ORIGINAL RESEARCH

Morphological, chemical and growth patterns characterization in shells of *Phorcus* species along the northeastern coasts of Tunisia

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ABSTRACT. This work is a characterization of the morphometry, the relative growth and the metal contamination degree of the shells of two species belonging to *Phorcus* genus along the north-eastern rocky coasts of Tunisia. The gastropods were sampled from nine locations during the winter 2017. Our findings suggested a spatial morphometric variability probably linked to the extent of the continental shelf, the coasts' geomorphology, the dynamics of water masses, the predation and the competition effects. Furthermore, the shell investigation allowed the study of the growth pattern and indicated that the development in shell size was faster than the increase in weight. Moreover, the use of *Phorcus* shells as bioindicators of long-term metal contamination seems to be of growing interest, due to their abundance, high longevity, easy sampling and ecobiological features, both in a scientific and ecosystem management perspective that aims to the establishment of conservation measures targeting marine coastal environments.

Key words: Rocky shoreline, shell contamination, morphometry, relative growth.

Caracterización de patrones morfológicos, químicos y de crecimiento en conchas de especies de *Phorcus* a lo largo de las costas nororientales de Túnez

RESUMEN. Este trabajo es una caracterización de la morfometría, el crecimiento relativo y el grado de contaminación metálica de las conchas de dos especies pertenecientes al Género *Phorcus* a lo largo de las costas rocosas del noreste de Túnez. Los gasterópodos fueron muestreados en nueve ubicaciones durante el invierno de 2017. Nuestros hallazgos sugirieron una variabilidad morfométrica espacial probablemente relacionada con la extensión de la plataforma continental, la geomorfología de las costas, la dinámica de las masas de agua, la depredación y los efectos de la competencia. Además, la investigación de la concha permitió estudiar el patrón de crecimiento e indicó que el desarrollo del tamaño de la concha fue más rápido que el aumento de peso. Además, el uso de conchas de *Phorcus* como bioindicadores de contaminación por metales a largo plazo, parece ser de creciente interés debido a su abundancia, alta longevidad, fácil muestreo y características ecobiológicas, tanto desde una perspectiva científica como de gestión ecosistémica, que apunta al establecimiento de medidas de conservación dirigidas a entornos marinos costeros.

Palabras clave: Costa rocosa, contaminación de la concha, morfometría, crecimiento relativo.

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INTRODUCTION

Mollusks form the second most abundant phylum of animals after arthropods and before chordates, comprising about 70,000-76,000 currently described species and more than 200,000 calculated living species with Gastropoda as the major group (Halanych 2004). They have a huge morphological and physiological diversity, mediated by phenotypic plasticity or adaptation, facilitating their biological success (Michalek 2019). Thereby they have colonized nearly all ecological niches from terrestrial habitats to coastal ecosystems and deep-sea hydrothermal vents, coping with opposing environmental conditions as wave exposure, desiccation periods and increased temperatures due to solar radiation, salinity, pH and CO_2 fluctuations and even excessive levels of pollution (Moreira et al. 2019). A main part of this success is claimed to be their bio-mineralized shells, although some groups, including most cephalopods and some gastropods (e.g. nudibranchs) have lost their external shell (Rosenberg 2014). Indeed, the basic shell production process is common among shelled mollusks wherein a fleshy mantle secretes the calcareous husk comprising layers (2 or more) of hierarchically arranged bio-composite materials in which a rigid mineralized phase is embedded in a softer organic matrix (Clark et al. 2020). This mineralized component is always $CaCO_3$ either calcite (trigonal) or aragonite (orthorhombic) and sometimes a mixture of them or also pathologically the unstable polymorph vaterite (μ-$CaCO_3$) in patches or repairs (Loftus et al. 2015; Clark et al. 2020). This biogenic calcium carbonate mainly exists in mollusk shells with more of 95% added by small amount of SiO_2, protein and polysaccharide (Addadi et al. 2006). Then, it has an important sorption of metal ions similarly to the geological $CaCO_3$, one among the most abundant minerals on the earth existing mainly in two polymorphs (CAL and ARA) and recognized to adsorb a definite amount of metals in the aqueous environments (Garcia-Sánchez and Alvarez-Ayuso 2002; Godelitsas et al. 2003). Thereby, the biogenic form of the mineral is abundant in the natural environments and would be an ideal alternative substitute of the geological $CaCO_3$ for metals assessment programs in contaminated waters (Liu et al. 2009; Du et al. 2011).

Intertidal mollusks, especially gastropods, generally exhibit bigger and more ridged and robust shells providing larger surface area for convective heat loss compared with sub-tidal conspecifics and better response to predation threat by *Stramonita haemastoma* (Linnaeus, 1767); *Conus ventricosus* Gmelin, 1791; *Carcinus* spp. Leach, 1814, etc. (Harley et al. 2009; Boulajfene et al. 2015; Boulajfene and Tlig-Zouari 2016). For example, we quote the species belonging to *Phorcus* genus Risso, 1826 recognized as good indicators of water quality due to their reduced mobility, easy sampling, adequate size, widespread distribution, abundance all year-round and ability to adsorb and accumulate high metal concentration by their shells and tissues (Boulajfene et al. 2017, 2019, 2021). This genus gathers nine valid species, namely *P. articulatus* (Lamarck, 1822); *P. atratus* (Wood, 1828); *P. lineatus* (da Costa, 1778); *P. mutabilis* (Philippi, 1846); *P. punctulatus* (Lamarck, 1822); *P. richardi* (Payraudeau, 1826); *P. sauciatus* (Koch, 1845); *P. turbinatus* (Born, 1780) and *P. mariae* Templado and Rolán, 2012 all characterized by a sub triangular to globose shell shape with rounded to angular whorls and a convex base (Templado and Rolán 2012; Affenzeller et al. 2017; Sousa et al. 2018). Their shells are either smooth or show thick spiral striae and the umbilicus narrows with age and can even be closed completely by a columellar fold in large species (Affenzeller et al. 2017). This structure is formed, in topshells, during the embryonic stage, with the secretion of protein fibers from the outer skin of the visceral mass and from the mantle, while they are free-swimming larvae, and is followed by the secretion of calcium carbonate from the same cells. Next to this embryonic phase, the shell continues to grow through the addition of a protein mesh and $CaCO_3$ mostly on its margins but also on its interior offering refuge both from predators and from desiccation being impermeable to liquids and gasses and resistant to crushing (Affenzeller et al. 2017). In the Mediterranean, the most frequent species of the genus *Phorcus* are *P. mutabilis*, *P. richardi*, *P. articulatus* and *P. turbinatus* while on the Tunisian coasts, only the three last species were encountered and reported (Tlig-Zouari et al. 2010; Boulajfene et al. 2016). Note that the majority of the works executed on topshells were devoted to their soft body and only some researches have interested their shells namely the studies of oxygen isotopes (Prendergast et al. 2013; Parker et al. 2017; García-Escárzaga et al. 2019) and of their morphological variability (Boulajfene et al. 2016; Cabral 2020; Vasconcelos et al. 2022).

