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Research Article

Frequency histograms, condition parameters and size-depending metal accumulation in two species belonging to *Phorcus* genus

Wafa BOULAJFENE* & **Sabiha TIIG-ZOUARI**

University of Tunis El Manar. Faculty of Sciences - Department of Biology. Research Laboratory of Biodiversity. Parasitology and Ecology of Aquatic Ecosystems. Tunis 1068. Tunisia

***Correspondence:** wboulajfene@gmail.com

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Abstract:

This work is a biological and ecotoxicological investigation in two Trochidae species *Phorcus turbinatus* and *Phorcus articulatus* taken from four stations, monthly for biological study and seasonally for size-depending metal monitoring. Our results related to biological characterization suggested mostly polymodal frequency histograms associated with a continual renewal of juveniles indicating a laying period spread over several months.

The condition index revealed similar values slightly decreasing during hot seasons probably due to the coincidence of the spawning period with the spring and the summer. As for the condition factor, it also presented close values with a slight increase during the periods of March-April, June-July and even September-November probably resulting from algal proliferation and trophic availability during those months.

The estimation of metal pollution indicators proposed an increasing classification of contamination related to the amounts of waste ejections at each locality coming from industrial activity, large fishing ports and mooring areas. Size-depending metal analyses revealed that despite the strong contamination, a moderate concentration decrease was associated with size growth. This life strategy would allow these species to counteract unfavorable conditions and ensure their persistence and thus justify their frequent use as bio-indicators of the state of health in coastal ecosystems.

Keywords: ecotoxicology; biological analysis, monitoring systems, size, pollution control, condition factor.

1. Introduction

The rocky intertidal areas are especially subject to numerous changes of physicochemical conditions that conduct to morphological, physiological and behavioral adaptations in many taxa increasing the ability of organisms to survive in various habitats (Underwood, 1985). These ecosystems are mainly influenced by waves' contravention, tides swaying, and strong temperature oscillations (Bertness et al., 2006).

However, they are sheltering a diverse fauna between decapods, barnacles (crustaceans), bivalves and gastropods (mollusks). More particularly, the rocky shores of Tunisia are known to harbor a wide range of taxa even they are strongly affected by xenobiotics namely organostannic compounds (tributyltin TBT), hydrocarbons and metals (Boulajfene et al., 2015; Boulajfene et al., 2017; Mzoughi and Chouba, 2011; Trabelsi and Driss, 2005). Note that contaminations are not limited to the sediment but also affect benthic organisms inhabiting those coasts. In fact, chemical analysis showed that the tissues of the mussel *Mytilus galloprovincialis* Lamarck, 1819 collected from the Gulf of Tunis and Galite station were strongly affected by total PAHs (68.6 and 69.7 g/kg) (Galgani et al., 2011). These authors suggested that the waters of the Gulf of Tunis are contaminated with benzo [a] anthracene (8.0-9.2 g/kg), chrysene (7.3-9.7 g/kg), fluoranthene (10.1-11.0 g/kg), phenanthrene (8.8-10.3 g/kg) and pyrene (9.5 g/kg) (Galgani et al., 2011). Moreover, the soft tissue of the muricidae *Stramonita haemastoma* (Linnaeus, 1758) collected from the Gulf of Tunis proved to be highly enriched in organotins provoking imposex phenomenon (Boulajfene et al., 2015). In addition, Boulajfene et al. (2017; 2019) reported a high metal contamination in species belonging to the *Phorcus* genus.

Those species, especially *P. turbinatus* (Born, 1778) and *P. articulatus* (Lamarck, 1822) are often considered as good bioindicators since they are directly linked to the bottom, widespread along the rocky shores and sedentary (low movement) (Belhaouari et al., 2011; Boucetta et al., 2016; Boulajfene et al., 2017).

The gastropod *P. turbinatus* occurs amply on the rocky intertidal shorelines of Tunisia from Tabarka (Northwest) to Zarzis (South) (Boulajfene and Tlig-Zouari, 2016), and is often associated with the Rhodophycea *Nemalion helminthoides* Batters, 1902 and *Rissoella verruculosa* Agardh, 1849, characteristic algae of beaten modes (Boudouresque, 2013). As for *P. articulatus*, it is abundant in the intertidal zone from the northeastern to southeastern coasts of Tunisia and seems to be correlated to the low-risk microhabitats of these shores (Boulajfene and Tlig-Zouari 2016). Indeed, this gastropod is specific to the upper infralittoral of quiet mode, invaded by *Cladophora spp*. (Houlihan and Innes 1982). These two sedentary gastropods were generally considered as good bioindicators of metal pollution in the marine environment (Belhaouari et al., 2011; Conti and Cecchetti, 2003). They are also able to tolerate temperature and salinity fluctuations (euryhaline and eurythermal) and survive even with low oxygen rates (Houlihan and Innes, 1982).

