Observations on the ecology and reproductive biology of the sipunculan worm *Aspidosiphon muelleri* in temperate waters

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A population of the sipunculan worm Aspidosiphon muelleri, *located in temperate waters of the western Mediterranean Sea, was monitored monthly for a year. Some aspects related to its ecology and reproductive biology are shown in the present work. The sex-ratio for this population was close to 1:1 (54% females vs 46% males), thus indicating a dioecious reproduction, although showing a lack of sexual dimorphism. Oocytes were detected in females from 4–5 mm; this size might be reached by* A. muelleri *in a few months. The population density increased notably during the summer (June–August). The spawning event date was indirectly estimated from the average size of the oocytes and the percentage of females with free oocytes in the coelom. Both variables were significantly correlated to shallow water temperature (Pearson correlation; P = 0.003 and P = 0.001, respectively). Oocyte size was also significantly correlated to average irradiance level (Pearson correlation; P = 0.044). Spawning took place between August and September (when the water temperature is getting close to its annual maximum) and may last only a few weeks in these temperate waters. The abundance of* A. muelleri *decreased drastically in September, likely as a consequence of the spawning event effort. Bivalves of the species* Epilepton clarkiae *were collected together with specimens of* A. muelleri *living inside the polychaete tubes used as shelters by the sipunculans, with a prevalence of 11.64%. To a lesser extent some Foraminifera were also reported attached to the body of the sipunculans.*

Keywords: sipuncula, reproduction, Mediterranean Sea, gametocytes, *Aspidosiphon muelleri*, *Epilepton clarkiae*

INTRODUCTION

Aspidosiphon muelleri Diesing, 1851 is the most widespread species of the family Aspidosiphonidae and presents a cosmopolitan distribution in temperate to subtropical waters. *Aspidosiphon muelleri* is also the most eurytopic member of the genus and lives in a wide variety of temperatures and depths, being more abundant in littoral waters (Murina, 1975; Cutler, 1994). In the Mediterranean Sea, it is the predominant littoral sipunculan species in the western basin, and it is common in both soft and hard substrata (Saiz-Salinas, 1993; Papazacharias *et al.*, 1998; Pancucci-Papadopoulou *et al.*, 1999; Açik *et al.*, 2005; Açik, 2008, 2011; Ferrero-Vicente *et al.*, 2011, 2013a, b).

Aspidosiphon muelleri has been reported from many different habitats and associations within the Mediterranean Sea; associated with the algae *Padina* sp. and *Cystoseira crinite* Duby, 1830, associated with sponges (Koukouras *et al.*, 1985; Açik *et al.*, 2005), as associated infauna of the echiuran *Bonellia viridis* Rolando, 1821 (Schembri & Jaccarini, 1978), in bare rock, inside the rhizome of the seagrass *Posidonia oceanica* and on coralligenous substrata (*Cladocora caespitosa* (Linnaeus, 1767) and *Pinna nobilis* Linnaeus, 1758) (Açik

et al., 2005; Açik, 2008). Drilling activity has been observed in individuals inhabiting chalk rock from the coast of Israel, even if the drilling mechanism is unknown (Por, 1975). *Aspidosiphon muelleri* has also been reported in soft substrata, from sandy to muddy bottoms along the Mediterranean Sea, often inhabiting gastropod shells or polychaete tubes (Saiz-Salinas, 1986, 1993; Saiz-Salinas & Villafranca-Urchegui, 1990; Papazacharias *et al.*, 1998; Somaschini *et al.*, 1998; Murina *et al.*, 1999; Klaoudatos *et al.*, 2006; Açik, 2008; Ferrero-Vicente *et al.*, 2011, 2013a, b). It is known that *A. muelleri* prefers to use empty tubes of the serpulid polychaete *Ditrupa arietina* (Müller, 1776) as a shelter in soft sediment, and its abundance could be limited by the availability of this particular resource (Ferrero-Vicente *et al.*, 2013b). *Ditrupa arietina* is a widespread endobenthic species, common in the Mediterranean where it achieves high densities. Although *D. arietina* is considered temporal fauna in altered sediment (Picard, 1965), there is evidence that its abundance has increased in some areas of the western Mediterranean (Grémare *et al.*, 1998a, b; Bolam & Fernandes, 2002; Labrune *et al.*, 2007; Morton & Harper, 2009), showing seasonal peaks of abundance in May–June (Sardá *et al.*, 1999). *Ditrupa arietina* lives for approximately 2 years, and after its death (presumably predated by a prosobranch naticid), its tube becomes available for secondary colonization by *A. muelleri* (Morton & Harper, 2009; Morton & Salvador, 2009).

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Although external signs of sexual dimorphism are lacking, sipunculans are typically dioecious, but cases of hermaphroditic species have been reported (Gibbs, 1975; Cutler & Cutler, 1986). Gametes first develop in the coelomic fluid, before being taken up into the nephridia. They are stored for a short time in the nephridia, and finally released by way of nephridiopores after a short period of storage. Fertilization is external in all cases (Rice, 1975a, 1989; Cutler, 1994; Maiorova & Adrianov, 2005, 2007). Asexual reproduction is known to occur within the genus *Aspidosiphon* (Rice, 1970), in *Aspidosiphon elegans* (Chamisso & Eysenhardt, 1821), which is considered to be an alien species in the Mediterranean Sea (Açik, 2008). Nonetheless, this reproductive ability has not been reported in *A. muelleri*, and little is known about the reproductive cycle of this species. Most of sipunculan species with a well-known life cycle show abbreviated development, omitting some stage of the Sipuncula development (trochophore stage, pelagosphera stage or having a lecithotrophic pelagosphera) (Rice, 1976). Species with a known life cycle are *Apionsoma misakianum* (Ikeda, 1904) (Rice, 1981), *Nephasoma pellucidum* (Keferstein, 1865) (Schulze & Rice, 2009), *Phascolion strombus strombus* (Montagu, 1804) (Åkesson, 1958; Wanninger *et al.*, 2005), *Phascolion cryptum* Hendrix, 1975 (Rice, 1975b), *Phascolosoma turnerae* Rice 1985 (Rice *et al.*, 2012), *Siphonosoma cumanense* (Keferstein, 1867) (Rice, 1988), *Themiste pyroides* (Chamberlin, 1919) (Rice, 1967; Adrianov *et al.*, 2011), *Themiste lageniformis* Baird, 1868 (Pilger, 1987), *Themiste alutacea* (Grube, 1858) (Rice, 1975b) and *Thysanocardia nigra* (Ikeda, 1904) (Rice, 1967; Adrianov & Maiorova, 2010).

