Scientia Marina 80(3)
September 2016, 359-368, Barcelona (Spain)
ISSN-L: 0214-8358
doi: http://dx.doi.org/10.3989/scimar.04312.09A

Gametogenic cycle of the rough cockle *Acanthocardia tuberculata* (Mollusca: Bivalvia) in the M'diq Bay (SW Mediterranean Sea)

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Summary: The reproductive cycle of *Acanthocardia tuberculata* (Linnaeus, 1758) was studied in M'diq Bay. The gonadal development was determined by means of standard histological techniques, mean oocyte diameters and a condition index. Rough cockle is a gonochoric species with synchronous gonadal development and spawning in males and females. The sex ratio obtained was not significantly different to 1:1. Gametogenesis began in late winter (November) coinciding with the temperature drop. In June, with increasing sea water temperature, most of the population was spent and resting oocytes appeared dispersed in the gonad. Resting stage occurred from August to October, during which time sex could not be determined in 100% of the population. *A. tuberculata* showed a clear seasonality in its gametogenic cycle, with one spawning peak per year in June. Quantitative measurements of 6318 oocyte diameters indicated the patterns of development observed in the qualitative staging. The results obtained revealed the direct influence of temperature on the reproductive cycle. First sexual maturity occurred at 42.77 mm shell length. The information gathered in this study allowed preliminary management measures to be suggested for the fishery of this species, including a closed season during the main spawning season (May-June) and the establishment of a minimum landing size (at least 50 mm shell length) for *A. tuberculata* from the Moroccan Mediterranean coast.

Keywords: Cardiidae; reproductive cycle; gametogenesis; image analysis; cockle; first sexual maturity.

Ciclo gametogénico de *Acanthocardia tuberculata* (Mollusca: Bivalvia) en la Bahía de M'diq (mar Mediterráneo)

Resumen: El ciclo reproductivo de *Acanthocardia tuberculata* (Linnaeus, 1758) se estudió en la bahía de M'diq. El desarrollo gonadal se determinó por medio de técnicas histológicas estándar, diámetros medios de ovocitos y un índice de condición. El corruco es una especie gonocórica con el desarrollo gonadal y desove sincrónico en machos y hembras. La proporción de sexos obtenida no fue significativamente diferente a1: 1. La gametogénesis comenzó a finales del invierno (noviembre) coincidiendo con la bajada de temperatura. En junio, con el aumento de la temperatura del agua de mar, la mayoría de la población era ovocitos gastados y dispersos en la gónada. La etapa de reposo entre agosto y octubre, tiempo durante el cual el sexo no se pudo determinar en el 100% de la población. *A. tuberculata* presentó una clara estacionalidad en su ciclo gametogénico, con un pico de desove en junio. Las mediciones de diámetros de 6.318 ovocitos dan los patrones de desarrollo observados desde el punto de vista cualitativo. Los resultados obtenidos revelaron la influencia directa de la temperatura en el ciclo reproductivo. La primera madurez sexual se produjo a los 42.77 mm de longitud de concha. La información obtenida en este estudio permitió sugerir medidas preliminares de gestión para la pesquería de esta especie, incluyendo una temporada de reposo durante el desove principal (mayo-junio) y el establecimiento de una talla mínima de recogida (de al menos 50 mm de longitud de concha) para *A.tuberculata* de la costa mediterránea marroquí.

Palabras clave: Cardiidae; ciclo reproductivo; gametogénesis; análisis de imagen; berberecho; primera madurez sexual.

Citation/Como citar este artículo: Rharrass A., Talbaoui M., Gaspar M., Kabine M., Rharbi N. 2016. Gametogenic cycle of the rough cockle *Acanthocardia tuberculata* (Mollusca: Bivalvia) in the M'diq Bay (SW Mediterranean Sea). Sci. Mar. 80(3): 359-368. doi: http://dx.doi.org/10.3989/scimar.04312.09A

Editor: J. Templado.

Received: July 2, 2015. **Accepted**: April 22, 2016. **Published**: September 23, 2016.

INTRODUCTION

The cockle *Acanthocardia tuberculata* (Linnaeus, 1758) is an Atlantic-Mediterranean species whose habitat ranges in the Atlantic from the southwestern British Isles to Morocco. It lives in fine, clean sand from low tide down to 100 m (Zavodnik and Šimunović 1997, Poppe and Goto−2000). In Morocco *A. tuberculata* mainly occupies the depth ranged between isobaths 5 and 15 m and currently inhabits the shell-sand sediments admixture with gravel to muddy sand (Rharrass et al. 2011). This fishery is located of the northwest coast of Morocco, involves more than five hundred fishermen and is very important for the local economy. This species is mainly exploited in this area by a fleet of 107 boats using the manual Portuguese dredge, which has remained unchanged throughout time. It consists of a small, heavy semi-circular iron structure with a net bag and a toothed lower bar at the mouth. To be efficient, this tough method demands three fishermen at least as crewmembers per vessel. In the north of Morocco, as at another localities (Spain, Portugal, Croatia, Greece), *A. tuberculata* is usually caught for human consumption along with the smooth clam (*Callista chione*). Both species often dominate the benthic macrofauna in M'diq Bay (Rharrass et al. 2011). On the other hand, as reported by Peharda et al. (2012) among others, in the areas of the Adriatic Sea these bivalves are collected by Scuba diving and *A. tuberculata* is not harvested for human consumption, though empty shells and occasional live specimens are collected sporadically for decorative purposes.

