Determination of biometric and somatic parameters of *Rhyssoplax olivacea* (Polyplacophora: Chitonidae) on the Algerian west coast, Mediterranean Sea: Implication for management and conservation

Nacima Mesli^{1,*}, Yassine Guendouzi², Zoheir Bouchikhi-Tani¹

Abstract

A detailed description of *Rhyssoplax olivacea* biometry, sampled over two seasons in 2019 at five sites on the Algerian west coast, was provided for the first time: total and shell length, total and shell width, total animal weight, soft tissue and shell weight. With a total length ranging from 15.09 mm in the cold season to 14.23 mm in the hot season and a total weight varying from 0.45 g in the cold season to 0.42 g in the hot season, the chiton of the Algerian west coast is intermediate between the larger chiton of the western Mediterranean and the smaller one of the eastern regions. The relationship between the parameters studied highlighted the effects of site and season on growth performance. Several somatic indices (i.e. condition index, body shape and body mass indices) were used to assess the chiton's overall health and physiological status, highlighting its adaptability to its environment, as well as the quality, and availability of nutritional resources and its reproductive performance.

Keywords

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Chiton; Rhyssoplax olivacea; Ecophysiology; Condition Index; Body Shape Indices; Body Mass Indices

Received: 23 January 2025; revised: 12 May 2025; accepted: 28 October 2025

1. Introduction

The ecological, economic, and social importance of marine ecosystems is well established (Costanza et al., 1997; Salomidi et al., 2012), accounting for 63% of the total global value of all ecosystem services, with coastal ecosystems providing more than half (Costanza et al., 1997); they are prime areas for economic development, particularly port and industrial activities, urban agglomerations and tourist resorts (Allain et al., 2006; Amara, 2011). They also provide numerous ecological and biological services, such as essential habitats for various organisms, nurseries and areas for purification, storage and treatment of terrigenous inputs (Amara and Paul, 2003; Selleslagh et al., 2009; Amara, 2010).

Nevertheless, this zone is affected by anthropogenic activities (pollution, ocean acidification, fishing, etc.) and

species invasions (Halpern et al., 2007, 2008), exacerbated by climate change (Drobenko, 2010), thus causing a loss of biodiversity, reducing the adaptive capacities of organisms and consequently weakening and destabilizing the biosphere overall (Amara, 2011).

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That applies to the Algerian west coast, where economic development has accelerated in recent decades and which boasts port infrastructures among the most important in the country, vast industrial zones, seaside resorts and galloping urbanisation (Ghodbani and Berrahi-Midoun, 2013; Ghodbani et al., 2016); coastal dynamic disruption and landscape and biodiversity erosion are issues as in other Mediterranean regions (Ghodbani and Bougherira, 2019); Several species are used to assess the level of alteration by measuring specific parameters, notably physiological and morphological parameters (Kaiser, 2001; Markert et al., 2003), such as benthic macrofauna, due to several characteristics, including their diet and sedentary lifestyle (Bakalem and Romano, 1989; Oehlmann and Schulte-

¹ University of Tlemcen Abou Bekr Belkaid, Laboratoire Valorisation des actions de l'Homme pour la protection de l'environnement et application en santé publique (VAHPEASP), Department of Biology, BP 119, 13000 Tlemcen, Algérie

² Laboratory Management and Valorization of Agricultural and Aquatic Ecosystems, Science Institute, University Center of Tipaza Morsli Abdallah, Oued Merzoug, 42200, Tipaza, Algeria

^{*}Correspondence: meslinacima74@gmail.com (N. Mesli)

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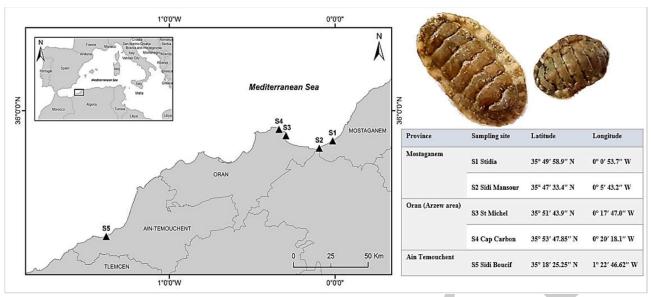


Figure 1. Sampling sites on the Algerian west coast (southwestern Mediterranean Sea): S1: Stidia, S2: Sidi Mansour, S3: St Michel, S4: Cap Carbon, S5: Sidi Boucif.

Oehlmann, 2003; Richir and Gobert, 2014; Guendouzi et al., 2018, 2020; Elias, 2021).

Indeed, besides serving as intermediate links between various trophic levels, marine invertebrates are valuable bioindicators, reflecting the conditions of their environment (Richir and Gobert, 2013; Nieto et al., 2017; Rouane-Hacene et al., 2018). This applies to chitons (Polyplacophora: Chitonida), which are exclusively marine molluscs (Córdoba et al., 2021) and endemic to the Mediterranean Sea (Kaas and Knudsen, 1992; Koukouras and Karachle, 2005) They are characterised by low mobility and living on natural or artificial hard substrates (Gracia et al., 2005), and known for being relatively conservative in their morphological variability (Sigwart et al. 2015), their body structures have been preserved for more than 300 million years, thus, they are considered to be living fossils (Sigwart et al., 2013). No study substantiates that the chiton R. olivacea is at risk of extinction.

Chitons have a shell composed of eight valves encased in a resistant and flexible mantle called the girdle and a large and muscular foot that allows them to attach to hard substrates (Eernisse et al., 2007). The shell of *R. olivacea*, like that of most chitons, is characterized by a combination of eight superimposed hard plates and surrounded by a girdle, which allows a variety of movements (Dell'Angelo, 1982; Del'Angelo and Schwabe, 2010; Schwabe, 2010; Connors et al., 2019), enabling them to move and adapt to irregular surfaces, and thus playing a crucial role in protecting them from desiccation (Connors et al., 2019) and attacks by potential predators (Bruet et al., 2008; Amini and Miserez, 2013; Connors et al., 2012).

Although they are not necessarily edible in the regional culinary culture, they are nonetheless important given their trophic position as intertidal herbivores, thus playing a key role in the protection and management of coastal ecosystems (Crocetta et al., 2014; Liversage and Kotta, 2018). Indeed, they graze by scraping algae from the substrate, thereby intervening in algal succession and distribution, as well as in the process of coastal bioerosion resulting from the abrasion of rocks by the action of scraping (Fernández et al., 2000; Sampedro et al., 2012). They are also a basibiont maintaining the biodiversity of the area by generating substrates for other organisms (Avila-Poveda, 2020).

