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Conceived and designed the experiments: F.R., C.P. and R.C. Performed the experiments: F.R., R.M., A.S. and C.P. Analysed the data: F.R., E.F.S., C.P. and R.C. Contributed reagents/materials/analysis tools: E.F.S. and R.C. All authors wrote and reviewed the manuscript.

Cost-efficiency improvement of bivalves shells preparation when tracing their geographic origin through ICP-MS analysis of elemental fingerprints

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Abstract

Developing methodologies employed to trace the geographic origin of seafood as accurate and fast as possible can help to speed-up the delivery of results to legal authorities, reduce associated costs and minimize environmental impacts (associated with the residues generated). The present study evaluated if trace element fingerprints (TEF) of a small homogenized subsample of Manila clams (*Ruditapes philippinarum*) right valve yielded a representative elemental signature of the whole shell. Four elemental ratios (Ba/Ca, Mg/Ca, Mn/Ca and Sr/Ca) commonly employed to trace the

geographic origin of bivalves were determined from subsamples of 0.2 and 3 g of the homogenized right valve and the whole left valve (4 g). A Canonical Analysis of Principal Coordinates developed for the subsamples of small portions (0.2 g) of the homogenized right valve revealed an accuracy of 100%, that led to the correct classification of the subsample of 3 g to their respective valve and that of 4 g to its matching shell. Results achieved indicate that TEF of a small homogenized portion of a bivalves valve is representative of the whole shell and can be employed to provide an accurate, fast, reliable and environmentally safer method to trace its geographic origin.

1. Introduction

The production of bivalves plays a key role in global fisheries and aquaculture worldwide, with commercial catches/production exceeding 33 million tons in 2017 and yielding over 26 million euros (FAO, 2018). Due to market globalization and recurrent alerts on food safety issues, a growing awareness of consumers on the need of seafood traceability (i.e. the authenticity origin of species) is emerging (Leal, Pimentel, Ricardo, Rosa, & Calado, 2015). The mislabeling of seafood geographic origin is particularly relevant for bivalves due to their trophic ecology (Maloy, Culloty, & Slater, 2009). Bivalves are recognized for their potential to accumulate pathogenic microorganisms which represents a risk to human health when consumed raw or lightly cooked (Rippey, 1994). Thus, the determination of bivalves geographic origin is crucial for controlling their quality and safeguarding the interest of consumers.

Although bivalve shells are primarily composed by calcium carbonate, other minor elements are incorporated during their growth (Becker, Fodrie, McMillan, & Levin, 2004; Poulain et al., 2015), reflecting in large amount the surrounding environmental information in their ecosystem of origin (Schöne & Gillikin, 2013; Thorrold, Zacherl, &

Levin, 2007; Wanamaker, Kreutz, Schöne, & Introne, 2011). This feature allows researchers to use trace element fingerprints (TEF) present in bivalve shells as a proxy to assess their geographic origin (Sorte, Etter, Spackman, Boyle, & Hannigan, 2013; Honig, Etter, Pepperman, Morello, & Hannigan, 2020). Indeed, the use of TEF for bivalve's traceability can be performed through inductively coupled plasma-mass spectrometry (ICP-MS) considering the concentration of a wide range of element/calcium ratios commonly recorded in bivalve shells (e.g. Ba, Cd, Cu, Cr, Mg, Mn, Pb, Sr, U and Zn) (Bennion, et al., 2019; Ricardo, et al., 2015; Ricardo, Pimentel, Génio, & Calado, 2017). The differences in trace element concentrations between bivalve shells from different locations can at times be subtle and, as such, using a technique as ICP-MS that allows a multi-element analysis is preferred.

Before performing ICP-MS analysis, trace elements must be made available for detection by extracting them from bivalve shells using a digestion method. The basic configuration for the ICP-MS analysis requires the introduction of the sample as a liquid and, therefore, for solid matrices, an acid digestion procedure is necessary. The selection of the acid digestion method is crucial in the elemental analysis, so it is important that the dissolution of the matrices and of any remaining organic component are complete, avoiding analytical interferences (Enamorado-Báez, Abril, & Gómez-Guzmán, 2013). The most common reagent used to digest bivalve shells is HNO_3, having already been successfully used in shells from multiple species, such as *Mytilus edulis* (Bennion, et al., 2019) and *Cerastoderma edule* (Ricardo et al., 2015; Ricardo, Pimentel, Génio, & Calado, 2017).

Previous studies have used TEF of whole valve (Ricardo, et al., 2015; Ricardo, Pimentel, Génio, & Calado, 2017), or a small piece of the outer most part of the valve (Bennion, et al., 2019; Morrison, Bennion, Gill, & Graham, 2019) to trace their

geographic origin and never tested the use of a small subsample of the whole homogenized shell. The use of the whole bivalve valve is a time-consuming approach and requires the use of higher volumes of nitric acid to perform a suitable digestion for posterior ICP-MS analysis. The present study aimed to evaluate if TEF from a subsample of the homogenized valve could be successfully used as a representative proxy for the TEF of the whole shell of this commercially important bivalve. This cost-efficiency optimization of TEF, as a tool for tracing the geographic origin of bivalves, can be paramount to more readily deliver results to legal authorities fighting fraudulent practices that mislabel the place of origin of seafood (particularly Manila clams) and puts consumers health at risk. This optimization will also allow to reduce processing costs associated with these methods and minimize environmental impacts associated with the residues generated when digesting shells for ICP-MS analysis.

