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Comparative analysis of the proximate and elemental composition of the blue crab *Callinectes sapidus*, the warty crab *Eriphia verrucosa*, and the edible crab *Cancer pagurus*

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Abstract

The proximate composition and element contents of claw muscle tissue of Atlantic blue crabs (*Callinectes sapidus*) were compared with the native warty crab (*Eriphia verrucosa*) and the commercially edible crab (*Cancer pagurus*). The scope of the analysis was to profile the chemical characteristics and nutritive value of the three crab species. Elemental fingerprints showed significant inter-specific differences, whereas non-significant variations in the moisture and ash contents were observed. In the blue crab, protein content was significantly lower than in the other two species, while its carbon content resulted lower than that characterizing only the warty crab. Among micro-elements, Ba, Cr, Cu, Li, Mn, Ni, and Pb showed extremely low concentrations and negligible among-species differences.

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Significant inter-specific differences were observed for Na, Sr, V, Ba, Cd and Zn; in particular, cadmium and zinc were characterized in the blue crab by concentrations significantly lower than in the other two species. The analysis of the available literature on the three species indicated a general lack of comparable information on their elemental composition. The need to implement extended elemental fingerprinting techniques for shellfish quality assessment is discussed, in view of other complementary profiling methods such as NMR-based metabolomics.

Keywords: Food science, Food chemistry, Food constituents, Food analysis

1. Introduction

The native habitats of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 extend in Western Atlantic from Nova Scotia to Uruguay, and in the USA the species supports an important fishery (Kennedy and Cronin, 2007). The blue crab was introduced in Europe at the start of the century (Nehring, 2011) and appeared in Mediterranean waters between 1935 and 1945 into the Aegean Sea (Artüz, 1990). To date the species is recorded almost ubiquitously in the Mediterranean and Black Seas (Nehring, 2011; Pashkov et al., 2012; Castejón and Guerao, 2013); yet, established populations have been reported, besides the eastern Mediterranean Sea (Kevrekidis et al., 2013; Sumer et al., 2013), only in the Adriatic Sea (Mancinelli et al., 2013a, 2013b; Cilenti et al., 2015).

The species is starting to penetrate the southern European shellfish markets (Ribeiro and Veríssimo, 2014); however, important blue crab fisheries are located only in the Eastern Mediterranean. For example, annual landings of 17–77 tons of blue crabs have been recorded in Turkey in 2008 and 2009, respectively (Ayas and Ozogul, 2011) while 50–80 tons were landed between 2010 and 2011 in northern Greece (Kevrekidis et al., 2013).

The chemical composition of the blue crab has been extensively investigated worldwide (Kennedy and Cronin, 2007). Specifically, several studies have been performed on Mediterranean populations in Turkish waters, in order to assess the nutritive value of the meat (e.g., Küçükgülmez et al., 2006), and contamination by heavy metals (Mutlu et al., 2011). In contrast, information on populations from other European and Mediterranean locations are virtually non-existent.

In the last decade, multi-element fingerprinting – i.e., the use of the elemental profile of an organism as a natural tag of its origin – has been proven to be a powerful technique to assess the nutritional quality and to increase the geographical traceability of agricultural goods and related products (Gonzálvez et al., 2009; Al Chami et al., 2014; Pandotra et al., 2015). However, to date no

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attempts have been made to apply the technique to crustacean species, and element fingerprinting of shellfish products is to date limited to mussels and other bivalves (Ricardo et al., 2015).

The general aim of the present study is to provide an advanced resolution of the quality of *C. sapidus* as a shellfish product for the European fish market. Molfese et al. (2014) indicated that during the 1920–2010 period in northern European countries an increase in the harvest of low-trophic level invertebrate species, including crustaceans, corresponded to a decreasing abundance of finfish resources. Moreover, this increase is destined to continue in the future (see also Stentiford et al., 2012). Noticeably, among the species listed in Green et al. (2014) the edible crab *Cancer pagurus* from northern Atlantic Ocean is the only valuable species constantly found in European fish markets. Given the current critical conditions of most of the stocks of crustacean species of commercial interest (Vasilakopoulos and Maravelias, 2015), new sustainable crustacean fishery species require to be identified, tested for their nutritional value, and compared with those currently exploited.