The chiton is well studied across various topics, i.e. biology (Fischer et al., 1990; Emam and Ismail, 1993; Eernisse, 2008; Schwabe, 2010; Shaw and al., 2009, 2010; Lord, 2012; Lord and Shanks, 2012; Avila-Poveda and Abadia-Chanona, 2013; Sigwart et al., 2015; Connors et al., 2019; Brito et al., 2020; Quintana and Hernández, 2021; Ramirez-Santana et al., 2023; Koc-Bilican and Çakmak, 2024), and ecology (Glynn, 1970; Soliman et al., 1996; Aguilera and Navarrete, 2012; Aguilera et al., 2013; Ramirez-Santana et al., 2019; Hernández-P et al., 2023), including its potential as a good bioindicator of trace metals and a biointegrator of environmental conditions (Mesli et al., 2023).

However, very little knowledge exists on the biometric characteristics and evolution of ecological characteristics of *Rhyssoplax olivacea*.

This study aims to (1) provide for the first time the data on the morphometric parameters (size and weight of body parts) of *R. olivacea*; (2) establish the relationships between the biological parameters; (3) assess the possibility of energetic trade-offs between soft tissue and shell by adopting an ecophysiological approach; (4) identify changes in the somatic state of *R. olivacea* between cold

and hot seasons in the five sites across the Algerian west coast using condition index, body shape and body weight indices.

2. Material and methods

2.1 Study area

The study sites are located all along the Algerian west coast (Figure 1). In the province of Mostaganem two sites were selected: Stidia site (S1), which hosts the Macta wetland, a RAMSAR class site, known for its economic activities (e.g. agriculture, fishing, tourism, urbanisation, etc., DGF, 2005), as well as its growing demography and the contamination of its coastal and ground water (Taleb et al., 2015). At Stidia (site S1), the bedrock substrate was rich, supporting various algal communities, making the site a very favourable environment for the organisms living there.

The second site, Sidi Mansour (S2), is a relatively unspoilt sandy beach bordered by cliffs frequented during the summer season by tourists (Taibi et al., 2016). The substrate is predominantly soft, scattered with a few rocks covered with a thin algal layer, however, the living organisms are sheltered from human activity, particularly during the cold season.

The St Michel (S3) and Cap Carbon (S4) sites are located in the Gulf of Arzew in the province of Oran, where the largest oil and gas industrial platform is situated (Sahnoun et al., 2010). At St Michel (site S3), chitons lived on algae covered rocks. In contrast, at Cap Carbon (S4), food resources were more limited and less readily available, the plant cover being sparse and composed of seasonal algae.

Sidi Boucif (S5), located in the province of Ain Temouchent, boasts several tourist resorts and hotels and is home to one of Algeria's largest cement factories; the region is also known for its large port infrastructure (previously used for the iron ore trade) and its fishing and naval construction activities. The beach of Sidi Boucif is a hotspot in terms of pollution caused by the wastewater discharges from the surrounding areas. Nevertheless, the algae cover was dense and provided shelter for a large community of molluscs.

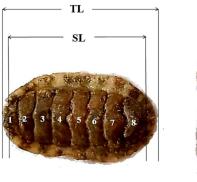
The selection of sites was determined to assess intraspecific morphological variation significantly: reflecting different habitat types, levels of environmental quality, and degrees of anthropic impact.

2.2 Sampling and samples preparation

One hundred specimens of the Mediterranean Polyplacophora $R.\ olivacea$ were collected from the intertidal zone at each site during the cold season (January to March) and hot season (June to September) of 2019. The individuals collected were washed with seawater at the site and immediately transported in an isothermal box at a temperature of $+5^{\circ}C$. In the laboratory, the individuals were left in seawater from their respective sampling sites for 24 to 36 hours.

2.3 Biometric parameters

After depuration (emptying of the gastrointestinal tract), the total individual wet weight was recorded to the nearest 0.01 g. According to Baxter (1982) and Avila-Poveda, (2013), Total Length including the girdle (TL), Shell Length without the girdle (SL), Total Width with the girdle (TWd) and Shell Width at the fourth valve without the girdle (SWd) were measured using a 0.01 mm precision calliper (Figure 2). Each individual was dissected using stainless steel instruments, and the soft tissue (STWg) and shell (SWg) were collected and weighed to the nearest 0.01 g.



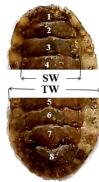


Figure 2. The morphometric measurements of *Rhyssoplax olivacea* considered in the study. The numbers (1–8) indicate the position of the shell valves from the anterior valve to the posterior valve; TL: Total Length [mm]; SL: Shell Length [mm]; TWd: Total Width [mm]; SWd: Shell Width [mm].

Several ratios and coefficient of determination R^2 were calculated using a linear function (Y = aX + b) without data treatments for Weight-Weight and Size-Size relationships (Elleboode and Mahe, 2024), and a logarithmic function $(y = ax^b)$ for Size-Weight relationships (Tokeshi et al. 2000; Elleboode and Mahe, 2024).

2.4 Somatic indices

2.4.1 Condition index

The verification of the physiological state of the organisms, Condition index 'CI' in the case of our study, is required for the biomonitoring of environmental quality (EPA US, 2019). The CI is the ratio between soft tissue wet weight and total wet weight multiplied by $100 \ (n=10 \ \text{in each site})$ for each season) (AFNOR, 1985; Amiard et al., 1998).

This index, which reflects the occupancy rate of the shell by soft tissues (Bodoy et al., 1986), is used to assess seasonal variations in nutrient reserves, tissue quality, and the global condition of the organism (Crosby and Gale, 1990, 2010).

2.4.2 Body shape indices

According to Schwabe (2010) and Avila-Poveda, (2013), the body shape BS (i.e. body outline) of *R. olivacea* is the

ratios between total length and total width and between shell length and shell width:

$$BS_1 = \frac{TL}{TW_d} \tag{1}$$

$$BS_2 = \frac{SL}{SW_d} \tag{2}$$

Standardisations to describe the outline of chitons were proposed by Von Middendorff (1847), and modified by Bergenhayn (1930) to obtain the ratio between the maximum length of the animal and its maximum width, thus illustrating three types of outlines (Schwabe, 2010), from 0 to 1.5 (wide oval and/or short oval), from 1.5 to 3.5 (oval), and >3.5 (elongated oval).

2.4.3 Body mass indices

According to Stevenson and Woods (2006), body mass indices (BMI) or body fat indices were calculated as follows:

$$BM_1 = \frac{TW_g}{TL^2} \tag{3}$$

$$BM_2 = \frac{TW_g}{SL^2} \tag{4}$$

$$BM_3 = \frac{TW_g}{TWd^2} \tag{5}$$

$$BM_4 = \frac{TW_g}{SWd^2} \tag{6}$$

The assessment of body variations is considered a key element in ecophysiological exchanges in chitons (Stevenson and Woods, 2006). This is because it requires less experimentation and presents a non-destructive, easily applicable method for quantifying the state of health of organisms and their interaction with their environment. Furthermore, comparing BMI with other somatic indices could provide a better understanding of corporal changes (i.e. growth and reproduction efforts, etc.).