2. Material and methods

2.1 Samples collection and ICP-MS analysis

Fresh Manila clams *Ruditapes philippinarum* (*n*=5) were collected in the Tagus estuary (38º 41.456´ N 9º 17. 430´ W), the most important commercial fishing area for this species in Portugal. All specimens were collected by hand-raking, stored in aseptic plastic bags and kept refrigerated until being processed in the laboratory. Valves were separated and all organic tissues were removed using ceramic coated blades and tweezers. Valves were carefully washed with tap water and distilled water to remove mud and any debris, air-dried and stored for further analysis.

In order to remove organic matter from the shell, prior to elemental analysis valves were transferred to falcon centrifuge tubes (®VWR Metal-free Centrifuge Tubes) , and soaked in high-purity H_2O_2 (30% w/v) (AnalaR NORMAPUR, VWR Scientific

Products) overnight (14–16 h) (Ricardo, et al., 2015). Five right valves were individually homogenised using a mortar grinder (RM 200, Retsch, Hann, Germany), that was carefully cleaned with silicate followed by alcohol (70%) between samples to avoid cross-contamination. The following subsamples of each right valve were weighed: five of 0.2 g and one of the remaining fraction for a total of 3 g (1 species X 5 right valves X 6 samples = 30 samples). Left valves (4 g) were not homogenized in order to test in which way TEF response could be influenced by this procedure (1 species X 5 left valves = 5 samples) (Figure 1). The digestion of the 0.2 and 3 g subsamples of the right valve, and of the whole left valve (4 g) was performed through the addition of 1, 3 and 5 mL of high-purity concentrated HNO_3 (70% w/v). Average times required to achieve a full digestion were as follows: 1 minute for a 0.2 g subsample, 3 minutes for a 3 g subsample and overnight (14-16 h) for the whole valve (Figure 1). After digestion, the resulting solution was diluted with Milli – Q (Millipore) water to a final acid concentration of 1-2% HNO_3. Barium (Ba), calcium (Ca), magnesium (Mg), manganese (Mn) and strontium (Sr) concentrations were analysed using an Agilent 7700 ICP-MS equipped with an octopole reaction system (ORS) collision/reaction cell technology to minimize spectral interferences using the operation conditions summarized in Table 1. A rigorous quality control program was implemented for the determination of elements, including method blanks, certified reference materials, and replicate samples. The accuracy of the proposed method was evaluated through the analysis of certified reference material BCS-CRM-513 (SGT Limestone 1), with results being within certified values, ranging from 88 to 108 %. Precision was estimated using the relative standard deviation (RSD) of five replicate samples and was ≤ 10% (Table S2). The detection limits (DL) were calculated as three times the standard deviation of blanks (n=10), with results being summarized in Table

S2. In order to evaluate the cost-efficiency based on the volume of HNO_3 consumed and disposed and the time needed for the digestion process, two different scenarios were set up: 1) a set of 30 samples and, 2) mimicking a real traceability study scenario to pinpoint the geographic origin of seafood as described in project TraSeafood (https://www.rjcalado.com/traseafood) for Manila clams (*R. philippinarum*), where 30 specimens were collected per sampling area in each location, over ten different locations (2 areas X 10 locations X 30 specimens = 600 samples).

2.2 Data and statistical analysis

Prior to all statistical analysis, Ba, Mg, Mn and Sr concentrations determined for *R. philippinarum* valves were converted to element/Ca ratios (mmol/mol) in order to minimize total mass effects (Thorrold, Jones, Swart, & Targett, 1998). A Canonical Analysis of Principal Coordinates (CAP) is a constrained ordination tool that was performed to visualize inter-individual spatial differences in TEF among different procedures and to evaluate the classification accuracy (leave-one-out diagnostic) of matching each individual subsample with its original shell (Anderson & Willis, 2003). Based on a calibration dataset, the CAP permit to built a reference model that could be used to classify new samples. This classification is based on the resemblaces between the new samples and the groups used to built the reference model (Anderson & Robinson 2003). Briefly, a CAP predictive model was built using 25 samples (5 samples of 0.2 g from each right valve) as a calibration set, being evaluated with cross-validation (leave-one-out) method (Anderson, Gorley, & Clarke, 2008). The representativeness of the small portion was evaluated by classification of each individual sample on this model (3 g subsample from the right valve and the whole left

valve (4 g)). All statistical analyses were performed using Primer v7 with add-on PERMANOVA+ (Clarke & Gorley, 2015).

