Nova Acta Científica Compostelana (Bioloxía), **25**: 1-8 (2018) - ISSN 2340-0021

Validation of lateral visibility of the ligament as a characteristic for fast discrimination between juveniles of *Cerastoderma edule* and *C. glaucum* (Mollusca, Bivalvia)

Validación de la visibilidad lateral del ligamento como característica para la discriminación rápida de los juveniles de *Cerastoderma edule* y *C. glaucum* (Mollusca, Bivalvia)

José Manuel Parada

Rúa Santiago, 76, Padrón, 15900, A Coruña, Spain

paradajm@gmail.com

(Recibido:12/10/2017; Aceptado 27/11/2017; Publicado on-line: 11/01/2018)

Abstract

Many different methods have been used to differentiate *Cerastoderma edule* (L., 1758) from *C. glaucum* (Bruguière, 1789). However, very few of them are fast and valid for specimens less than 10 – 12 mm length. Although the lateral visibility of the ligament has been described as a discriminating trait, it is seldom used despite the fact that it can be applied immediately. To determine the validity of this characteristic at the juvenile stage, a group of specimens from both species was identified using both this and other methods based on the relationships between different valve sizes. In all of the specimens identified with these methods as *C. glaucum*, the ligament could not be detected in lateral view and vice versa, whereas the contrary is true for *C. edule.*

Key words: *Cerastoderma edule*, *Cerastoderma glaucum*, juvenile identification

Resumen

Se han usado muchos métodos diferentes para diferenciar *Cerastoderma edule* (L., 1758) de *C. glaucum* (Bruguière, 1789). Sin embargo sólo unos pocos son válidos y rápidos para diferenciar especímenes menores de 10 – 12 mm de longitud. Aunque la visibilidad lateral del ligamento se ha descrito como un rasgo diferenciador, rara vez se usa, a pesar de que se puede aplicar inmediatamente. Para determinar la validez de esta característica en la etapa juvenil, se identificó un grupo de especímenes de ambas especies, utilizando éste y otros métodos basados en las relaciones entre diferentes dimensiones de las valvas. En todos los especímenes identificados con estos métodos como *C. glaucum*, el ligamento no se pudo detectar en vista lateral, pero si se detectó, por el contrario, en *C. edule.*

Palabras clave: *Cerastoderma edule*, *Cerastoderma glaucum*, identificación de juveniles

INTRODUCTION

Cerastoderma edule (Linnaeus, 1758) and *Cerastoderma glaucum* (Bruguière, 1789) are two sympatric species that coexist in areas of the Northeast Atlantic. The study of these two species has generated a large number of papers aimed at differentiating them both taxonomically and phylogenetically (Pohlo, 1963 and Freire *et al.*, 2005, among others). By using the direct observation of a set of different characteristics such as the profile and asymmetry of the valves, the thickness of the periostracum and the shape of the junction between the ventral and posterior valve, it is relatively easy to differentiate between adult specimens of the two species (Machado & Costa, 1994). However their morphological traits vary widely and this has been linked to the variability of different environmental variables (Rygg, 1970; Mariani *et al.*, 2002).

Apart from molecular techniques, the different identification methods that use morphological traits were only effective for specimens over 10 or 15 mm in length. Pohlo (1963) proposed a morphometric method that applies for specimens from 2 mm in width, based on the relationship between width and ligament length. Brock (1978) proposed a method to determine the posterior curvature of the shell in the vicinity of the umbo by means of the number of contacts between the shell and a needle (one contact for *C. edule*; two for *C. glaucum*), but this was only applicable to specimens larger than 10 mm in width. This author also suggested using a curvature index based on total length and width (Brock, 1991; in Mariani *et al.*, 2002). Labourg & Lasserre (1980) used the relationship between total length and ligament length. However, in both species, the regressions of these variables overlapped in specimens measuring less than 15 mm length. Otero Llovo *et al.* (1984) reported regressions with significant differences between total valve height and ligament length in both species, but these authors only used specimens greater than 12 mm height. Machado & Costa (1994) arranged the different morphological characteristics in order of discrimination validity. However, the characteristics with the greatest discrimination capacity show allometric growth and prove to be difficult to use with juvenile specimens. In fact, out of all the different morphological characteristics reviewed by these authors, the only canonical function that was found to be capable of distinguishing between specimens measuring less than 10 mm in length was the one related to rib height and width along with the furrow width (see materials and methods for more details).