In Tunisia, those species have only been the subject of some works limited to their abundance and biomass at Kerkennah Islands (Cheour and Aloui-Bejaoui 2010; Cheour et al. 2014a; Cheour et al. 2014b), their morphological plasticity (Boulajfene and Tlig-Zouari, 2016) and their metal contamination (Boulajfene et al., 2017; 2019; 2021). However, no work even if interested in their bioaccumulator feature, has focused on their metal concentration in relation to body size. Furthermore, their biological traits remain little studied (Sousa et al., 2018) and even unprocessed and unknown in Tunisia. In fact, aquatic animals can accumulate and biomagnify these substances and transfer them across the food web, constituting risks for both predators and humans.

Many factors can affect the metal concentrations namely the level of environmental contamination and the duration of exposure (Has-Schön et al. 2015). Consequently, as specimens grow, it can be predictable that larger or older individuals accumulate higher metal concentrations than smaller or younger ones.

Thus, the present work aims to (i) study some biological traits of the two species *P. turbinatus* and *P. articulatus* collected from four rocky stations, (ii) categorize the study stations according to a classification of metal pollution and (iii) perform a seasonal assessment of metal accumulation depending on samples' body size in both species.

2. Materials & Methods

2.1. Frequency histograms and condition parameters

In order to assess some biological traits in *P. turbinatus* and *P. articulatus*, monthly monitoring was conducted from March 2014 to February 2015 at four stations (La Goulette, Korbous, Sidi Daoued and Kelibia) located at the northeastern and eastern coasts of Tunisia (Fig. 1). The choice of stations was based on the abundance of species and the presence or absence of a potential source of metal pollution: industrial zones (Sidi Daoued); fishing ports (Sidi Daoued, Kelibia), marinas and anchorage areas (La Goulette). As for Korbous station (hydrothermal source), it was distinguished due to its low pollution given that it shelters many rare and protected species such as *Patella ferruginea* Gmelin, 1791 and *Pinna nobilis* Linnaeus, 1758 (Boukhicha et al., 2014; Rabaoui et al., 2008 ; Tlig-Zouari et al., 2010). Thus, it was considered as a reference station in this study.

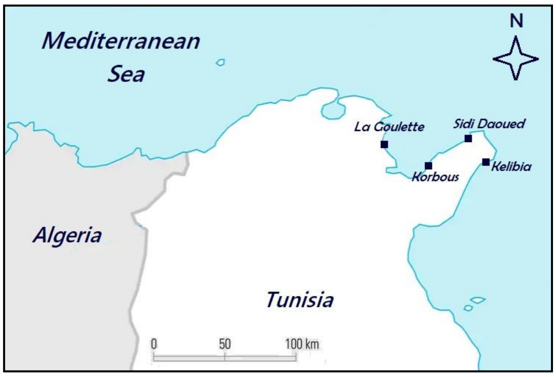

Figure 1. The location of the sampling stations along the northeastern coasts of Tunisia

A sample of 30 individuals of each species was taken monthly and randomly at each station and divided into size classes, which percentages were calculated and represented by frequency histograms.

The delimitation of the reproduction period is mainly based on the variations of the biometric index CI (condition index) mainly used in shellfish farming to assess animals' filling degree (Merzouki et al., 2009) and determined by the ratio between the weight of the flesh and the weight of the shell. To do so, the total length of each individual (in mm) was measured using an electronic caliper (at 1/100 mm pre) and the total weight (in g) was weighed using a "Pioneer" balance. Subsequently, the specimens were dissected to estimate the mass of the flesh (in g).

The index used in this study, is the AFNOR index (NF V 45056, Sep 85) expressed by the following equation: IC= (Fresh weight) / (Total weight) x 100.

The condition factor (CF) is an allometric index, based on a linear relationship between the weight and the cube of the size. It is an initial selection biomarker allows to check the variations in the metabolic balance of individuals (Van der Oost et al., 2003). It is often used to indicate the effects induced by exposure to environmental toxicants or provide information on energy storage varying according to physicochemical factors and to the nutritional level of the organisms (Van der Oost et al., 2003). The condition factor (CF) was calculated as follows (Fulton, 1911):

$$CF = (\text{Total weight})/\text{size}^3$$

2.2. Metal contamination

Data from Boulajfene et al. (2017, 2019) obtained via seasonal analyses of five metals (Cu, Zn, Pb, Cd and Hg) in the biological tissue of *P. turbinatus* and *P. articulatus*, allowed calculating the metal contamination indicators (CR, PI and MPI) for the four study stations. Metal pollution ratios are based on reference values (Meybeck et al., 1999). In the present study, these values were obtained from Korbous station (reference).