However, to our knowledge, the genus *Aspidosiphon* presents a larval development with two pelagic stages: trochophore and planktotrophic pelagosphera larvae. This pelagosphera larva increases notably in size during its long period in the water (1–6 months) before its metamorphosis to a vermiform juvenile, which could make possible an inter-oceanic communication between different populations. (Scheltema & Hall, 1975; Cutler, 1994). Although planktotrophic pelagosphera larvae of many Aspidosiphonidae have been collected in plankton tows, their complete life cycles are still unknown (Hall & Scheltema, 1975; Rice, 1981).

The aim of the present study is to describe some aspects related to the reproductive biology and ecology of *Aspidosiphon muelleri* in temperate waters.

MATERIALS AND METHODS

Study area and sampling design

The study area is located near the shoreline of San Pedro del Pinatar (south-eastern Spain) (Figure 1). Samples were randomly taken around the coordinates $37°48.85'$N $0°42.37'$W (always keeping less than 50 m away from this point). The area of study was selected due to its suitable features for the study of *Aspidosiphon muelleri*. On one hand, the sipunculan assemblages at this bottom are well characterized, being *A. muelleri* an abundant species in this area (Ferrero-Vicente *et al.*, 2011, 2013a), thus ensuring the presence of individuals which is irregular in many areas of the western Mediterranean Sea (Ferrero-Vicente *et al.*, 2013a). On the other hand, there is a high density of empty tubes and shells available to be used as shelter by *A. muelleri*, which allows the assessment of some
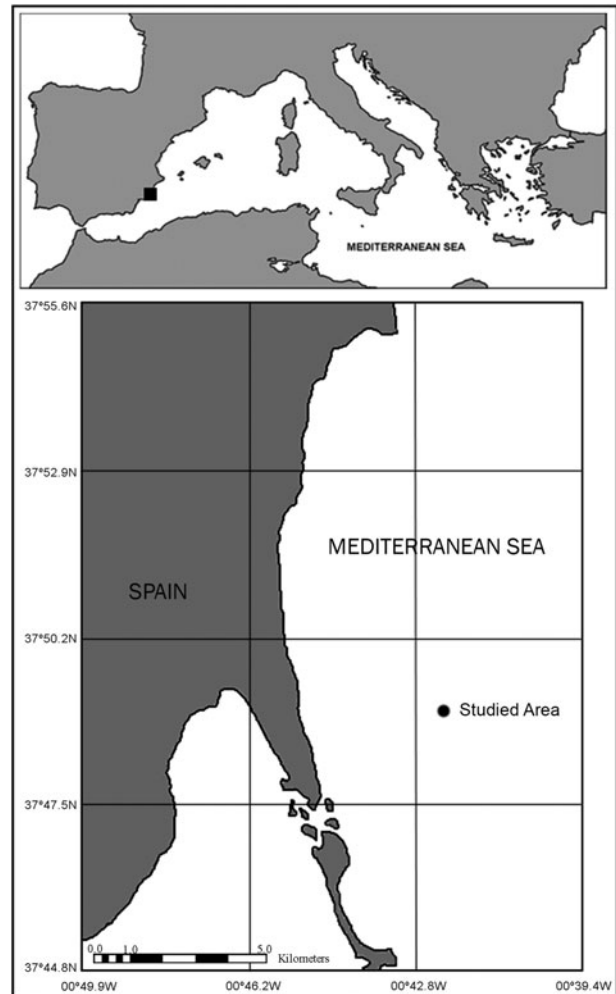

Fig. 1. Map showing the location of the studied population of *Aspidosiphon muelleri*.

aspects of the ecology of *A. muelleri* related to these resources (Cutler & Cutler, 1989; Saiz-Salinas, 1993; Ferrero-Vicente *et al.*, 2013a, b).

Eleven monthly samples were taken from June 2011 to May 2012 using a Van Veen grab (0.028 m²) for a total of 132 samples. One of these samples was assigned to sediment analysis (granulometry, organic matter, pH and Eh). The ten remaining samples were sieved (500 μm) and used to study the ecology and reproductive biology of *A. muelleri*. Depth, temperature and bottom salinity were also measured using a CTD sensor (RBR-XR-420/620).

Laboratory work and data process

Redox potential (Eh) and pH were measured in the samples designated to the sediment analysis immediately after collection. Organic matter content was measured from a 30 g subsample, taken from the shallow layer of the sediment (0–5 cm), through weight loss on ignition after 4 h at 500°C inside a muffle furnace. The granulometric analysis was carried out following the methodology described by Buchanan (1984), sorting the samples in five categories: gravel; coarse sand; medium sand; fine sand; and silt and clays.

Ten replicates from every month were used for the fauna analysis (120 samples). Samples were sieved through a 500 µm mesh screen to separate the macrofauna and then fixed in 10% buffered formalin. Afterward, the fauna was preserved in 70% ethanol and sorted into different taxa. Abundances of other ecologically related species were also monitored and grouped into shelter suppliers (gastropods and *Ditrupa arietina*) or shelter dwellers (hermit crabs, polychaetes and tanaids). Sipunculans were identified to the species level (Cutler, 1994) through analysis of their internal and external anatomy using a binocular scope and identifying characteristics of taxonomically-relevant structures. Length and trunk thickness of each specimen and shelter were logged. Specimens of *A. muelleri* were dissected; gender was determined when it was possible, and oocytes present in the coelom were photographed and their size measured by image analysis techniques. We used 50 oocytes of each female specimen to calculate average size in every month.

Shelter availability (shelters m^{-2}) was calculated for every one of the samples used for the fauna analysis. Shelters usually habited by sipunculans (according to previous observations since 2005) were sorted into empty tubes of *D. arietina* and empty shells of gastropods.

Univariate techniques and descriptive statistics were used to analyse the population dynamics of *A. muelleri* and its reproductive behaviour, moreover to define its possible relation with the abiotic factors of the environment. Relation between specimen size and shelter size was evaluated (Pearson's *r* correlation) as well as relation between reproductive variables (% females with presence of free oocytes in the coelom and oocyte size) and possible trigger factors for reproductive activity (surface/bottom temperature and irradiance).

