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Cuttlebone morphometrics and sex identification of *Sepia bertheloti* (d'Orbigny, 1835) from the central-east Atlantic

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Abstract

Analysis of 322 cuttlebones of *Sepia bertheloti* caught in the waters of the Northwest Africa showed significant differences in growth between males and females. Morphometric analysis revealed a relatively different cuttlebone growth pattern between sexes, with males presenting faster growth in length to reach larger sizes and females displaying greater growth in width. This difference in cuttlebone growth is related to female gonadal development and the subsequent increase of the paleal cavity, providing more support and space to accommodate a higher number of oocytes.

Keywords: African cuttlefish, *Sepia bertheloti*, Cuttlebone, Fragmacone

Introduction

The use of morphometric analysis to define basic characters of growth or development of some structures in cephalopods has been applied from the first contributions of Hoyle [1]. These morphometric analyses, called “Traditional morphometry” [2], compare the linear dimensions of different parts of an organism to define statistical relationships with objectives, such as the taxonomic differentiation of species or intra-specific morphometric differences between sexes. These characteristics have been used for the Sepiidae family, mainly for taxonomic differentiation [3], as well as to analyse morphometric differences within the same species [4].

Within the cephalopods context, the Sepiidae family represents a special interest for studies of hard structures due to the presence of the cuttlebone [5]. The cuttlebone is a complex structure composed of calcium carbonate in its aragonite polymorph mixed with a small amount of organic matter and a complex of β -chitin and protein [6, 7] that provides internal skeletal support and buoyancy

to the individual [8]. The cuttlebone shows a structure finely laminated (lamellas). According to Bettencourt and Guerra [9] and Chung and Wang [10], the periodicity of lamellar deposition depends on a combination of physiological and environmental factors (i.e., water temperature).

Sepia bertheloti and *Sepia hierredda* are by-catch species in the bottom trawl fishery targeting *Sepia officinalis* along the coast of the western Sahara [11]; There are no separate statistics of catches/landings for both species because they are caught and marketed together, but *S. bertheloti* represents between 11 and 35% of catches reported for both by-catch species [12]. In addition, probably due to the low commercial interest for *S. bertheloti*, most knowledge on this species has been obtained from the analysis of stomach contents of their natural predators [13, 14] by exploratory fishing to assess the status of the resources [15, 16] or for analyses of metal concentrations [17].

In this context, any contribution to the biology and ecology of *S. bertheloti* would contribute to its conservation and fishery management. The morphologic study of the cuttlebone could provide useful information for the assessment and estimation of mortality, particularly when the individuals are broken and only the cuttlebone

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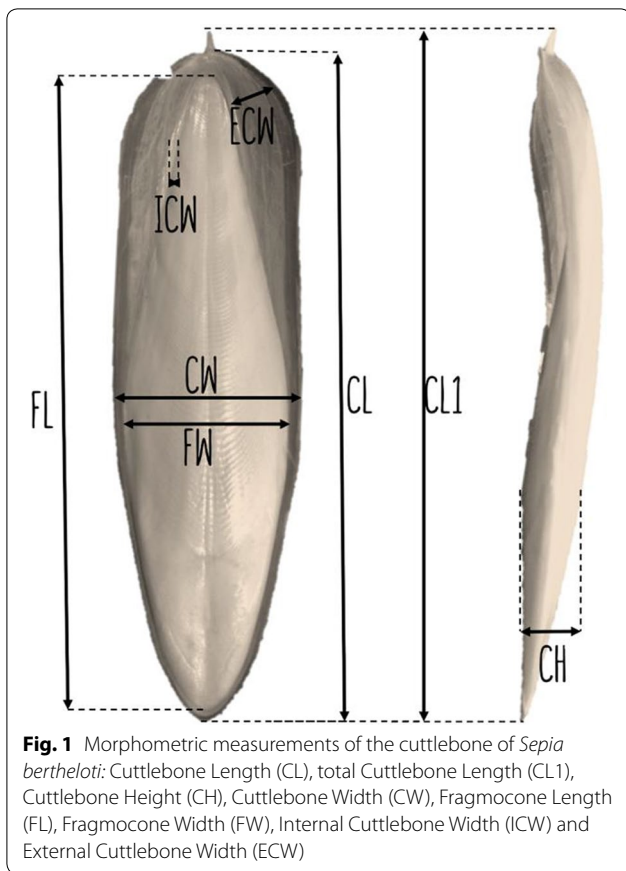