Few studies have been conducted on the reproduction of *A. tuberculata* (Marano et al. 1980, Tirado et al. 2002b), and research has mainly focused on the accumulation of biotoxins (Berenguer et al. 1993, Tagmouti et al. 2000, Vale and Sampayo 2002, Sagou et al. 2005, Vale and Taleb 2005, Takati et al. 2007). Some studies have also focused on analysing metal concentrations (Hornung 1989), shell structure (Zolotoybko and Quintana 2002) and gene organization of the mitochondrial genome (Dreyer and Steiner 2006). Distribution data have been provided in several publications (Jukić et al. 1998, Zenetos et al. 2005, Siletić 2006, Rufino et al. 2010). A recent study on the age, growth and population structure of *Acanthocardia tuberculata* was carried out by Peharda et al. (2010) in the eastern Adriatic Sea. In the northwest of Morocco a previous study on the depth segregation of *Acanthocardia tuberculata* revealed a correlation between mean length and depth segregation, in which adults are positioned higher but without exceeding a depth of 15 m (Rharrass et al. 2011). From the work of Boutaib et al. (2011) on the faecal contamination of the red cockle at M'Diq and Oued Laou, shellfish can be sold more safely after purification under heat treatment.

Acanthocardia tuberculata is being intensively exploited on the north coast of Morocco, leading to overexploitation of the natural banks. The lack of suitable management measures such as a closed season in the north of Morocco is due to the absence of biological studies on the reproductive cycle of this species in the area. Hence, the aim of this paper is to provide information on the reproductive cycle of *A. tuberculata* on the west Mediterranean coast of Morocco and to suggest a closed season and the minimum commercial size for a more sustainable management of this fishery. A correct estimate of the size at first maturity is a common tool for adopting the legal minimum size allowed for fishing and was succesfuly suggested by several authors to implement suitable fishery management measures (Moura et al. 2008, Elhassni et al. 2010, Galimany et al. 2015).

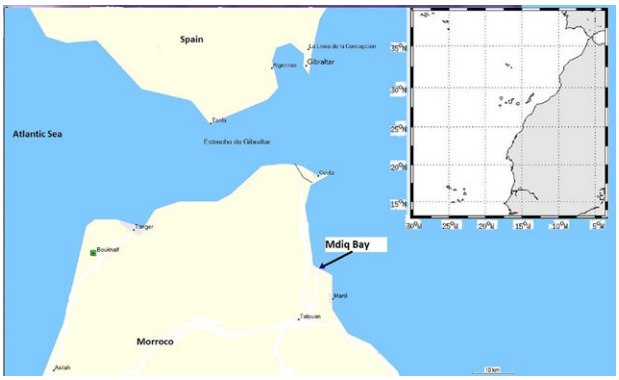

Fig. 1. – Location of M'diq Bay on the northwest coast of Morocco (near Strait of Gibraltar, SW Mediterranean Sea), where *Acanthocardia tuberculata* were collected.

MATERIALS AND METHODS

The rough cockle (*Acanthocardia tuberculata*) was collected monthly on the western Mediterranean coast of Morocco (northwest Morocco, on the western part of its Mediterranean facades in proximity to the Strait of Gibraltar). The sampling operations were carried out for 16 months between November 2009 and April 2011 at inshore sites of M'diq Bay (Fig. 1) representing the highest density distribution of the local *A. tuberculata* population. The samplings were undertaken using a commercial fishing boat with an artisanal dredge. Because of the climatic conditions sampling of two months (December 2009 and September 2010) was missed. The specimens were collected using a dredge with a toothed aperture of 16×18 cm. Additionally, in order to catch smaller individuals, the usual net bag used by the fishermen of the area (6 cm mesh), was replaced by a smaller, stretched mesh size (2.5 cm) (Rharrass et al. 2011).

In order to study the sex ratio, 783 specimens measuring between 32.7 and 70.3 mm in shell length (SL) were analysed. In most cases, the sex was identified macroscopically by the colour and consistency of the gonad, which differs between sexes. Female gonads are whitish with a milky consistency, while male gonads are yellowish-orange with a granular consistency. Whenever the sex could not be identified macroscopically, a smear of the gonad was taken and examined under a microscope (Moura et al. 2008).

Maturation stage of the gonad was determined for 30 individuals sampled each month throughout a 16-month period (from November 2009 to April 2011) from a site off M'diq Bay. Specimens with SL greater than 50 mm

were used, because individuals of this size consistently contain gametogenic material. A sample of the gonad of each of the individuals sampled monthly (≥50 mm) was also prepared for histological examination.

After a 24-h Davidson fixation, in preparing slides for microscopic examination, tissues were dehydrated with serial dilutions of alcohol and embedded in paraffin. Using a microtome, 2- to 3-μm-thick sections were cut to produce very thin sections that were placed on a microscope slide ready for clearing. After clearing, only the tissue remained adhering to the slide. The mounted sections were treated with hematoxylin and eosin histology stain. The final step in this procedure was to permanently mount the sections under a cover slip using Eukitt mounting reagent Roti® Histokitt (Roth).