3. Results and discussion

Trace element fingerprints (TEF) from the right valve sub-sets and the left valve of five shells of *Ruditapes philippinarum* are shown in Table S1 (see supplementary information). The most abundant elements considered in the present work and recorded in right valve sub-sets and the left valve were Sr and Mg, contrarily to Ba and Mn, with their concentration ranging between 1.38-1.52 and 0.57-0.87 mmol/mol, respectively.

In line with the high concentrations of Sr, followed by Mg, Mn and Ba in the TEF of *R. philippinarum* shells, previous studies targeting other bivalve species have already reported similar patterns, such as on the common cockle *Cerastoderma edule* (Ricardo, et al., 2015; Ricardo, et al., 2017), in New Zealand cockle *Austrovenus stutchburyi* (Norrie, Dunphy, Baker, & Lundquist, 2016) and blue mussel *Mytilus edulis* (Sorte, Etter, Spackman, Boyle, & Hannigan, 2013). At present, most studies available on the TEF of bivalve shells are focused on the analysis of the whole valve and not in subsamples of one single valve (Bellotto & Miekeley, 2007; Phung, et al., 2013; Ricardo, Pimentel, Génio, & Calado, 2017).

The first two canonical discriminant functions of CAP model explained 91.68% of TEF variation in the data set (CAP 1: 55.63%, CAP 2 36.05%; Figure 2), with results revealing an overall accuracy of 100% for the smaller subsamples (0.2 g) of the homogenized right valve (Figure 2 and Table 2). The classification using the remaining homogenized right valves (3 g) and the entire left valves (4 g) to the respective shells was assigned with a success of 100% (Figure 2 and Table 2). These findings suggest

that TEF of only 0.2 g subsamples of right valves are highly representative of the TEF of entire shells and, therefore, any significant shift in the composition of the whole shell will be reproduced in these subsamples.

TEF of biogenic carbonates have been successfully used as "natural tags" to discriminate the geographic origin of bivalves (Norrie, Dunphy, Baker, & Lundquist, 2016; Ricardo, et al., 2015; Ricardo, Pimentel, Génio, & Calado, 2017; Sorte, Etter, Spackman, Boyle, & Hannigan, 2013). Indeed, the ratios monitored in the present study (Mg/Ca, Mn/Ca, Sr/Ca and Ba/Ca) have been reported to display significant variations in bivalve shells, likely as a consequence of shifting environmental conditions (Poulain, et al., 2015; Thébault, et al., 2009; Zhao, Schöne, & Mertz-Kraus, 2017). However, Ricardo et al. (2015) showed that TEF can be successfully employed to discriminate the geographic origin of *Cerastoderma edule* at a high spatial resolution using whole valves. Moreover, Bennion et al. (2019) and Morrison et al. (2019), used smaller pieces of shells to discriminate specimens from geographically close populations (6-220 km). Nonetheless, the methodology used in these two studies is not easy to replicate and safeguard that identical subsamples of each shell can indeed be retrieved, as replicates. Cutting exact subsamples of the outer most annuli along the whole shell of a bivalve using a ceramic blade is prone to error, as what looks to the naked eye as distinct annuli in the shell are indeed a multitude of annuli. It is likely that accuracy to perform this task may likely only be possible through the use of a laser cutter coupled to a scanning electron microscope (SEM). The present study advances the state of the art by evaluating the use of a much smaller subsample (0.2 g) of the whole valve to achieve the same goal.

The costs associated with each procedure tested in the present work (sub-samples of 0.2 and 3 g, as well as the whole valve (4 g)) are shown in Table 3, mimicking a realistic

sampling scenario as described on TraSeafood research project (referred above). Optimizing ICP-MS analysis by reducing the amount of valve used to produce the homogenate reduces the amount of nitric acid, as well as time, used for digestion. Consequently, the environmental impact related to the use of acids and associated waste disposal is also optimized. The costs associated with using 0.2 or 4 g samples ranged from € 12.60 to € 60.75 for 30 samples, which is of little relevance when compared with the processing of 600 samples. In this case, the costs associated with the different approaches differ significantly, ranging from € 245.40 to € 1215.00 for a sample of 0.2 or 4 g, respectively. The same trend is recorded for nitric acid consumption, as it ranges from 30 to 150 mL when processing 30 samples of 0.2 or 4 g, respectively. These figures are even more contrasting if one considers the digestion of 600 samples of 0.2 or 4 g, as it requires 600 and 3000 mL of nitric acid, respectively. It is worth highlighting that if one considers bivalves of considerable larger sizes, such as the Pacific oyster (*Crassostrea gigas*), the Mediterranean mussel (*Mytilus galloprovincialis*) or the great scallop (*Pecten maximus*) with commercial sizes ranging from 100 to 400 mm, the consumption of nitric acid to digest a whole valve is significantly higher. Thus, as the present study revealed, the TEF of only a smaller subsample (0.2 g) of the valve can be used as a proxy of the fingerprint present in the whole shell, making this approach cheaper, faster and as reliable.