Specifically, multi-element information complemented with conventional proximate composition analyses was used here to i) profile the chemical characteristics and nutritive value of blue crabs captured in a coastal habitat located in the Salento Peninsula on the Adriatic Sea (SE Italy), and to ii) compare them with a native crab species, the warty crab *Eriphia verrucosa* Forskål, 1775, and a north Atlantic species, the edible crab *Cancer pagurus* Linnaeus, 1758. The edible crab supports an important fishery in English and Irish waters, and it is widely exported to Southern European countries (Barrento et al., 2008; Barrento et al., 2009a). *Eriphia verrucosa* has a potentially high commercial value in Mediterranean countries, and to date it is episodically found in local fish markets (Kaya et al., 2009).

2. Material and methods

2.1. Ethical approval

Sampling permits were obtained from all relevant Institutions in Italy. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

2.2. Sample collection

Callinectes sapidus and *Eriphia verrucosa* specimens were captured in June 2014 in the Acquatina Lagoon (SE Italy), a non-tidal brackish-water basin

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located in SE Italy on the Adriatic Sea (see Mancinelli et al., 2013c; Longo and Mancinelli, 2014 for details). Crab traps were identical to those described in Carrozzo et al. (2014). After collection, specimens were transferred alive in refrigerated containers to the laboratory, where they were sexed. For each crab species, six intact males were randomly chosen, and had their carapace width (in mm) and wet weight (in g) measured (see Table 1 in results). Crabs were then euthanized by thermal shock (-20 °C for 10 min).

From each collected specimen, two samples of muscle tissue per claw weighting 2.0 g and 5.0 g were removed using a ceramic scalpel, frozen (-20 °C), and used for total carbon/nitrogen content and moisture/ash content and other element analysis, respectively. Simultaneously, six male *Cancer pagurus* captured in Weymouth Bay (Dorset, UK) were purchased live from local fishermen. After transfer to the laboratory under refrigerated conditions, crabs were measured and euthanized; muscle sample collection was performed according to the aforementioned procedures.

2.3. Proximate composition analyses

Moisture content was determined by oven-drying the muscle samples at 105 °C until constant weight was attained (AOAC 2010). Ash content was estimated as the difference in mass before and after incinerating oven-dried samples for 24 h at 525 °C in a muffle furnace (AOAC 2010). Total carbon and nitrogen contents (expressed as % dry mass) were determined by combustion (AOAC 2010) on previously freeze-dried and pulverized samples using an elemental analyzer (Thermo Scientific Flash EA 1112). Crude protein content was estimated on each sample as N×6.25, where N was the total nitrogen content.

2.4. Element analyses

The total concentrations of B, Ba, Ca, Cd, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, V, and Zn were determined by wet digestion (Raab et al., 2005). Samples were mixed with 4 ml H_2O_2 and 6 ml HNO_3 at 180 °C for 10 min using a microwave digestion system (Milestone START D). Samples were consequently cooled, diluted with ultrapure water to a final volume of 25 ml, filtered through Whatman No. 42 filter papers, and finally measured for element content using an inductively coupled plasma atomic emission spectrometer (ICP-AES; Thermo Scientific iCap 6000 Series). Results were ultimately expressed as mg 100 g^{-1} tissue wet weight.

2.5. Statistical analysis

Principal component analysis (PCA) and Permutational multivariate ANOVA (PERMANOVA) were used to explore the multivariate structure and to test

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Table 1. Biometric data, proximate composition and mineral elements of claw muscle tissues of the three crab species under analysis. Means, SE in brackets ($n = 6$); if < 0.005, SE are not shown. Macro- and microelements are expressed as g 100 g^{-1} wet weight. Among macro-elements, the C:N ratio is included. Results of 1-way ANOVAs (fixed factor = species) followed by post-hoc bivariate comparisons (Tukey HSD test) are reported. Abbreviations: Cs = *Callinectes sapidus*; Ev = *Eriphia verrucosa*; Cp = *Cancer pagurus*.