X-ray fluorescence spectrometry (XRF) is an elemental global analysis technique used to identify and quantify chemical elements in a sample. The samples are irradiated by X-rays obtained either by electrons formed through a heated filament (Joule effect) and accelerated on a metal target via a potential difference of several thousand volts, or by a radiation from a radioisotope (Marcoen et al. 2000). Those rays excite (when they have a sufficient energy) the few electrons of the orbitals closest to the atom's nucleus, not influenced by chemicals bonds, which passes to a higher energy level. When returning to equilibrium, the atom is 'de-excited' by transition of a lower level electron giving an inferior potential energy. The energy excess DE is released as secondary X-rays of precise energy, characteristic of each chemical element (Marcoen et al. 2000). The intensity of X-ray fluorescence emitted by the sample is proportional to the concentration of the considered element. Thus, the collection of the X-rays fluorescence on a detector and the measurement of their energies and intensities, make possible to recognize and enumerate the elements contents of the sample (Marcoen et al. 2000).

The continuous deterioration of the biodiversity, the weakening of the Tunisian coastal ecosystems and the fragmentary and punctual data relating to *Phorcus* genus shells, lead us to undertake a general characterization of the calcareous husk of two species along the rocky coasts of the northeastern part of Tunisia. Main objectives of this work were to (i) study the spatial inter and intra-specific morphometric variability through specific metric measurements, (ii) examine the relative growth pattern by checking two length-weight relationships, and (iii) assess the metal shells content using the X-ray fluorescence spectrometry in order to further investigate negative effects of metal pollution on marine gastropods shell properties.

MATERIALS AND METHODS

Sampling

During winter 2017, 10 locations were chosen depending on the existence of one or both species, abundances and the presence or absence of potential sources of pollution. *Phorcus turbinatus* was collected from nine stations namely Bizerte Grottes 37° 20′ 03.13″ N-9° 51′ 03.36″ E, Jarzouna 37° 15′ 52.16″ N-9° 53′ 36.05″ E, Cap Zebib Grottes 37° 16′ 05.84″ N-10° 03′ 55.00″ E, Cap Zebib Port 7° 15′ 46.36″ N-10° 04′ 04.87″ E, La Goulette 36° 49′ 08.37″ N-10° 18′ 38.49″ E, Korbous 6° 50′ 31.52″ N-10° 34′ 09.90″ E, Sidi Daoued 37° 02′ 40.03″ N-10° 91′ 09.69″ E, Kelibia1 36° 83′ 31.71″ N-11° 11′ 66.47″ E and Monastir Marina 5° 77′ 34.61″ N-10° 83′ 75.78″ E. As for *P. articulatus*, it was taken from Cap Zebib Grottes, La Goulette, Korbous, Sidi Daoued and Keliba2 36° 83′ 33.52″ N-11° 11′ 58.72″ E (Figure 1). Potential sources of pollution are differentiated according to the presence of industrial areas (Zarzouna, Sidi Daoued, Monastir); fishing ports (Zarzouna Sidi Daoued, Kelibia, Cap Zebib port); marinas (La Goulette, Monastir) and mooring areas (La Goulette). Korbous station was characterized by low

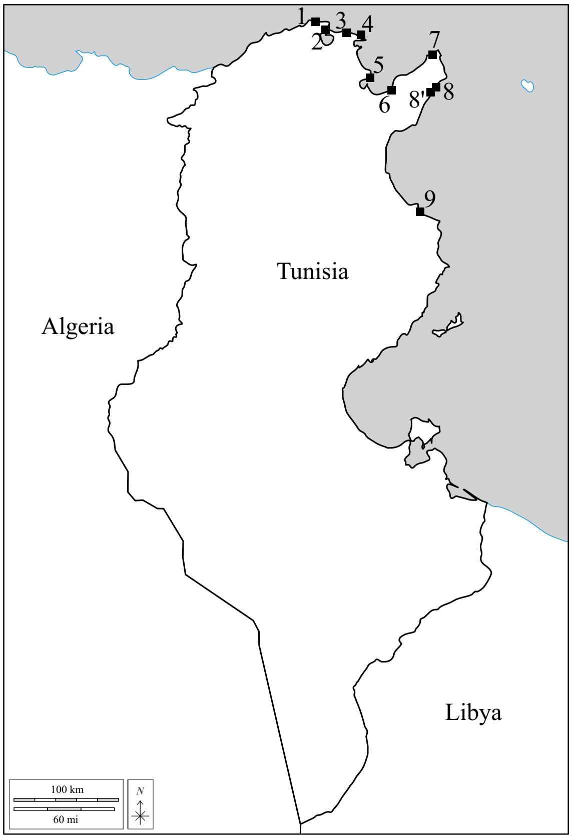

Figure 1. Sampling stations along the northeastern and eastern coasts of Tunisia. 1: Bizerte Grottes, 2: Jarzouna, 3: Cap Zebib Grottes, 4: Cap Zebib Port, 5: La Goulette, 6: Korbous, 7: Sidi Daoued, 8: Kelibia1, 8': Kelibia2, 9: Monastir.

pollution levels and by the presence of some rare and endangered species such as *Patella ferruginea* Gmelin, 1791 and *Pinna nobilis* Linnaeus, 1758 (Tlig-Zouari et al. 2010; Boulajfene et al. 2015, 2017). Note that Keliba1 is an exposed site while Kelibia2 is sheltered. At each station, a sample (n = 30) was collected randomly from the mediolittoral at a depth < 50 cm and was transported alive to the laboratory in plastic boxes.