4. Conclusions

The present study confirms that TEF of a small portion of a single valve can be used as a reliable proxy of their whole shell. In spite of the small number of samples employed, this new approach can play a key role in reducing the time required to process samples and deliver results to legal authorities. Moreover, the consumption of nitric acid

employed is significantly reduced, improving the sustainability of this practice. Future studies should try to apply these methodologies to the shells of other bivalve species, namely those that display larger commercial sizes, as well as ascertain their suitability to differentiate them if they are sourced from different of geographic locations. Also, try to optimize the use of hydrogen peroxide employed to eliminate the organic matter associated with bivalve shells prior to their digestion. Overall, it is likely that there is still room to optimize associated costs with the processing of large numbers of samples of bivalve shells for ICP-MS analysis to determine their TEF and verify the claims associated with their geographic origin.

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5. References

Anderson, M.J., & Willis, T.J. (2003). Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology, 84*, 511-525.

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

Becker, B.J., Fodrie, F.J., McMillan, P., & Levin, L.A. (2004). Spatial and temporal variation in trace elemental fingerprints of mytilid mussel shells: A precursor to invertebrate larval tracking. *Limnology and Oceanography, 50,* 48-61.

Bellotto, V., & Miekeley, N. (2007). Trace metals in mussel shells and corresponding soft tissue samples: a validation experiment for the use of *Perna perna* shells in pollution monitoring. *Analytical and bioanalytical chemistry, 389*, 769-776.

Bennion, M., Morrison, L., Brophy, D., Carlsson, J., Abrahantes, J. C., & Graham, C. T. (2019). Trace element fingerprinting of blue mussel (*Mytilus edulis*) shells and soft tissues successfully reveals harvesting locations. *Science of The Total Environment, 685,* 50-58.

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

Enamorado-Báez, S., Abril, J., & Gómez-Guzmán, J. (2013). Determination of 25 trace element concentrations in biological reference materials by ICP-MS following different

microwave-assisted acid digestion methods based on scaling masses of digested samples. *ISRN Analytical Chemistry*, 2013, 851713.

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 4 November 2019)

Honig, A., Etter, R., Pepperman, K., Morello, S., & Hannigan, R. (2020). Site and age discrimination using trace element fingerprints in the blue mussel, *Mytilus edulis*. *Journal of Experimental Marine Biology and Ecology*, 522, 151249.

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, 331-336.

Maloy, A. P., Culloty, S.C., & Slater, J.W. (2009). Use of PCR-DGGE to investigate the trophic ecology of marine suspension feeding bivalves. *Marine Ecology Progress Series*, 381, 109-118.

Morrison, L., Bennion, M., Gill, S., & Graham, C.T. (2019). Spatio-temporal trace element fingerprinting of king scallops (*Pecten maximus*) reveals harvesting period and location. *Science of the Total Environment*, 697, 134121.

Norrie, C., Dunphy, B., Baker, J., & Lundquist, C. (2016). Local-scale variation in trace elemental fingerprints of the estuarine bivalve *Austrovenus stutchburyi* within and between estuaries. *Marine Ecology Progress Series, 559*, 89-102.

Phung, A.T., Baeyens, W., Leermakers, M., Goderis, S., Vanhaecke, F., & Gao, Y. (2013). Reproducibility of laser ablation–inductively coupled plasma–mass spectrometry (LA–ICP–MS) measurements in mussel shells and comparison with micro-drill sampling and solution ICP–MS. *Talanta, 115*, 6-14.

Poulain, C., Gillikin, D.P., Thébault, J., Munaron, J.-M., Bohn, M., Robert, R., Paulet, Y.-M., & Lorrain, A. (2015). An evaluation of Mg/Ca, Sr/Ca, and Ba/Ca ratios as environmental proxies in aragonite bivalve shells. *Chemical Geology, 396*, 42-50.

Ricardo, F., Génio, L., Leal, M.C., Albuquerque, R., Queiroga, H., Rosa, R., & Calado, R. (2015). Trace element fingerprinting of cockle (*Cerastoderma edule*) shells can reveal harvesting location in adjacent areas. *Scientific. Reports, 5*, 11932.

Ricardo, F., Pimentel, T., Génio, L., & Calado, R. (2017). Spatio-temporal variability of trace elements fingerprints in cockle (*Cerastoderma edule*) shells and its relevance for tracing geographic origin. *Scientific reports, 7*, 3475.

Rippey, S.R. (1994). Infectious diseases associated with molluscan shellfish consumption. *Clinical Microbiology Reviews, 7*, 419-425.

Schöne, B.R., & Gillikin, D.P. (2013). Unraveling environmental histories from skeletal diaries—advances in sclerochronology. *Palaeogeography, Palaeclimatology, Palaeoecology, 373*, 1-5.