The stages of gonad development were scored according to the scale proposed by Gaspar and Monteiro (1998) and Moura et al. (2008) as follows: Stage 0, inactive/resting; Stage I, early active; Stage II, late active; Stage III, ripe; Stage IV, partially spawned; and Stage V, spent. The reproductive period was considered as the time when the clams were in Stage IV. When more than one developmental stage occurred simultaneously within a single individual, the decision about the staging criteria was based upon the condition of the majority of the section.

A quantitative analysis was also carried out by measuring the oocyte diameter. Only slides of female gonad are used for this analysis because, according to the comparative analysis, no differences were detected between male and female in terms of either gametogenic development or spawning. The follicle images were recorded with a Sony DKC-CM30 camera and processed using the Zeiss-KS100 image analysis software (release 3.0). From each month, 10 slides where chosen and follicles were randomly selected from each one: the second follicle above, below, on the left and on the right of the follicle initially chosen in the middle, in which all oocytes were counted and measured (Moura et al. 2008). The same procedure was followed for an additional five follicles.

The results of monthly oocyte measurements by image analysis were used to determine the average number of oocytes by individuals in female gonads. Histograms of oocyte size frequency were analysed to track the temporal evolution of oocyte cohorts for each of the sampled females throughout 16 months. The results were converted into curves for better understanding.

Additionally, a total of 480 specimens were used for the analysis of a condition index (CI) for each sample, calculated as mean ash-free dry weight/dry shell weight (Walne and Mann 1975). The length of 30 specimens was measured monthly, and the soft parts were then pulled out of the shell, placed in the drying stove at 105°C for 24 h, and weighed to the nearest milligram. This index was calculated for a standard sample of 60 mm in order to suppress the effect of growth so that accumulation or loss of organic matter associated with reproduction could be shown.

In order to determine the size at first maturity, defined as the SL at which 50% of the population is mature, SL_{50}, an additional sample of 250 specimens (ranging between 23.96 and 65.56 mm SL) was analysed in June 2012. This month was selected because, according to the histological analysis made in the same month of the previous year, all adult specimens were either at the ripe or spawning stages. The gonads of these specimens were monitored using the histological methods described above. The collected individuals were classified as mature and immature individuals. Mature individuals with gonads in stages III and IV were classified by size class at an interval of 10 mm (Moura et al. 2008). The percentage of mature individuals was calculated for each sex and size class, and then the STATISTICA software (Pauly 1984) was used to fit a logistic model to the data, through the following equation:

$$P = 1 / 1 + e^{-r\,(SL - SL_{50})}$$

where P is the proportion of mature individuals, SL the shell length (mm), SL_{50} the shell length (mm) at which 50% of the individuals are mature, and r a constant (Pauly 1984).

To evaluate the possible influence of environmental factors on the gametogenic cycle, seawater samples were taken for determination of temperature and chlorophyll *a* (Chl *a*). The samples were collected in the water column near the bottom (0.3 m above the seabed). A water sample (2 L) was taken from the bottom for determination of Chl *a*. Pigment analyses were carried out by filtering the water through Whatman GF/C glass filters and the pigments of the retained cells were then extracted with acetone for 12 h in cool, dark conditions, following the recommendations of Lorenzen and Jeffrey (1980). Concentrations of Chl *a* were calculated using the monochromatic equations (Lorenzen 1967).

Sex-ratios were checked against a 1:1 ratio with a χ_2 test. Sexual and seasonal variations in histological parameters, CI and oocyte diameter (OD) were analysed by ANOVA. To assess the influence of the environmental factors on the reproductive cycle, correlations between different environmental parameters (temperature and Chl *a*) and the monthly gametogenic variables studied (percentage of spawning, CI and OD) were determined. Assumptions of normality were verified prior to selecting a correlation coefficient (Spearman or Pearson).

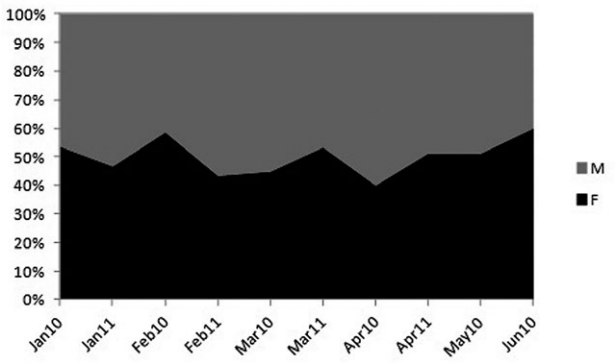

Fig. 2. – Relative frequency (%) of sexes during the study. χ^2-test, P >0.05 (M, male; F, female).