		Callinectes sapidus	*Eriphia verrucosa*	*Cancer pagurus*	$F_{2,15}$	*Tukey test*
Biometric	Wet weight (g)	173.00 (26.78)	118.98 (23.35)	615.69 (38.72)		
	Carapace width (mm)	136.00 (7.58)	64.00 (4.06)	151.50 (1.50)		
Proximate composition	Moisture (%)	80.12 (1.90)	78.03 (3.63)	77.96 (4.64)	0.12	
	Ash (%)	1.63 (0.20)	1.81 (0.12)	2.25 (0.29)	2.15	
	Protein (%)	15.13 (0.32)	18.13 (0.49)	17.02 (0.77)	7.32*	Ev = Cp > Cs
Macro-elements	C	7.36 (0.15)	9.38 (0.28)	8.37 (0.20)	23.01***	Ev = Cp > Cs
	C:N	3.04 (0.04)	3.24 (0.11)	3.14 (0.05)	1.73	
	Ca	119.66 (67.43)	456.76 (89.68)	128.61 (29.50)	8.23**	Ev > Cp = Cs
	K	302.06 (8.74)	278.43 (7.10)	388.19 (33.30)	8.10**	Cp > Ev = Cs
	Fe	0.30 (0.07)	0.46 (0.09)	0.57 (0.15)	1.63	
	Mg	35.86 (1.59)	66.40 (5.10)	42.03 (2.40)	22.83***	Ev > Cp = Cs
	Na	188.34 (8.55)	325.90 (8.09)	212.05 (30.09)	15.54**	Ev > Cp = Cs
Micro-elements	B	0.06 (0.02)	0.11 (0.01)	0.05	4.63*	Cs = Ev > Cp
	Ba	0.02 (0.01)	0.04 (0.01)	0.14 (0.13)	0.74	
	Cd	0.01	0.02	0.02	28.24***	Ev = Cp > Cs
	Cr	0.01	0.02	0.01	3.30	
	Cu	0.90 (0.26)	1.33 (0.29)	0.71 (0.03)	2.04	
	Li	0.00	0.01	0.01 (0.01)	1.31	
	Mn	0.03 (0.01)	0.10 (0.04)	0.03	3.11	
	Ni	0.02 (0.01)	0.01	0.01	0.62	
	Pb	0.01	0.01	0.01	0.69	
	Sr	1.30 (1.10)	5.92 (1.39)	0.85 (0.41)	7.16*	Ev > Cp = Cs
	V	0.07	0.12 (0.01)	0.06	29.58***	Ev > Cp = Cs
	Zn	4.76 (0.96)	9.40 (0.27)	7.45 (1.41)	5.49*	Ev = Cp > Cs

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.0001$.

occurrence of among-species differences, respectively. Specifically, Euclidean distance similarity matrices were constructed on mean-centered data, and PERMANOVA analyses were performed assuming "species" as a fixed factor and with *P* values calculated using 999 unrestricted permutations of raw data. Pair-wise comparisons were performed when a significant main effect was

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detected. PCA and PERMANOVA analyses were performed using PERMANOVA+ (v1.0.3; PRIMER-E Ltd., UK). Univariate tests (1-way ANOVA followed by bivariate comparisons based on Tukey HSD tests) were performed to test among-species differences of proximate composition, micro- and macro-elements, after the assumption of homogeneity of variances was checked using Cochran's C-test; log- or square root transformations were used when necessary.

