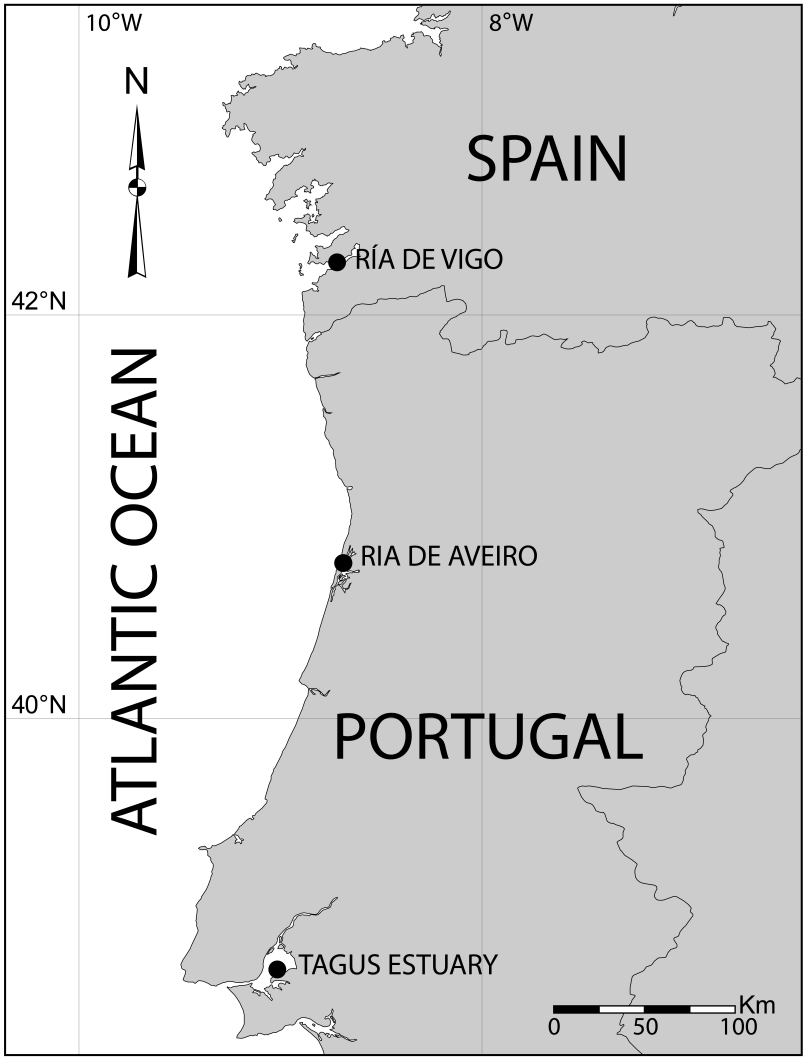
	Original Group	Allocation Group			Total per Group	% Correct Allocation
		Te	RAv	RV		
Reference model Evaluation	Te	10	0	0	10	100
	RAv	0	10	0	10	100
	RV	0	0	10	10	100