RESULTS

Abiotic factors

Granulometry of the sediment was homogeneous throughout the studied period (Figure 2). Sediment in the area is dominated by fine sand (43.8 ± 2.17%) and medium sands (25.9 ± 1.6%), with a presence of bioclasts, including gastropod shells and serpulid polychaete tubes. Depth ranged from 34.2 to 36.5 m. Presence of shelters in the sediment was high and constant for the whole study period, with a greater density of tubes than gastropod shells (Figure 3B). Average shelter
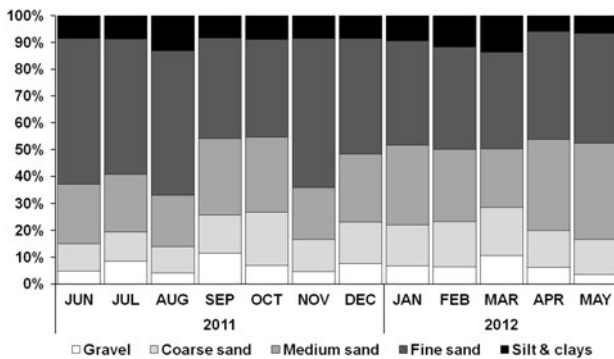

Fig. 2. Granulometry in the studied area for each studied month.

densities were 4725 ± 174 tubes m^{-2} and 1934 ± 99 gastropod shells m^{-2}, respectively. Tubes represented 70.9% of the total shelters available, while gastropod shells represented the remaining 29.1%. Organic matter content ranged between 0.76–1.95% (Figure 4F), and the redox potential (Eh) was also variable, but lower values were recorded during the warmer months (Figure 4D). Eh values were negative throughout the year, which shows the reductive environment of this sediment.

Water temperature varied seasonally, following the previous months' irradiance levels (Figure 4A, E). Seabed temperature fluctuated annually, reaching the interannual peak (24.5°C) in October 2011 and the interannual low in March 2012 (13.46°C).There is a clear stratification in the water column that synchronizes with the months of maximum irradiance and weather stability, and there may be as much as 6.8°C difference detectable between surface temperature and bottom temperature in August 2011. The bottom salinity ranged from 37.2 to 37.9 psu, with a slight drop in October 2011, at the beginning of autumn (Figure 4B).

Biology of *Aspidosiphon muelleri*

A total of 156 sipunculans were collected, with *Aspidosiphon muelleri* being the dominant species (146 specimens, 93.6% of the total). All specimens were dissected to analyse their external and internal anatomy. The remaining individuals corresponded to *Thysanocardia procera* (Möbius, 1875) (two specimens, 1.3%), *Sipunculus nudus* Linnaeus, 1766 (one specimen, 0.6%), *Phascolion strombus strombus* (one specimen, 0.6%) and six unidentified specimens (3.9%). *Aspidosiphon muelleri* sexual identification was possible in 72 of the cases, of which 54.2% were females whereas 45.8% were males. Sexual dimorphism was not detected and sexual identification was only possible through the observation of gametocytes in the coelom. In some cases, gametocytes were present in small specimens (3.7 mm and greater). Sipunculans were found inhabiting *Ditrupa arietina* tubes in 95% of the cases, in gastropod shells in 3.6% of the cases, and into bare substratum in the remaining 1.4% of cases. A significant correlation was detected between the sipunculan body width and tube width (Pearson correlation; $r = 0.707$; $P = 0.000$) and between sipunculan body length and tube length (Pearson correlation; $r = 0.478$; $P = 0.000$) (Figure 5). In all cases, tube length was longer than the body length of the animal, which means that the animal was always totally covered by the tube. Abundance of *A. muelleri* was not significantly related to shelter abundance (Pearson correlation; $P = 0.684$) and yielded an average density of 46.5 ± 6.0 ind m^{-2} for the whole study period, with an average body length of 6.6 ± 0.2 mm (mean ± standard error (SE)), and average body width of 0.7 ± 0.0 mm.

Skin colour was highly variable, but generally dull white, turning orange in some specimens. Anal and caudal shields displayed a darker tonality, usually dark orange. Juvenile specimens usually exhibited a translucent skin. Both types of hooks (uni and bidentate) were present in the introvert (Figure 6D). Individuals presented two elongate nephridia, as long as 20–60% of the trunk length (36.7 ± 2.0%). The number of intestine coils was significantly correlated with the length of the body (Pearson correlation; $r = 0.582$; $P = 0.011$) and ranged between 12 and 21 coils.
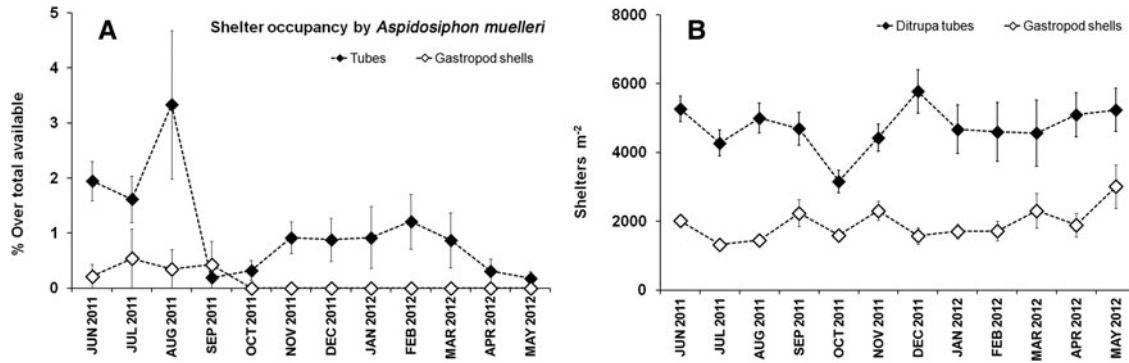
Fig. 3. (A) Ratio of shelter occupancy by *Aspidosiphon muelleri* for each month; (B) shelters density for each month in the studied area.

Maximum abundances of *A. muelleri* occurred in the summer period, with a peak in August (135.7 ± 37.9 ind m^{-2}; mean ± SE). After this month the population decreased drastically, with a posterior slight recovery during the winter months (Figure 7A).