Recent studies have been carried out on the population structure and biology of *C. glaucum* (Derbali *et al.*, 2012; Kandeel *et al.*, 2013); on the population dynamics and growth of this species (Malham *et al.*, 2012) and on stock assessments prior to the commercial exploitation of bivalves (Parada & Molares, 2013). This kind of studies manage a high number of specimens that require easy-to-use techniques for the identification of juvenile specimens (Magalhães *et al.*, 2016), especially in areas where *C. glaucum* coexist with *C. edule*. Moreover, the recent collapse of the populations of *C. edule* owing to the presence of the parasitic cercozoan *Marteilia cochillia,* which has been recorded recurrently since 2012 in several of the most important shellfish beds of Galicia (Villalba *et al.*, 2014; Iglesias *et al.*, 2015), could favor a greater relative presence of *C. glaucum* in exploitation areas previously dominated by *C. edule*. Thus, a fast and simple method to discriminate between recruits belonging to these two species is essential not only for the efficient processing of the samples used in stock assessments, but also to effectively monitor the possible differential evolution of their populations in relation with changes in environmental and pathological conditions.

While the methods proposed by Pohlo (1963) and Machado & Costa (1994) are valid for use with juvenile specimens, both require subsequent calculations in accordance with the variables. Therefore, their application is difficult and time-consuming in stock assessments, which calls for fast processing of numerous samples involving the immediate identification of a large number of specimens of different species and sizes. This paper reviews the validity of the lateral visibility of the ligament as a characteristic for fast discrimination which can be applied to the different size ranges. This morphological characteristic was highlighted by Boyden & Russell (1972) as the most useful in identifying these species, but it has not been used subsequently, except by Machado & Costa(1994), who relegated it to one of the lowest positions in terms of discrimination capacity, at least for the populations of the coast of Portugal.

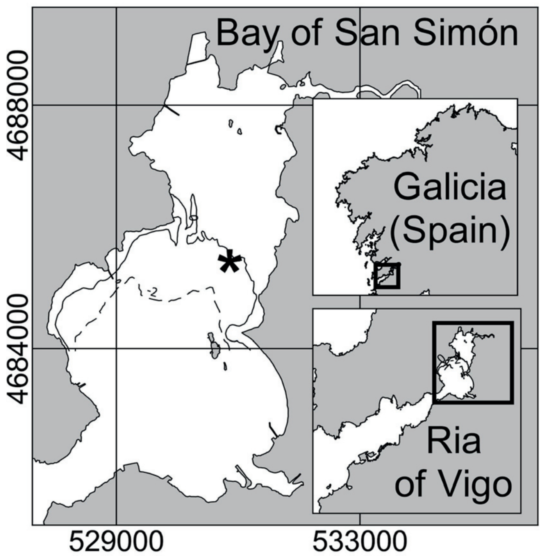

Figure 1. Study area. The asterisk shows the area where the sample was taken. Continuous and dashed lines show isobaths of 0 and -2 m respectively.

Figura. 1. Área de estudio. El asterisco muestra el área donde fue recogida la muestra. Las líneas continua y discontinua muestran las isóbatas de 0 y -2 m, respectivamente.

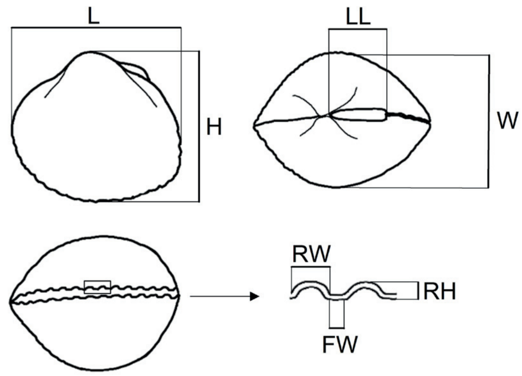

Figure 2. Measured variables. L, total length; H, total height; W, total width; LL, ligament length; RW, rib width; FW, furrow width and RH, rib height.

Figura 2. Variables medidas. L, longitud total; H, altura total; W, ancho total; LL, longitud del ligamento; RW, anchura de la costilla; FW, anchura del surco; RH, altura de la costilla.