2.2.1. Metal Contamination Ratio: CR

A dimensionless number calculated for each sample j and for each element i (Meybeck et al., 1999):

$[CR]_{ij} = [Mmes]_{ij}/[Mref]_{ij}$

With Mmes: measured levels; Mref: reference contents

When contamination is equivalent to zero, CR is theoretically equal to 1. This index highlights the great variability of contamination depending on the stations and the metals.

2.2.2. Pollution index: PI

A dimensionless parameter was also calculated for each sample and each element (Meybeck et al., 1999):

$$[PI]_{ij} = ([Mmes]_{ij} - [Mref]_{ij}) / [Mref]_{ij}$$

PI is equal to zero in the absence of contamination. It can be negative when the theoretical reference level is greater than the measured level. This index does not provide additional information compared to CR but allows for the calculation of the Metal Pollution indicator MPI.

2.2.3. Metal Pollution Indicator: MPI

This indicator is based on the five elements most sensitive to human activities that are Cd, Cu, Hg, Pb, Zn (Meybeck et al., 1999):

$$[MPI]_j = \sum [PI]_{ij}$$

With PI_{ij}: pollution indexes of the sample (j) for the metals Cd, Cu, Hg, Pb and Zn.

A second set of 30 specimens was taken seasonally from two stations (La Goulette & Kelibia) and the same five metals (Cu, Zn, Pb, Cd and Hg) were analyzed in individuals of both species belonging to different size classes [<12.99mm / 13-19.99mm / >20mm] by atomic absorption spectrometry as explained by Boulajfene et al. (2017).

2.3. Statistical analysis

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.

3. Results

3.1. Frequency histograms and condition parameters

This part was devoted to the representation of size structures and the estimation of the condition indexes and factors per month and per station for both species.

At La Goulette station, the frequency histograms of *P. turbinatus* were unimodal for the majority of the populations (March, April, May, June, October and February 2015). Samples taken in September, November, December 2014 and January 2015 were bimodal while those taken in July and August showed polymodal frequency histograms. The sets collected during November and December 2014 contained essentially small individuals (<13mm) (70% and 63.33% respectively). Large individuals (>20mm) are mainly encountered in the samples of July (86.66%), August (40%), October (53.33%), November (30%) and February 2015 (50%) (Tab. 1). Samples of the same species collected from Korbous displayed unimodal frequency histograms in July and August while those obtained during April, June, September, November and February 2015 were bimodal. The remaining sets showed a polymodal distribution. Small individuals constituted the majority of the samples collected during November 2014 (46.66%), December 2014 (40%) and January 2015 (30%). Nevertheless, the samples from May, August and January 2015 were highly marked by large individuals (40%, 23.33% and 20% respectively). Frequency histogram relating to the sample taken from Sidi Daoued in May was bimodal although those corresponding to the samples of July, August and October were unimodal. The individuals collected during the remaining months presented a polymodal frequency distribution. Small individuals (<13mm) were especially common during April (16.66%), September (16.66%) and November (30%). Regarding large specimens (size >20mm), they were found in abundance in samples taken in May (20%), August (26.66%), October (43.33%) and February 2015 (50%). All the frequency histograms representative of Kelibia station were polymodal whatever the month. The samples taken during September and November were marked by a strong presence of small individuals (40% and 36.33% respectively) while large individuals (>20mm) were especially frequent in the samples of July (43.33%), August (26.66%), October (53.33%), December 2014 (23.33%), January 2015 (26.66%) and February 2015 (33.33%).

As for *P. articulatus*, the histograms corresponding to La Goulette locality were polymodal for the majority of the samples except those of July and September (bimodal type). The sets collected during April, July and August showed a considerable presence of small individuals (26.66%, 30% and 16.66% respectively) whereas large individuals were mainly encountered in the samples of March (60%), April (50%), May (60%), August (60%), October (66.66 %), January 2015 (40%) and February 2015 (56.66%).

Korbous station exposed unimodal histograms during August, October and November and bimodal ones obtained in June, July, September, December and January-February 2015. The distributions of the individuals' sets for the remaining months were polymodal. Small individuals

were very rare, present only during April with low frequency (16.66%). Large individuals were common in the samples of May (70%), August (56.66%), November (73.33%), December (70%) and February 2015 (43.33%). The histograms relating to Sidi Daoued samples in September and January 2015 were bimodal and those of July, August and October were unimodal.