3. Results and discussion

The claw muscle tissues of the three crab species showed negligible differences in terms of proximate composition (Table 1). The only exception was represented by the protein content, as the blue crab was characterized by values significantly lower than those determined for both the warty crab and the edible crab (19.8 and 12.5%, respectively; Table 1). Information for other Mediterranean blue crab and warty crab populations from the Aegean Sea and Black Sea (see references in Table 2) are fully consistent with the present results, corroborating the lack of inter-specific differences in proximate composition observed among the three crab species analysed (Table 2). In particular, the blue crab showed proximate values comparable with those determined for commercially-exploited populations in both their native (USA) and Mediterranean habitats (Turkey); similarly, the proximate composition of the warty crab resulted substantially similar to that determined for Turkish populations from the Mediterranean and Black Sea. Noticeably, the protein content estimated for *C. pagurus* was the only exception, resulting lower than those estimated for populations of both the English Channel and Scotland (Table 2). It is worth noting that in this study protein content was estimated using total nitrogen concentration. Although considered a standard method by the AOAC in food chemistry, to date alternative, more effective estimation methods are adopted, including numerous chromogenic assays based on the use of specific protein-binding fluorophores (e.g., the biuret, Lowry, or Bradford assays: Sapan et al., 1999 and literature cited; see also Noble and Bailey, 2009) or recently-developed methods based on enzyme hydrolysis followed by derivatisation with *o*-phthaldialdehyde and fluorescence detection (eOPA: Bennett et al., 2016). Thus, the variations in protein content (or lack of) observed in this study among crab species need to be further confirmed by future analyses involving more advanced quantification procedures. In addition, electrophoretic techniques (e.g., sodium dodecyl sulfate polyacrylamide gel electrophoresis, or SDS-PAGE) are becoming popular in food quality investigations for the comparison of protein profiles (e.g., Yi et al., 2013) and will provide an advanced resolution of inter-specific differences.

In general, other comparative studies of proximate parameters among crab species found little or no differences (e.g., *Callinectes sapidus vs. Portunus pelagicus*: Gökoğlu and Yerlikaya, 2003; *Callinectes pallidus vs. Cardisoma*

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Table 2. Proximate composition of claw muscle tissues of the three crab species under analysis: comparison with literature data. Mean values, SE not reported. For literature data, means ± 95% confidence intervals are reported in italics. For the sake of comparison, literature information on other crab species of economic interest are reported at the bottom of the table.

Species	Moisture(%)	Protein (%)	Ash (%)	References
Callinectes sapidus	80.12	15.13	1.63	*Present study*
	81.20	16.10	1.60	Thompson and Farragut, 1982
	82.05	15.55	1.55	Wheaton and Lawson, 1985[a]
	82.34	14.86	1.64	Gökoðlu and Yerlikaya, 2003[b]
	78.02	19.55		Küçükgülmez et al., 2006
	80.9 ± 2.75	*16.51 ± 2.9*	*1.6 ± 0.08*	
Eriphia verrucosa	78.03	18.13	1.81	*Present study*
	76.13	19.66	2.35	Kaya et al., 2009
	72.24	17.12	2.34	Altinelataman and Dincer, 2007
		22.6		Demirbaş et al., 2013[c]
	74.19 ± 8.37	*19.79 ± 5.04*	*2.35 ± 0.02*	
Cancer pagurus	77.96	17.02	2.25	*Present study*
	76.77	18.4	2.1	Barrento et al., 2010
	76.3	19.1	2.2	Maulvault et al., 2012
	76.54 ± 1.01	*18.75 ± 1.51*	*2.15 ± 0.22*	
Carcinus maenas	78.7	17.1		Skonberg and Perkins, 2002
Carcinus mediterraneus	79–81	17.8–18.2		Cherif et al., 2008
Eriocheir sinensis	78.8	18.9	1.89	Chen et al., 2007
Maja brachydactyla	79.2	15.7	2.55	Marques et al., 2010
Scylla serrata	84.4	14.3	1.67	Benjakul and Sutthipan, 2009

[a] averages of 77.4–86.7 (%moisture) 11.9–19.2 (%protein) and 1.3–1.8 (%ash).
[b] average of 81.58–83.1 (%moisture) 14.71–15 (%protein) and 1.39–1.89 (%ash).
[c] average of 19.4–25.8(%protein).

armatum: Elegbede and Fashina-Bombata, 2013). Additionally, data available for other crab species (besides those tested in this study) indicate a scant variability (Table 2; moisture: range 78.7–84.4%; protein content: 14.3–18.9%; ash: 1.67–2.55%). Here, among-species comparisons were performed using adult males captured in the same season; thus, it is likely that sex- or season-related differences in proximate composition may even weaken the possibility of detecting inter-specific differences in brachyuran species (Ozogul et al., 2013;

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Baklouti et al., 2013), and proximate composition *per se* – expressed in terms of % moisture, ash content or protein content – may not represent an effective tool for inter-specific, or, at the species scale, inter-site comparisons.