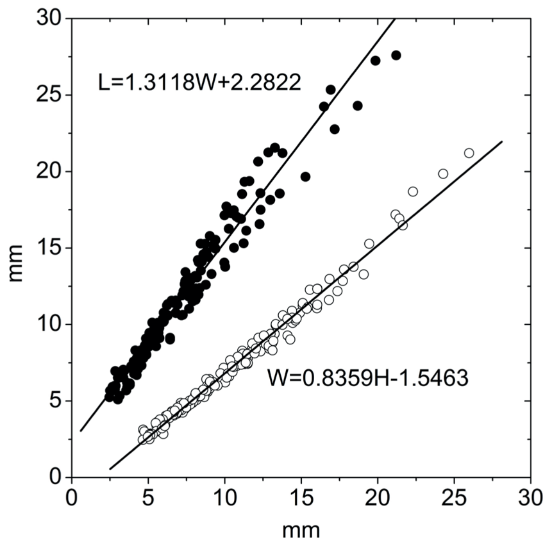

Figure 3. Linear regressions width – height (○) and length – width (●) for all the individuals studied.

Figura 3. Regresiones lineales entre el ancho y la altura (○) y entre la longitud y el ancho (●) para todos los individuos estudiados.

MATERIALS AND METHODS

In July 2015, 176 specimens were collected from the infralittoral zone of the Bay of San Simón (Ria of Vigo) (Fig. 1) after the most important recruitment peak took place, in order to ensure that juvenile specimens would be predominant. The length ranged from 5.1 to 27.6 mm, with 77.3% of the specimens studied measuring less than 15 mm. The total length (L), width (W) and height (H) (Fig. 2) of all the specimens were measured with a digital caliper (± 0.01 mm). Based on the relationships between the three variables (Fig. 3), it was possible to determine that a height of 12 mm (the lower limit for the application of the method of Otero Llovo *et al.*, 1984) corresponded theoretically to a width of 8.48 mm, which is 1.52 mm below the lower limit for the application of the needle method proposed by Brock (1978). Similarly, a width of 2 mm (the lower limit for the application of Pohlo's (1963) method corresponded to a length of 4.9 mm, 0.2 mm less than the minimum size of the specimens studied. Three methods were used to identify the specimens as either *C. edule* or *C. glaucum*: 1) Pohlo (1963) for specimens in the entire studied length range. This method uses the following regression:

$$y = 0.395910 \log(W) - 0.385956 \log(LL)$$

where LL = ligament length (Fig. 2), and W = total width of the specimen. Following this method, we identified the specimens with y less than 0.25992 as *C. edule* and those above this value as *C. lamarcki* (a species associated with the *C. lamarcki*/*C. glaucum* complex;

MACHADO & COSTA, 1994). 2) OTERO LLOVO *et al.* (1984) for specimens greater than 12 mm height. This method uses a regression between specimen height (H) and ligament length (LL) that differs significantly in each of the species:

$$LL_1 = 0.264H_1 + 0.5484$$
$$LL_2 = 0.215H_2 - 1.0171$$

where the subscripts 1 and 2 refer to *C. edule* and *C. glaucum*, respectively. After applying each H and LL value, specimens were identified as *C. edule* or *C. glaucum* depending on how close the values fitted into the equations. 3) MACHADO & COSTA (1994) for specimens less than 10 mm length. This method uses the canonical function:

$$D_s = -0.08+19.15RH' - 20.55RW'+17.92FW'$$

$$RH' = \text{In } RH + 1$$
$$RW' = \text{In } RW + 1$$
$$FW' = \text{In } FW + 1$$

where RH = rib height, RW = rib width and FW = furrow width of the ventral area of the valves (Fig. 2). Values of D_s lower than -0.6 correspond to *C. edule.* The values of LL, RH, RW and FW were measured with a binocular magnifying glass equipped with a micrometer eyepiece (± 0.01 mm).

To determine the lateral visibility of the ligament, each specimen was observed laterally with non-stereoscopic vision to ensure the visualization of only one plane parallel to the longitudinal axis of the specimens (Fig. 4). A 2x power hand-held magnifying glass was used to view the smallest specimens. The needle method of BROCK (1978) was not applied since it is applied within a size range that was already included in the method of OTERO LLOVO *et al*. (1984). Given the relationship between the variability of these species and their geographic distribution (TARNOWSKA, *et al*., 2012) the method of OTERO LLOVO *et al*. (1984) was considered more appropriate than BROCK's (1978) method since it was developed using specimens from Galicia.