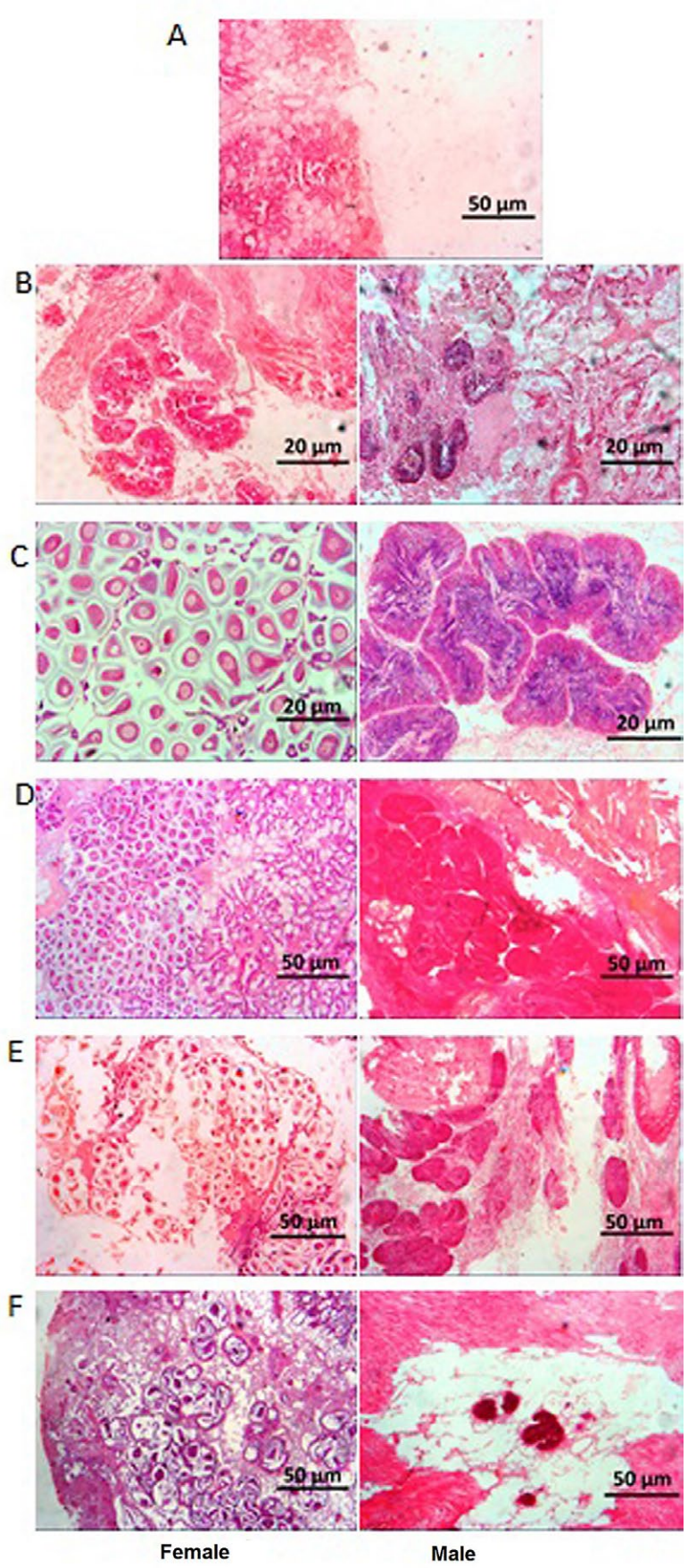

Fig. 3. – Photomicrographs of developmental stages of *Acanthocardia tuberculata* female and male gonads: A, Stage 0, resting; B, Stage I, early active; C, Stage II, late active; D, Stage III, ripe; E, Stage IV, partially spawned; and F, Stage V, spent.

RESULTS

The sex ratio obtained from monthly gonad samples for *A. tuberculata* is given in Figure 2. A month-by-month analysis showed that it was, however, impossible to determine the sex between August and October, so the months with more than two individuals in cytolized stage were not considered for the sex ratio estimation. From the remaining individuals analysed, 49.45% were females and 48.98% males. According to the χ^2 test the sex ratio obtained from the total number of individuals sampled (χ^2=0.57; P>0.05) and from comparisons by months was not significantly different from 1:1.

Histological examinations of the gonads from the 16-month sampling demonstrated that in the reproductive cycle of *A. tuberculata* at the sexes were clearly separated and no simultaneous hermaphrodites were found (Fig. 3). The results indicate synchronous gonadal development and spawning in males and females. No significant differences between the frequencies of the same stages in males and females were found (ANOVA, P>0.05). A synchrony was also detected among individuals. The gonadal cycle (as seen in Figure 4) showed that the phases of the gametogenesis were well delimited.

The smallest specimen of rough cockle identified in gametogenesis phases measured 48.81 mm in length and was a male in early active stage collected in November; the remaining samples varied by up to 68.10 mm in a male. By November (both in 2009 and 2010), the gametogenic cycle had already begun and 20% to 30% of the population was in an early active stage (Fig. 4). However, the remainder of the population still remained in the resting stage (stage 0) with empty follicles, so it was impossible to sex the individuals.

From the first half of January 2010, early active and late active stages of gonadal development (43.4% and 50.0% respectively) coexisted similarly. The gametogenesis was slow until February, when mature gonads were recognized. The ripening stage lasted longer, because sexual maturity began in the first months of spring and 66.7% of the specimen reached full ripeness in April. Spawning was found to start in early summer 2010, with one peak in June. Towards the end of July 2010 about 40% of the population was in the resting stage.

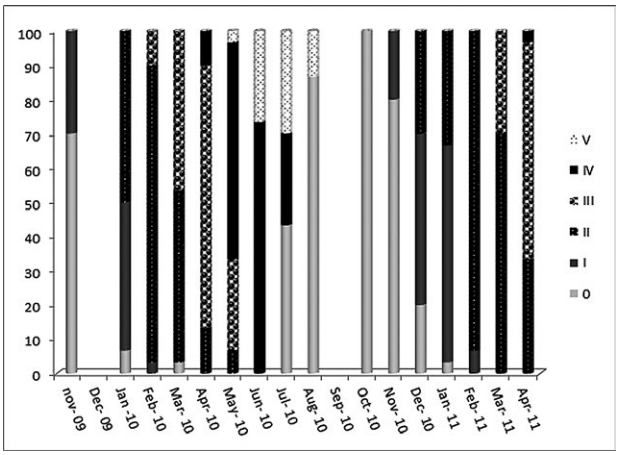

Fig. 4. – Monthly frequency of gonad developmental stages of *Acanthocardia tuberculata*. 0, resting; I, early active; II, late active; III, ripe; IV, partially spawned, and V, spent.