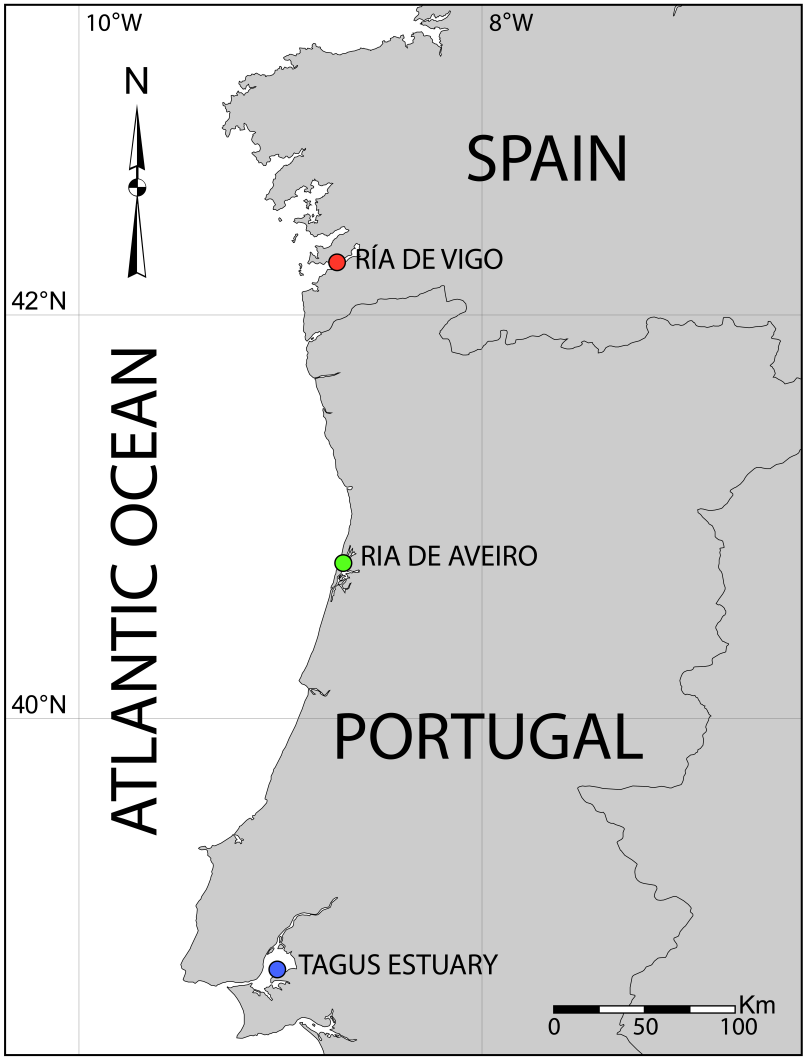
Table 4. Allocation success (by sampling group) of the canonical analysis of principal coordinates (CAP) based on the fatty acid profiles of the adductor muscle of Manila clams *Ruditapes philippinarum*. Te: Tagus estuary; RAv: Ria de Aveiro; RV: Ría de Vigo; Rt: Retail; Ws: Wholesaler; DC1: Depuration center tank 1; DC2: Depuration center tank 2.

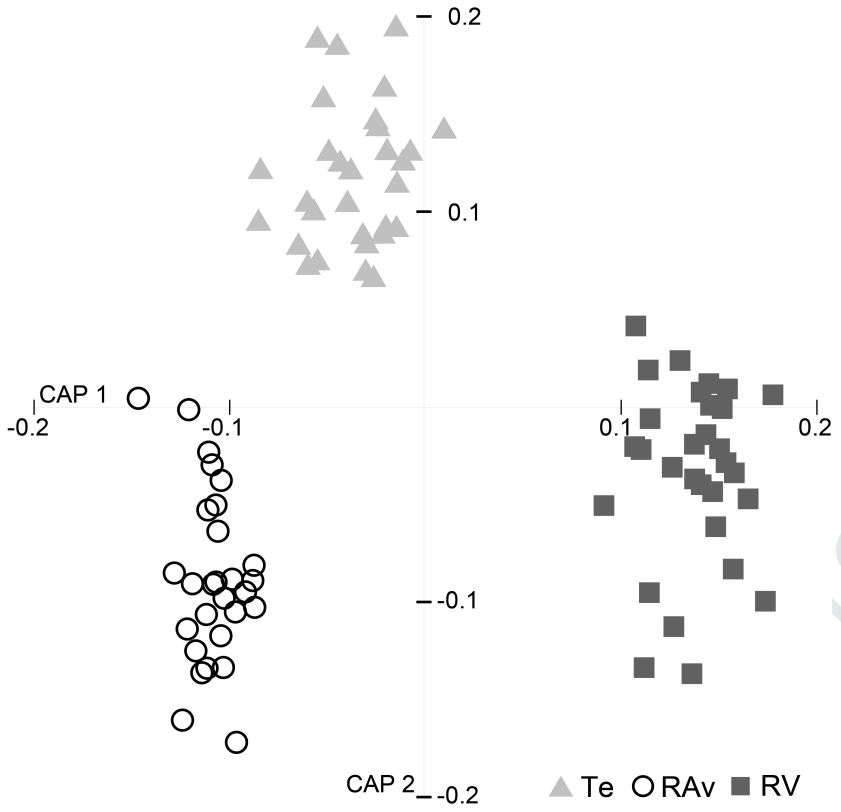
	Original Group	Allocation Group			Total per Group	% Correct Allocation (One-leave-out cross-validation)	% Allocation to TE
		Te	RAv	RV			
Reference Model	Te	30	0	0	30	100	-
	RAv	0	30	0	30	100	-
	RV	0	0	30	30	100	-
Pinpoint of harvesting location	Rt	11	0	1	12	-	91.7
	Ws	7	0	0	7	-	100
	DC1	5	25	0	30	-	16.7
	DC2	27	3	0	30	-	90