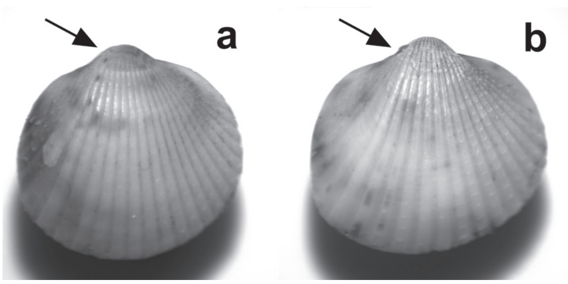

Figure 4. *Cerastoderma glaucum* (a) and *C. edule* (b) of 7.3 and 7.8 mm in length in lateral non stereoscopic view. The arrows show the position of the ligament.
Figura 4. *Cerastoderma glaucum* (a) y *C. edule* (b) de 7.3 y 7.8 mm de longitud en vista lateral no estereoscópica. Las flechas señalan la posición del ligamento.

A comparison between the regressions obtained in this study was carried out using the linear regression comparison method (ZAR, 2010). These regressions were also compared with those reported by other authors using the slope comparison method (FOWLER & COHEN, 1994). According to the priority classification of MACHADO & COSTA (1994), the most effective methods are the ones based on valve profile. Therefore after we had identified the specimens using the methods described above, the curvature index (C) developed by BROCK (1991; in MARIANI *et al*., 2002) was employed to check its validity as a quantitative integrator of the general morphology of the shell for the whole size range. C is defined as:

$$C = W/2L$$

RESULTS

The application of the method proposed by POHLO (1963) resulted in the identification of 39 specimens as *Cerastoderma glaucum* and 137 as *Cerastoderma edule*. In none of those identified as *C. glaucum* was the ligament visible, whereas in 18 of those considered to be *C. edule*, the ligament was not visible in lateral view (Table I). These 18 discordant specimens account for a misclassification of 10.2% of the 176 specimens examined. All the discordant specimens measured less than 7 mm in width (< 11.6 mm in length), except one, which measured 7.3 mm (Fig. 5). Taking into account only specimens wider than 7 mm, the regressions W – LL for each species were significant (Table II). The slope of regression W – LL for *C. glaucum* differed significantly from that of *C. edule* ($t = 35.67$; $p = 5.97E-48$).

Table I. Number of individuals of all sizes, and below and above 7 mm in width identified as *C. edule* or *C. glaucum* by each of the methodologies. Number of individuals misclassified are shown in brackets.
Tabla I. Número de individuos de todas las tallas, y mayores y menores de 7 mm de ancho identificados como *C. edule* o *C. glaucum* con cada una de las metodologías empleadas. El número de individuos identificados en discordancia con el método de la visibilidad lateral del ligamento se muestra entre paréntesis.

Identification method	Identification	All sizes	<7 mm W	>7 mm W
Lateral visibility of the ligament	*C. edule*	119	65	54
	C. glaucum	57	33	24
Pohlo (1963)	*C. edule*	137 (18)	82 (17)	55 (1)
	C. glaucum	39 (-18)	16 (-17)	23 (-1)
Otero Llovo *et al.* (1984)	*C. edule*	119 (0)	65 (0)	54 (0)
	C. glaucum	57 (0)	33 (0)	24 (0)
Machado & Costa (1994)	*C. edule*	119 (0)	65 (0)	54 (0)
	C. glaucum	57 (0)	33 (0)	24 (0)

In none of the specimens identified as *C. glaucum* by means of the H – LL relationship for individuals having H > 12 mm, following Otero Llovo *et al.* (1984), was the ligament visible. By the same token, the ligament was visible in all the specimens considered to be *C. edule* with this method. Total agreement was found between this identification method and the one based on ligament visibility (Table I). When only specimens of H > 12 mm were considered, a significant H – LL regression was obtained for each species (Fig. 6; Table II), resulting in slopes that differed significantly (t = 20.19; p = 2.12E-25).