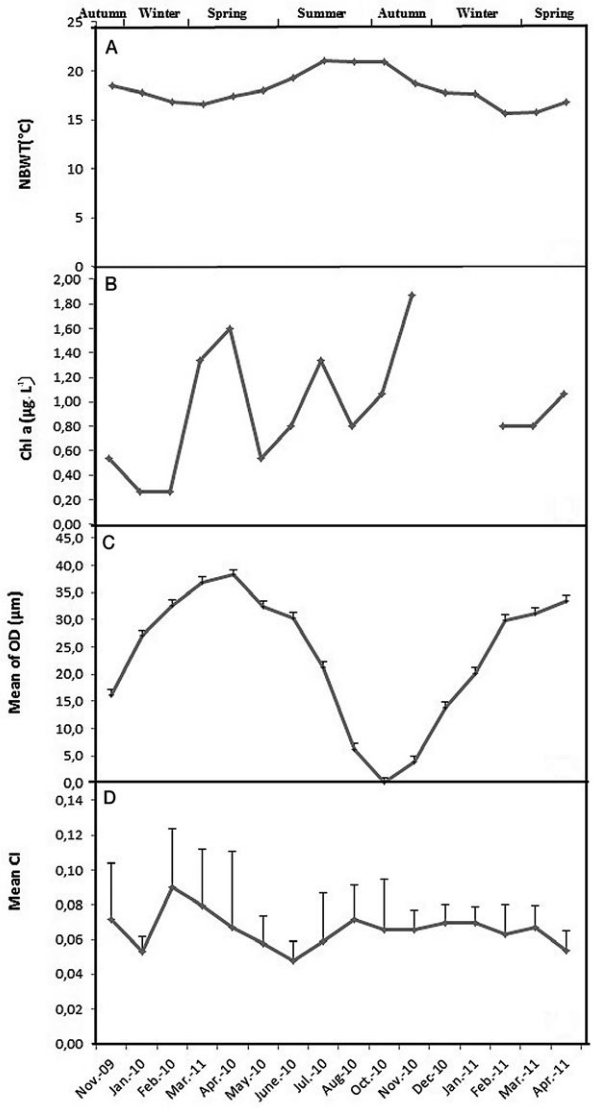

Fig. 5. – Correlations between monthly environmental parameters and gametogenic descriptors for *A. tuberculata* at M'diq Bay (SW Mediterranean Sea): A, near-bottom water temperature; B, changes in concentration of chlorophyll *a* in near-bottom sea water; C, mean oocyte diameter (OD); D, mean condition index (CI).

Between August and October all the individuals examined were found to be in the resting stage, with their gonads completely empty and contracted, and sexes were hard to determine either macroscopically or microscopically. No apparent differences were observed between the sexes in the same months in 2009 and 2011. Figure 5 shows the evolution of near-bottom water temperature (NBWT), OD, Chl *a* and CI during the study period. NBWT registered in M'diq Bay ranged between 15.6°C and 21.1°C, with low and high values in winter and summer, respectively. In general, a decrease in the NBWT was observed between November and April, whereas this environmental parameter began to increase progressively from May to reach its maximum in June, and stabilized during the summer months (June to August). Small variations

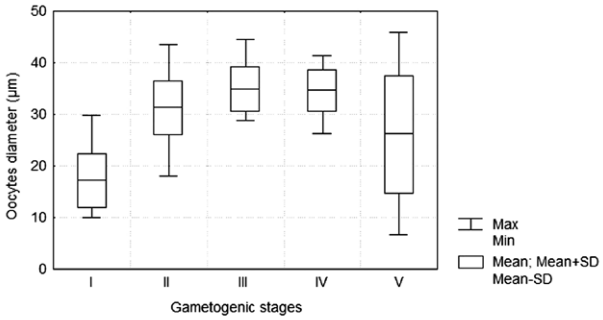

Fig. 6. – Average oocyte diameters relating to each stage of development of *A. tuberculata:* I, early active; II, late active; III, ripe; IV, partially spawned; V, spent.

were observed between consecutive years; in 2011 the mean seabed temperature was slightly lower in winter months. The Chl *a* content (Fig. 5) showed a maximum peak in March, April and November 2010 and a lower peak in July 2010 and April 2011. Between these extremes we observed minimum levels of Chl *a*.

In the main, CI decreased with the ripeness of the gonad and with the onset of the spawning period in both years (Fig. 5). However, this trend was reversed in February (in both 2010 and 2011) and at the beginning of the resting period. Results of correlations between CI and the other descriptors of the reproductive cycle tested (Table 1) showed a significant reverse correlation between CI and spent percentage (r=-0.59; P=0.022; P<0.05).