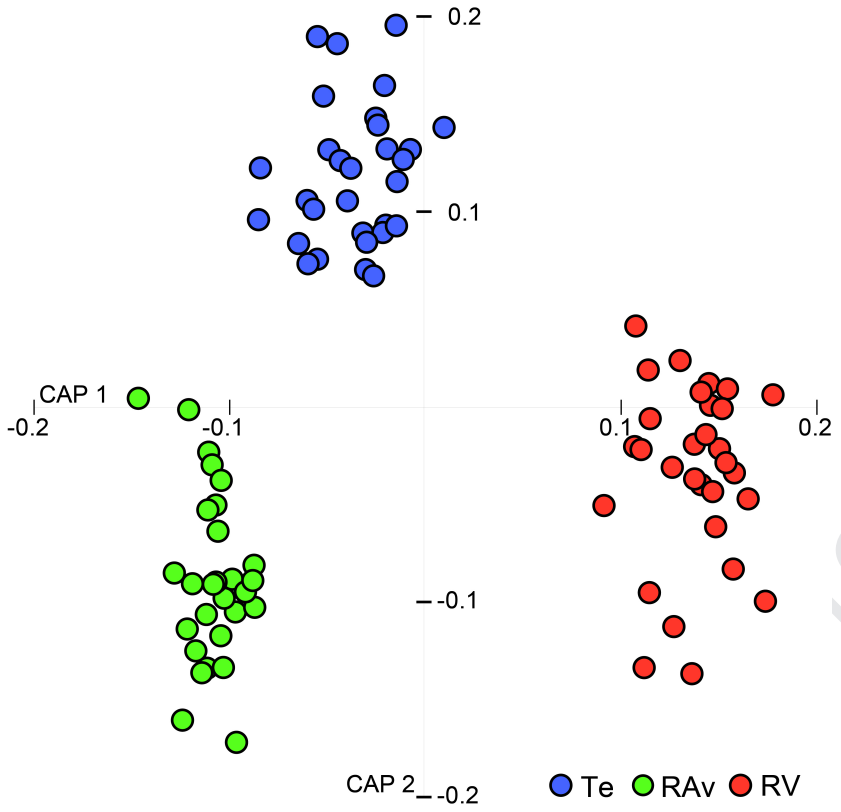
Table 5. Nemeney test results performed for each fatty acid to assess significant differences between sample groups. Values of p highlighted in light grey are < 0.05. Te: Tagus estuary; RAv: Ria de Aveiro; RV: Ría de Vigo; Rt: Retail; Ws: Wholesaler; DC1: Depuration center tank 1; DC2: Depuration center tank 2.