Morphometry

On each shell of the two studied species, nine metric measurements were taken using an electronic caliper (at 1/100 mm pre), referring to the work of Boucetta et al. (2010). The taken variables are the maximum and the minimum length of the shell (Lmax/Lmin); the width of the shell (l); the base length and width (Lb/lb); the thickness of the shell (Ep); the length of the last whorl (S) and finally the length and the width of the aperture (Lou/lou). The measurement (L) was noted for relative growth study (Figure 2).

In order to eliminate the size effect, all the morphometric variables were log-transformed according to Reist (1985):

$$M_{trans} = \log M - \beta (\log LC - \text{mean} \log LC)$$

with Mtrans: transformed measure, M: Original measure, β: Slope of the regression, LC: total shell length, and mean LC: average length.

Growth patterns

Relative growth rates of both species were estimated by checking the relationships between the shell length (L) (Figure 2) and the total weight (TW), as well as between the shell length (L) and the shell weight (SW), using Huxley (1924) equation:

$$Y = ax^b$$

being the two variables, a (ordinate at the origin) and b (slope of the line), computed by the least square method. The significance of the regression was verified by Student's test at 5% error threshold and the ln of the theoretical value of the test table was used, namely ln (1.701) = 0.531. The rate of allometry b was compared to 3:

- If b = 3: the allometry is isometric (growths in length and in weight are proportional).
- If b < 3: the allometry is minor or negative (the length increases faster than the weight).
- If b > 3: the allometry is major (the weight increases faster than the length).

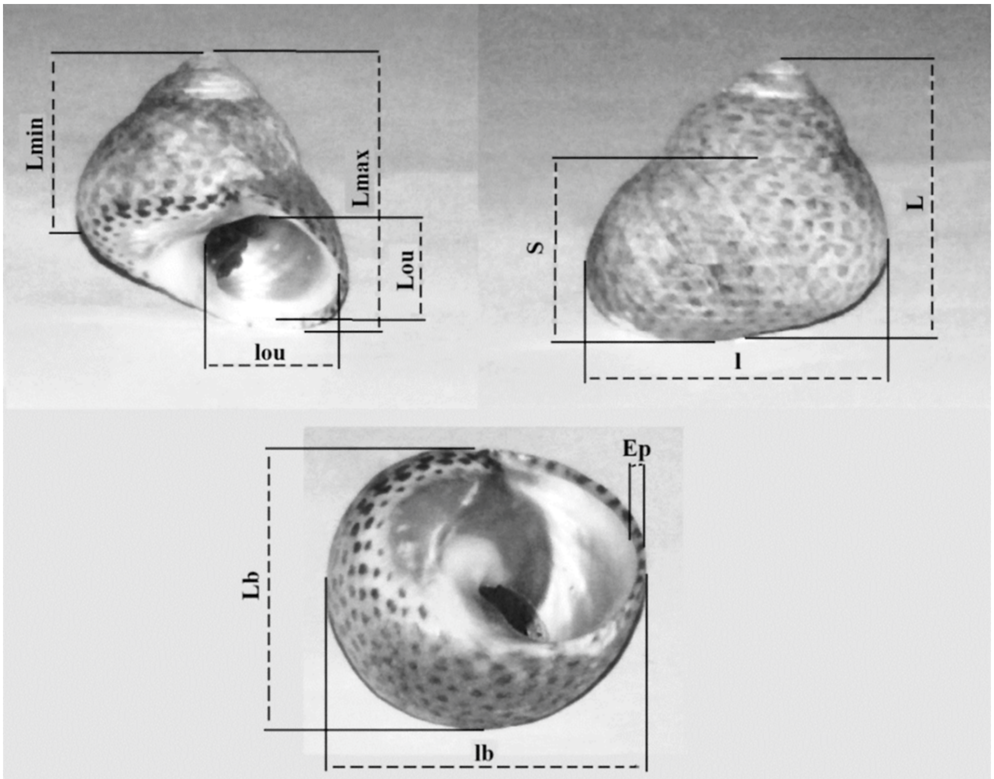

Figure 2. Metric measurements taken on both study species. Lmax: maximum length, Lmin: minimum length, l: width, Lb/lb: base length and width, Ep: thickness, S: length of the last whorl, Lou/lou: length and the width of the aperture. L: relative growth study.

Metal content

After the decortication of the gastropods, the shells were dried for 48 h at 55 °C, and subsequently grounded and homogenized using a Planetary mono mill (190 t s^{-1}) (Pulverisette 6-Fritsch). Hereafter, samples were returned to the oven for 12 h (at 62 °C). A fraction of 5 g from each ground material was used to which 1.25 g of candle powder was added. Mixtures were compressed in the 'Herzog' pressure machine (200 kPa) to obtain fine pellets. Trace element concentrations were measured by X-ray fluorescence spectrometry. Relative elemental concentrations in the shells were measured at high resolution (2 mm), using an Avaatech X-ray fluorescence (XRF) base-scanner at the Hellenic Center for Marine Researches (Hillesheim, 2005).

Statistical analysis

Factorial Discriminant Analysis was performed on the metric variables of both species using the R2.14.1 software (ade4 package) to discriminate different groups from graphical representations (Thioulouse and Dray 2007). Lambda Wilks test was also carried out to test the significance of difference between the averages of those groups obtained by the FDA. Given the lack of normality and homogeneity of variances of the studied variables, the significance of differences was verified by Kruskal-Wallis test using Statgraphics software. Then, a non-parametric correlation statistic (Spearman rank correlation) was executed using the same software to estimate correlations between Zn and Cu contents in shells, soft bodies and sediment.

RESULTS

Morphometry

Contributions of metric variables taken on the shells in the formation of the two first discriminant functions were investigated. The first factorial component constituted 29.02% of the total inertia with an eigenvalue of 0.8, while the second one had an eigenvalue equal to 0.653 and attracted 23.7% of the total inertia (Figure 3 A). The comparison of the morphometry of the two studied species suggested a morphological divergence with the presence of some overlapping (Figure 3 B). Factorial discriminant results distinguished two major groups: the majority of *P. turbinatus* samples were located in the positive side of axis 1, with the shift of samples from La Goulette and Korbous, respectively, towards the positive and the negative side of the second principal component, and samples of *P. articulatus* shifted all towards the negative side of the first axis. Wilks test revealed a very significant difference between the averages of the groups discriminated by the FDA (wilks = 0.01366; approximate F = 19.822; P = 2.2e-16 at a threshold of 1%) showing a strong morphometric variability between those groups.