Ditrupa tubes occupancy rate by *Aspidosiphon muelleri* was also higher in the summer months, with a peak in August (3.33 ± 1.34%) matching with the maximum abundance of sipunculans (Figure 3A). Gastropod shells were only used as
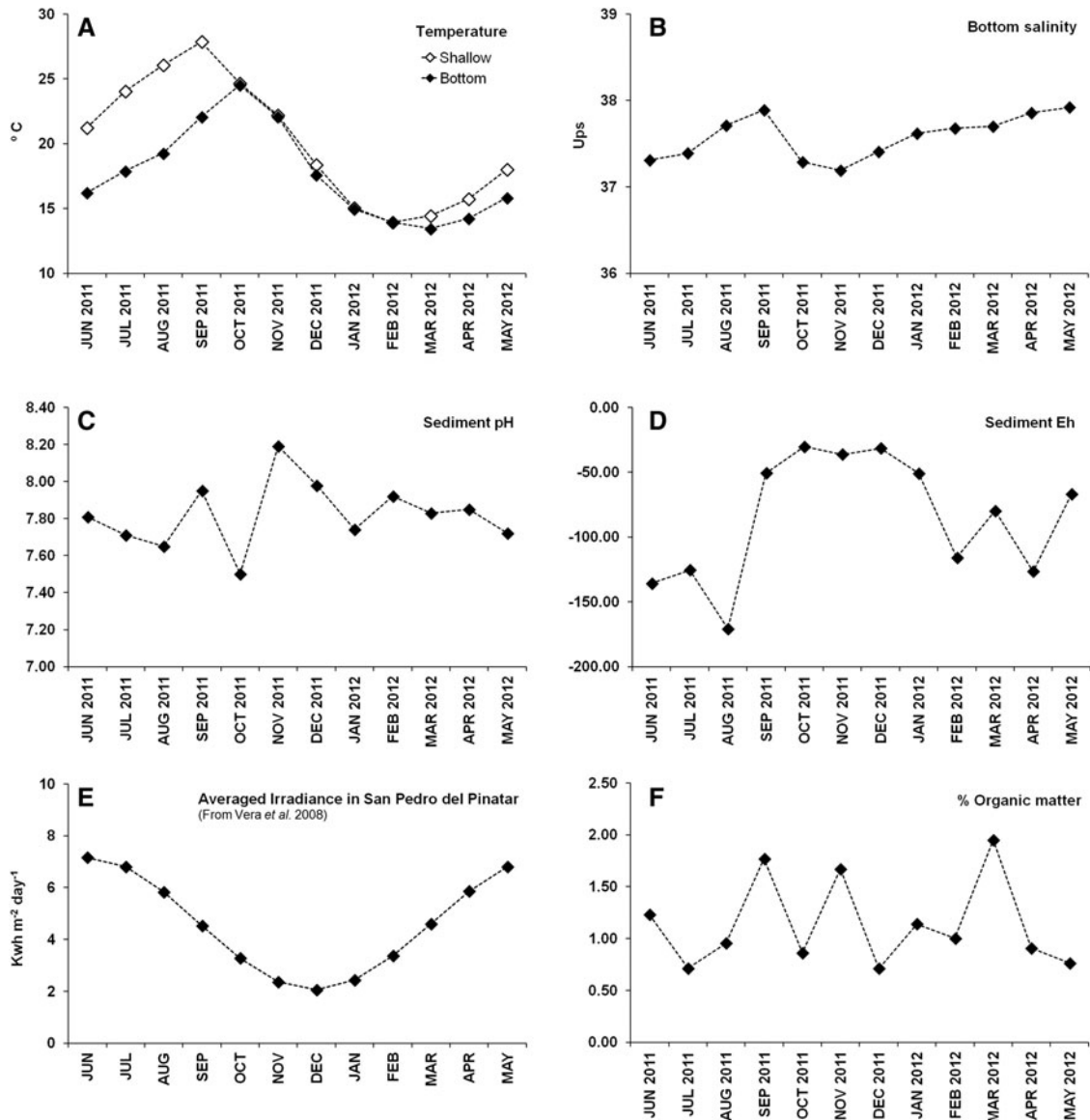

Fig. 4. Charts from the different parameters measured at the studied area for each month.
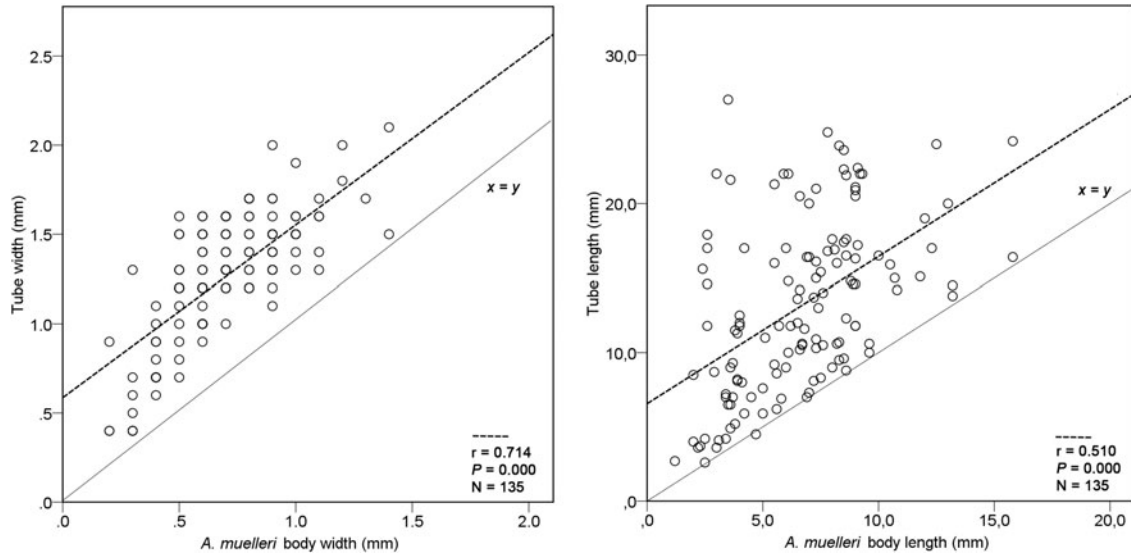
Fig. 5. Dispersion charts showing the correlations between sipunculan body size and shelter size.

a shelter in the summer period, when the maximum demand occurred. The rest of the months the occupancy rate for gastropod shells decreased to 0%.

Gametocytes development takes place on the retractor muscle base and is eventually released into the coelomic fluid. Reproductive activity occurred mainly during the summer, beginning in May and extending through October, with a maximum peak in August (Figure 8). Both, the oocyte size and the percentage of females with free oocytes in the coelom, reached their maximum during that month. Average oocyte size was significantly related to shallow water temperature (Pearson correlation; $r = 0.808$; $P = 0.001$) as well as to irradiance (Pearson correlation; $r = 0.589$; $P = 0.044$). Percentage of females with free oocytes was also related to shallow water temperature (Pearson correlation; $r = 0.776$; $P = 0.003$) and showed a non-significant relation to irradiance (Pearson correlation; $r = 0.562$; $P = 0.057$).

Oocytes presented an asymmetric-ovoid shape, with a pointed pole in most of the cases (Figure 9). Oocytes started to grow in May and August—just prior to spawning—and they had an average size of 118 ± 23 μm along its longer axis.

Although density of coelomocytes was not measured, a remarkable increase in abundance was observed in October, matching the final stage of the spawning event.

Sampling occurred once a month, and gametes into the nephridia (presumably where they would be hours before being released to the seawater) were not observed.