Fig. 1 Morphometric measurements of the cuttlebone of *Sepia bertheloti*: Cuttlebone Length (CL), total Cuttlebone Length (CL1), Cuttlebone Height (CH), Cuttlebone Width (CW), Fragmocone Length (FL), Fragmocone Width (FW), Internal Cuttlebone Width (ICW) and External Cuttlebone Width (ECW)

appear in the captures [4]. Almonacid-Rioseco [18] indicated that in the small-scale trap fishery off the Canary Islands, approximately 50% of *S. officinalis* caught were represented only by cuttlebones and obviously not reported in the landings. This characteristic makes it necessary to study these hard structures to estimate not only the actual catches by the different fishing gears but also the productivity of the area where the diverse fishing sectors operate.

In this context, the objective of this study was to describe the morphometric relationships of the cuttlebone of *S. bertheloti* and detect eventual differences between males and females caught in the northwest African waters.

Material and methods

A total of 322 individuals (247 males and 74 females) of *S. bertheloti* were obtained from commercial catches landed in the port of Tanger (Morocco) and by the bottom trawl fishery in the central-east Atlantic (CECAF area 34.1.11) from July to September 2018. All samples were immediately frozen after being caught. The specimens of *S. bertheloti* were identified following the key proposed by Nesis [19].

After defrosting, the Dorsal Mantle Length (DML), Total wet Weight (TW) and Gonad Weight (GW) were recorded for each specimen. Moreover, each cuttlebone was removed, weighted and measured according

Table 1 Parameters of the morphometric relationships established between the dorsal mantle length (DML) and cuttlebone measurements in male and female *Sepia bertheloti*

Variable	Sex	a	a standard error	b	b standard error	R	F
FL	F	0.139	0.020	0.933	0.033	0.968	1778.5
	M	0.255	0.050	0.796	0.045	0.939	1315.1
FW	F	0.476	0.059	0.939	0.040	0.954	2538.1
	M	3.321	0.186	0.799	0.024	0.931	1063.3
CW	F	0.211	0.036	1.147	0.054	0.946	2452.1
	M	3.814	0.179	0.794	0.020	0.949	1500.2
ECW	F	0.941	0.126	1.094	0.067	0.913	1266.4
	M	0.402	0.280	1.182	0.030	0.951	1543.1
CL	F	0.915	0.028	0.148	0.018	0.977	1056.9
	M	0.192	0.036	0.856	0.043	0.951	3398.6
CL1	F	0.957	0.082	0.119	0.043	0.880	436.9
	M	0.181	0.061	0.863	0.075	0.888	530.9
CH	F	0.212	0.044	1.614	0.098	0.914	2272.1
	M	0.305	0.025	1.378	0.036	0.949	1472.8
TW	F	0.591	0.094	2.001	0.075	0.972	1716.8
	M	0.326	0.049	2.327	0.176	0.939	4191.5

Parameters of the potential equations between morphometric variables of the cuttlebone and the dorsal mantle Length (DML) of *Sepia bertheloti* females

parameters described by Roper and Voss [20]: Cuttlebone Length (CL), Total Cuttlebone Length (CL1), Cuttlebone Height (CH), Cuttlebone Width (CW), Fragmacone Length (FL), Fragmacone Width (FW), Internal Cuttlebone Width (ICW), and External Cuttlebone Width (ECW) (Fig. 1). All length measurements were taken to the nearest millimetre (0.001), and the weights were taken to the nearest centigram (0.01). For each specimen, sex was determined macroscopically, and the maturity stage was assigned based on the scale described by Nigmatullin [21]. Total cuttlebone length (CL1) was not taken in individuals with totally or partially fractured spines due to damages during trawling or handling on board.

To observe differences between the parts of the cuttlebone and the DML, a regression analysis was performed using the linear regression model (Table 1). These functions were performed individually for males (247 individuals) and females (74 individuals). Analysis of covariance (ANCOVA) was performed to detect significant differences between male and female cuttlebones. The first test determined whether the regression slopes for each size class are statistically homogeneous. If the slopes are homogeneous, the second test was performed to verify whether there were differences between sexes in each size class. The mean values of the dependent variables (cuttlebone measurements) for each size class predict differences between sexes. Moreover, the gonadosomatic index ($GSI = GW/TW \times 100$) of the individuals was calculated and plotted against cuttlebone measures to determine eventual differences between sexes. All statistical analyses were performed using R (v 3.4.2) IBM-SPSS Statistics® package.