Growth patterns

The analysis of the relative growth between the shell length (L), the total weight (TW) and the shell weight (SW) revealed important correlation coefficients r that varied between 0.949 and 0.992 for the relation L/TW, and between 0.917 and 0.989 for the relation L/SW in *P. turbinatus* (consecutively in Monastir and Kelibia1 for both relations) (Figure 4). As for *P. articulatus*, correlation coefficients varied between 0.860 (Cap Zebib Grottes) and 0.977 (Sidi Daoued) for the relation L/TW, and

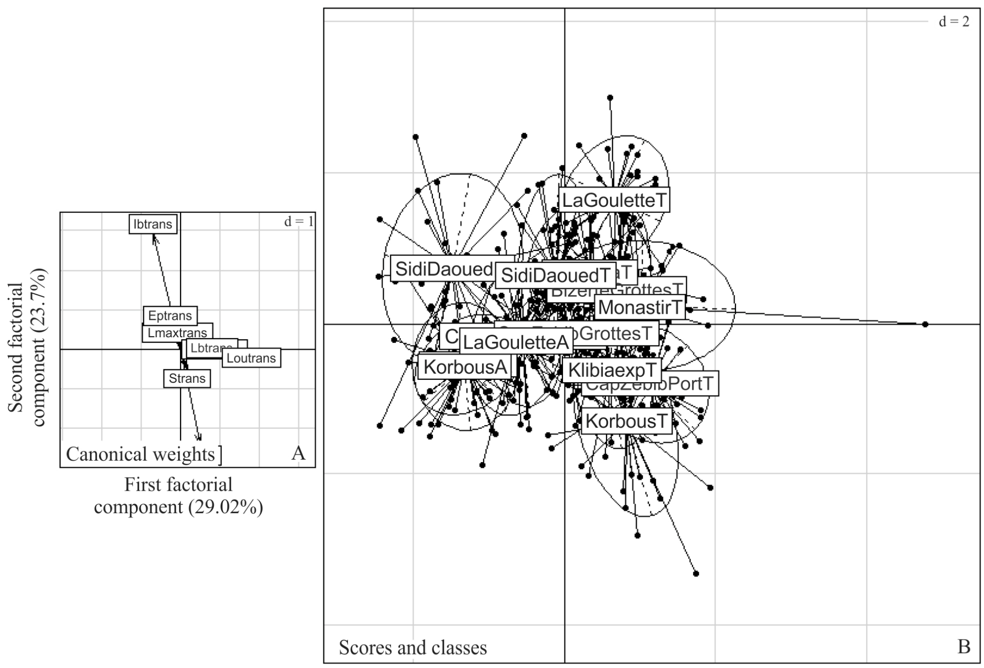

Figure 3. A) Contribution of the transformed measurements in the formation of the two first discriminant functions. B) Factorial Discriminant Analysis of the studied populations (T for *Phorcus turbinatus* populations, and A for *P. articulatus* populations).

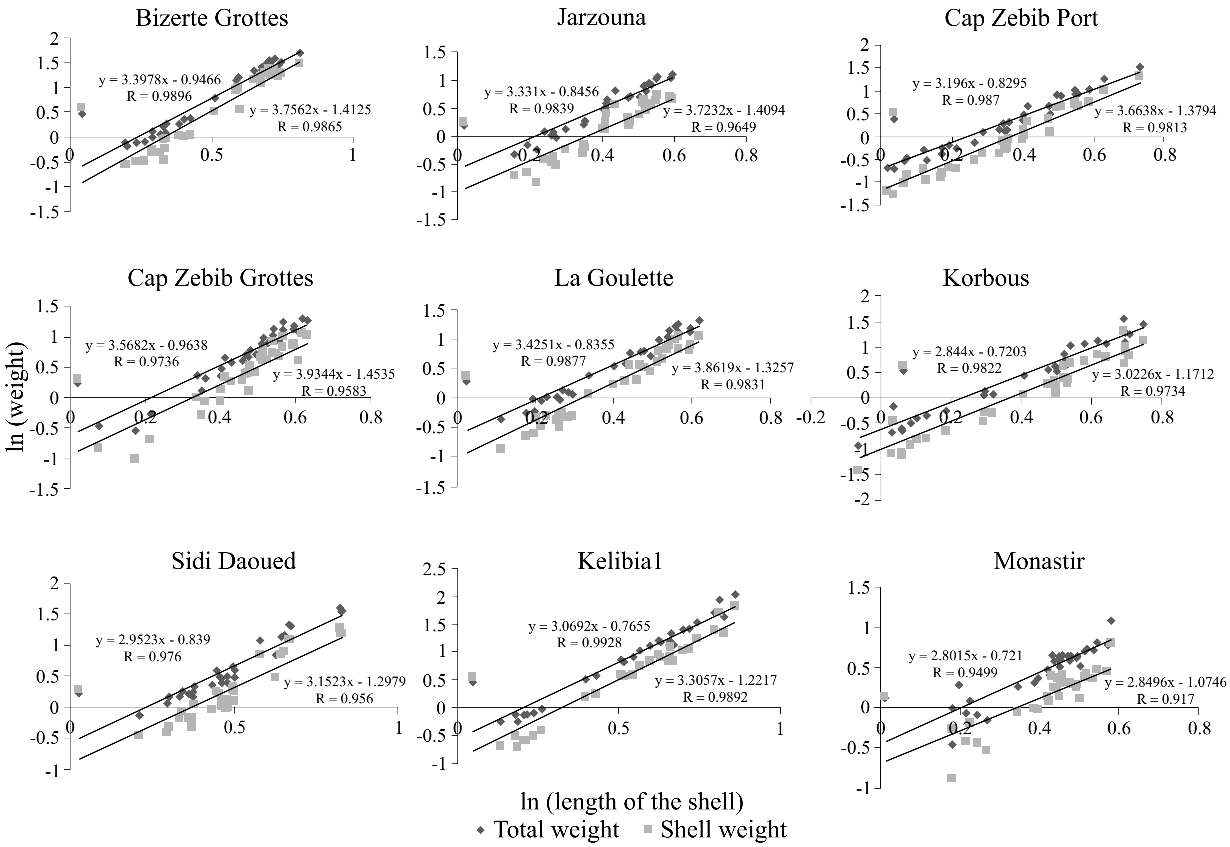

Figure 4. Total length (L)/total weight (TW) and total length (L)/weight of the shell (SW) relationships in *Phorcus turbinatus*.

between 0.799 (Korbous) and 0.958 (Sidi Daoued) for the relation L/SW (Figure 5). Calculated t values for both species were all greater than the ln of the theoretical value (= 0.531), indicating a strong relationship between the variables size (L) and weight (Tables 1 and 2). The comparison of those values with 'b' given by the slope, suggested a minor allometry at all stations showing faster increase in length versus weight.