In regard to shelter supplier organisms (*Ditrupa*, Gastropoda and Scaphopoda), population density of *D. arietina* reached its maximum in the month of June, then slowly descended until March when it began to increase again. Abundance of *D. arietina* was much higher than abundances of Gastropoda or Scaphopoda (Figure 7B, C, E). Gastropoda tripled its population during the month of November (43.6 ± 18.5 ind m^{-2}), and the rest of the year remained a stable population (~ 7–10 ind m^{-2}); scaphopod abundance was low and generally higher in the summer months, reaching a maximum of 14.3 ± 5.8 ind m^{-2} in May 2012.

Regarding other shell-dwelling organisms (not Sipuncula), tanaid abundance increased notably, with its population reaching 200.0 ± 40.5 ind m^{-2} in August 2011. Tanaid abundance was high during the summer and autumn months, although the number of individual tube-dwellers did not increase in relation to total individuals during this period (Figure 7D). Hermit crabs did not yield a high density of individuals and abundance was variable, but more stable from February to June (Figure 7G). Its maximum abundance was 10.7 ± 3.4 ind m^{-2} in the month of June 2011. Polychaete presence (mainly Syllidae) inside the tubes was high throughout the year, with a peak in April 2012, reaching a maximum density of 156.2 ± 81.5 ind m^{-2} (Figure 7F). The presence of the commensal bivalve *Epilepton clarkiae* (Clark, 1852) living together with *Aspidosiphon muelleri* and
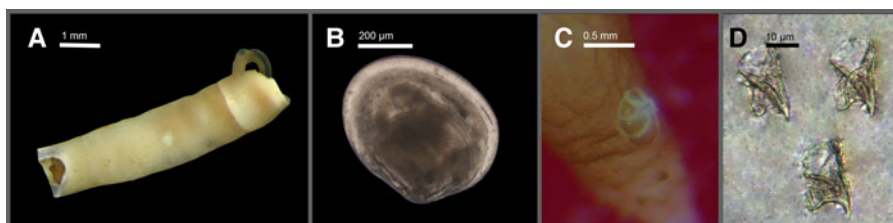

Fig. 6. (A) Specimen of *Aspidosiphon muelleri* inside of a *Ditrupa arietina* tube; (B) specimen of *Epilepton clarkiae* collected inside a *D. arietina* tube inhabited by *A. muelleri*; (C) foraminifer species attached to the anal shield of *A. muelleri*; (D) unidentate pyramidal-shaped hooks from the introvert of *A. muelleri*.
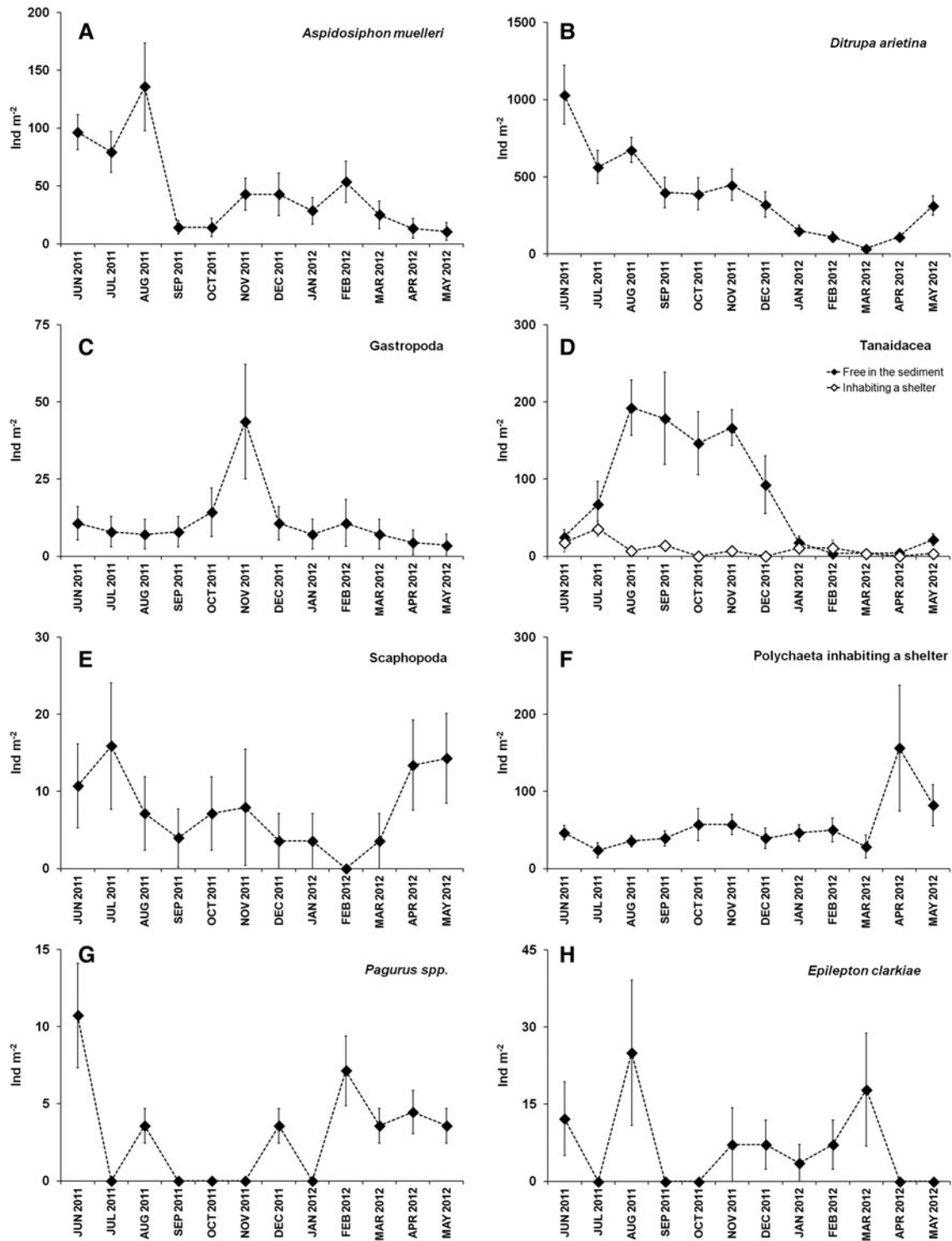
Fig. 7. Abundances of the different species and taxonomic groups recorded for each month (mean $\pm$ standard error).

sharing the *Ditrupa arietina* tube was constant throughout the year (Figure 7H). Bivalves were found in the tubes with a prevalence of 11.64%; one bivalve (7.53% of the cases), two bivalves (3.42%) and even three bivalves (0.69%) were found in the same tube attached to the body of the sipunculans. Bivalves were not found in any gastropod shell inhabited by *A. muelleri*. Foraminifers were also encountered inside some of the tubes attached to the body of the sipunculans (Figure 6C).