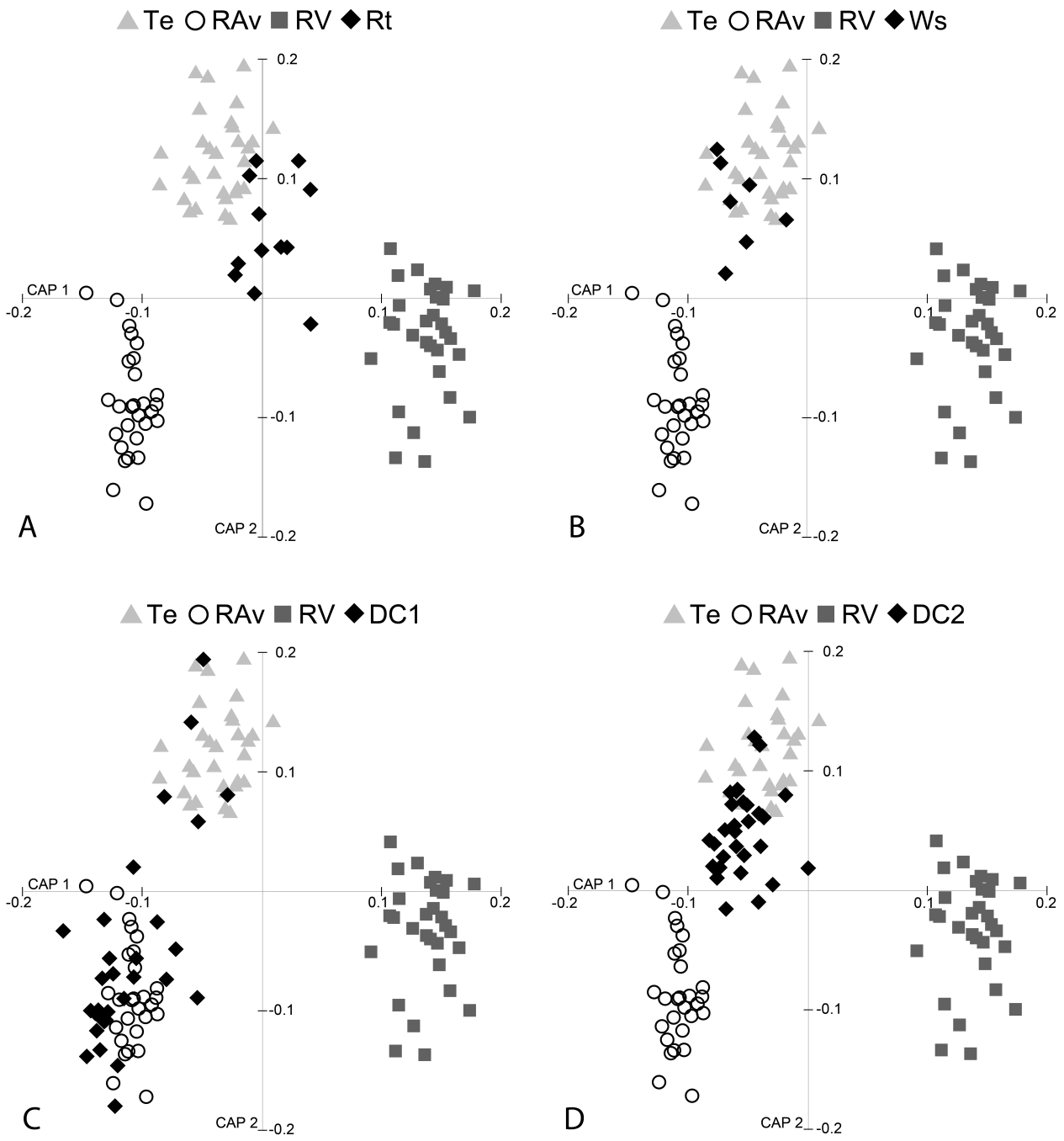
	Te vs RAv	Te vs RV	RAv vs RV	Te vs Rt	RAv vs Rt	RV vs Rt	Te vs Ws	RAv vs Ws	RV vs Ws	Te vs DC1	RAv vs DC1	RV vs DC1	Te vs DC2	RAv vs DC2	RV vs DC2
14:0	0.0011	< 0.0001	0.0361	0.0169	> 0.9999	0.3161	0.9941	0.0249	< 0.0001	0.9122	0.0558	< 0.0001	0.9235	< 0.0001	< 0.0001
15:0	0.3396	0.0259	< 0.0001	> 0.9999	0.6450	0.2193	0.9996	0.5919	0.7275	< 0.0001	< 0.0001	0.7653	0.1673	0.0001	0.9933
16:0	0.2062	0.0020	0.7841	0.5540	> 0.9999	0.9147	0.8666	0.0884	0.0048	0.0931	< 0.0002	< 0.0001	0.3056	0.0001	< 0.0001
16:1*n*-9	0.0048	< 0.0001	0.7561	0.5602	0.9497	0.3121	0.9395	0.0154	0.0004	0.0521	0.9876	0.24698	> 0.9999	0.0100	< 0.0001
16:1*n*-7	0.0525	< 0.0001	< 0.0001	0.9364	0.0172	0.1876	0.9827	0.9530	0.0046	0.0001	0.7544	< 0.0001	0.9999	0.1265	< 0.0001
17:0	0.9964	< 0.0001	< 0.0001	0.9981	0.9523	0.0310	0.9890	0.9998	0.0036	0.0129	0.0967	< 0.0001	0.0624	0.2914	< 0.0001
18:0	< 0.0001	0.9973	< 0.0001	0.9970	0.0004	> 0.9999	0.9950	0.0163	> 0.9999	0.0013	0.1801	0.0118	0.0346	0.0145	0.1634
18:1*n*-9	> 0.9999	< 0.0001	< 0.0001	0.5994	0.7233	0.2578	> 0.9999	> 0.9999	0.0136	0.9855	0.9979	0.0001	0.9998	> 0.9999	< 0.0001