Sorte, C.J., Etter, R. J., Spackman, R., Boyle, E.E., & Hannigan, R.E. (2013). Elemental Fingerprinting of Mussel Shells to Predict Population Sources and Redistribution Potential in the Gulf of Maine. *PloS one, 8*, e80868.

Thébault, J., Chauvaud, L., L'Helguen, S., Clavier, J., Barats, A., Jacquet, S., Pécheyran, C., & Amouroux, D. (2009). Barium and molybdenum records in bivalve shells: Geochemical proxies for phytoplankton dynamics in coastal environments? *Limnology and Oceanography, 54*, 1002-1014.

Thorrold, S.R., Jones, C.M., Swart, P.K., & Targett, T.E. (1998). Accurate classification of juvenile weakfish Cynoscion regalis to estuarine nursery areas based on chemical signatures in otoliths. *Marine Ecology Progress Series, 173*, 253-265.

Thorrold, S.R., Zacherl, D.C., & Levin, L.A. (2007). Population connectivity and larval dispersal: using geochemical signatures in calcified structures. *Oceanography, 20*, 80-89.

Wanamaker, A.D., Kreutz, K.J., Schöne, B.R., & Introne, D.S. (2011). Gulf of Maine shells reveal changes in seawater temperature seasonality during the Medieval Climate Anomaly and the Little Ice Age. *Palaeogeography, Palaeoclimatology, Palaeoecology, 302*, 43-51.

Zhao, L., Schöne, B.R., & Mertz-Kraus, R. (2017). Controls on strontium and barium incorporation into freshwater bivalve shells (*Corbicula fluminea*). *Palaeogeography, Palaeoclimatology, Palaeoecology, 465*, 386-394.

Figure Captions

Figure 1. Outline of experimental design and the protocols used for acid digestion.

Figure 2. Canonical Analysis of Principal Coordinates (CAP) based on Trace element fingerprints (TEF) from *Ruditapes philippinarum* shells.

Table 1. Operating conditions of the Agilent 7700 ICP-MS.

RF power	1550 W
Plasma gas flow rate	Ar 15 L min^{-1}
Auxiliary gas flow rate	Ar 0.9 L min^{-1}
Carrier gas flow rate	Ar 1.05 L min^{-1}
Sample depth	10.0 mm
Interface	Pt sampler cone, Ni skimmer cone
CeO+/Ce+	1.0%

Collision gas/flow rate	He 2.8 mL min^{-1}
Octopole bias	-18 V
Octopole RF	200 V
Energy Discrimation	5.0 V
Internal standards	^{72}Ge, ^{103}Rh, ^{192}Tb

343 **Table 2.** Classification success (by shell; S1-S5) of canonical analysis of principal coordinates (CAP) for *Ruditapes philippinarum* subsamples
344 based on trace element fingerprints and classification success from remaining subsamples of right valves (3 g) (R1-R5) and whole left valves (4
345 g) (L1-L5) homogenates.

Original shell	Predicted shell					total per shell	% correct shell	% Classification				
	S1	S2	S3	S4	S5			R1 and L1	R2 and L2	R3 and L3	R4 and L4	R5 and L5
S1	5					5	100	100				
S2		5				5	100		100			
S3			5			5	100			100		
S4				5		5	100				100	
S5					5	5	100					100

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350 **Table 3.** Estimated costs (consumables and services) for processing 30 and 600 samples. Note: costs associated with manpower (ICP-MS and
351 laboratory technician time) were considered to be the same for all biomasses.

	Consumables and services							
Biomass (g)	**Nitric acid (mL/sample)**	**Digestion (minutes)**	**Nitric acid/sample (€/mL)**	**Nitric acid disposal/sample (€/mL)**	**Total cost (€)**		**Total nitric acid (mL)**	
					30 samples	600 samples	30 samples	600 samples
0.2	1	1	0.390	0.019	12.60	245.40	30	600
3	3	3	1.170	0.057	36.81	736.20	90	1800
4	5	960	1.930	0.095	60.75	1215.00	150	3000