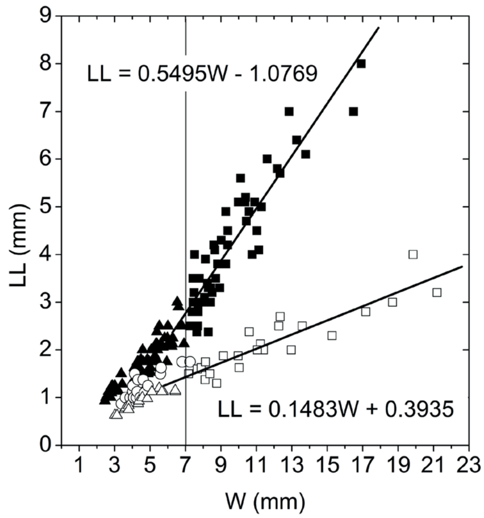

Figure 5. Relationship shell width - length of ligament for specimens identified following Pohlo (1963) and through the lateral visualization of the ligament. Linear regressions are shown for *C. edule* (■) and *C. gl*aucum (□) > 7 mm width, and *C. edule* (▲) and *C. glaucum* (Δ) < 7 mm width. Misidentified *C. edule* (○) are also shown.
Figura 5. Relación entre el ancho total y la longitud del ligamento en los ejemplares identificados siguiendo el método de Pohlo (1963) y mediante la visualización lateral del ligamento. Se muestran las regresiones lineales para *C. edule* (■) y *C. glaucum* (□) de más de 7 mm de ancho, y *C. edule* (▲) y *C. glaucum* (Δ) menores de 7 mm de ancho. También se muestran los ejemplares de *C. edule* discrepantes (○).

In all the specimens identified as *C. glaucum* using the canonical function D_s of Machado & Costa (1994) for specimens less than 10 mm in length by mean of method 3, the ligament could not be seen on the lateral view, whereas it was visible in all those classified as *C. edule* (Fig. 7). Total agreement was found between these two methods (Table I). The

Table II. Regression analysis for individuals identified by means of (1) Pohlo (1963) and (2) Otero Llovo *et al.* (1984) methods. n, number of observations; y, dependent variable; x, independent variable; x range in mm; a (SD), intercept and standard deviation; b (SD), regression coefficient and standard deviation; R^2_{adj}, adjusted coefficient of determination; p, level of significance.
Tabla II. Análisis de regresión entre los individuos identificados con los métodos de (1) Pohlo (1963) y (2) Otero Llovo *et al.* (1984). n, número de observaciones; y, variable dependiente; x, variable independiente; x range, rango de x en mm; a (SD), constante y su desviación estándar; b (SD), pendiente y su desviación estándar; R^2_{adj}, coeficiente de determinación ajustado; p, nivel de significación.

Species	Method	n	y	x	x range	a (SD)	b (SD)	R^2_{adj}	p
C. edule	1	54	LL	W	7 – 17	-1.077 (0.327)	0.55 (0.033)	0.836	< 0.001
C. glaucum	1	23	LL	W	7 – 21	0.394 (0.185)	0.148 (0.015)	0.82	< 0.001
C. edule	2	37	LL	H	12 – 22	-1.827 (0.56)	0.445 (0.038)	0.795	< 0.001
C. glaucum	2	16	LL	H	12 - 26	-1.221 (0.477)	0.232 (0.027)	0.832	< 0.001

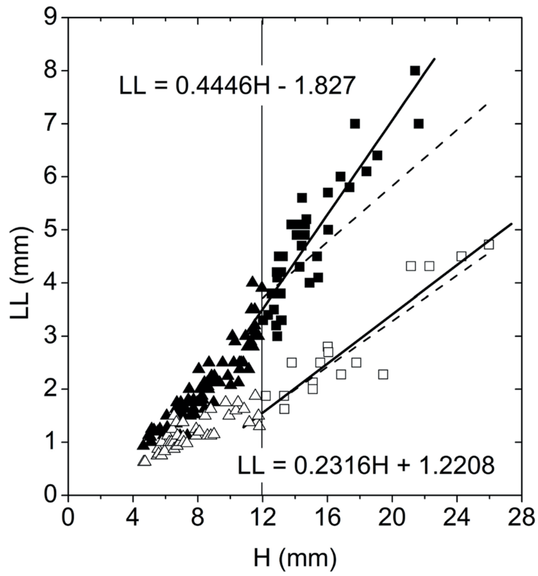

Figure 6. Relationship shell height – length of ligament for specimens identified following OTERO LLOVO *et al.* (1984) and through the lateral visualization of the ligament. Linear regression (continuous lines and equations) are given for *C. edule* (■) and *C. glaucum* (□) > 12 mm height. Dashed lines represent regressions obtained by OTERO LLOVO *et al*. (1984). *C. edule* (▲) and *C. glaucum* (Δ) < 12 mm height are also shown.