The PCA analysis performed on the elemental concentrations of the three crab species revealed that the first 5 principal components explained 83.4% of the total variance of the data set; in particular, the first one explained 36.9% and the second one 23.5% (Fig. 1). Contrary to what observed for the proximate characteristics, considerable among-species differences were indicated by the PCA plot (Fig. 1), and were corroborated by a PERMANOVA test (Pseudo-$F_{2,15} = 8.27$, $P = 0.001$). Further bivariate comparisons indicated that significant multivariate differences occurred among all the species pairs tested (i.e., *Callinectes vs. Eriphia*: $t = 3.72$, $P = 0.002$; *Callinectes vs. Cancer*: $t = 2.39$, $P = 0.002$; *Eriphia vs. Cancer*: $t = 2.63$, $P = 0.006$).

Among macro-elements, *C. sapidus* showed a significantly lower carbon content compared to the other two species; in addition, both calcium and magnesium

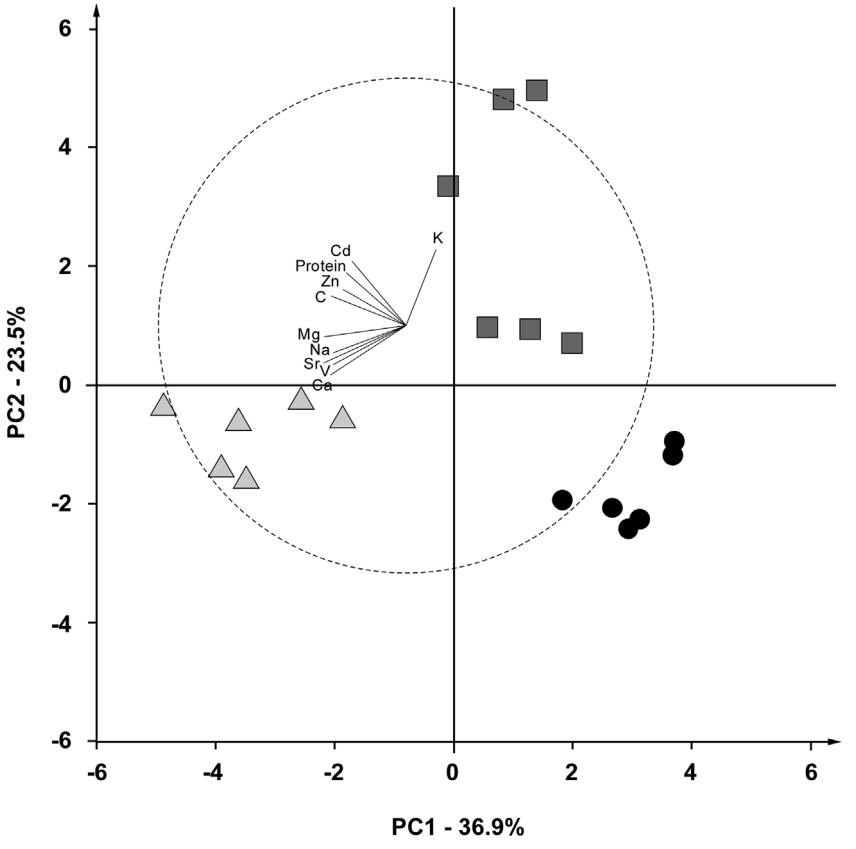

Fig. 1. Score plot of the first two components of the principal components analysis (PCA) conducted with the proximate and element composition data characterizing *Callinectes sapidus* (circle symbol), *Eriphia verrucosa* (triangle), and *Cancer pagurus* (square) claw muscletissues. The most significant correlations with proximate parameters and chemical elements are shown.