DISCUSSION

Aspidosiphon muelleri is a common species within the macrobenthic fauna of soft bottoms from the western Mediterranean, being often a highly abundant species (Saiz-Salinas, 1986; Ferrero-Vicente *et al.*, 2011, 2013a, b). Densities of 1675 ind m^{-2}, the maximum known for this species (Ferrero-Vicente *et al.*, 2013a) have been recorded in this area. The area of study was selected due to its known
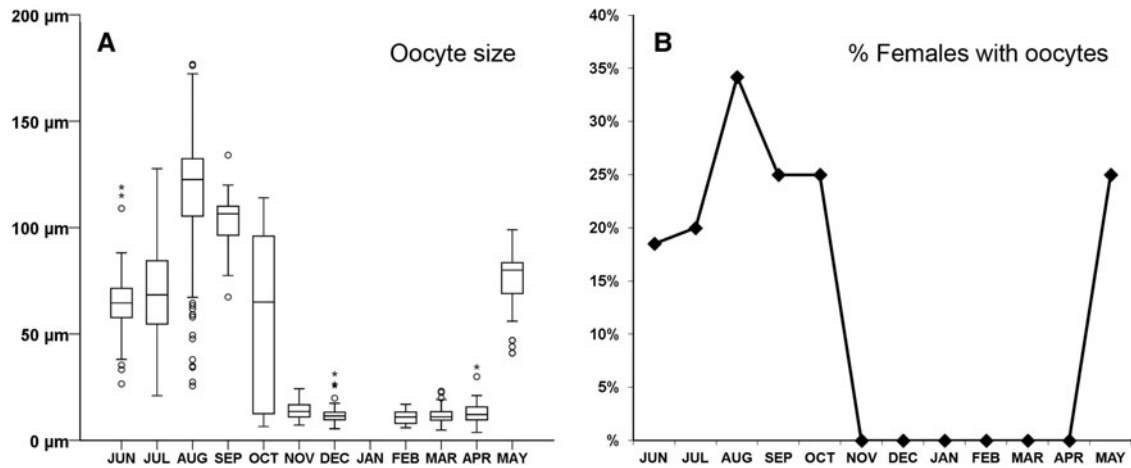
Fig. 8. (A) Averaged oocytes size in each month; (B) percentage of females with presence of free oocytes in the coelom.

high abundance of *A. muelleri*. The averaged abundance of individuals (46.5 ± 6.0 ind m^{-2}) was higher than recorded in other nearby areas of the western Mediterranean (13.78 ± 3.93 ind m^{-2} in Ferrero-Vicente *et al.*, 2013a), whereas the relative abundance—93.6% of the total sipunculans collected were *A. muelleri*—was similar to other studies

Fig. 9. (A–C) Ovoid-shaped coelomic oocytes of different size; (D) coelomic sperm plates. The abnormal appearance of the cytoplasm, pulled away from the overlaying egg envelope (A–B), could be an artefact due to fixation process in formalin.

from the area: 89.06 and 74.07% (Ferrero-Vicente *et al.*, 2011, 2013a).

Sex-ratio was close to 1:1 (54.2% females *vs* 45.8% males), which fits with a dioecious reproduction typical in Sipuncula (Rice, 1989). Nonetheless not all sipunculans present this pattern, and biased sex-ratios are not uncommon in some species; Rice *et al.* (2012) recorded a 74.0% of females for *Phascolosoma turnerae* (a deep-sea wood-dwelling sipunculan), and Pilger (1987) recorded 96% females and 4% males of the parthenogenic species *Themiste lageniformis*. Many other species present sex-ratios in which females outnumber the males (Pilger, 1987). Even asexual reproduction has also been described for one species of the genus, *Aspidosiphon elegans* (Rice, 1970).

Gametocytes were observed in specimens from 3.7 mm, and 4–5 mm appears to be the approximate size for the gametocytes development in this population of *A. muelleri*. This size could be reached by *A. muelleri* in a few months, since growth from larval fixation to adult size (even more than 10 mm) could take less than six months (Ferrero-Vicente *et al.*, 2013b).

According to Ferrero-Vicente *et al.* (2013b), approximately 82% of the available shelters in this area correspond to *Ditrupa* tubes whereas 18% are gastropod shells. This work also suggests a shelter limitation for this species (getting abundances 10 times higher by adding shelters in the sediment) and a preference of *A. muelleri* to use tubes as shelter instead of snails. Shelter partitioning in the present work was similar; approximately 71% of available shelters were tubes and 29% were gastropod shells. Shelter preference of tubes over gastropod shells fit also well with the present results, since 95% of individuals inhabited a tube and only 3.6% a snail. The remaining 1.4% of individuals was encountered bare into the sediment, but they presumably left the shelter in the fixation process. A linear relation between shelter abundance and sipunculan abundance was not detected due to high shelter availability throughout the whole study period (Figure 3B), thus indicating that shelter availability is not driving the maximum density of *A. muelleri* in this area. However, there may be some limitation during the summer, matching the maximum population density. At this point, sipunculans start to use gastropod shells as resource in spite of their preference for using tubes, and it might be that *Ditrupa* tubes are not so easy to find at that point of the year, probably due to the
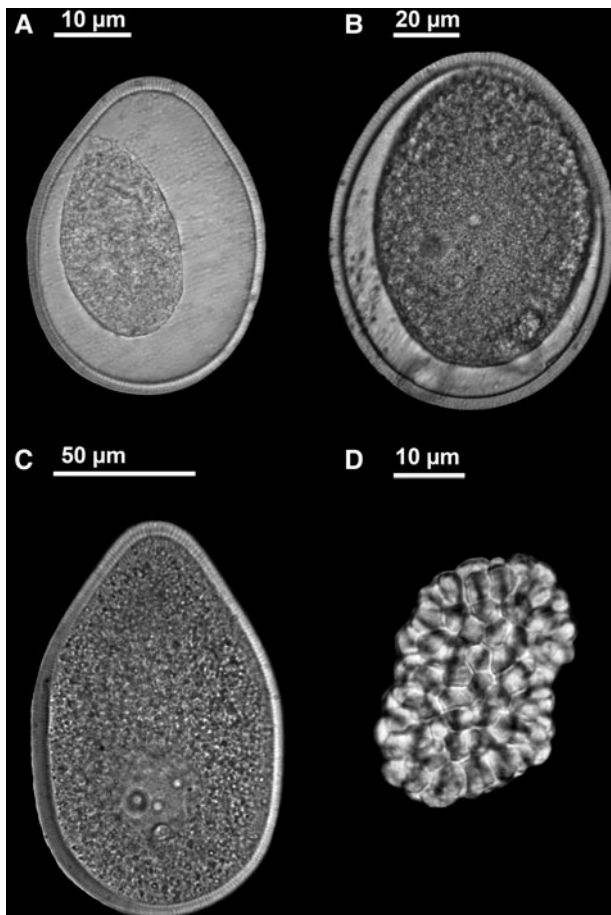
higher demand of this resource by tanaids and *A. muelleri*. After reproduction, when population density decreased drastically, sipunculans used preferably tubes as shelter. Correlation between body and shelter size was strong, and it is known that *A. muelleri* left its shelter just when all available space of the shelter—in a contracted state of the animal—is occupied for its body, ensuring that the animal can always be covered by the tube (Ferrero-Vicente *et al.*, 2013b). This preference for *Ditrupa* tubes was not found for other shell/tube-dwelling species of similar size from the western Mediterranean (*Phascolion caupo* Hendrix, 1975 and *Phascolion strombus strombus*) (Ferrero-Vicente *et al.*, 2012, 2013b).