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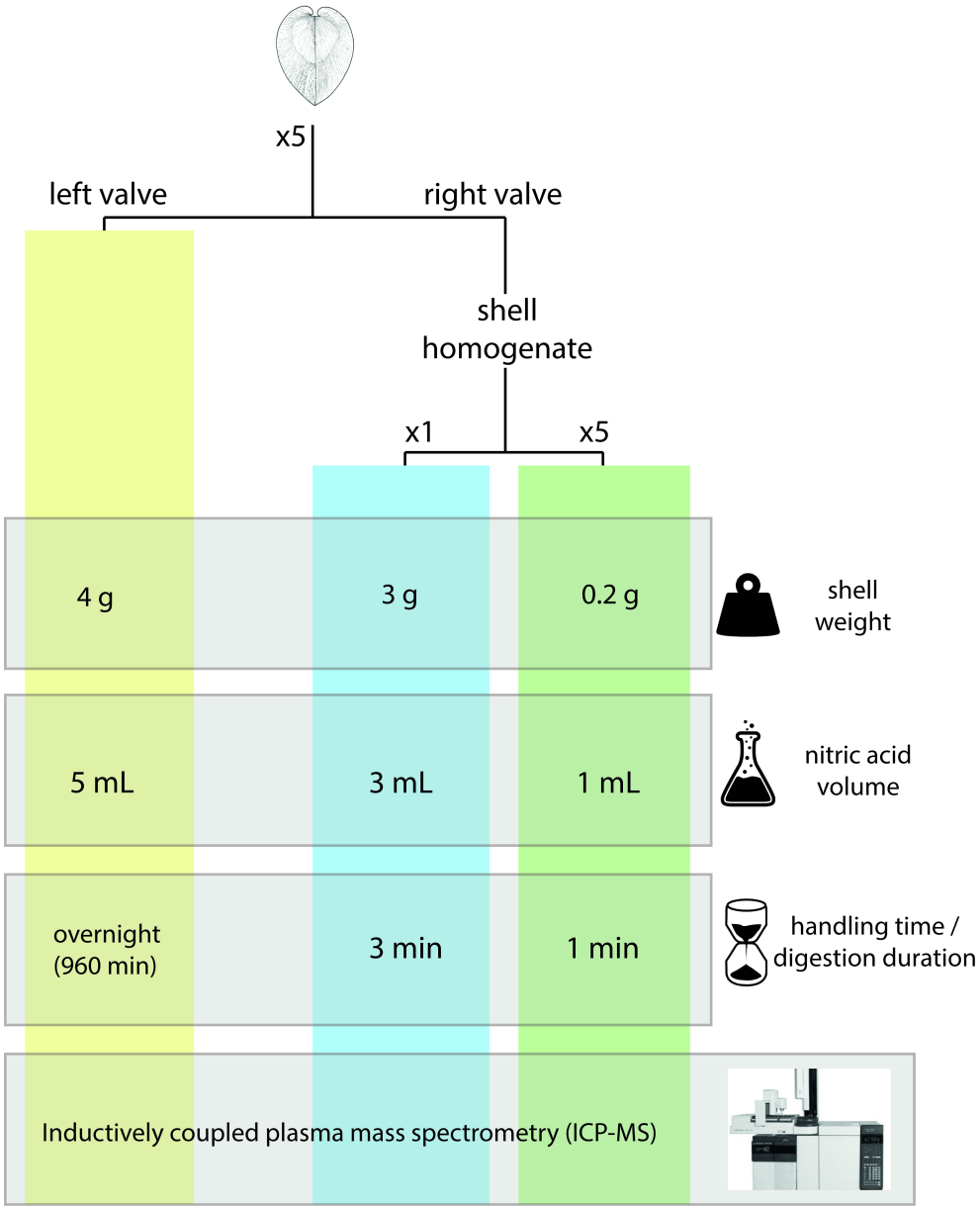
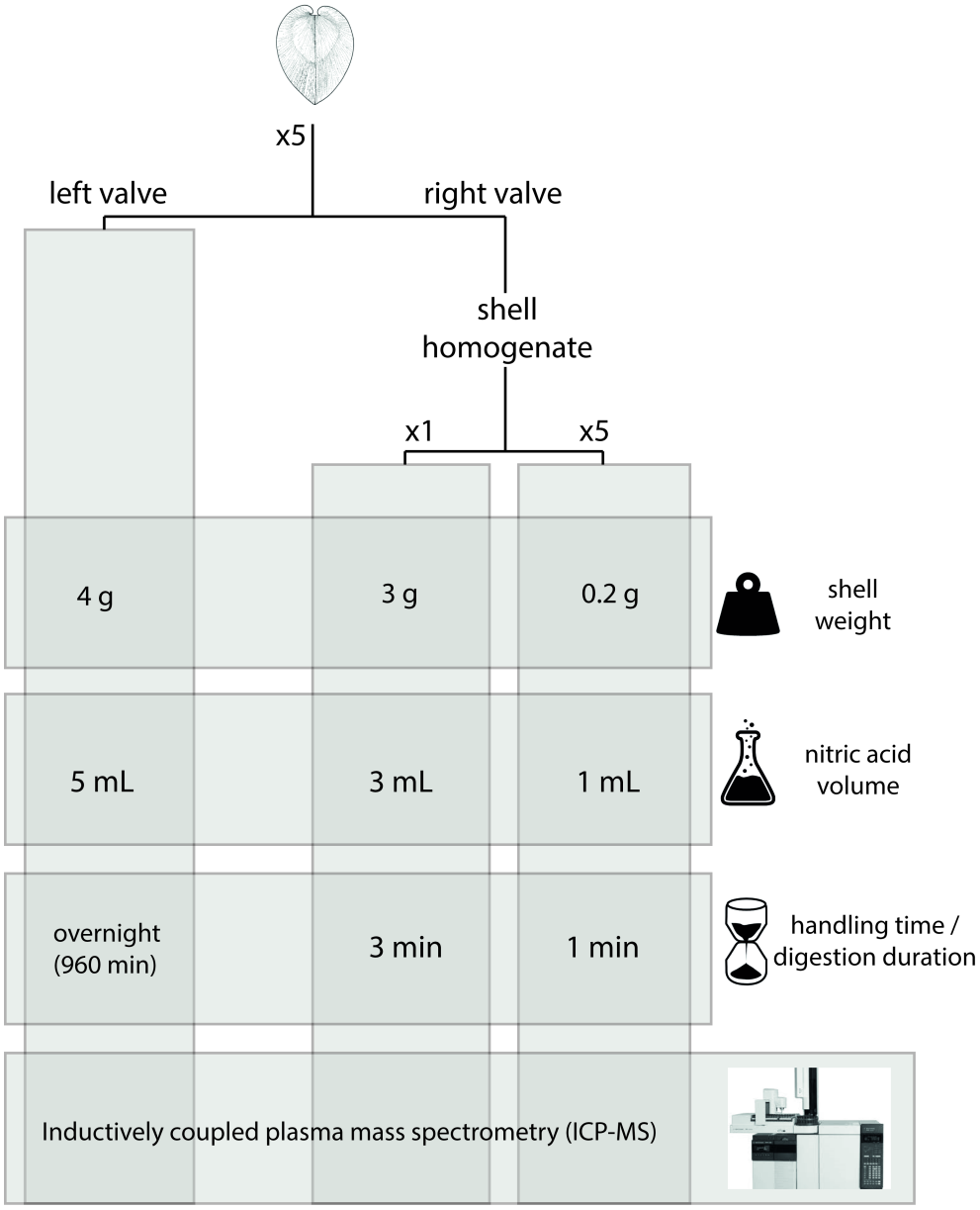
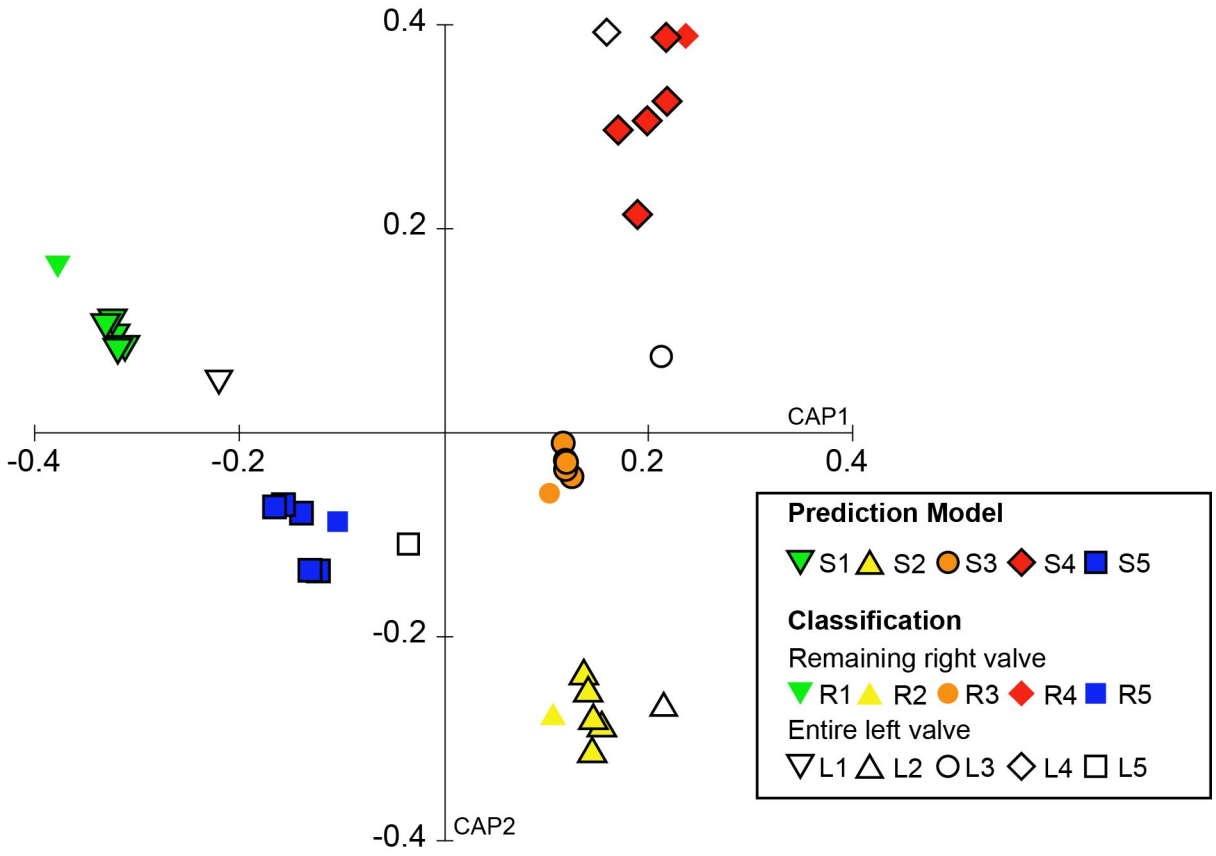
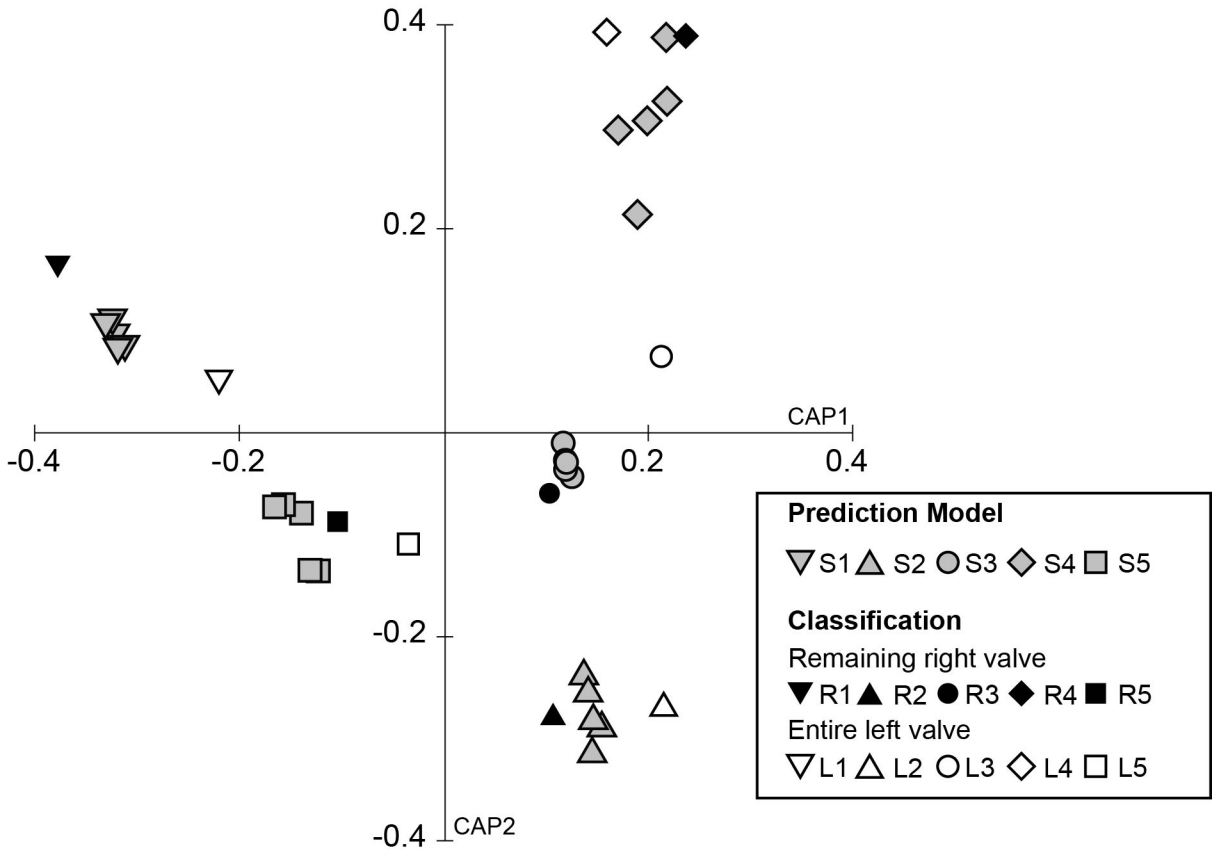

x5
left valve
right valve
shell homogenate
x1
x5
4 g
3 g
0.2 g
shell weight
5 mL
3 mL
1 mL
nitric acid volume
overnight (960 min)
3 min
1 min
handling time / digestion duration
Inductively coupled plasma mass spectrometry (ICP-MS)


x5
left valve
right valve
shell homogenate
x1
x5
4 g
3 g
0.2 g
shell weight
5 mL
3 mL
1 mL
nitric acid volume
overnight (960 min)
3 min
1 min
handling time / digestion duration
Inductively coupled plasma mass spectrometry (ICP-MS)


0.4
0.2
-0.2
-0.4
CAP1
-0.4
-0.2
0.2
0.4
CAP2
Prediction Model
S1 S2 S3 S4 S5
Classification
Remaining right valve
R1 R2 R3 R4 R5
Entire left valve
L1 L2 L3 L4 L5


0.4
0.2
-0.2
-0.4
CAP1
-0.4
-0.2
0.2
0.4
CAP2
Prediction Model
S1 S2 S3 S4 S5
Classification
Remaining right valve
R1 R2 R3 R4 R5
Entire left valve
L1 L2 L3 L4 L5

Conflict of Interest Form

Competing interests: The authors declare no conflict of Interest.