Quantitative measurements of 6318 oocyte diameters support the patterns observed in the qualitative staging (Fig. 4). The number of oocytes per follicle in November (2009 and 2010) was the lowest of the year, showing the smallest mean oocyte diameter (16.12±3.0 µm) and indicating that spawning had occurred. Due to the ripening of gonads, the monthly mean diameter of oocytes started to increase from January and by April it had reached its maximum value (38.32±3.6 µm). The decrease of mean OD in May corresponded with the onset of the spawning event in some individuals and the oocyte diameter kept decreasing until July. The vanishing of oocytes by August indicates the start of the inactive period, which extended until October. After the resting period, another reproductive cycle started again from November and was noted by an enhancement of OD.

Table 1. – Results of correlations (Spearman coefficient) between the descriptors of the reproductive cycle of *A. tuberculata* in M'diq Bay. The variances of the statistical test and those of the probability are mentioned [NS :(P>0.05); S:(P<0.05)].

CI/OD		CI/% spent		OD/% spent	
r	p	r	p	r	p
0.018	0.95 NS	–0.59	**0.022 S**	0.39	0.145 NS

Average ODs associated with stages of development (Fig. 6) showed that they were related to each other. The early active stage was characterized by a mean OD of 17.1±5.2 µm, which increased during gonadal maturation, attaining 31.2±5.3 and 34.9±4.3 µm in late active and ripe gonads, respectively. In the partially spawned stage the mean oocyte diameter decreased slightly to 34.6±4.1 µm. The mean oocyte diameter continued its decrease in the spent stage (26.0±11.4 µm) but remained high due to the presence of degenerating oocytes.

The frequency distributions of oocyte size in females of *A. tuberculata* displayed a noteworthy monthly oscillation during the study of gonadal activity (Fig. 7). The seasonal variation in the frequen-

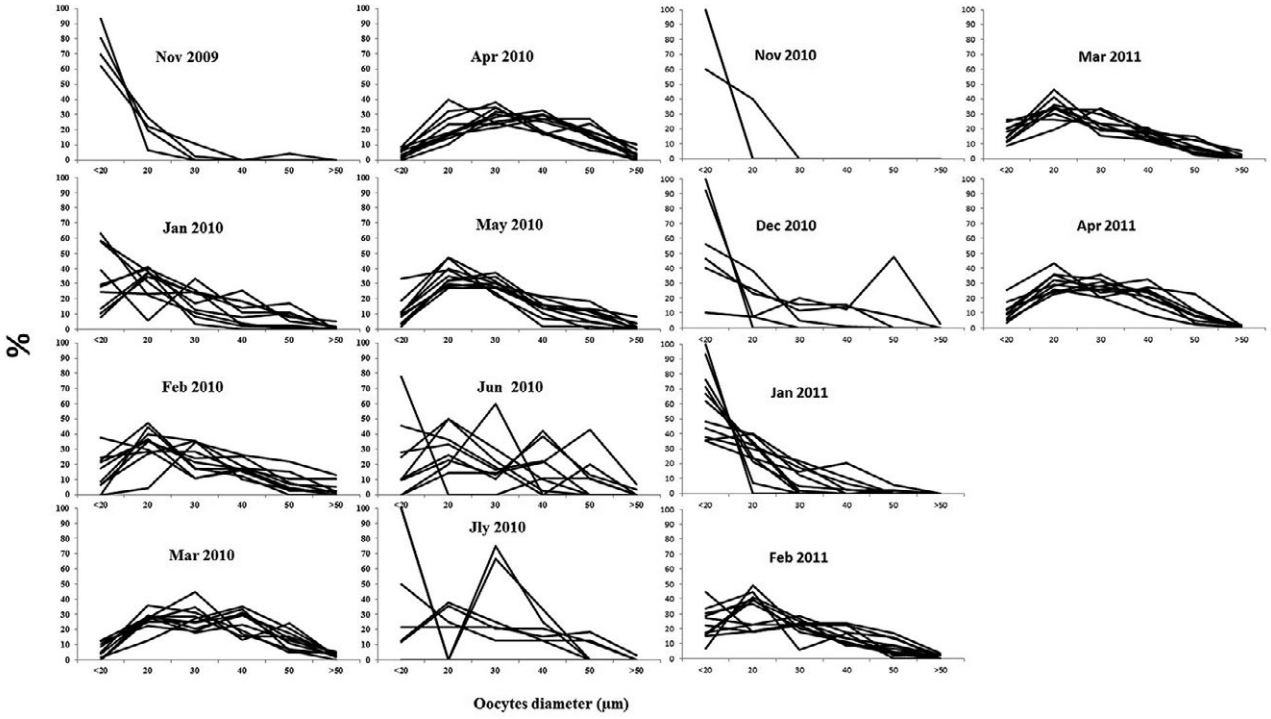

Fig. 7. – Monthly variation in the frequency distributions of oocyte diameter in females of *A. tuberculata.*

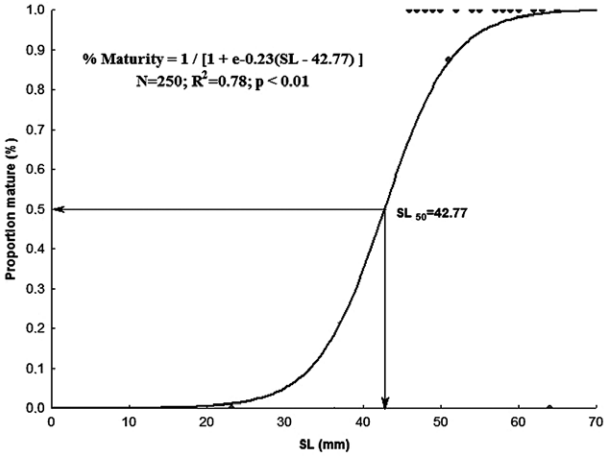

Fig. 8. – Shell length at first maturity for *A. tuberculata*. Proportion of mature individuals in May 2012 in relation to shell length.