18:1*n*-7	0.0167	0.0004	< 0.0001	0.6899	0.0009	0.6449	0.9995	0.6692	0.0342	< 0.0001	0.4208	< 0.0001	0.2145	0.9585	< 0.0001
18:2*n*-6	0.0067	< 0.0001	0.1214	0.2028	0.9999	0.2143	0.9999	0.4838	0.0072	< 0.0001	0.0896	> 0.9999	0.1103	0.9632	0.0052
18:3*n*-3	< 0.0001	< 0.0001	0.1481	0.1348	0.0146	0.7863	> 0.9999	< 0.0001	0.0122	< 0.0001	> 0.9999	0.1281	0.9431	< 0.0001	0.0004
18:4*n*-3	< 0.0001	< 0.0001	0.3207	0.5155	0.2360	0.0013	> 0.9999	0.0120	< 0.0001	< 0.0001	0.9821	0.0387	0.0497	0.1437	< 0.0001
20:1*n*-9/11	> 0.9999	0.0003	0.0002	0.9711	0.9859	0.0006	0.6977	0.6461	0.9361	< 0.0001	< 0.0001	0.0066	0.8630	0.8031	0.0393
20:1*n*-7	0.01664	< 0.0001	< 0.0001	0.0221	< 0.0001	0.9993	> 0.9999	0.3436	0.0521	0.0513	0.9996	< 0.0001	0.9403	0.0003	0.0026
20:2*n*-6	0.2171	0.8041	0.95739	0.9989	0.2484	0.7105	> 0.9999	0.8894	0.9967	< 0.0001	< 0.0001	< 0.0001	0.9991	0.0703	0.4975
20:3*n*-6	< 0.0001	> 0.9999	< 0.0001	0.9167	0.0004	0.8315	0.9704	< 0.0001	0.9896	0.0081	0.0058	0.00295	0.0002	0.1046	< 0.0001
20:4*n*-6	0.5552	< 0.0001	0.0242	0.9997	0.9622	0.0111	> 0.9999	0.9045	0.0251	0.0007	< 0.0001	< 0.0001	0.9987	0.2459	< 0.0001