Figura 6. Relación entre la altura total y la longitud del ligamento en los ejemplares identificados siguiendo el método de OTERO LLOVO *et al.* (1984) y mediante la visualización lateral del ligamento. Se muestran las regresiones para *C. edule* (■) y *C. glaucum* (□) mayores de 12 mm de altura (ecuaciones y líneas continuas). Las líneas discontinuas representan las regresiones obtenidas por OTERO LLOVO *et al.* (1984). También se muestran los ejemplares de *C. edule* (▲) y *C. glaucum* (Δ) menores de 12 mm de altura.

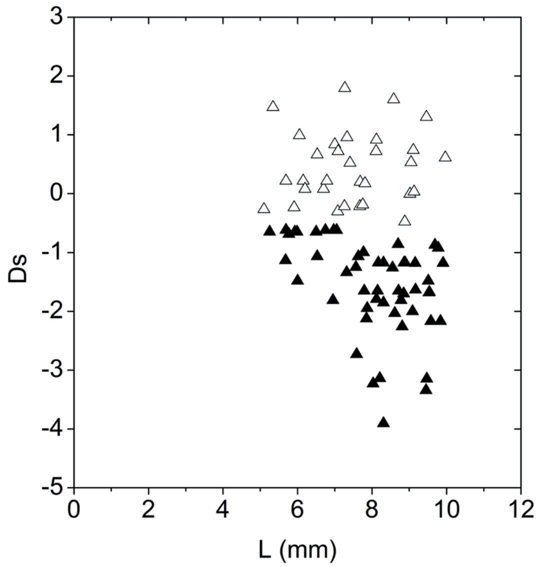

Figure 7. D_s for specimens of *C. edule* (▲) and *C. glaucum* (Δ) < 10 mm length identified following MACHADO & COSTA (1994) and through the lateral visualization of the ligament.

Figura 7. D_s de los ejemplares de *C. edule* (▲) y *C. glaucum* (Δ) menores de 10 mm de longitud identificados siguiendo el método de MACHADO & COSTA (1994) y mediante la visualización lateral del ligamento.

minimum value of D_s for specimens with a non-visible ligament (*C. glaucum*) was -0.476 and the maximum value for specimens with a visible ligament (*C. edule*) was -0.617. Mean D_s values were 0.442 and -1.562 for *C. glaucum* and *C. edule*, respectively and they were significantly different from each other ($t = 12.117$; $p = 6.02E-21$).

Specimens greater than 10 mm in length with a non-visible ligament identified as *C. glaucum* by the methods applied presented an overall curvature index that was higher than those whose ligament was visible (identified as *C. edule*). Specimens measuring less than 10 mm were not clearly separated by the curvature index (Fig. 8) reaching values between 0.26 and 0.36. However, 96% of those less than 10 mm and whose ligament could not be seen in lateral view (also identified as *C. glaucum* according to MACHADO & COSTA (1994)), presented a curvature index greater than 0.29, while 39% of those with visible ligament (also identified as *C. edule* according to POHLO, (1963)) had a curvature index greater than 0.29.

DISCUSSION

Contrary to what was reported by MACHADO & COSTA (1994) for populations of the coast of Portugal, and in keeping with BOYDEN & RUSSELL (1972), the visibility of the ligament on the lateral plane has proven to be an effective tool for distinguishing between specimens of *C. edule* and *C. glaucum* of 5 mm in length or larger in Galicia. Identification based on this method was consistent with the identification resulting from the three other methods used. Only a few discrepancies were found with the method based on the W-LL relationship (POHLO, 1963). However, the method proposed by POHLO (1963), was not efficient for specimens less than 7 mm in