Results

DML ranged between 63 and 133 mm in males and 67–110 mm in females, whereas TW was 35.5–192.9 g in males and 37.3–103.5 g in females. The morphometric relationships established through regression analyses between DML and cuttlebone measurements in males and females are compiled in Table I. The relationship DML-CL1 displayed the lowest Pearson correlation coefficient (r) for both sexes, which could be due to the low number of specimens without broken spines.

ANCOVA revealed that FW ($F = 124.417$, $p < 0.0001$), CW ($F = 449.185$, $p < 0.0001$), and ECW ($F = 148.228$, $p < 0.0001$) were the morphometric measurements with statistically significant differences between sexes. These differences indicated that cuttlebone width, fragmacone width and external width are proportionally larger in females compared with males for a given length class (Figs. 2, 3a–c). Female cuttlebones also presented greater



Fig. 2 Dorsal and ventral views of the cuttlebone of a mature female (DML = 96.0 mm) and male (DML = 95.3 mm)

weights than male cuttlebones for the same length class (Fig. 3d).

All individuals were mature and showed a high development of the gonads, being ready to spawn. Significant differences were recorded between the GSI of males and females in relation to FW, CW and ECW (Fig. 4b–d).

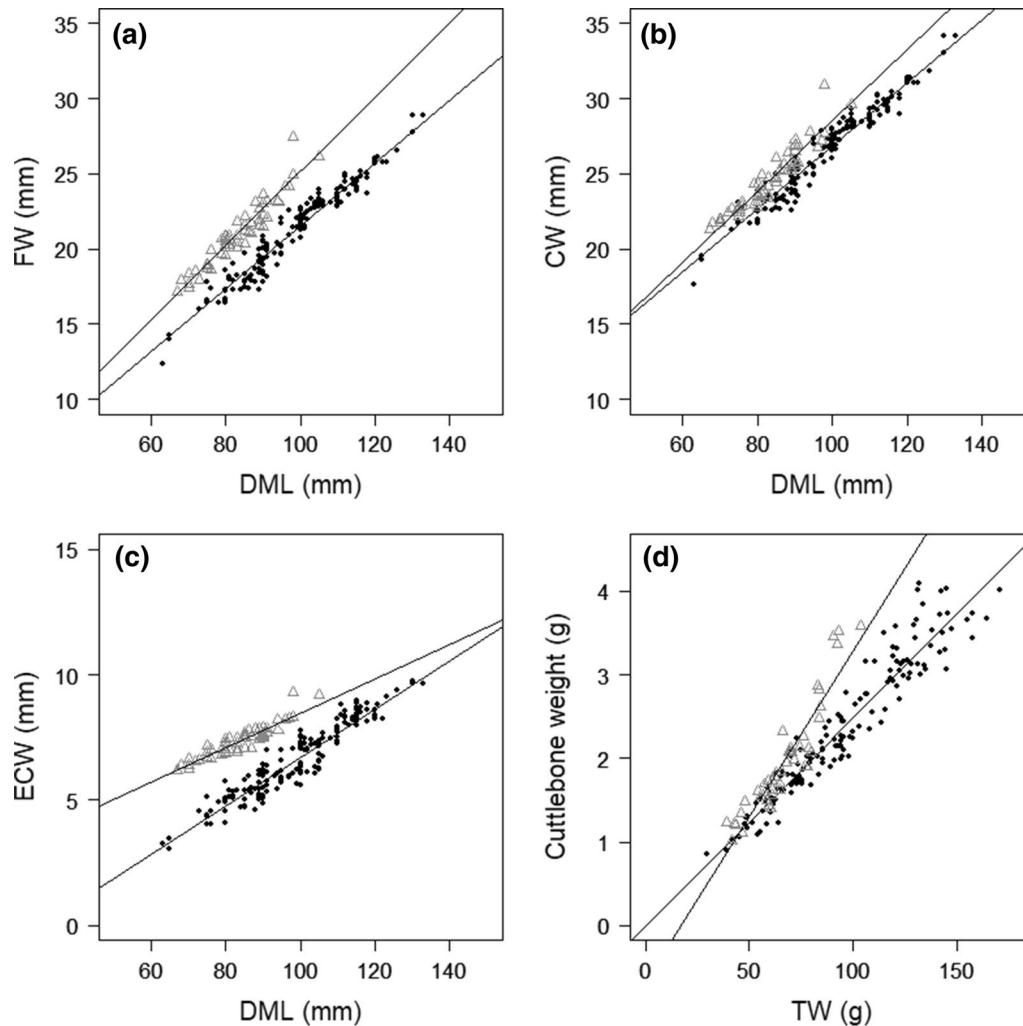
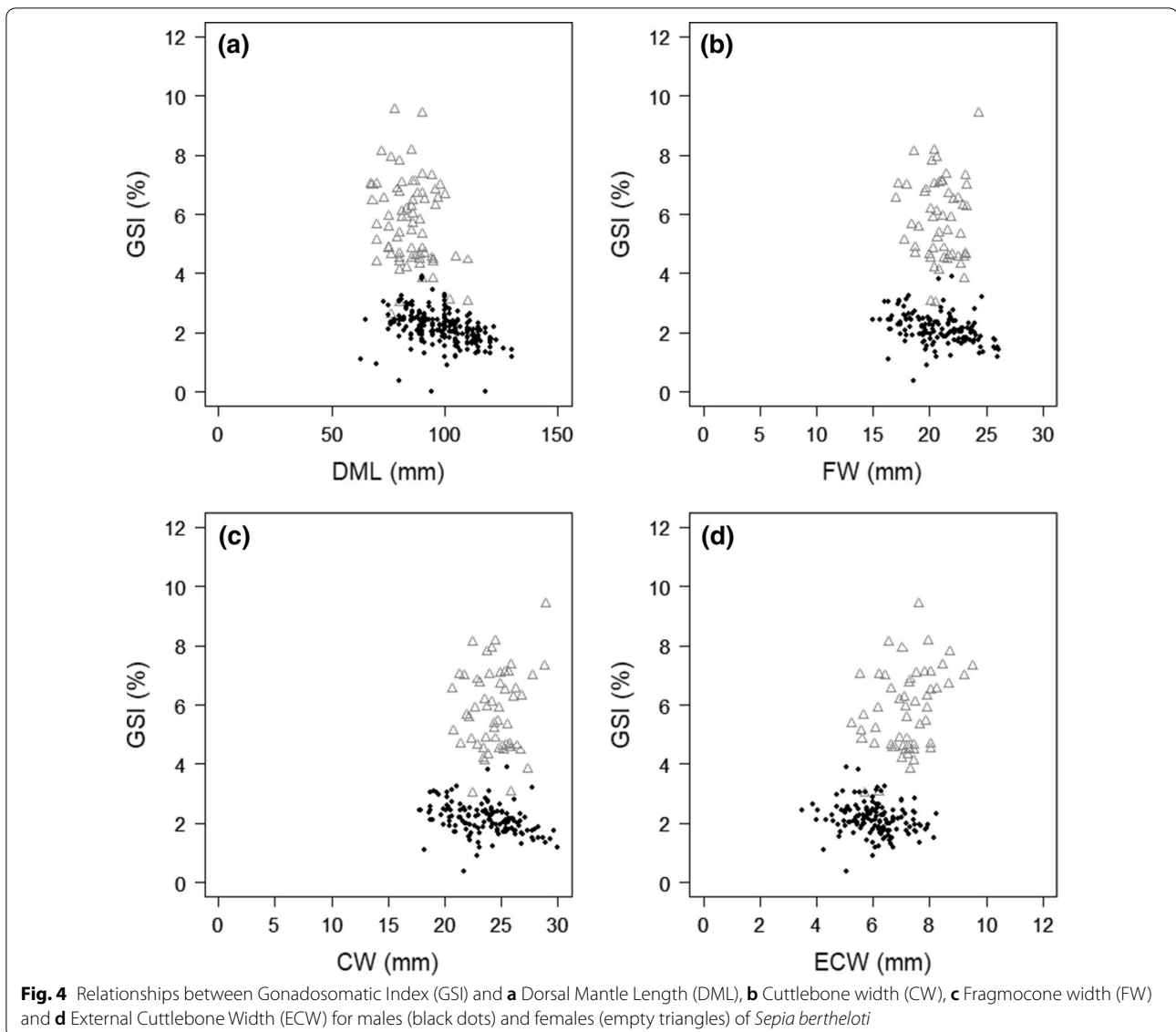


Fig. 3 Morphometric relationships between Dorsal Mantle Length (DML) and **a** Fragmacone Width (FW), **b** Cuttlebone Width (CW), **c** External Cuttlebone Width (ECW) and between **d** Total wet Weight (TW) and Cuttlebone weight (CW) for males (black dots) and females (empty triangles) of *Sepia bertheloti*

Discussion

External sexual dimorphism of *Sepia bertheloti* was mainly based on the fact that males present median arm suckers with greater diameter than marginal suckers and hectocotylus in the left ventral arm. Apart from sexual structures, females internally show the buccal membrane extending ventrally with two spermathecae [12]. However, minimal attention has been paid to cuttlebone as a potential element to differentiate males from females. This study showed that female cuttlebones are significantly wider probably due to the need for a bigger volume of the paleal cavity to increase the capacity to accommodate the gonads. Jereb and Roper [12] remarked that the mantle is relatively wider in females than in males.