Internal anatomy did not yield differences with regard to other specimens from other areas (Saiz-Salinas, 1993; Cutler, 1994; Pancucci-Papadopoulou *et al.*, 1999; Açik *et al.*, 2005; Açik, 2011), but specimens showed plasticity in some characters (e.g. skin took different tonalities from translucent skin in juvenile specimens to white or dark orange skin in adult specimens). Presence of unidentate and bidentate hooks, with pyramidal shape in the proximal part of the introvert was also noted. Some specimens presented pointed spines in the anal shield, in the grooves close to the introvert. This characteristic was not discriminative since different degrees of spine length and edge shape were detected, from almost flat to very pointed spines.

The number of intestine coils was significantly correlated with the length of the body (Pearson correlation; $r = 0.582$; $P = 0.011$), which indicates that intestine grows in length as long as the animal keeps growing.

Sediment in the area has a negative redox potential, ranging from -30.2 to -170.8 mV, which indicates a reducing environment. This condition does not seem to be relevant for this species, likely due to the fact that *A. muelleri* lives in the shallowest and most oxygenated layer of the sediment, protected from predation by its shelter.

In temperate waters, marine invertebrates usually exhibit a two- or three-month peak in reproductive activity during the summer or early autumn (depending on latitude) (Cutler, 1994; Ying *et al.*, 2009). In the case of *A. muelleri,* the highest density of individuals occurred during July and August, and then decreased drastically in September. This increase in population density coincides with the development of oocytes, which increase its size along these months. Female oocytes average size in its longer axis (118 ± 23 μm) was similar to that logged by Açik (2008) for this species ($117.5 – 145$ μm).

Trigger factors for reproduction in sipunculans are poorly understood, but in our case the development of the gametocytes and spawning event might be stimulated by an increase in irradiation and water temperature, as usual in many marine invertebrates from temperate waters. The first females with presence of oocytes appeared in the month of May (following the increase in water temperature). We can indirectly estimate spawning took place between August and September (just before the annual maximum temperature) because the percentage of females with oocytes and oocyte size both reached their maximum in August. This spawning event may last a few weeks, but it has been observed in other species that in colder waters at high latitude as well as in equatorial waters, spawning may be extended for several months (Rice, 1975b; Amor, 1993; Cutler, 1994; Adrianov & Maiorova, 2010). Moreover a higher water temperature

induces a higher larval survival, but on the other hand, this might mean a shorter larval stage, which implies a lesser dispersion (O'Connor *et al.*, 2007).

It is known that macroinvertebrates present seasonal life cycles in order to take advantage of optimal environmental conditions or avoid unfavourable conditions (Johnson *et al.*, 2012). Fluctuations in sipunculan spawning period are also known to occur due to differences in their conditions of habitation, showing an extended spawning period in a more stable situation (Rice, 1967). This fact led us to think that different *A. muelleri* populations can exhibit different reproductive strategies for different areas, and they could have different spawning timing in areas with a less pronounced seasonality, like high latitude or equatorial areas.

It is known that lifespan can be long for some species (e.g >20 years in *Phascolosoma turnerae* (Rice *et al.*, 2012)). In our case, the dramatic decrease in abundance of individuals from August to September suggests that many adult individuals might die after the spawning, maybe as a consequence of this event. Although the abundance in September was low, the percentage of females with oocytes was still high. In October, the oocyte size was smaller and highly variable; a high presence of coelomocytes was observed during this period, and likely their function is the phagocytosis of the oocytes, which were not expelled to the seawater after the spawning (Amor, 1993; Adrianov & Maiorova, 2010).

As with many other sedentary groups, sipunculans attract a variety of associated fauna. Some small bivalves with a known association to sipunculans are *Kurtiella bidentata* (Montagu, 1803), reported together with *Golfingia vulgaris* (de Blainville, 1827) (Gibbs, 1977), *Montacuta phascolionis* Dautzenberg & Fischer, 1925, and *Pythinella cuneata* (Verrill & Bush, 1898) together with *Phascolion strombus* (Gage, 1968, 1979; Troncoso & Urgorri, 1992). To our knowledge, previous data about commensalism between bivalves and *Aspidosiphon muelleri* are limited to the relation with the bivalve *Jousseaumiella concharum* Knudsen, 1944 (Saiz-Salinas, 1993). This is the first report of *Epilepton clarkiae* associated with *A. muelleri*, although it is not the first record of this bivalve in association with a sipunculan, since it was reported together with *Golfingia vulgaris* inside the burrows, which this sipunculan digs in the sediment (Gibbs, 1977), but other associations have been found with *Phascolion strombus* (Troncoso & Urgorri, 1992) and *Golfingia elongata* (McMillan, 1968). The collected specimens of *E. clarkiae* were juveniles, and an adult specimen might not be able to inhabit the tube since its regular size is bigger than the tube diameter. The fact that *E. clarkiae* was not found in the gastropod shells inhabited by *A. muelleri* is probably due to the low number of sipunculans recorded inside gastropod shells. This commensal relationship might play an important role in the distribution of *E. clarkiae* since any individuals living bare into the sediment, or even inside a shelter not occupied by *A. muelleri,* were not found.

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REFERENCES

Adrianov A.V. and Maiorova A.S. (2010) Reproduction and development of common species of peanut worms (Sipuncula) from the sea of Japan. *Russian Journal of Marine Biology* 36, 1–15.