cy distributions of oocytes corroborated the previous data and showed five oocyte cohorts corresponding to the different gonadal stages. The seasonal evolution of oocyte cohorts followed a unimodal model, confirming a preparation for a single spawning event. From November (2009 and 2010), a resurgence of oocytes was recognized, and there was a clear dominance of the modal diameter <20 μm, indicating that the reproductive cycle had started. Growth of oocytes continued monthly until April, when it reached the highest values (30≤OD≤50 μm), indicating a ripening period. In May a slight decrease in the frequency of mature oocytes was recorded due to spawning of some individuals. The diameter of oocytes decreased significantly in June, certifying that spawning had occurred completely, but in July the frequencies of mature oocytes underwent a higher decrease. An absence of oocytes between August and October indicated that all females had released their genital products and entered the resting phase.

Spearman correlation coefficients were estimated between the descriptors of the reproductive cycle (OD, CI and % spent) and environmental parameters (temperature and Chl *a*) and were calculated simultaneously. Only the temperature was directly and negatively correlated with OD (Spearman; r=–0.62; P=0.006; P<0.05).

The estimation of the size at sexual maturity (Fig. 8) was based on the microscopic examination of gonads of 250 individuals collected in M'diq Bay on June 2012. This month was selected because, according to the histological analysis made in the same month of the previous year, all adult specimens were either at the ripe or spawning stages, with their sizes ranging between 23.9 and 65.56 mm. In order to determine the size at first maturity in the *A. tuberculata* population, the individuals were grouped into size-classes of 5 mm SL and the relative frequency of ripe and spawning organisms per size class was fitted to a logistic model (r=0.87, Fig. 8). The smallest matured individual found in the population was a male measuring 32.94 mm SL, whereas the largest individual identified was a female measuring 65.56 mm. Even when some individuals reached their first sexual cycle at 30-35 mm, the size at first sexual maturity (SL_{50}) for *A.tuberculata* was estimated at 42.77 mm SL

DISCUSSION

A clear sexual dimorphism was observed during the study, since differences were observed in the colour of the male and female gonads. The sex ratio was not significantly different to 1:1. Contrary to our result, Tirado et al. (2002b) from southern Spain observed no sexual dimorphism in the gonad colour in the population of *A. tuberculata* and also reported that the sexes could not be determined by the colour. No other studies on the reproductive cycle of *A. tuberculata* were found in the literature.

The present investigation of rough cockle gonads suggests only one reproductive event per year from April to July and showed a period of sexual repose from August to November. Both sexes showed a synchronism in gonadal development and spawning. The gonadal development of this species has been previously studied once through histological analysis in the Mediterranean Sea (Tirado et al. 2002b). In this study, populations of *A. tuberculata* from southern Spain exhibited a reproductive event between April and July, and a resting stage between August and December. The existence of a sexual resting period has also been described for other temperate bivalves (*Spisula solida*: Gaspar and Monteiro 1999, Joaquim et al. 2008; *Scrobicularia plana:* Rodríguez-Rúa et al. 2003; *Mesodesma mactroides*: Herrmann et al. 2009).

Monthly mean oocyte diameter has proven to be a useful tool for studying the gonad development of the rough cockle, and has also been used previously in several bivalves (*Argopecten irradians*: Sastry 1979; *Eurhomalea exalbida*: Morriconi et al. 2002; *Callista chione*: Metaxatos 2004, Moura et al. 2008; *Spisula solida*: Joaquim et al. 2008; *Mesodesma mactroides*: Hermann et al. 2009). Likewise, findings from this study revealed a progressive increase in the average OD as reproductive development progresses: 17.1±5.2 μm in early gametogenesis, 38.32±3.6 μm in April, and 26.0±11.4 μm in the spent stage.

The number of spawning events and the duration of the spawning period can vary greatly between species, geographic area, and environmental conditions (Gosling 2003) such as water temperature, salinity and food availability (Sastry 1970, De Villiers 1975, Peredo et al. 1986, Penchaszadeh et al. 2000, Kraeuter and Castagna 2001, Laudien et al. 2001). These parameters are known to directly affect the physiological status of bivalves, regulating gametogenic cycles and spawning, among other physiological processes (Galimany et al. 2015). In agreement with these studies, a strong negative correlation between temperature and seasonal variation of the OD (r=–0.67; P<0,005) was observed in this study. The highest temperature recorded was 21.1°C, coinciding with a period of sexual repose, and the beginning of gametogenesis coincided with a decrease in temperature in November. Spawning in late spring has the advantage of rela-

tively high temperature and phytoplankton concentration in the water column, and therefore larval growth can be fast (Cardoso et al. 2006).