Metal content

Metal analysis in the shells of *P. turbinatus* revealed concentrations that did not vary significantly among stations ($p > 0.05$) (Table 2). The lowest contents of Cu and Zn were respectively 4.6 ppm (Monastir) and 2.1 ppm (Korbous), while their maximum-recorded values were 10.2 ppm (Korbous) and 21.9 ppm at Bizerte Grottes. Lanthanum and Mn were only measurable at a few localities.

As for *P. articulatus*, concentrations of Cu were slightly higher, ranging between 6.5 and 10.9 ppm at Sidi Daoued and Kelibia2, respectively (Table 3). Zinc reached high amounts in the shells collected at Sidi Daoued (15.1 ppm) and La Goulette (16.2 ppm).

Spearman test showed the absence of significant correlation between the metals contents and the variables average size, average weight and average shell weight (Table 4). Furthermore, the comparison of the amount of metals picked up by the shell of each species along the common sampling stations (Kruskal-Wallis test) revealed that there was not a statistically significant difference ($p > 0.05$).

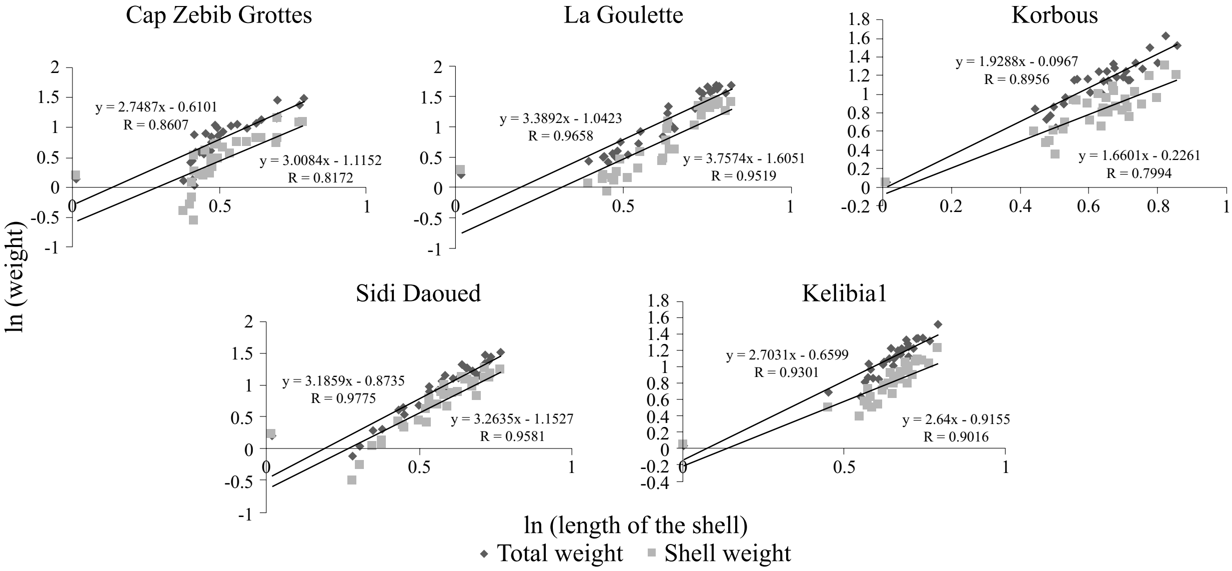

Figure 5. Total length (L)/total weight (TW) and total length (L)/weight of the shell (SW) relationships in *Phorcus articulatus*.

DISCUSSION

The shell in *Phorcus* genus is an exoskeleton that serves for muscle attachment and calcium storage, but that also protects from predators, mechanical damage and dehydration. Moreover, these organisms have evolved morphological and behavioral traits that increase their tolerance to hard environmental conditions as large thermal fluctuations, hydro dynamism, pH and oxygen variations. Morphometry is quite beneficial in distinguishing groups and establishing phylogenic relationships being the genetic expression of the cumulative adaptations of living organisms to their long-term changing environment. Indeed, the thickness and microstructure of shells is highly variable among areas, which appears to be predominately driven by environmental conditions, as it is not explained by genetic differences (De Noia et al. 2020). The spatial shell shape variation noted in this work, especially between the samples of La Goulette and Korbous and the others populations in *P. turbinatus*, is probably linked to simultaneous factors such as the predation, the competition effects, the extent of the continental shelf, the geomorphology of the coast and the dynamics of water masses. In fact, the northern part of Tunisia is subject to intense coastal current arriving from the Atlantic side through the Gibraltar Strait that fades when progressing towards the south (Béranger et al. 2004; Sorgente et al. 2011). In this same context, Jørgensen (2002) explained the shell morphological variations in *Lacuna parva* (da Costa, 1778) collected from 23 European localities by the current rising and tides swaying. Furthermore, Boulajfene and Tlig-Zouari (2016) related the morphological diversity, noted in the same study species using the geometric morphometry, to the population size and the competition for algae, which recovery depends on the atmospheric pressure and hydro dynamism. More recently, Faidallah et al. (2021) described the morphological plasticity of *P. turbinatus* sampled from the eastern Libyan shores as a difference of adaptation to the harsh environmental conditions in the littoral zone specifically desiccation, overheating/overcooling and exposure to extreme salinities. Our interspecific shell shape comparison revealed a clear morphological disparity with some

Table 1. Parameters of L/TW and L/SW relationships in *Phorcus turbinatus* and *P. articulatus* (N = 30).