20:4*n*-3	0.0007	0.0193	< 0.0001	0.9999	0.0090	0.3382	> 0.9999	0.0818	0.5475	< 0.0001	0.1597	< 0.0001	0.9989	0.0050	0.0033
20:5*n*-3	< 0.0001	0.0016	< 0.0001	0.9429	< 0.0001	0.4619	0.9980	0.2105	0.0464	< 0.0001	0.4538	< 0.0001	0.6174	0.0394	< 0.0001
22:2*n*-9	0.8074	< 0.0001	< 0.0001	0.0173	0.0003	0.9995	0.9743	0.5836	0.3296	< 0.0001	0.0076	< 0.0001	0.7745	0.0726	0.0093
22:2*n*-6	0.0103	0.3711	0.7957	0.1724	> 0.9999	0.9727	0.9974	0.7041	0.9930	< 0.0001	< 0.0001	< 0.0001	0.9975	0.0012	0.1132
22:3*n*-6	< 0.0001	0.0128	< 0.0001	0.9980	0.0004	0.0309	> 0.9999	0.0037	0.2497	0.0044	0.0975	< 0.0001	0.8929	< 0.0001	< 0.0001
22:4*n*-6	0.1632	0.0002	< 0.0001	> 0.9999	0.5327	0.0118	0.9852	0.2247	0.4713	0.7068	0.0011	0.0667	0.0004	< 0.0001	> 0.9999
22:5*n*-6	< 0.0001	< 0.0001	0.0860	0.0042	0.9891	0.0612	0.9996	0.0239	< 0.0001	> 0.9999	< 0.0001	< 0.0001	0.6549	0.0006	< 0.0001
22:5*n*-3	0.3022	< 0.0001	< 0.0001	0.7513	0.0281	0.0761	0.9955	0.9911	0.0007	< 0.0001	< 0.0001	0.9975	0.9997	0.1334	< 0.0001
22:6*n*-3	< 0.0001	0.0005	< 0.0001	0.9265	< 0.0001	0.3531	0.6376	0.7638	0.0004	0.0008	0.9545	< 0.0001	0.2630	0.0749	< 0.0001