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content were close to those determined for *C. pagurus*, in turn considerably lower than those characterizing *E. verrucosa* (Table 1). Among micro-elements, Ba, Cr, Cu, Li, Mn, Ni, and Pb showed negligible among-species differences and were generally characterized by concentrations close to the detection limit of the instrument (Table 1). The negligible contents in chromium, nickel, and lead in *Callinectes sapidus* and *Eriphia verrucosa* confirm the pristine environmental conditions of the Acquatina Lagoon, and that the heavy metals episodically characterizing its sediments (Tramati et al., 2012) are not transferred along the food chain. Data for *Cancer pagurus,* similarly, indicate that exploited populations of the edible crab in eastern Atlantic are from unpolluted sites.

Sodium, strontium, and vanadium contents in the blue crab were similar to those determined in the edible crab, and significantly lower than those characterizing the warty crab. Negligible differences were observed in the barium content of *C. sapidus* and *E. verrucosa*, both significantly higher than that characterizing *C. pagurus*. In contrast, the concentrations of cadmium and zinc in the blue crab were significantly lower than those observed for the other two species (Table 1). However, in none of the species Cd exceeded the limits identified by the European Union (0.05 mg 100 g^{-1}; EC 2008). Cd and Zn have similar chemistries and therefore share uptake pathways into aquatic invertebrates (Rainbow, 1997). The difference between *C. sapidus* and *C. pagurus* may be ascribed to the influence of inter-site variations in the sources of the two metals; conversely, the dissimilarity observed with the coexisting *E. verrucosa* is likely to be related to a wide spectrum of factors related to trophic habits or, alternatively, to inter-specific metabolic differences in the uptake and elimination kinetics of the two metals (Marsden and Rainbow, 2004).

Noticeably, for *E. verrucosa* no literature information are available on macro- and micro-element contents. The species has a potentially high commercial value in Mediterranean countries (Kaya et al., 2009), and it is advisable that future studies will reduce the knowledge gap and provide more complete information on other Mediterranean or Black Sea populations. For *Cancer pagurus*, notwithstanding its high commercial value, literature information are scant and focused on a reduced set of elements (Barrento et al., 2009a, 2009b; Maulvault et al., 2012). In contrast, for *Callinectes sapidus* several studies on both north Atlantic and Mediterranean populations are available (Ayas and Ozogul, 2011; Gökoğlu and Yerlikaya, 2003; Jop et al., 1997; Küçükgülmez et al., 2006); however, none of them performed an elemental analysis of blue crab claw muscle tissues as comprehensive as the one included in the present study.

The results of the present investigation are fully consistent with those obtained in a recent study comparing the ^{1}H-NMR metabolomic profile of lipid and

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aqueous extracts of raw claw muscle (Zotti et al., 2016), suggesting that multi-elemental fingerprinting may represent a complementary method for shellfish quality profiling, versatile and relatively low-cost.

4. Conclusions

Here is presented a previously unattempted description of the quality of claw muscle tissue of *Callinectes sapidus* collected from the Adriatic Sea in terms of proximate composition and elemental contents, The analysis encompassed a wide spectrum of macro- and micro-elements, ultimately producing an elemental fingerprinting of the blue crab and allowing a comprehensive comparison with the native crab *Eriphia verrucosa* and the commercially exploited *Cancer pagurus*. This study complemented the information provided by more advanced biochemical screening methods (e.g., ^{1}H-NMR metabolomics: Zotti et al., 2016), and contributed to the definition of standardized procedures for shellfish quality profiling, and for the implementation of a "foodomics approach" for the advanced investigation of human food and the nutrition domains (Zotti et al., 2016).

Declarations

Author contribution statement

Maurizio G. Zotti, Sandra A De Pascali, Laura Del Coco, Danilo Migoni, Salvatrice Vizzini, Giorgio Mancinelli, Francesco P. Fanizzi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Conflict of interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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