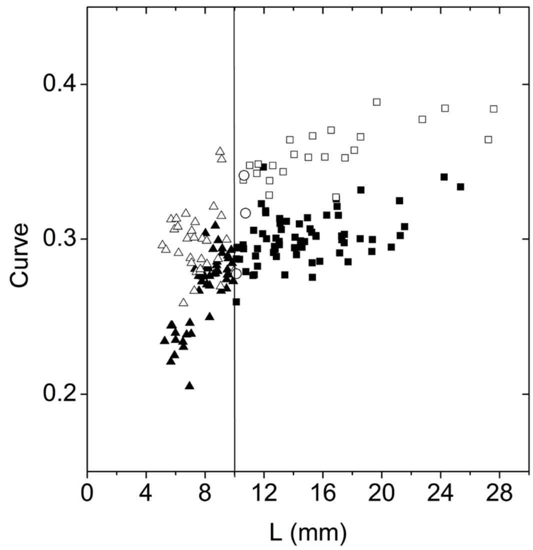

Figure 8. Curvature index of individuals studied. Black symbols represent *C. edule* and white ones *C. glaucum* identified following the four methods. Triangles correspond to individuals less than 10 mm long identified following Machado & Costa (1994) and lateral visibility of the ligament. Circles are the three individuals > 10 mm length misclassified following Pohlo (1963).

Figura 8. Índice de curvatura de los ejemplares estudiados. Los símbolos negros representan a *C. edule* y los blancos a *C. glaucum* identificados mediante los cuatro métodos. Los triángulos se corresponden con individuos menores de 10 mm de longitud identificados mediante el método de Machado & Costa (1994) y la visibilidad lateral del ligamento. Los círculos representan a los tres individuos mayores de 10 mm de longitud con identificación discordante siguiendo el método de Pohlo (1963).

width, unless ligament visibility was taken into account. Pohlo (1963) reported a 10% error in his method, consistent with 10.2% of the discordant specimens in this work, if identification is based solely on ligament visibility. Considering only specimens larger than 7 mm wide, only 3 out of the 176 specimens studied (1.7%) were misclassified. If identification by means of the lateral visibility of the ligament is considered valid, regressions for both species between shell height and ligament length were found to be significantly different. In keeping with Otero Llovo *et al.* (1984) these regressions are only applicable when differentiating specimens greater than 12 mm height. The slope of the regression found for *C. glaucum* did not differ significantly from the one described by these authors (p = 0.567 for the slope difference test). However, the regression found for *C. edule* presented a slope, which was significantly different (p = 0.0015) from the one reported by Otero Llovo *et al.* (1984). Identification based on ligament visibility fully coincided with the identification resulting from the application of the canonical function D_s of Machado & Costa (1994). Coinciding with the findings of these authors, separation by means of this function was unambiguous for specimens measuring 10 mm length or less. Discrimination based on the curvature index (Boyden & Rusell, 1972) was useful, although not infallible, for specimens over 10 mm length.

A combination of function D_s of Machado & Costa (1994) for specimens less than 10 mm with the methods for large-sized specimens proposed by Pohlo (1963) and/or the curvature index of Brock (1991; in Mariani *et al.*, 2002), could be used to differentiate between these species throughout the entire range of sizes. However, these methods are too slow to use in stock assessment tasks in which large numbers of specimens must be processed. In contrast, the visualization of the ligament with non-stereoscope vision in lateral view (Boyden & Rusell, 1972) results in identifications that coincide with the previous methods, and moreover it may be performed at the same time as each specimen is being measured, thus allowing for the quick identification and processing of the samples.

ACKNOWLEGMENTS

The suggestions from the independent referees have improved the final version of this article.

REFERENCES

Boyden, C.R. & Russell, P.J.C. (1972). The distribution and habitat range of the brackish water cockle (*Cardium* (*Cerastoderma*) *glaucum*) in the British Isles. *Journal of Animal Ecology*, 41(3): 719-734.

Brock, V. (1978). Morphological and biochemical criteria for the separation of *Cardium glaucum* (Bruguière) from *Cardium edule* (L.). *Ophelia*, 17(2): 207-214.

Derbali, A., Elhasni, K., Jarboui, O. & Ghorbel, M. (2012). Distribution, abundance and biological parameters of *Cerastoderma glaucum* (Mollusca: Bivalvia) along the Gabes coasts (Tunisia, Central Mediterranean). *Acta Adriatica*, 53(3): 363 – 374.