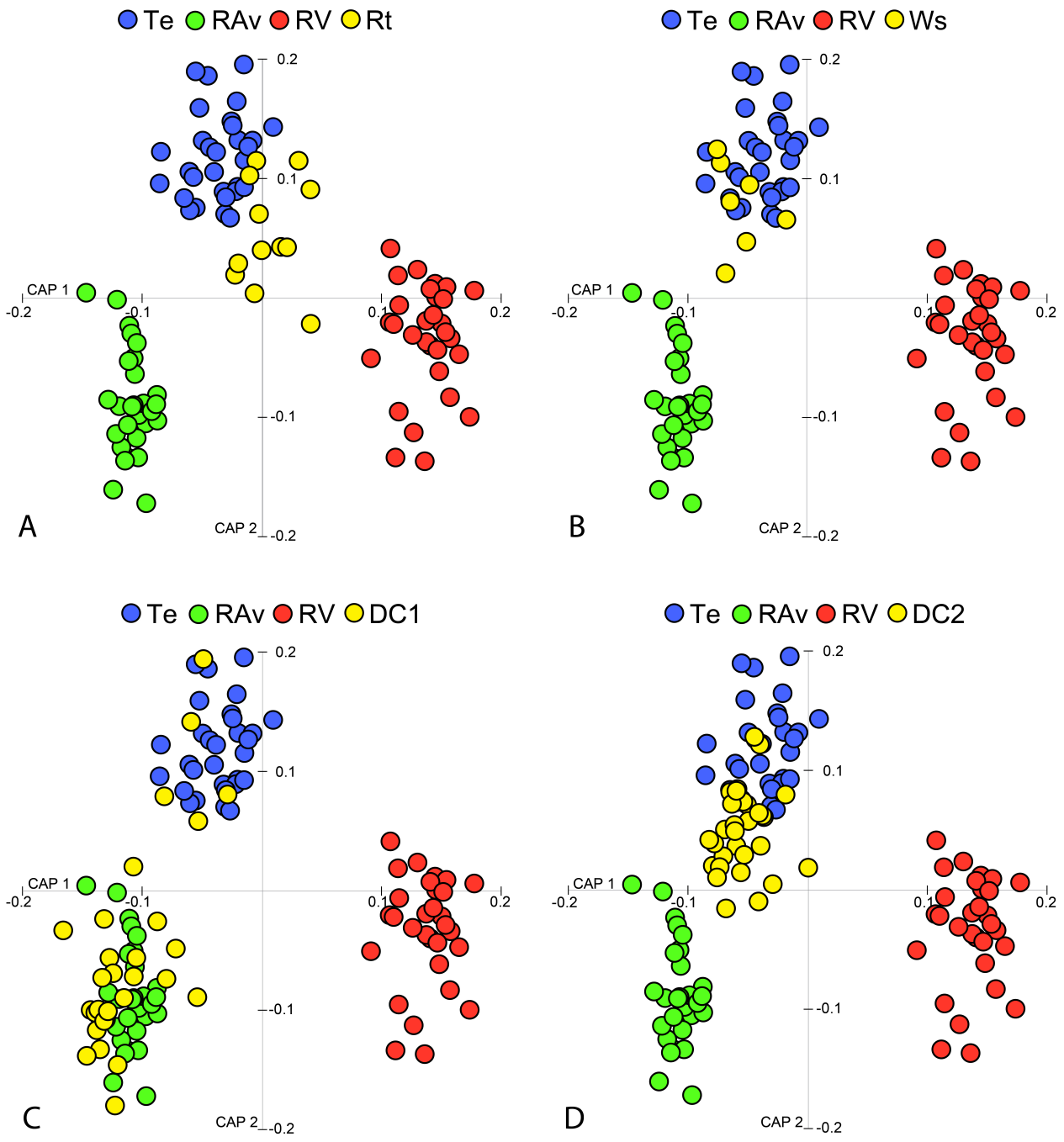

10°W
8°W
N
SPAIN
RÍA DE VIGO
42°N
ATLANTIC OCEAN
RIA DE AVEIRO
40°N
PORTUGAL
TAGUS ESTUARY
Km
0
50
100


10°W
8°W
N
SPAIN
RÍA DE VIGO
42°N
ATLANTIC OCEAN
RIA DE AVEIRO
40°N
PORTUGAL
TAGUS ESTUARY
Km
0
50
100






Te RAv RV Rt
Te RAv RV Ws
Te RAv RV DC1
Te RAv RV DC2
CAP 1
CAP 2
0.2
0.1
-0.1
-0.2
A
B
C
D


Te RAv RV Rt
Te RAv RV Ws
Te RAv RV DC1
Te RAv RV DC2
CAP 1
CAP 2
0.2
0.1
-0.1
-0.2
A
B
C
D

Highlights (maximum 85 characters, including spaces, per bullet points):

- Fatty acid (FA) profiles of the adductor muscle trace the harvesting site of Manila clams
- The dominant FAs were the 22:6*n*-3 (DHA), 16:0 (PA) and 20:5*n*-3 (EPA)
- Collection site of Manila clams with unknown origin was pinpointed
- The illegal harvesting of Manila clams from interdicted areas was uncovered

Declaration of interest:

The authors declare that they have no conflict of interest.