Regarding the individuals collected during March, April, May, November and December 2014, they showed a polymodal form of their frequencies' distribution. Small individuals (<13mm) are especially recurrent during April (16.66%) and November (13.33%). As for large specimens (>20mm), they were found in abundance during March (60%), April (46.66%), May (33.33%), November (36.66%), December (33.33%) and February (43.33%). The majority of the obtained histograms corresponding to Kelibia station were bimodal (July, November, December, January and February 2015).

The remaining histograms were unimodal (March, April, May and October) and polymodal (June, August and September). The samples taken during July and August were marked by great presence of small individuals (23.33% and 20% respectively) when large individuals were especially common in April (33.33%), May (60%), June (60%), December (33.33%), January (40%) and February (40%) (Tab. 1).

Table 1 Average, minimum and maximum sizes in the four stations during the sampling period (mm)

	Phorcus turbinatus			*Phorcus articulatus*		
	Average size	Minimum	Maximum	Average size	Minimum	Maximum
La Goulette	16.45 ± 2.7	6 November 2014	26.84 July 2014	18.04 ± 1.7	8.89 November 2014	26.45 June 2014
Korbous	15.43 ± 2.52	6.08 September 2014	31.35 April 2014	19.89 ± 1.27	6.1 April 2014	25.7 May 2014
Sidi Daoued	17.17 ± 2,57	6.68 April 2014	26.5 August 2014	18.01 ± 2.12	10.61 March 2014	31.88 April 2014
Kelibia	16.24 ± 3.33	7.08 May 2014	26.81 July 2014	18.18 ± 1.72	6.5 April 2014	25.64 December 2014

The condition index, monthly estimated in *P. turbinatus* at the four study stations, displayed a noticeable decrease of its values essentially during the period comprised between April and August to reach minima generally in June-July (Fig. 2a). Concerning the second species *P. articulatus*, their condition indexes diminished considerably between April and October and the minima were reached later than *P. turbinatus* during July-September (Fig. 2a).

Regarding the condition factor (CF), evaluated monthly in *P. turbinatus*, it showed close values throughout the study period with slight rises during the periods between April-June and September-November (Fig. 2b). In *P. articulatus*, the CF was also almost of close values but increased in March (Sidi Daoued, Kelibia) or in April (La Goulette, Korbous) and then in October (Korbous, Sidi Daoued) or November (La Goulette, Kelibia) (Fig. 2b).