For this purpose, Avila-Poveda (2013) tested the body mass index in Chiton articulatus for the first time, in order to assess underweight or overweight related to fat accumulation in the gonads, of which lipids are the main component.

2.5 Statistical analysis

Data were expressed as means \pm standard deviation (S.D.) with (n=10), for both measured parameters (morphometric parameters and somatic indices). Analysis of variance

(ANOVA) was used to compare means of the measured parameters (morphometric parameters and somatic indices) among sites (5 levels) and seasons (2 levels), after testing for normality and homoscedasticity. When ANOVA was significant (p < 0.05), a post-hoc comparison of means was made using Fisher's LSD test. Principal component analysis (PCA) was used to define possible correlations between the fourteen variables [TL, SL, TWd, SWd, TWg, STWg, SWg, CI, BSI₍₁₎, BSI₍₂₎, BMI₍₁₎, BMI₍₂₎, BMI₍₃₎, BMI₍₄₎]. Statistical analysis was performed using the software STATISTICA (Statsoft STATISTICA version 10).

3. Results and discussion

The present study aimed to provide the first biometric measurements of the chiton *R. olivacea*, in order to highlight the existence of intraspecific morphological variations. The environmental quality of the sites monitored and the conservation of biodiversity were also assessed.

3.1 Biometric parameters

There are few studies focusing on the biometric measurements of *R. olivacea*, such as weights, total length, and width (Flores-Campaña et al., 2007), which makes our results among the earliest studies on the Algerian coast.

Total wet weight was higher in the cold season compared to the hot season, notably in S2 (Sidi Mansour), S4 (Cap Carbon) and S5 (Sidi Boucif). However, S1 (Stidia) and S3 (St Michel) showed opposite patterns (Table 1), but with a higher maximum value during the cold season in S3 (0.68 g). The ANOVA indicated a highly significant site effect (p < 0.001); while the season effect and the 'site × season' interaction were not significant.

Knowledge of the weight of chitons was not a factor of interest, as this species is not considered an economically potential source for human consumption. However, most biological studies have focused on molluscs and bivalves (e.g., Soto et al., 2000; Zorita et al., 2007; Richir and Gobert, 2014; Rouane-Hacene et al., 2015, 2018; Guendouzi et al., 2018, 2020; Bouiba Yahiaoui et al., 2024).

The proportional relationship between body weight and ecological impact reflects the importance of body size as a significant factor in food webs (Rall et al., 2011; Berg et al., 2011; Mulder et al., 2011; Digel et al., 2011; Webb et al., 2011; Lord and Shanks, 2012). Indeed, several recent studies have underlined chitons among the most linked organisms within intertidal and subtidal food webs, affecting or affected by a non-negligible number of ecologically important species (Kéfi et al., 2015; Pérez-Matus et al., 2017; Brito et al., 2020); according to personal observations, *R. olivacea* shared the ecological niche of several gastropod species and echinoderms, as well as potential predators such as crabs, octopus and others.

The comparison between the weights of the two compartments (i.e., soft tissue and shell) allowed us to indirectly understand the exchange of energy between them in

Table 1. Mean morphometric parameters of Rhyssoplax olivacea from five sampling sites located along the west coast of Algeria (Mediterranean).

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Site	Season	TL [mm]	SL [mm]	TWd [mm]	SWd [mm]	TWg [g]	STWg [g]	SWg [g]
S1	Cold	$16.28 \pm 1.47c$ (14.28–18.16)	$15.03 \pm 1.48c$ (12.53-17.12)	10.47 ± 0.55de (9.55-11.22)	$8.55 \pm 0.44c$ (7.93–9.32)	0.44 ± 0.08 bc (0.30-0.60)	$0.09 \pm 0.03b$ (0.04-0.13)	0.31 ± 0.06 bc $(0.22-0.43)$
(Stidia)	Hot	$15.88 \pm 1.14c$ (13.76–17.13)	$14.84 \pm 1.06c$ (13.21–16.18)	9.82 ± 0.84bd (8.50–11.27)	8.24 ± 0.71bc (7.28-9.40)	$0.53 \pm 0.14c$ (0.34-0.75)	$0.13 \pm 0.05c$ (0.08-0.22)	$0.36 \pm 0.11c$ (0.24-0.51)
S 2	Cold	19.25 ± 1.60d (17.32-21.65)	17.95 ± 1.49e (16.13-19.80)	12.32 ± 1.22 f $(10.39-14.50)$	10.19 ± 1.22d (8.53-12.57)	$0.83 \pm 0.24e$ (0.51-1.25)	$0.19 \pm 0.06d$ (0.14-0.28)	$0.59 \pm 0.21d$ (0.30-0.98)
(Sidi Mansour)	Hot	$17.31 \pm 1.34c$ (14.93–20.19)	$16.07 \pm 1.17c$ (14.09–18.33)	10.96 ± 0.96e (9.72–13.09)	9.54 ± 0.73d (8.33–10.88)	$0.67 \pm 0.21d$ (0.47-1.06)	$0.14 \pm 0.03c$ (0.08-0.17)	0.52 ± 0.16 d (0.36–0.84)
S3	Cold	12.55 ± 2.23 ab $(10.53-17.49)$	$11.24 \pm 1.68a$ (8.87–14.24)	8.47 ± 2.07a (6.16–12.86)	6.96 ± 1.03a (5.69-9.20)	0.32 ± 0.18 ab (0.12-0.68)	$0.06 \pm 0.04a$ (0.01-0.14)	0.23 ± 0.15 ab (0.08–0.54)
(St Michel)	Hot	13.02 ± 1.86 ab (10.47–17.12)	11.91 ± 1.74ab (9.87–15.99)	8.73 ± 0.99ab (7.50–11.25)	7.47 ± 0.63 ab (6.54–8.70)	0.35 ± 0.12 ab $(0.23-0.63)$	$0.05 \pm 0.02a$ (0.03-0.08)	0.26 ± 0.08 abc (0.16–0.47)
S 4	Cold	$13.84 \pm 2.04a$ (12.03-19.03)	$12.94 \pm 1.61b$ (11.22–16.87)	9.06 ± 1.41ab (7.17–12.02)	7.55 ± 0.97 ab $(6.43-9.43)$	0.34 ± 0.16 ab (0.12-0.65)	$0.05 \pm 0.03a$ (0.01-0.10)	0.26 ± 0.13 abc (0.10-0.49)
(Cap Carbon)	Hot	$13.34 \pm 2.09a$ (10.53–17.98	12.37 ± 1.91ab (9.81–16.45)	8.81 ± 1.27ab (6.80–11.39)	7.47 ± 1.25ab (5.81–10.39)	0.31 ± 0.14 ab (0.10-0.61)	$0.05 \pm 0.03a$ (0.01-0.09)	0.23 ± 0.11 ab (0.09–0.47)
S 5	Cold	$13.55 \pm 1.45a$ (11.42–16.39)	12.55 ± 1.45ab (9.99–15.34)	8.13 ± 0.93 ac (6.99–10.21)	7.36 ± 0.87a (6.64–9.34)	0.34 ± 0.14 ab (0.19-0.61)	0.07 ± 0.03 ab $(0.02-0.13)$	0.24 ± 0.09 ab (0.15-0.44)
(Sidi Boucif)	Hot	11.57 ± 2.59b (8.82–17.77)	9.76 ± 2.23d (7.04–14.15)	7.11 ± 1.31c (5.57-9.56)	$5.59 \pm 1.49e$ (3.92–8.25)	$0.24 \pm 0.15a$ (0.10-0.60)	$0.05 \pm 0.03a$ (0.02-0.12)	$0.17 \pm 0.12a$ $(0.07-0.45)$