Sepia bertheloti males are larger than females [12], which is also noted in *S. latimanus* [9, 22], *S. koilados*, *S. rhoda*, and *S. subplana*. However, in most cuttlefish species (i.e., *S. acuminata*, *S. australis*, *S. bidhaia*, *S. braggi*, *S. elegans*, *S. filibranchia*, *S. grahami*, *S. hedley*, *S. latimanus*, *S. limata*, *S. mestus*, *S. orbignyana*, *S. plana*, *S. senta*, *S. smithi*, *S. sulcata*, *Sepiella inermis*, and *S. weberi*) females growth larger than males [12]. Regarding *S. latimanus*, Dan et al. [22] observed that there were no morphometric differences in the growth pattern of both sexes during the initial life stages, but males become larger than females after 1 year of age. Males and females of *S. bertheloti* caught more northwards than the distributional range given by Jereb and Roper [12] presented a maximum size of 133 and 100 mm DML, respectively,



which is clearly smaller than the maximum sizes reported by Jereb and Roper [12] for the Eastern Atlantic (175 and 134 mm DML, respectively). However, females were usually wider, and this aspect could be associated with the wider morphometric structure of their cuttlebone for a given mantle length.

This differentiation of the cuttlebone between sexes with females with a broader cuttlebone has also been described in other cuttlefish species, such as *S. officinalis* [4], *S. prabahari*, *S. weberi* and *Sepiella ornata* [12]. Therefore, although *S. bertheloti* males and females cannot be differentiated macroscopically (except for the presence of a hectocotylus), they show sexual differences in the morphometric structure of the cuttlebone.

The present results are comparable to those obtained by Almonacid-Rioseco et al. [4] for *S. officinalis*, which also showed a differentiation between sexes for the same measurements of the cuttlebone (FW, CW, ECW). These results reveal that sexes have a relatively different growth pattern. Specifically, males exhibit faster growth in length to reach larger sizes, while females exhibit greater growth in the cuttlebone width in preparation for reproduction (Fig. 3a–c). Vasconcelos et al. [23] also describe this sexual dimorphism for *S. officinalis* in the Algarve coast (southern Portugal). On the other hand, the gonadosomatic index allowed comparison of gonadal development with the cuttlebone measurements that presented differences between sexes, revealing that the faster growth in width of female cuttlebone

is related to an increase in gonadal mass. Accordingly, this relationship can be interpreted as an adaptation of the female body to the gonadal development, which always exhibits increased occupation compared with the male gonad. In this sense, Akyol et al. [24] reported that the total number of oocytes produced by *S. officinalis* varied from 49 to 828 with an average of 261 ± 27 per female, whereas Laptikhovskiy et al. [25] reported a range of large yolk oocytes between 130 and 839. This body adaptation hypothesis was previously proposed by Hewitt and Stait [26] and Sainz [27], who mentioned that these changes in *S. officinalis* were due to phylogenetic features where females adapted to their reproductive stage by developing a wider cuttlebone than males. The volume of oocytes could justify a larger capacity of the female paleal cavity and therefore the adaptation of female cuttlebone to provide the required support and space.

Due to the lack of immature individuals, we are not able to determine whether the differentiation between sexes based on cuttlebone morphometrics is only possible when analysing ripe individuals prior to spawning (i.e., when the cuttlebone morphology adapts to lodge the voluminous gonad), which would decrease the usefulness of this “tool” for gender identification in juveniles stages.

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Authors' contributions

AG-M performed data collection and analysis and interpretation of the results. LC-M performed data collection and analysis. DJ-A performed data collection and interpretation of results. JJC-H performed data analysis and interpretation of the results. The manuscript was written and substantially revised by all the authors, who also approved the version submitted to the journal. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analysed during the current study are not publicly available because data are being used for another research, but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The scientific study was conducted with animals from commercial fishing without any treatment of live animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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