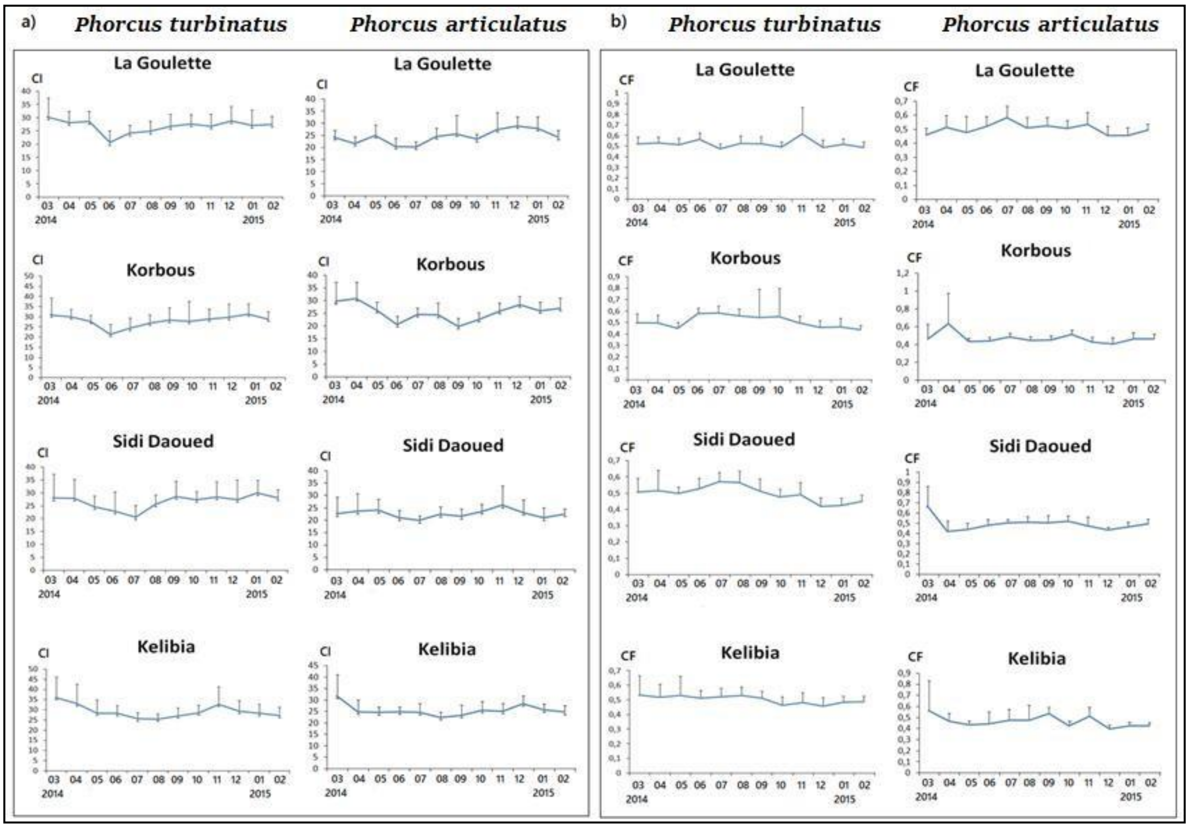

Figure 2. a) Condition index (CI) and b) Condition Factor (CF) monthly estimated in *Phorcus turbinatus* and *Phorcus articulatus* in the four study stations during the period March 2014-February 2015

3.2. Metal contamination

Metal pollution indicators were estimated seasonally in order to compare the excretory efficiency of the two species living in the same localities.

3.2.1. *Metal Contamination Ratio: CR*

The estimation of the metal contamination ratio in *P. turbinatus* at the four study stations compared to Korbous (reference station) suggested a considerable contamination of Sidi Daoued locality by cadmium throughout the four seasons and by mercury during autumn and winter. La Goulette station showed a great metal contamination essentially during spring and summer. As for Kelibia station, it was severely contaminated by lead (CR=4.3) principally in summer. The concentration of this metal decreased afterward with the cooling of the climate. The same ratio estimated in *P. articulatus*, showed close degrees of pollution by Cu, Zn, Cd and Hg between all the sites during spring and summer. Those rates increased in cold periods, especially at Kelibia. Lead represented the same rate during the four seasons. It was minimal at Korbous, more concentrated at La Goulette and Sidi Daoued and greater at Kelibia essentially in summer.

3.2.2. *Pollution index: PI*

The pollution index showed the same spatial and temporal evolution as the metal contamination ratio. Note that this index indicated contaminations by Cu and Pb in Sidi Daoued station, greater than those estimated at Korbous, during the winter for both species.

3.2.3. Metal Pollution Indicator: MPI

The comparison of the obtained results, in both species, revealed that the metal contamination of all the stations was mainly accentuated during summer compared to the other seasons (Fig. 3). Furthermore, the MPI estimation brought to the same general classification during all seasons except the autumn 2014 and winter 2015 for *P. turbinatus* (Tab. 2).

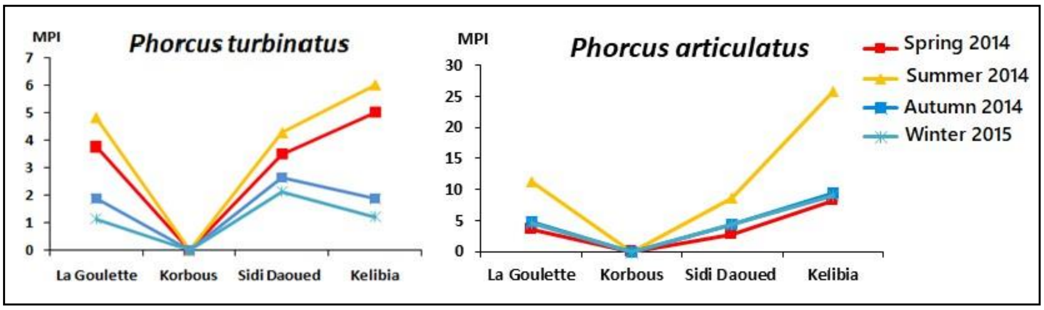

Figure 3. Metal Pollution Indicator (MPI) seasonally estimated in the four study stations using *Phocus turbinatus* and *Phorcus articulatus*

Table 2. Increasing classification of metal pollution in the study stations according to the measured MPI

+ Sidi Daoued		++ La Goulette		+++ Kelibia	
P. turbinatus [2.0 - 4.0]	*P. articulatus* [2.5 - 8.0]	*P. turbinatus* [1.1 - 4.9]	*P. articulatus* [4.8 - 10.1]	*P. turbinatus* [1.4 - 6.0]	*P. articulatus* [8.0 - 26.0]

The estimated MPI, using *P. turbinatus* species, showed no significant differences between seasons (p-value = 0.11) and between stations (p-value = 0.8) while MPI calculated using *P. articulatus* exhibited a significant difference between stations (p-value = 0.032) and a non-significant difference between seasons (p-value = 0.39).