The results show as mean \pm s.d. (min-max) values with n=10. TL: Total Length; SL: Shell Length; TWd: Total Width; SWd: Shell Width; TWg: Total Weight; STWg: Soft Tissues Weight; SWg: Shell Weight. For each parameter, letters indicate significant differences (Fisher's LSD test, p < 0.05) among sites and seasons.

the growth and reproduction efforts deployed by chitons during the two seasons, especially with the very strong correlation (r=0.98) during the cold season (see Table 6); this confirmed the results established by Mesli et al., (2023) in their study of trace metals bioaccumulation in *R. olivacea* and its impact on the condition index (CI), where concentrations of essential trace metals (e.g., Fe, Cu, and Co) followed opposite trends during the two seasons, in shell and soft tissue which showed a highly significant correlation with CI during the cold season (see Condition Index Section), suggesting that this was the reproductive season.

A highly significant 'site effect' (p < 0.001, ANOVA) was observed for all biometric parameters. A 'seasonal effect' is statistically significant (p < 0.05) for TL, SL, TWd and SWd, and not significant (p < 0.05) for TWg, STWg and SWg. The 'site × season effect' was highly statistically significant (p < 0.001) for STWg, statistically significant (p < 0.05) for SL and SWd, and not significant for TL, TWd, TWg and SWg.

The highest values of all biometric parameters (e.g., TL, SL, TWd, SWd, TWg, STWg and SWg; Table 1) were recorded in S2 (Sidi Mansour) over the two seasons (Table 1), which was considered as the reference site according to the TEPI (trace element pollution index) values published by Mesli et al. (2023). In fact, this site is far from the various anthropogenic discharges. During the cold season, the individuals have the lowest values of TL (12.55 \pm 2.23 mm),

SL $(11.24 \pm 1.68 \text{ mm})$, SWd $(6.96 \pm 1.03 \text{ mm})$ and TWg $(0.32 \pm 0.18 \text{ g})$ at the St Michel site (S3), the lowest values of STWg $(0.05 \pm 0.03 \text{ g})$ are recorded in Cap Carbon site (S4) and TWd $(8.13 \pm 0.93 \text{ g})$ in Sidi Boucif site (S5). In fact, at site S3, which is characterised by the lowest biometric values, the shape and nature of the substrate may be other factors affecting the body shape and large size of these organisms, allowing them to better resist strong waves by adhering strongly to the rocks (Watters, 1991). During the hot season, unlike the STWg values, the biometric parameters have the lower value in site S5 (p < 0.001), which may be due to the status of this site, which was considered a hotspot site in terms of pollution (Mesli et al., 2023).

The first description of *R. olivacea* (common name: *Chiton olivaceus*) was provided by Lorentz Spengler in 1794 (Kaas and Knudsen, 1992), who identified three varieties (Table 2): variety "A" which was sampled along the North African coast and characterized by a mean value of 32 mm of total length (TL) and 16 mm of total width (TWd); variety "B" and variety "C" which were sampled at Mogador (Atlantic coast of Morocco) and characterized by mean values of 22 and 27 mm for TL, respectively; and 12 and 16 mm for TWd, respectively (Table 2). The measurements of the present study were even closer to variety "B". The total length (TL) of *R. olivacea* measured was smaller than that found by Koukouras and Karachle (2005) across the Mediterranean (Tangiers, Morocco) and the Atlantic Eastern coast (Southern Portugal), with 34 mm of TL for males

Table 2. Studies on the morphometric parameters of Rhyssoplax olivacea (common name: Chiton olivaceus) (Spengler, 1797) from different regions of distribution.

Reference	Location	Sampling period	TL [mm]	TWd [mm]	TWg [g]
	Variety "A" The coast of North Africa (Mediterranean)	1797	32	16	-
Kaas and Knudsen (1992)	Variety "B" Mogador, Morocco (Atlantic)		22	12	-
	Variety "C" Mogador, Morocco (Atlantic)		27	16	-
Koukouras and Karachle (2005)	Mediterranean	2004	34	26.7	-
Varkoulis et al. (2023)	Agios Stefanos, the Pagasitikos Gulf, Hellas (central Aegean, Mediter- ranean)	From April 2022 to April 2023	15.06	8.88	0.42
(2020)	Plakes, the Pagasitikos Gulf, Hellas (central Aegean, Mediterranean)		13.45	7.95	0.31
Mygdalias et al. (2024)	The Aegean Sea, (eastern Mediterranean Sea)	Summer 2023 and 2024	13.71 (20.03–14.	40)	-
December 1	Alexander and Other Company	Cold season (January to March) 2019	15.09	9.69	0.45
Present study	Algerian west coast (Mediterranean)	Hot season (June to September) 2019	14.23	9.09	0.42

Morphometric parameters are expressed as mean values. TL: Total Length; TWd: Total Width; TWg: Total Weight.

and 26.5 mm for females (Table 2). However, our results are close to the TL values of *R. corallinus* (Risso, 1826), another Mediterranean endemic species, with 12.5 mm of TL for males and 14 mm for females (Koukouras and Karachle, 2005). On the other hand, the mean length, width and total weight of *R. olivacea* in the present study were higher than those reported by Varkoulis et al. (2023), collected at Plakes (central Aegean, eastern Mediterranean), measuring 13.45 mm TL, 7.95 mm TWd and 0.31 g TWg, but similar to those collected at Agios Stefanos with 15.06 mm TL, 8.88 mm TWd and 0.42 g TWg. The total length (TL) of *R. olivacea* in this study was also close to that described by Mygdalias et al. (2024) in five distinct regions of the Aegean Sea, ranging from 20.03 to 14.40 mm (Table 2).