	L/TW					L/SW				
Stations	a	b	r^2	t_{cal}	Allometry	a	b	r^2	t_{cal}	Allometry
Phorcus turbinatus										
Bizerte Grottes	3.397	-0.946	0.979	12.225	Negative	3.756	-1.412	0.973	9.800	Negative
Jarzouna	3.331	-0.845	0.968	9.947	Negative	3.723	-1.409	0.931	6.372	Negative
Cap Zebib Port	3.196	-0.829	0.974	11.985	Negative	3.663	-1.379	0.962	8.528	Negative
Cap Zebib Grottes	3.568	-0.963	0.947	6.781	Negative	3.934	-1.453	0.918	4.295	Negative
La Goulette	3.425	-0.835	0.975	10.674	Negative	3.861	-1.325	0.966	8.043	Negative
Korbous	2.844	-0.72	0.964	12.375	Negative	3.022	-1.171	0.947	9.944	Negative
Sidi Daoued	2.952	-0.839	0.952	10.134	Negative	3.152	-1.297	0.913	7.091	Negative
Kelibia1	3.069	-0.765	0.984	16.480	Negative	3.305	-1.221	0.978	13.485	Negative
Monastir	2.801	-0.721	0.902	7.231	Negative	2.849	-1.074	0.840	5.5819	Negative
Phorcus articulatus										
Cap Zebib Grottes	2.748	-0.610	0.740	3.673	Negative	3.008	-1.115	0.667	2.784	Negative
La Goulette	3.389	-1.042	0.932	6.660	Negative	3.757	-1.605	0.906	5.101	Negative
Korbous	1.928	-0.096	0.802	7.937	Negative	1.660	-0.226	0.639	6.588	Negative
Sidi Daoued	3.185	-0.873	0.955	9.095	Negative	3.263	-1.152	0.917	6.572	Negative
Kelibia2	2.703	-0.659	0.865	6.239	Negative	2.640	-0.915	0.812	5.572	Negative

overlapping between the groups since both species are belonging to the same genus. Boulajfene and Tlig-Zouari (2016) reported that the main shape difference is that *P. articulatus* has a more conical shaped shell than *P. turbinatus*. Authors suggested that a less voluminous shell could better annoy the removal by the action of waves, preferundum of *P. turbinatus*, while a higher and a stronger shell would better resist against predator attacks.

P. turbinatus shells sampled in this work had an average size equal to 15.87 ± 0.1 mm all stations combined. They are moderately bigger than the topshells collected from eastern Libyan coasts by Faidallah et al. (2021) who estimated average sizes in two sampling stations to 12.81 and 13.72 mm and smaller than the samples recorded by Boucetta (2017) from the eastern coasts of Algeria whose sizes oscillated between 24.14 and 27.96 mm. As for the average size of *P. articulatus* noted in this work, it was valued to 18.41 ± 0.1mm, as the shell is more conical such previously mentioned. Concerning species filling, the average total weight was equal to 2.118 ± 0.47 g for *P. turbinatus* and to 2.95 ± 0.37 g for *P. articulatus* compared to an average ranging between 3.17-3.76 g and between 6.34-14.47 g in *P. turbinatus* collected from Libyan and Algerian shores (Boucetta 2017; Faidallah et al. 2021). Our average shell weights, all stations combined, are respectively 1.55 ± 0.35 g and 2.19 ± 0.29 g, corroborating with results of Boulajfene and Tlig-Zouari (2016) that the shell of *P. articulatus* is thicker and stronger. The comparison of the allometric growth relationships in the two study species revealed a strong correlation between the length and the total weight and between the length and the shell weight variables. Moreover, the fact

Table 2. Metal content in the shell of *Phorcus turbinatus* (ppm/< dl: below detection limit). Two replicates by metal.

Stations	As	Br	Ce	Cr	Cu	Hf	La	Mn	Nd	Rb	Sr	Th	Zn
Bizerte Grottes	8 ± 1.46	16.7 ± 3.62	12.6 ± 3.09	6.6 ± 1.68	7.8 ± 1.61	3.6 ± 1.2	6.6 ± 1.74	3.7 ± 1.18	1.6 ± 0.5	6.5 ± 1.27	1,362.7 ± 28.63	25 ± 2.6	21.9 ± 2.36
Jarzouna	7.9 ± 1.63	15.1 ± 3.11	3 ± 1.21	8.6 ± 2.17	6.3 ± 1.47	7.2 ± 2.45	3.7 ± 1.1	2 ± 0.6	1.4 ± 0.32	6.5 ± 1.34	1,387.3 ± 25.09	23.7 ± 1.44	2.8 ± 0.4
Cap Zebib Port	8.7 ± 2.15	16.6 ± 2.48	9.4 ± 2.88	12.7 ± 2.48	6.8 ± 1.63	0.9 ± 0.2	2.7 ± 0.96	< dl	4.6 ± 1.24	5.9 ± 1.18	1,373.2 ± 33.7	26.1 ± 2.5	4.6 ± 0.7
Cap Zebib Grottes	4.9 ± 1.22	18.1 ± 4.16	18.8 ± 4.42	10 ± 1.61	7.9 ± 1.5	6.6 ± 2.14	16.8 ± 3.43	3.1 ± 1.07	11.3 ± 3.29	6.4 ± 1.09	1,325.5 ± 32.34	24.5 ± 1.67	3.9 ± 0.32
La Goulette	7.7 ± 2.28	14.8 ± 2.32	17.3 ± 3. 61	11.5 ± 2.31	9.5 ± 2.13	4.9 ± 1.35	< dl	< dl	1.1 ± 0.3	5.5 ± 1.13	1,456.2 ± 41.19	26.5 ± 2.41	3.5 ± 0.4
Korbous	9.4 ± 2.91	15.9 ± 3.26	9.9 ± 3.12	11.6 ± 2.27	10.2 ± 2.78	7.6 ± 2.16	8.3 ± 1.07	< dl	5.1 ± 1.37	5.5 ± 0.97	1,349.2 ± 37.62	24.4 ± 1.3	2.1 ± 0.2
Sidi Daoued	4 ± 1.39	14.5 ± 2.81	19.2 ± 4.03	11.2 ± 1.93	6.1 ± 1.9	0.5 ± 0.1	0.2 ± 0.1	4.6 ± 1.8	4.9 ± 1.12	7.5 ± 1.22	1,405.3 ± 36.3	25.4 ± 2.07	2.3 ± 0.4
Kelibia1	3.7 ± 1.27	13.9 ± 1.72	13.1 ± 2.26	9.8 ± 1.44	6.8 ± 1.04	4.6 ± 1.28	4.3 ± 0.8	< dl	4.8 ± 0.9	6.1 ± 1.46	1,283.6 ± 21.72	23 ± 1.22	12.3 ± 1.57
Monastir	6.5 ± 1.67	18.3 ± 3.62	0.8 ± 0.17	8.5 ± 1.37	4.6 ± 0.65	7.9 ± 1.74	< dl	< dl	3.8 ± 0.5	7.4 ± 1.26	1,376.2 ± 27.32	24.4 ± 1.56	3.5 ± 0.8
Average	6.75 ± 2.09	15.98 ± 1.56	11.56 ± 6.55	10.05 ± 1.91	7.33 ± 1.73	4.86 ± 2.77	6.08 ± 5.39	3.35 ± 1.09	4.28 ± 3.09	6.36 ± 0.72	1,368.8 ± 48.66	24.77 ± 1.10	6.32 ± 6.61