The size-depending metal analyses (Cu, Zn, Pb, Cd and Hg) in individuals of both species belonging to different size classes showed that the metal contents decreased non-significantly (p-value > 0.05) with the increase of the individual size (negative relationship) during the four seasons (Fig.4).

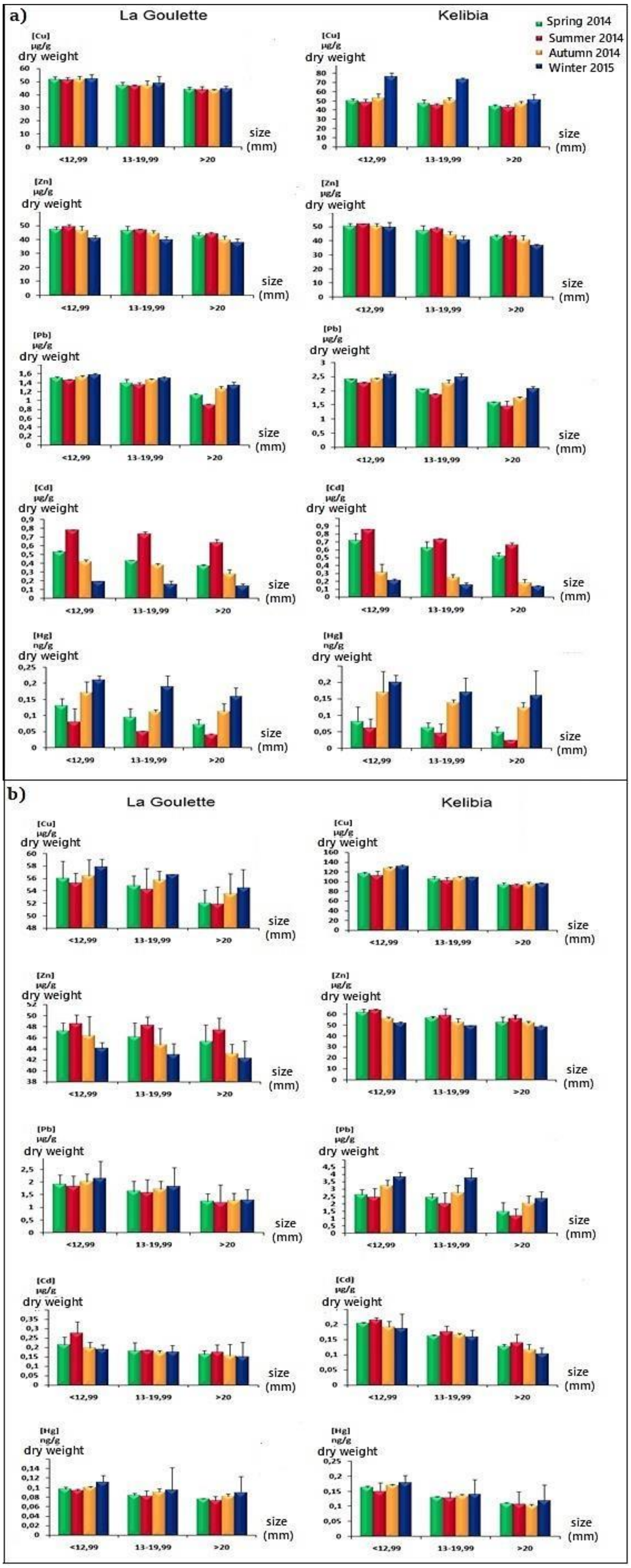

Figure 4. Seasonal size-depending metal analyses of a) *Phorcus turbinatus* and b) *Phorcus articulates* in two stations (La Goulette/Kelibia)

4. Discussion

Gastropod mollusks living in the intertidal zone are subject to many critical conditions like thermal variations, desiccation, and dislodgement by strong hydrodynamics and wave action, predation and competition (Melatunan et al., 2013). Some species show plasticity in their responses to environmental conditions fluctuations and develop adaptation strategies, namely behavioral (migration, selection of microhabitats), metabolic (oxygen consumption and reduction in metabolic level) and morphological (morphometric modification of the shell) (Alyakrinskaya, 2010; Melatunan et al., 2013).

The Trochidae constitute a good example of organisms resistant to the stressful conditions of coastal fringe, as they are able to survive in the air for a long time thanks to their capacity to store a large volume of water in their shells (Langerhans and Dewitt, 2002). However, there still a lack of information on their biological traits and their metal accumulation patterns.

Monthly assessment (12 months) of *P. turbinatus* and *P. articulatus* populations revealed polymodal size distributions, during the majority of months at the four study stations indicating the coexistence of successive cohorts. The Hot period was generally marked by the presence of large specimens. As for small individuals, they r to be introduced continuously but irregularly into the populations (during winter, spring and autumn). Polymodal frequency histograms associated with continual renewal of juveniles seem to reflect a laying period spread over several months (from spring to autumn) and the absence of synchronization between the clutches of individuals in the same population.