The local variations in biometric parameters (lengths, widths and weights) of *R. olivacea* could be attributed to the quality and quantity of food available at each site, their diet consisting of algae scraped from the substrate, as well as invertebrates such as sponges, hydroids and bryozoans (Aguilera and Navarrete, 2007), which gives them a key role in the intertidal community, by affecting the succession and distribution of algae and certain invertebrates (Fernández et al., 2000).

The spatiotemporal variation of the following parameters STWg (p < 0.001), SL (p < 0.05) and SWd (p < 0.05), could be due to the reproductive performance and physiological state of the organisms. In fact, dietary consumption would directly fuel the development of the gonads; however, some constituents of the different compartments of reproductive adults can be mobilised in the short term towards the gonads (which are situated in the soft tissues)

Table 3. Results of the analyses of variance (F-test) on morphometric parameters of *Rhyssoplax olivacea* and on somatic indices (CI: condition index; BS: body shape index; BMI: body mass index). The factors are the site, the season and their interaction (Site × Season).

Parameter	F (Site)	F (Season)	F (Site × Season)
TL	35.972**	5.66*	1.68 ^{ns}
SL	46.48**	8.65*	3.63*
TWd	32.26**	6.12*	1.35 ^{ns}
SWd	35.03**	5.41*	3.69*
TWg	28.25**	1.03 ^{ns}	2.02 ^{ns}
STWg	38.53**	1.96 ^{ns}	5.02**
SWg	24.10**	0.56 ^{ns}	1.06 ^{ns}
CI	6.83**	0.21 ^{ns}	0.88 ^{ns}
BSI ₍₁₎	4.74**	0.01 ^{ns}	0.65 ^{ns}
BSI ₍₂₎	4.69**	0.26 ^{ns}	1.04 ^{ns}
$BM\dot{l}_{(1)}$	5.83**	0.91 ^{ns}	1.82 ^{ns}
BMI ₍₂₎	4.61**	2.29 ^{ns}	0.75 ^{ns}
BMI ₍₃₎	8.04**	1.81 ^{ns}	4.05^{*}
BMI ₍₄₎	7.37**	1.93 ^{ns}	2.71*

 $^{\rm ns}$ not significant, *significant (p < 0.05), **highly significant (p < 0.001).

 $BS_{(1)}$: TL/TWd; $BSI_{(2)}$: SL/SWd; $BMI_{(1)}$: TWg/TL²; $BMI_{(2)}$: TWg/SL²; $BMI_{(3)}$: TWg/TWd²; $BMI_{(4)}$: TWg/SWd².

TL: Total Length; TWd: Total Width; SL: Shell Length; SWd: Shell Width; TWg: Total Weight; STWg: Soft Tissues Weight; SWg: Shell Weight.

to meet the nutritional and energy requirements for reproduction (Avila-Poveda, 2013).

Chitons, being ectotherms, do not have to maintain a constant body temperature. Bergmann's rule, which links increased body size to decreased seawater temperatures

and consequently reduced body size in the hotter seawater (Blackburn et al., 1999), could explain the inter-seasonal variations in morphometric parameters (i.e. Lengths and widths, with and without girdle), with slightly higher measurements during the cold season compared to the hot season (Table 1), furthermore, the ANOVA revealed a significant season effect (p < 0.05; Table 3). According to Ibáñez et al. (2021), the body size of several species of chitons from the South-East Pacific increased in cold environments, following Bergmann's rule. This rule was confirmed by Hernández-P et al. (2023), for *Stenoplax limaciformis* with the largest body size are found in the coldest regions.

The relationships between the different biometric parameters showed strong values of determination correlations (Figure 3), with a decrease order as: SWg vs. TWg ($R^2 \geq 97\%$) > SL vs. TL ($R^2 \geq 94\%$) > TWd vs. TL ($R^2 \geq 85\%$) > SWd vs. SL and TWd vs. TWg ($R^2 \geq 84\%$) > TL vs. TWg ($R^2 \geq 82\%$) > STWg vs. TWg ($R^2 \geq 74\%$). A modest correlation was observed for STWg vs. SWg with $R^2 \geq 64\%$ (Figure 3), which may be explained by the dependency of organism growth processes on: (1) exogenous factors, such as environmental factors (Emam and Ismail, 1993), notably the availability and composition of food, and (2) the physiological characteristics and dietary requirements affecting food assimilation (Lora-Vilchis et al., 2004; Rivero-Rodríguez et al., 2007).

3.2 Somatic indices

3.2.1 Condition index

A highly significant 'site effect' on condition index (p < 0.001; ANOVA) was observed (Table 3), while the 'season effect' and the 'site × season effect' on condition index were not significant (p > 0.05).

The 'CI' (condition index) followed the same fluctuations at all sites (Table 4), with the highest values in the cold season, except for Stidia site (S1), where the highest values were calculated during the hot season (24.55). The Sidi Mansour site (S2) was the site with the highest values during the cold season (23.88), and S1 during the hot season (24.59). However, the Cap Carbon site (S4) was the site reporting the lowest values of CI with 14.81 and 14.45 during the cold and hot season, respectively.

The CI values displaying maximum at S2, the reference site according to the trace metals contamination (Mesli et al., 2023). The comparison with reference sites enhances the ecological interpretation of data for management decisions (Silva-Cavalcanti et al., 2018).

The condition index allows predictions of habitat quality, as well as reproductive performance (Stevenson and Woods, 2006). In fact, the site S1 recorded the highest CI values during the hot season, where the domestic discharges are accentuated by summer visitors to this tourist site, the algal diversity of the site could suggest an enrichment of the environment by nutrients. According to

Table 4. Somatic indices of *Rhyssoplax olivacea* during the cold and hot season, sampling along the west coast of Algeria (Mediterranean).