Table 3. Metal content in the shell of *Phorcus articulatus* (ppm/< dl: below detection limit). Two replicates by metal.

Stations	As	Br	Ce	Cr	Cu	Hf	La	Mn	Nd	Rb	Sr	Th	Zn
Cap Zebib Grottes	3.2 ± 0.7	15.5 ± 1.31	2.2 ± 0.3	9.8 ± 1.61	9.4 ± 1.36	7.4 ± 1.66	10.2 ± 1.83	6.8 ± 1.14	< dl	5.2 ± 0.67	1,250.6 ± 25.47	20.6 ± 2.17	2 ± 0.2
La Goulette	3.1 ± 0.64	13.3 ± 1.69	9.5 ± 1.73	10.2 ± 1.72	7.6 ± 1.2	2.7 ± 0.7	0.9 ± 0.2	13.7 ± 2.06	3.7 ± 1.1	5.4 ± 0.88	1,191.9 ± 18.3	23 ± 3.13	16.2 ± 2.37
Korbous	10.2 ± 1.26	14.6 ± 2.12	8.8 ± 1.38	9.4 ± 1.64	9.2 ± 1.44	9.7 ± 1.9	1.7 ± 0.46	< dl	< dl	5.6 ± 0.8	1,253.2 ± 26.41	25.2 ± 2.37	11 ± 1.62
Sidi Daoued	2.3 ± 0.6	11.5 ± 1.86	2.2 ± 0.43	7.1 ± 0.78	6.5 ± 0.8	7.5 ± 1.12	< dl	< dl	5.6 ± 1.24	6.8 ± 0.73	1,288.2 ± 23.69	21.5 ± 2.16	15.1 ± 1.24
Kelibia2	5.8 ± 1.11	15.8 ± 2.4	17.6 ± 3.17	10.7 ± 1.08	10.9 ± 1.5	6.7 ± 0.68	3.8 ± 0.72	7.8 ± 1.76	5.3 ± 0.8	5.3 ± 0.61	1,133.3 ± 14.6	21.9 ± 1.7	5.5 ± 0.48
Average	4.92 ± 3.23	14.14 ± 1.76	8.06 ± 6.37	9.44 ± 1.7	8.72 ± 1.7	6.8 ± 2.55	4.15 ± 4.21	9.43 ± 3.72	4.86 ± 1.02	5.66 ± 0.65	1,223.44 ± 61.1	22.44 ± 1.76	9.96 ± 6.11

Table 4. Spearman Correlation Rank between [Cu]/[Zn] in shells, soft tissues and sediment, and averages size, weight and shell weight for *Phorcus turbinatus* and *P. articulatus* (first line: parameter, second line: p-values).

	[Cu] shell	[Zn] shell
Phorcus turbinatus		
[Zn] shell	-0.0857	
	0.8480	
[Cu] tissue	-0.6000	0.4857
	0.1797	0.2774
[Zn] tissue	-0.5429	0.7714
	0.2248	0.0845
[Cu] sediment	-0.1160	0.6377
	0.7954	0.1539
[Zn] sediment	-0.8857	-0.0857
	0.476	0.8480
Average size	0.3143	0.3143
	0.4822	0.4822
Average weight	-0.6000	0.4857
	0.1797	0.2774
Average shell weight	-0.4857	0.6000
	0.2774	0.1797
	[Cu] shell	[Zn] shell
Phorcus articulatus		
[Zn] shell	-0.8000	
	0.1659	
[Cu] tissue	0.2000	-0.4000
	0.7290	0.4884
[Zn] tissue	-0.8000	0.4000
	0.1659	0.4884
[Cu] sediment	0.2000	-0.4000
	0.7290	0.4884
[Zn] sediment	-0.8000	0.6000
	0.1659	0.2987
Average size	1.0000	-0.8000
	0.4884	0.1659
Average weight	0.8000	-0.6000
	0.1659	0.2987
Average shell weight	0.8000	-0.6000
	0.1659	0.2987
	[Cu] shell	[Zn] shell

that the length increases faster than the weight, verified by the negative allometry in all stations, can be caused by several factors as food supply, population density and competition, wave action, water cooling, among others. (Crothers 2012; Sousa et al. 2018). Indeed, Mannino et al. (2008) observed that the diminution of water temperature in winter promotes a metabolic slowing in *P. turbinatus*, which growth interrupts temporarily then continues rapidly through the year, slowing only in the next winter. This growth rate coupled with limited trophic resources and strong competition in all sampling stations (Boulajfene and Tlig-Zouari 2016) could explain the growing in shell size more quickly than in weight. Our results corroborate those of Faidallah et al. (2021) who reported minor allometry in Libyan topshells, but differ from findings of Boucetta (2017) who suggested proportional growths in length and in weight in *P. turbinatus* collected in Algeria (isometric allometry). Samely as our outcomes, Sousa (2020) reported a negative allometry in *P. sauciatus* (Koch, 1845) sampled from the archipelago of Madeira in the North Atlantic Ocean, and Vasconcelos et al. (2022) suggested mostly a minor allometry in three topshell species from the Algarve Coast (Southern Portugal) namely *P. lineatus*, *P. sauciatus* and *Steromphala umbilicalis* (da Costa, 1778).