Fowler, J. & Cohen, L. (1994). Practical statistics for field biology. Wiley, J. & Sons (eds.) 227 pp. Chichester.

Freire, R., Insua, A. & Méndez, J. (2005). *Cerastoderma glaucum* 5S ribosomal DNA: characterization of the repeat unit, divergence with respect to *Cerastoderma edule*, and PCR-RFLPs for the identification of both cockles. *Genome*, 48: 427-442.

Iglesias, D., Villalba, A., No, E., Darriba, S., Mariño, C., Fernández, J. & Carballal, M.J. (2015). Cockle C*erastoderma edule* marteiliosis first detected in Ría de Arousa (Galicia, NW Spain) has spread to other Galician rías causing mass mortality. 17th EAFP International conference on diseases of fish and shellfish. 1: 124.

Kandeel, K.E., Mohammed, S.Z., Mostafa, A.F. & Abd-alla, M.E. (2013). Reproductive biology of the cockle *Cerastoderma glaucum* (Bivalvia:Cardiidae) from Lake Qarun, Egypt. *Egyptian Journal of Aquatic Research*, 39: 249–260.

Labourg, P.J. & Lasserre, G. (1980). Dynamique des populations de *Cerastoderma glaucum* dans une lagune aménagée de la région d'Arcachon. *Marine Biology*, 60: 147-157.

Machado, M.M. & Costa, A.M. (1994). Enzymatic and morphological criteria for distinguishing between *Cardium edule* and *C. glaucum* of the Portuguese coast. *Marine Biology*, 120: 535-544.

Magalhães, L., Freitas, R. & Demontaudouin, X. (2016). Cockle population dynamics: recruitment predicts adult biomass, not the inverse. *Marine Biology*, 163(16). DOI 10.1007/s00227-015-2809-3.

Malham, S.K., Hutchinson, T.H. & Longshaw, M. (2012). A review of the biology of European cockles (*Cerastoderma* spp.). *Journal of the Marine Biological Association of the UK*, 92(7), 1563–1577.

Mariani, S., Piccari, F. & De Matthaeis, E. (2002). Shell morphology in *Cerastoderma* spp. (Bivalvia: Cardiidae) and its significance for adaptation to tidal and non-tidal coastal habitats. *Journal of the Marine Biological Association of the UK*, 82: 483-490.

Otero Llovo, J., De Coo Martin, A., García Fernández, A., López Gómez, M.C., Garrido Mauricio, S. & Lorenzo Miranda, J. (1984). Estudio biométrico de una población de *Cerastoderma edule* y *Cerastoderma glaucum* (Poiret, 1789) en la Ría de Arousa (Galicia). In Monteiro Marques, V. (Ed.) *Proceedings of IV Simposio Iberico de Estudos do Benthos Marinho*: 2: 307-314. Lisboa.

Parada, J.M. & Molares, J. (2013). Artisanal exploitation of natural clam beds: Organization and management tools. *In*: da Costa, (Ed.), *Clam Fisheries and Aquaculture*: 11: 273 – 289. Nova Science Publishers, Inc, Hauppauge NY,

Pohlo, R.H. (1963). Notes on the discrimination between *Cardium edule* L. and *Cardium lamarcki*, Reeve (Lamellibranchia). *Cahiers de Biologie Marine*, 4: 177-182.

Rygg, B. (1970). Studies on *Cerastoderma edule* (L.) and *Cerastoderma glaucum* (Poiret). *Sarsia*, 43: 65-80.

Tarnowska, K., Krakau, M., Jacobsen, S., Wolowicz, M., Féral, J.-P. & Chenuil, A. (2012). Comparative phylogeography of two sister (congeneric) species of cardiid bivalve: Strong influence of habitat, life history and post-glacial history. *Estuarine, Coastal and Shelf Science*, 107: 150-158.

Villalba, A., Iglesias, D., Ramilo, A., Darriba, S., Parada, J.M., No, E., Abollo, E., Molares, J. & Carballal, M.J. (2014). Cockle *Cerastoderma edule* fishery collapse in the Ría de Arousa (Galicia, NW Spain) associated with the protistan parasite *Marteilia cochillia*. *Diseases of Aquatic Organisms*, 109: 55–80.

Zar, J,H, (2010). *Biostatistical analysis*. 5ht Edition, Pearson International Edition, London, 960 pp.