Site	Season	CI	BS ₍₁₎	BS ₍₂₎
C1 (Ct:dia)	Cold	21.15 ± 4.82^{ab} (13.33–27.91)	$1.55 \pm 0.10^{ m abc} \ (1.42 - 1.71)$	1.76 ± 0.16^{ab} (1.57-2.06)
S1 (Stidia)	Hot	24.53 ± 4.95^{b} (12.50–29.33)	$1.62 \pm 0.09^{\text{bcd}}$ (1.45–1.75)	1.81 ± 0.14^{ab} $(1.52-1.98)$
C2 (C:d: Managery)	Cold	23.88 ± 5.17 ^b (16.80–31.37)	$1.57 \pm 0.10^{\text{abcd}} \\ (1.41 - 1.76)$	1.77 ± 0.14^{ab} (1.55–1.99)
S2 (Sidi Mansour)	Hot	20.56 ± 4.54^{ab} (16.04–29.17)	$1.58 \pm 0.09^{\text{abcd}} \\ (1.49 - 1.79)$	$1.69 \pm 0.12^{\text{abcd}} $ $(1.42 - 1.84)$
C2 (Ct Mishall)	Cold	18.03 ± 7.66 ^{acd} (3.45–28.57)	1.51 ± 0.18^{a} $(1.31-1.93)$	1.62 ± 0.14^{cd} (1.41–1.91)
S3 (St Michel)	Hot	$16.36 \pm 4.79^{\text{acd}} $ $(10.26-22.22)$	1.49 ± 0.13^{a} (1.25–1.63)	1.59 ± 0.14^{cd} $(1.35-1.84)$
CA (Care Carrie and)	Cold	14.81 ± 6.35^{cd} (6.25–25.93)	1.53 ± 0.11^{abc} $(1.36-1.70)$	1.72 ± 0.13^{abd} (1.53-1.89)
S4 (Cap Carbon)	Hot	$14.45 \pm 6.39^{\circ}$ (4.55–23.68)	$1.52 \pm 0.08^{ab} $ $(1.37-1.62)$	$1.66 \pm 0.09^{\text{acd}}$ (1.53-1.80)
SE (Sidi Bousif)	Cold	20.56 ± 8.20^{ab} (8.51–37.04)	1.67 ± 0.13^{d} (1.42–1.88)	1.71 ± 0.12^{abcd} (1.45–1.88)
S5 (Sidi Boucif)	Hot	19.76 ± 5.25^{abd} (11.76–30.00)	1.63 ± 0.16^{cd} (1.41–1.86)	1.76 ± 0.16^{ab} (1.58–2.15)

The results are expressed as means \pm standard deviation (SD) (n = 10 for each site in each season), with minimum and maximum values. CI: condition index; BS: body shape index; BS₍₁₎ = TL/TWd; BSI₍₂₎ = SL/SWd. For each index, letters indicate significant differences (Fisher's LSD test, p < 0.05) among sites for each season.

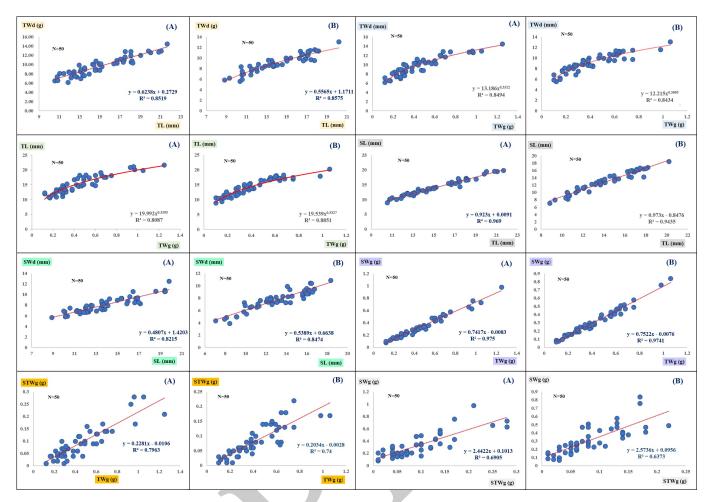


Figure 3. Relationships between the different morphometric parameters of *Rhyssoplax olivacea* collected during the cold and the hot seasons 2019 in the Algerian west coast (Mediterranean). (A: Cold Season; B: Hot Season.)

Hernández-P et al. (2023), larger chitons were observed in areas of high primary productivity. In St. Michel (S3) and Cap Carbon (S4), the populations occurred in an unfavourable environment, as food resources were relatively limited and less readily available, since the vegetation cover was sparse and composed of seasonal algae, consequently affecting CI values.

3.2.2 Body shape indices

The body shape indices (e.g., $BS_1 = TL/TW_d$ and $BS_2 = SL/SW_d$) of *R. olivacea*, have a high significant 'site effect' (p < 0.001: ANOVA, Table 3). While the 'season effect' and the 'site × season effect' were not statistically significant on body shape indices variation (p > 0.05: ANOVA, Table 3).

The BS $_1$ mean values in St. Michel site S3 (1.51 \pm 0.18), Cap Carbon site S4 (1.53 \pm 0.11) and Sidi Boucif site S5 (1.67 \pm 0.13) were higher during the cold season (Table 4). Conversely, Stidia site S1 (1.62 \pm 0.09) and Sidi Mansour site S2 (1.58 \pm 0.09) showed the highest values during the hot season. Regarding the BS $_2$ mean value, the stations S2 (1.77 \pm 0.14), S3 (1.62 \pm 0.14) and S4 (1.72 \pm 0.13) recor-

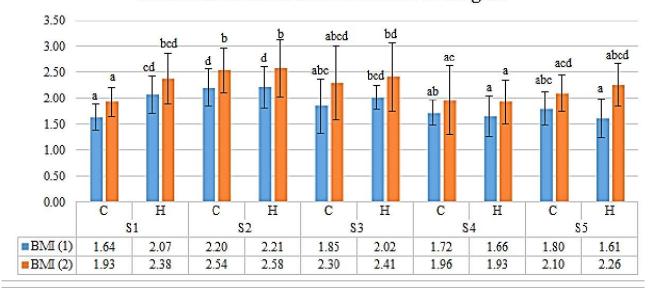
ded the highest values during the cold season. In contrast, S1 (1.81 \pm 0.14) and S5 (1.77 \pm 0.14) registered the highest results during the hot season. The site S3 show the lowest mean values of BS $_1$ (1.51 \pm 0.18 and 1.49 \pm 0.13) and BS $_2$ (1.62 \pm 0.14 and 1.59 \pm 0.14) during the cold and hot seasons, respectively.

Regarding the body shape indices, the *R. olivacea* were predominantly oval during both sampling seasons (BS < 1.5). However, a close relation to a short-oval body shape was noted during the cold season (1.49 < BS $_1$ < 1.56), particularly in S3 and S4 for BS $_1$ (Table 4).

According to the local variation of BS, chitons generally exhibited a variety of oval shapes, with some species being more or less elongated to suit habitat in holes and crevices (Schwabe, 2010); this indicated a strong phenological relationship between intertidal species such as *R. olivacea* and their habitat (Moore et al., 2011; Salloum et al., 2023).