The sizes recorded in *P. turbinatus* oscillated between a minimum of 6mm taken at La Goulette in November and a maximum of 31.35mm noted at Korbous in March. It seems that Korbous

station contains more favorable conditions than those present in the other stations and more advantageous habitats for individuals' growth namely the algal presence especially *Nemalion helminthoides* and *Rissoella verruculosa* (Boulajfene and Tlig-Zouari, 2016). As suggested by Bode et al. (1986), trophic and spatial competition could have a negative impact on the longevity of individuals. Note that no spat (< 6mm) was encountered during the entire study period probably linked to young specimens' isolation in different biotopes (crevices, cracks, crevices, etc.) from those of adults in order to take refuge away from predators and other stressors.

The average sizes, evaluated in the present work, ranged between 15.43 ± 2.52 (Korbous) and 17.17 ± 2.57 (Sidi Daoued). Those values are close to those recorded in Algeria [12.77-21.94mm] and slightly higher than those estimated in southern Corsica [10-15mm] (Boucetta et al., 2010; Vermeulen et al., 2011).

However, they are lower than those noted in Malta (between 15 and 38mm) and in Mallorca Spain where the size values of individuals can reach 43mm (Prendergast et al., 2013; Vidal et al., 2013). As for *P. articulatus*, the noted sizes oscillated between a minimum of 6.08mm taken in Korbous in April and a maximum of 31.88mm recorded in Sidi Daoued also in April.

The average size values ranged between 18.01 ± 2.12mm at Sidi Daoued and 19.89 ± 1.27mm at Korbous. The size values measured in the present work are lower than those recorded in Mallorca (Spain), reaching even 35mm (Vidal et al., 2013).

The monthly examination of the condition index in both species revealed similar values throughout the year but that decreased slightly during hot periods, generally from April to September in *P. turbinatus* and even October in *P. articulatus*. This decrease seems to be due to the coincidence of the spawning period with the hot periods (spring and summer) (Bode et al., 1986; Garwood and Kendall, 1985; Underwood, 1972). Our results corroborate those of Belhaouari et al. (2011) who reported that the condition index estimated during winter (29.02-29.90) is higher than that noted in summer (25.72-26.13) in *P. turbinatus* species, collected from the western Algerian coasts. These authors explained the decrease in this factor, in warm months, by its synchronization with spawning period. Indeed, this breeding period is marked by a strong loss of nutrient reserves. In this context, Deslous-Paoli et al. (1981) suggested that spawning in the oyster *Crassostrea gigas* (Thunberg, 1793) (claires de Marennes-Oleron) leads to a loss of 54% of flesh weight, of which 54% are proteins and 73% lipids. The autumn season subsequently constitutes a period of storage of lipid and carbohydrate reserves (more particularly glycogen) explaining the tissue growth of organisms (Deslous-Paoli et al., 1981). As such, breeding phases of a given species can change according to its geographical position. Indeed, in the northernmost range limit, breeding periods are shorter with a single spawning time, while in southern regions, the breeding period is longer with multiple spawning events (Sousa et al., 2018). For instance, in *P. lineatus* (da Costa, 1778) collected from Asturias, Spain, the gonadal development occurs from November to June and the breeding phases from June to September and might last until November in some individuals (Garwood and Kendall, 1985). As for spawning, it occurs between May and August (Graham, 1988). Further north in Wales, the same species is reported to have a shorter spawning season, lasting from July to August (Crothers, 2001). On the other hand, Schifano (1983) reported that *P. turbinatus* living along the Mediterranean Sea seems to have a longer breeding period with two spawning events in spring and autumn.

As for the condition factor, it is generally used as an initial assessment biomarker to indicate the effects induced by exposure to environmental toxic substances, or to provide information on energy storage varying according to physico-chemical factors and to the nutritional level of organisms (Van der Oost et al., 2003). Our results relating to this factor revealed close values almost throughout the study period with a slight increase during the months of March-April, June-July and even September-November. These elevations could be the result of algal proliferation and a trophic availability during spring and summer (Van der Oost et al., 2003).

Furthermore, the stability of this factor, observed in the present study, could be linked to the adaptive strategies developed by the species against specific xenobiotics. Indeed, exposure to contamination allows resistant species to acquire a certain tolerance and invest energy in cell defense, repair and regeneration mechanisms, thus eliminating the sinusoidal shape of the condition factor (Amiard and Amiard-Triquet, 2007; Van der Oost et al., 2003).