In bivalves, oocyte size depends on the energy available, the reproductive strategy of the species, and its life history, age, location and environmental stressors (Giese 1966, 1969, Honkoop and Van der Meer 1998, Toro et al. 2002, Maloy et al. 2003, Meneghetti et al. 2004). However, several authors have pointed out that the reproductive cycle of suspension feeders is not only influenced by physical parameters such as sea surface temperature (SST), but also by changes in phytoplankton biomass and its species composition (Sastry 1968, 1979, Carreto et al. 1995, Herrmann et al. 2009) reported that the increase in Chl *a* concentration in the Buenos Aires shelf region observed in winter and summer, with a main peak in spring and a secondary peak in autumn, related greatly to the dominance of *M. mactroides* in ripe and spawning stages. This finding suggests that availability and quality of phytoplankton may also have a direct impact on the reproductive cycle. Nonetheless, temperature is considered the main environmental factor that might influence the reproductive cycle of species, inducing gametogenesis and spawning (Gaspar and Monteiro 1998, 1999, Gaspar et al. 1999). This fact supports the idea stated by Ansel (1961) and Seed (1976) that seasonal changes in SST trigger gametogenesis and, in addition, short-term temperature changes may stimulate spawning.

The development of the gonad in bivalve molluscs fits one of two models according to Lammens (1967). In the first, a resting period is observed with the empty gonad during the months of winter, gametogenesis being initiated at the end of winter and/or the beginning of spring. Spawning takes place in the warmer months in response to seasonal changes in Chl *a*, SST, and phytoplankton. In the second, the energy reserves allocated for reproduction are stored during the summer and autumn and are maintained in winter, with spawning taking place between the following spring and summer. The reproductive pattern of *A. tuberculata* in the Mediterranean Sea is similar to that described by Hughes (1971) for *Scrobicularia plana*, and both of them follow the second model, with a clear seasonality described. In the same period and at the same locality (M'diq Bay), Rharrass (2015) recorded continuous spawning throughout the year in *Callista chione*. The differences in the onset of spawning suggest that each species has specific reproductive strategies, but it is known that the size of oocytes can also be affected by age, locality and environmental stress (Honkoop and Van der Meer 1998, Toro et al. 2002, Maloy et al. 2003, Meneghetti et al. 2004, Zvjezdana 2012). The study of the evolution of the average OD of the rough cockle (this study) and the smooth clam (Rharrass 2015) confirms some factors that regulate their metabolism. The comparison of the OD of both species showed that *C. chione* has bigger oocytes (up to 52.7±7.8 μm) than *A. tuberculata* (up to 38.23±3.61μm). Levitan (2006) pointed out that marine invertebrates with bigger eggs require a lower concentration of sperm cells for fertilization. Other studies have observed a potential relationship between the size of the oocyte and the success of the fertilization (Peharda et al. 2010, Zvjezdana 2012). Moreover, although the sexual cycle of bivalves is very dependent on environmental conditions (extrinsic factors), it is also under internal control (intrinsic factors) via hormonal elements (Deridovich and Reunova 1993, Osada et al. 1998, Bacca 2007). In situ, the rough cockle and smooth clam do not feed in the same way and on the same food sources. According to the differences in shape, the length of siphon and the burying depth capacity (Zwartz and Wanink 1989) *A. tuberculata* may act as suspension-and deposit-feeder, while *C. chione* is exclusively a suspension feeder; consequently they do not extract the same nutritional value. Indeed, even if both species share the same biotope, each single gender could have its own strategies for managing its energy, either via the food supply, or via the use of its reserves.

It has been noted that the CI is a good predictor of the gametogenic cycle, even in bivalves with a gonad not easily separable from the foot, such as *Donax trunculus* (Gaspar and Monteiro 1999), *Donax serra* (Laudien et al. 2001) and *Mesodesma donacium* (Riascos et al. 2008). However, for the present study, the CI was not useful for describing the spawning of the rough cockle. As mentioned by Tirado et al. (2002a), CI should not be interpreted without an accompanying histological study because this index may decrease without the release of gametes. Food supply maintained a direct relationship with the rate of gonadal development and with the total quantity of gonad generated (Delgado and Pérez-Camacho 2005). In general, during the period of sexual repose, it was observed that individuals showed an increase in their mean weight due to resorption of the gonad and formation of reserve tissue (Rodríguez-Rúa et al. 2003), but food supply restriction can limit gonadal recovery after spawning episodes (Delgado and Pérez-Camacho 2005). It is interesting to note that species of the genus *Cerastoderma* and *A. tuberculata* showed their fastest shell growth rate immediately prior to gamete release, indicating their ability to gain enough energy for both growth and gametogenesis when food resources are adequate (Peharda et al. 2012).

In the present study, the smallest matured rough cockle was an individual at 32.94 mm SL, which is in agreement with the finding of Tirado et al. (2002b) in southern Spain where, using histological analysis, they found that all individual of *A. tuberculata* larger than 30 mm had differentiated gonads. In the present study even when some individuals reached their first sexual cycle at 30-35 mm, the SL_{50} for *A. tuberculata* was estimated at 42.77 mm SL.

Determination of the size at first sexual maturity and the spawning period are the basic requirements for the protection and sustainable exploitation of stocks. Data on the reproductive characteristics obtained in this study show that *A. tuberculata* in the westernmost Mediterranean has one spawning peak per year, which occurs during the summer months, and that female and male gonad developments are synchronous. These results give an insight into the biology of *A. tubercu-*

lata. The information gathered in this study allowed preliminary management measures to be proposed for the fishery of this resource, including a closed season during the main spawning season (May-June) and the establishment of a minimum landing size (of at least 50 mm SL) for *A. tuberculata* from M'diq Bay.

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