For both BS_1 and BS_2 , the cold season appeared to have been marked by the most oval organisms (Table 4). The variations in shell size and shape could be caused by gonad growth during the reproductive cycle, tending to compress

Seasonal variation between BMI: in lengths



Seasonal variation between BMI: in widths

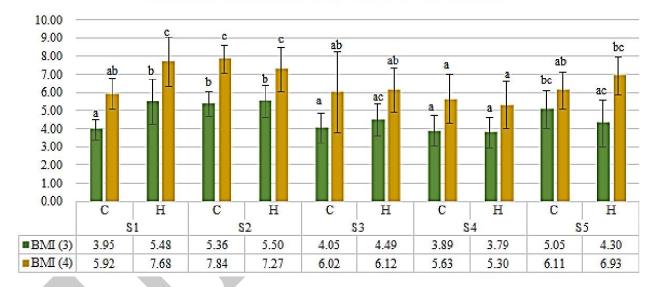


Figure 4. Seasonal and intersite variation of *Rhyssoplax olivacea* BMI (body mass index) calculated as (BMI \times 1000) in lengths and in widths. S1: Stidia; S2: Sidi Mansour; S3: St Michel; S4: Cap Carbon; S5: Sidi Boucif; C: Cold season; H: Hot season; BMI₍₁₎: TWg/TL2; BMI₍₂₎: TWg/SL²; BMI₍₃₎: TWg/TWd²; BMI₍₄₎: TWg/SWd². Bars represent means and SD (n=10). Letters (a–d) indicate significant differences (Fisher's LSD test, p < 0.05) among site for each season, respectively.

the visceral mass on which it sits, thus pushing the valves upwards and making the body shape more oval (Avila-Poveda, 2013; Abadia-Chanona et al., 2016), meaning that changes in reproductive characteristics would be synchronised with changes in growth parameters (Avila-Poveda et al., 2021). In fact, Ecology and reproductive performance are strongly linked by the body size of an organism (Lord and Shanks, 2012), with a significant impact on feeding, growth rate and other ecological aspects (Bonner and Pe-

ters, 1985; Sebens, 2002; Woodward et al., 2005).

According to Baxter and Jones (1986) and Avila-Poveda (2013), a large proportion of the animals were more oval in body shape than the small ones. The occurrence of chitons, including *R. olivacea*, in different habitats points to their high adaptability to environmental conditions (Mesli et al., 2023). According to Otaiza and Santelices (1985), the largest individuals occur in exposed habitats and the smallest in sheltered areas, where they have a better chance to

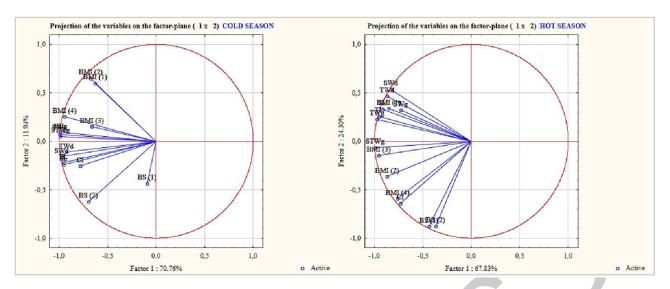


Figure 5. Principal component analysis of biometric parameters and somatic indices of Rhyssoplax olivacea sampled on the west coast of Algeria: plots of the variable loadings for the cold and hot seasons, respectively. TL: Total Length [mm]; SL: Shell Length [mm]; TWd: Total Width [mm]; SWd: Shell Width [mm]; TWg: Total Weight [g]; STWg: Soft Tissue Weight [g]; SWg: Shell Weight [g]; CI: Condition Index; BS: Body Shape indices; BMI: Body Mass Indices.

survive, because the environmental variability is lower (Saier, 2000). However, Horn (1982) stated that "narrow animals are located in the areas most exposed to waves", affirming the existence of a significant correlation between body shape and the intensity of wave action.

Furthermore, variations in food availability and quality between habitats, could significantly affect the morphology and physiology of molluscs (Kovačić et al., 2023).

3.2.3 Body mass indices

A highly significant 'site effect' on body mass indices BMIs variation (p < 0.001: ANOVA; Table 3), a non-significant 'season effect' for all BMIs variation (p > 0.05: ANOVA), and the 'site × season effect' was statistically significant (p < 0.05: ANOVA) for BMI₍₃₎ and BMI₍₄₎.

For all the BMIs calculated (in widths and in lengths), S4 showed the lowest values during the cold and hot season (p < 0.05). In contrast, S2 recorded the highest values in both seasons. The hot season was characterized by the highest values at most sites across the two seasons (p < 0.05).

The BMIs calculated using widths SW_d and TW_d showed higher values than those calculated using lengths SL and TL (Figure 4), thus illustrating more clearly the physical conditions of the organisms and revealed significant differences between sites for each of the two seasons (p < 0.05: Fisher's LSD test).

Local variations in BMI values (p < 0.001) highlighted the food quality and quantity across the sites. The BMI seasonal variations (p > 0.05) could indicate the requirements of organisms in terms of reproductive performance (Avila-Poveda, 2013). Indeed, in Polyplacophora, high re-

productive effort (intensity) is correlated with high body mass, constituted by the gonads (Avila-Poveda et al., 2021), resulting from an accumulation of lipids (Le Moullac et al., 2004).

3.3 Analysis of principal components

Results of the PCA showed that for the cold season, the two first components PC1 and PC2 represented 70.76% and 11.94% of the total variance, respectively (Figure 5). For the hot season, the two first components PC1 and PC2 represented 67.83 % and 24.30 % of the total variance, respectively (Figure 5).

During the two seasons (i.e., cold and hot season), all the parameters measured in *R. olivacea* (lengths, widths and weights) were strongly correlated with each other (Tables 5 and 6), r ranging from 0.8 to 0.99. The CI was positively correlated with:

- 1. STWg with r = 0.83 during the cold season and r=0.77 during the hot season;
- 2. $BMI_{(4)}$ with r = 0.79 during the cold season;
- 3. $BMI_{(2)}$, $BMI_{(3)}$, $BMI_{(4)}$ during the hot season with r = 0.81, 0.84 and 0.97, respectively;
- 4. and $BS_{(1)}$ and $BS_{(2)}$ with r = 0.84 and 0.82, respectively.

STWg showed a strong positive correlation with BMI₍₂₎ and BMI₍₄₎, during the cold season, with r=0.74 and 0.97, respectively; and with all BMIs during the hot season (r=0.80, 0.87, 0.94 and 0.77, respectively). Moreover, BMI₍₄₎

Table 5. Pearson Correlation matrix for variables of Rhyssoplax olivacea analysed during the cold season along the west coast of Algeria (Mediterranean).