Relating to the reproduction of two species of Trochidae, *Monodonta lineata* da Costa, 1778 and *Gibbula umbilicalis* (da Costa, 1778) collected from the northern coasts of Spain, Garwood and Kendall (1985) and Bode et al., (1986) reported continuous egg-laying and a presence of mature gametes throughout the year. These authors explained the duration of the egg-laying period, limited to summer and autumn, reported by Williams (1964) and by Underwood (1972) in the same species (*M. lineata* from the British Isles) by the difference in latitude and the impact of competition on the duration of reproductive activity. Note that this continued gonadal activity in both species could also be the origin of the puncture of large individuals. According to Bode et al. (1986), sexual maturity of *M. lineata* is reached at the age of one year and high temperatures are likely to accelerate the growth and maturity phenomena in these topshells. Note that in the Trochidae, reproduction can be seasonal or continuous throughout the year, planktonic larval development can be replaced by benthic development and hatching can take place after metamorphosis and directly produces juvenile individuals in certain species (*Margarites marginatus* Dall, 1919 and *Calliostoma ligatum* (Gould, 1849)) (Hickman, 1992).

On the other hand, in other species of the same family (*Trochus niloticus* Linnaeus, 1767 and *Umbonium vestiarium* (Linnaeus, 1758)), hatching is early (after 6 hours from fertilization) and releases lecithotrophic planktonic larvae. As for their metamorphosis, it takes place 2 to 3 days later.

These different modes of reproduction and mixed larval development constitute adaptive and evolutionary strategies and determine the range of larval dispersal (Hickman, 1992).

The estimation of metal pollution indicators proposed an increased sequence of contamination (Kelibia > La Goulette > Sidi Daoued) that may be related to the enormous amounts of waste ejections from industrial activity, large fishing ports, or marinas and mooring areas (Boulajfene et al. 2015). Furthermore, Boulajfene et al. (2017) correlated the high metal sedimentological content in these areas to ships and small tankers degassing. Metal analyses according to size classes, in both species, revealed that despite the strong contamination of the two localities (Kelibia and La Goulette), a moderate concentration decrease was associated with size growth. This seems to be related to the phenomenon of biological dilution (Smaoui-Damak et al., 2009). Indeed, the quantities of metals assimilated from the environment would be attenuated within large specimens.

This negative relationship could reveal faster metabolism in younger individuals compared to older ones (Léopold et al. 2015), a higher tissue growth speed than metal uptake rate (Dragun et al. 2016) or also a better metal bioregulation in adult specimens (Merciai et al. 2014). Dragun et al. (2016) and Jiang et al. (2022), confirmed that negative accumulation/size relationships are generally more frequent for alkaline elements (as Li, Na, and K), whereas positive relationships were more commonly found for transition elements (Mn, Fe, and Co…). Similarly to our results, Balzani et al. (2022) reported a negative relationship metal concentration-size for Cu in *Procambarus clarkii* (Girard, 1852) and Mg in *Ictalurus punctatus* (Rafinesque, 1818) and *Silurus glanis* Linnaeus, 1758 collected from the Arno River (Florence).

However, the same relationship was positive for Fe in *S. glanis*, Hg in *Lepomis gibbosus* (Linnaeus, 1758) and Cr in *I. punctatus* (Balzani et al., 2022). As for Cubadda et al. (2001), they reported positive correlations between metal content and individual size in four gastropods (*Monodonta mutabilis* (R. A. Philippi, 1851); *M. turbinata* (Born, 1778); *Patella caerulea* Linnaeus, 1758 and *P. lusitanica* Gmelin 1791) collected from Favignana Island (Sicily, Italy) with a partial exception in *M. mutabilis* and *M. turbinata* for Zn (negative correlations).

Furthermore, this size-depending metal analysis revealed that Kelibia station was more contaminated by Pb than La Goulette probably linked to the nature of the discharges from fishing activity in the large port of Kelibia characterized by important maritime traffic (Boulajfene et al., 2015).

5. Conclusion

In conclusion, the continued presence of dense populations of *P. turbinatus* and *P. articulatus* in the rocky intertidal of Tunisia would be related to the specific life traits of Trochidae species namely a planktonic larval life, wide larval dispersal on the coasts and adaptation to environmental conditions, accelerated metabolic activity especially in juveniles and increased activity continuous reproductive activity with a spread out laying period. This type of life strategy would allow them to counteract unfavorable environmental conditions (pollution, predation, competition, etc.) and ensure their persistence and would thus justifying their frequent use as bio-indicators of the state of health in coastal ecosystems. Nevertheless, the lack of understanding of metal accumulation patterns and interactions with size and body condition or health of these species, together with the deficiency of management, poses a simultaneous risk to human health and to the already stressed ecosystem.

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