Despite *Phorcus* species are recognized to accumulate high metal concentration in their shell and tissues (Sousa et al. 2018), this work constitutes the first investigation of metal content in the calcareous body part in this genus. The X-ray fluorescence was used to understand the geological conditions and ecological quality of the surrounding environment. Arsenic, for example, classified (along with Pb, Cd, Hg, C_2H_3Cl, and C_6H_6) as priority hazardous substances (Nordberg et al. 2002), was present with an average content of 6.75 ± 2.09 ppm in *P. turbinatus* and 4.92 ± 3.23 ppm in *P. articulatus* all stations combined. In marine environments, it is mainly generated by riverine inputs, which are contaminated by domestic sewage, industrial and agricultural chemicals (mining, smelting, materials beneficiation and wood preservation), leading to significant levels of As contamination in surface seawaters (Wang et al. 2022). Note that the mean concentration of arsenic in unpolluted seawater is 1.7 µg l^{-1} (ranging from 1 µg l^{-1} to 3 µg l^{-1}) (Mandal and Suzuki 2002; Wang et al. 2022). Even though zinc (Zn) is an essential trace element being a transition metal that participates in the modulation of regulatory proteins and cellular activities and playing antioxidant roles, its excess in living organisms gives rise to severe metabolic disorders (Trevisan et al. 2014). Indeed, higher zinc concentrations can induce apoptosis and oxidative stress, highlighting the dual role of this metal as oxidant/antioxidant and pro-apoptotic/anti-apoptotic agent (Trevisan et al. 2014). As for manganese, its toxicity generally occurs at dissolved concentrations above 1,000 µg l^{-1} (WHO 2004), which is usually well in excess of environmental concentrations. Our average concentrations of Mn and Zn (consecutively 3.35 ± 1.09 and 6.32 ± 6.61 ppm in *P. turbinatus*, and 9.96 ± 6.11 and 9.43 ± 3.72 ppm in *P. articulatus*) were close to those measured by Gawad (2018) in *Lanistes carinatus* (Olivier, 1804) from Lake Manzala, Egypt (Mn ranged between 5.124-6.768 µg g^{-1} and Zn between 2.762-4.562 µg g^{-1}). Nevertheless, Gawad (2018) noted relatively low Cu contents (0.350-1.240 µg g^{-1}) compared to an average of 7.33 ± 1.73 in *P. turbinatus* and 8.72 ± 1.7 in *P. articulatus*. As for Leppänen et al. (2021), they estimated high levels of Mn (going to ≈ 380 ppm) compared to our concentrations, but much lower contents of Sr (≈ 8 ppm) in the freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) sampled from the Mustionjoki River (South Finland) using the micro-X-ray fluorescence. The application of Spearman test displayed no significant correlation between [Cu] and [Zn] in the shells of both species and their concentrations in soft tissues and in the sediment reported by Boulajfene et al. (2017, 2019) ($p > 0.05$). The absence of significant correlation between [Cu] and [Zn] in shells and their concentrations in soft tissues and in the sediment, noted in the present study, can be explained by the fact that the integration of metals into mollusk shells occurs over a long period and is not simultaneous with tissue bioaccumulation nor sediment adsorption of metals. According to Jordaens et al. (2006), this incorporation occurs when elements, such as Pb, Zn and Mn, follow the same intracellular pathway as Ca during the biomineralization process, thus substituting for Ca when shells develop in contaminated areas. Consequently, metals may disrupt shell integrity, strength and microstructure formation and modify the activity of proteins during the crystallization process (Jordaens et al. 2006; Sharma et al. 2008). Yap et al. (2003) mentioned that the physiological condition of the mollusk could affect the soft tissue metal levels, but could hardly affect their concentrations in the shell. Other factors may affect bioaccumulation of metals in soft tissues like feeding habits, age and growth rate as well as the bioavailability of the metals based on pH and water hardness (Lau et al. 1998). Our findings corroborate with those of Gawad (2018), who reported the absence of significant direct relations among the concentrations of heavy metals in the water, sediment, soft tissue and shell of *L. carinatus*. This author suggested that those of water and bottom sediment might not directly affect metal contents in shell. As for Stewart et al. (2021), they implied a non-apical response to metal contamination (i.e. a response not directly linked to increased mortality) but that leads to shell weakening in marine scallops collected from the coastal waters around the Isle of Man, in the North Irish Sea. Those authors focused on the effects of metals on calcification in marine organisms and indicated that bivalve shell structure and strength may be a more sensitive, but very ecologically relevant, end-point or bio-indicator than those currently used in ecotoxicology assessments. As for Yap et al. (2003), they also reported an easy metal accumulation in the whole shells of *P. viridis* collected from the west coast of Peninsular Malaysia and supported the use of the total shell of the green-lipped mussel as a potential biomonitoring material for long-term contamination. In general, the assessment on mollusk shells, especially using XRF, can yield valuable information about the elemental composition of the shell material, which in turn can provide insights into the environmental conditions in which this organism lived (Stewart et al. 2021). This information can be particularly useful for studies related to past climates, ecosystems and archaeological sites especially since species belonging to *Phorcus* genus can have an important longevity going to 10 or even 15 years (Sousa et al. 2018).

The calcareous structure of shells of the two most widespread *Phorcus* species along the rocky coasts of Tunisia, seems to be a good bioindicator tool for many work subjects. Indeed, the shell demonstrated a spatial morphometric variability that was not proved by genetic analyzes but probably linked to simultaneous factors such as the extent of the continental shelf, the geomorphology of the coast, the dynamics of water masses, the predation and the competition effects. Then, the shell examination allowed getting an idea about the relative growth pattern and indicated that the development in shell size was more quickly than the increase in weight. Furthermore, the use of *Phorcus* shells as bioindicators of past and long-term metal contamination seems to be of growing interest, due to their abundance, easy sampling and ecobiological features, both in a scientific and ecosystem management perspective that aims to the establishment of conservation measures targeting marine coastal environments. Nevertheless, further studies on the biology and population dynamics of *Phorcus* species still required in order to guarantee the implementation of successful conservation strategies and a sustainable exploitation based on effective management measures.

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Author contributions

Wafa Boulajfene: conceptualization, methodology, software, investigation, resources, writing. Vassiliki-Angelique Catsiki: methodology, validation, visualization. Sabiha Tlig-Zouari: conceptualization and supervision.

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