	TWd	SWd	TL	SL	TWg	STWg	SWg	CI	$BS_{(1)}$	$BS_{(2)}$	$\mathrm{BMI}_{(1)}$	$\mathrm{BMI}_{(2)}$	$\mathrm{BMI}_{(3)}$	$\mathrm{BMI}_{(4)}$
TWd	1													
SWd	0.98	Н												
II.	0.98	0.99	1											
SL	0.97	0.99	0.99	1										
TWg	0.94	0.97	0.95	0.94	1									
STWg	0.91	0.95	0.94	0.92	66.0	⊣								
SWg	0.94	96.0	0.95	0.94	0.99	0.98	П							
I	0.67	0.75	0.76	0.74	0.76	0.83	0.72	1						
3S ₍₁₎	-0.19	0.01	0.02	0.05	0.01	0.08	-0.02	0.40	1					
$3S_{(2)}$	0.74	0.80	0.82	0.85	0.65	0.62	0.65	0.52	0.28	1				
$BMI_{(1)}$	0.50	0.53	0.47	0.49	99'0	09.0	69.0	0.11	-0.15	0.23	П			
$BMI_{(2)}$	0.50	0.52	0.47	0.44	0.72	0.74	0.71	0.56	-0.07	-0.04	89.0	П		
$BMI_{(3)}$	0.36	0.52	0.49	0.52	0.62	0.63	0.63	0.48	0.59	0.44	0.70	0.57	1	
BMI	0.80	0.85	0.82	0.81	0.95	0.07	0.05	0.70	0.11	7.7	090	0.07	0.72	-

TWd: Total Width [mm]; SWd: Shell Width [mm]; TL: Total Length [mm]; SL: Shell Length [mm]; TWg: Total Weight (g); STWg: Soft Tissue Weight (g); SWg: Shell Weight (g); CI: Condition Index; BS: BS: TL/TWd; BS: TL/TWd; BMI(1); TWg/TL²; BMI(2); TWg/SL²; BMI(3); TWg / TWd²; BMI(4); TWg/SWd².

Table 6. Pearson Correlation matrix for variables of Rhyssoplax olivacea analysed during the hot season along the west coast of Algeria (Mediterranean).

	TWd	SWd	TL	SL	TWg	STWg	SWg	ID	$BS_{(1)}$	$BS_{(2)}$	$\mathrm{BMI}_{(1)}$	$BMI_{(2)}$	$BMI_{(3)}$	$\mathrm{BMI}_{(4)}$
TWd	1													
SWd	66.0	П												
TL	0.97	0.95	П											
ST	66.0	0.97	66.0	1										
TWg	0.95	0.93	66.0	0.97	1									
STWg	0.85	0.81	0.95	0.91	0.95	1								
SWg	0.73	0.73	0.74	69.0	0.79	0.67	1							
CI	0.34	0.27	0.53	0.47	0.56	0.77	0.20	1						
BS (1)	-0.03	-0.10	0.20	0.11	0.23	0.49	0.13	0.84	1					
BS (2)	-0.04	-0.12	0.18	0.12	0.16	0.45	-0.08	0.82	0.95	1				
BMI (1)	98.0	98.0	0.85	0.84	0.90	0.80	0.63	0.48	0.01	-0.07	1			
BMI (2)	0.56	0.50	0.72	0.63	0.77	0.87	0.72	0.81	0.75	0.59	0.57	1		
BMI (3)	0.74	0.70	0.84	0.80	0.89	0.94	0.59	0.84	0.50	0.42	0.87	98.0	П	
BMI (4)	0.35	0.28	0.53	0.45	09.0	0.77	0.36	0.97	0.82	0.72	0.54	0.88	0.88	1

TWd: Total Width [mm]; SWd: Shell Width [mm]; TL: Total Length [mm]; SL: Shell Length [mm]; TWg: Total Weight (g); STWg: Soft Tissue Weight (g); SWg: Shell Weight (g); CI: Condition Index; BS: Body Shape Index; BMI: Body Mass Index; BS₍₁₎; TL/TWd; BS₍₂₎: SL/SWd, BMI₍₁₎; TWg/TL²; BMI₍₂₎; TWg/SL²; BMI₍₃₎; TWg / TWd²; BMI₍₄₎; TWg/SWd².

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was positively correlated with BS1 and BS2 during the hot season, with r = 0.82 and 0.72, respectively.

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Furthermore, the positive correlation between BMI₍₂₎ and BS₁ with r = 0.75, as well as BMI₄ with BS₍₁₎ and $BS_{(2)}$ (r = 0.82 and 0.72, respectively). This is because the bioaccumulation of the essential micronutrients required for many physiological processes, such as reproduction (Ponka et al., 2015), is highest before spawning and lowest after spawning, as nutritional reserves are depleted during gametogenesis (Radenac et al., 1997); indeed, weight variations associated with the reproductive cycle of the organism prevail over seasonality (Bryan, 1973; Boyden, 1974; Cossa, 1980; Amiard et al, 1986; Phillips and Rainbow, 1994; Wang and Fisher, 1997; Mourgaud et al, 2002).

The high significant correlation between the biometric parameters over the two seasons (Tables 5 and 6) revealed their close relationship with environmental quality; in fact, habitat structure and complexity, as well as biological productivity, species diversity and biological interactions impact on biological characteristics (Raffaelli and Hawkins, 1996). In addition, environmental contamination levels influence bioaccumulation and affect the weight of molluscs (Otchere, 2003).

4. Conclusion

The total length (TL) of Rhyssoplax olivacea collected on the Algerian west coast varied between 19.25 and 11.57 mm, the total width (TWd) ranged between 12.32 and 7.11 mm for a total weight (TWg) varying between 0.83 and 0.24 g. All the biometric parameters (TL, SL, TWd, SWd, TWg, STWg and SWg) showed the highest values at the reference site S2 (Sidi Mansour). The study also provides the first compilation of available data on the morphometric parameters of R. olivacea from different regions of the Mediterranean and adjacent Atlantic coasts.

A relationship between body size and seasonal temperatures was identified, following Bergmann's rule. Spatiotemporal variation in biometric parameters and somatic indices appear to reflect environmental and geomorphological conditions, as well as the life cycle of *R. olivacea*.

The study was able to validate non-destructive methods for quantifying the health of chitons and their somatic condition by comparing condition index (CI) with various other indices, e.g., body shape indices (BSI) and body mass indices (BMI). BS is proposed as a non-invasive and more direct alternative approach, measuring in situ the chitons still attached to the hard substrate. BMI is proposed to assess the quality and availability of food in the sites monitored, as well as the reproductive performance of chitons in association with BS and CI, notably because of the strong correlation between these indices. For example, the correlation between CI and $BMI_{(4)}$ was r = 0.79 during the cold season. During the hot season, very high correlations were observed between CI and $BS_{(1)}$, $BS_{(2)}$, $BMI_{(2)}$, $BMI_{(3)}$, and $BMI_{(4)}$ (r = 0.84, 0.82, 0.81, 0.84, and 0.97, respectively).

Acknowledgements

The authors would like to thank Prof. Rouane-Hacene Omar and Dr Ahlem Taleb Ben-Diab from the University of Oran 1, Ahmed Ben Bella (Algeria), for providing help during sample processing.

Supplementary material

Supplementary material associated with this article can be found here.

Conflict of interest

None declared.

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