Adrianov A.V., Maiorova A.S. and Malakhov V.V. (2011) Embryonic and larval development of the peanut worm *Phascolosoma agassizii* (Keferstein 1867) from the Sea of Japan (Sipuncula: Phascolosomatidea). *Invertebrate Reproduction & Development* 55, 22–29.

Açik S. (2008) Sipunculans along the Aegean coast of Turkey. *Zootaxa* 1852, 21–36.

Açik S. (2011) Sipuncula from the southern coast of Turkey (eastern Mediterranean), with a new report for the Mediterranean Sea. *Cahiers de Biologie Marine* 52, 313–329.

Açik S., Murina G.V., Çinar M.E. and Ergen Z. (2005) Sipunculans from the coast of northern Cyprus (eastern Mediterranean Sea). *Zootaxa* 1077, 1–23.

Åkesson B. (1958) A study of the nervous system of the Sipunculideae: with some remarks on the development of the two species *Phascolion strombi* Montagu and *Golfingia minuta* Keferstein. *Undersökningar över Öresund* 38, 1–249.

Amor A. (1993) Reproductive cycle of *Golfingia margaritacea*, a bipolar sipunculan, in subantarctic water. *Marine Biology* 117, 409–414.

Bolam S.G. and Fernandes T.F. (2002) Dense aggregations of tube-building polychaetes: response to small-scale disturbances. *Journal of Experimental Marine Biology and Ecology* 269, 197–222.

Buchanan J.B. (1984) Sediment analysis. In Holme N.A. and McIntyre A.D. (eds) *Methods for the study of marine benthos.* 2nd edition. Oxford: Blackwell Scientific Publications, pp. 41–46.

Cutler E.B. (1994) *The sipuncula: their systematics, biology, and evolution.* Ithaca, NY: Cornell University Press, 453 pp.

Cutler E.B. and Cutler N.J. (1986) A revision of the genus *Nephasoma* (Sipuncula: Golfingiidae). *Proceedings of the Biological Society of Washington* 99, 547–573.

Cutler E.B. and Cutler N.J. (1989) A revision of the genus *Aspidosiphon* (Sipuncula: Aspidosiphonidae). *Proceedings of the Biological Society of Washington* 102, 826–865.

Ferrero-Vicente L.M., Del-Pilar-Ruso Y., De-La-Ossa-Carretero J.A. and Sánchez-Lizaso J.L. (2013a) Distribution of Sipuncula in the Gulf of Valencia and Cape Nao (western Mediterranean). *Zootaxa* 3646, 235–250.

Ferrero-Vicente L.M., Loya-Fernández A., Marco-Méndez C., Martínez–García E. and Sánchez-Lizaso J.L. (2011) Soft–bottom sipunculans from San Pedro del Pinatar (Western Mediterranean): influence of anthropogenic impacts and sediment characteristics on their distribution. *Animal Biodiversity and Conservation* 34, 101–111.

Ferrero-Vicente L.M., Loya-Fernández A., Marco-Méndez C., Martínez–García E., Saiz-Salinas J.I. and Sánchez-Lizaso J.L. (2012) First record of the sipunculan worm *Phascolion (Phascolion)*

caupo Hendrix, 1975 in the Mediterranean Sea. *Mediterranean Marine Science* 13, 89–92.

Ferrero-Vicente L.M., Marco-Méndez C., Loya-Fernández A. and Sánchez-Lizaso J.L. (2013b) Limiting factors on the distribution of shell/tube-dwelling sipunculans. *Journal of Experimental Marine Biology and Ecology* 446, 345–354.

Gage J. (1968) The mode of life of *Mysella cuneata*, a bivalve 'commensal' with *Phascolion strombi* (Sipunculoidea). *Canadian Journal of Zoology* 46, 919–934.

Gage J. (1979) Mode of life and behaviour of *Montacuta phascolionis*, a bivalve commensal with the sipunculan *Phascolion strombi. Journal of the Marine Biological Association of the United Kingdom* 59, 635–657.

Gibbs P.E. (1975) Gametogenesis and spawning in a hermaphroditic population of *Golfingia minuta* (Sipuncula). *Journal of the Marine Biological Association of the United Kingdom* 55, 69–82.

Gibbs P.E. (1977) *British sipunculans. Synopses of the British Fauna No. 12.* London:The Linnean Society of London, pp. 1–35.

Grémare A., Amouroux J.M. and Vétion G. (1998a) Long-term comparison of macrobenthos within the soft bottoms of the Bay of Banyuls-sur-mer (northwestern Mediterranean Sea). *Journal of Sea Research* 40, 281–302.

Grémare A., Sardá R., Medernach L., Jordana E., Pinedo S., Amouroux J.M., Martin D., Nozais C. and Charles F. (1998b) On the dramatic increase of *Ditrupa arientina* O.F. Müller (Annelida: Polychaeta) along both the French and the Spanish Catalan coasts. *Estuarine, Coastal and Shelf Science* 47, 447–457.

Hall J.R. and Scheltema R.S. (1975) Comparative morphology of open-ocean Pelagospaera. In Rice M.E. and Todorović M. (eds) *Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura.* Belgrade: Naucno Delo Press, pp. 183–197.

Johnson R.C., Carreiro M.M., Jin H.S. and JacK J.D. (2012) Within-year temporal variation and life-cycle seasonality affect stream macroinvertebrate community structure and biotic metrics. *Ecological Indicators* 13, 206–214.

Klaoudatos S.D., Klaoudatos D.S., Smith J., Bogdanos K. and Papageorgiou E. (2006) Assessment of site specific benthic impact of floating cage farming in the eastern Hios island, Eastern Aegean Sea, Greece. *Journal of Experimental Marine Biology and Ecology* 338, 96–111.

Koukouras A., voultsiadou-koukoura E., Chintiroglou H. and Dounas C. (1985) Benthic bionomy of the North Aegean Sea. III. A comparison of the macrobenthic animal assemblages associated with seven sponge species. *Cahiers de Biologie Marine* 26, 301–319.

Labrune C., Grémare A., Guizien K. and Amouroux J.M. (2007) Long-term comparison of soft bottom macrobenthos in the Bay of Banyuls-sur-Mer (north-western Mediterranean Sea): a reappraisal. *Journal of Sea Research* 58, 125–143.

Maiorova A.S. and Adrianov A.V. (2005) The first finding of spermatozeugmata in sipunculids (Sipuncula), animals with external fertilization. *Doklady Biological Sciences* 402, 214–216.

Maiorova A.S. and Adrianov A.V. (2007) Ultrastructural observations on spermiogenesis in the peanut worm, *Themiste pyroides* (Chamberlin, 1920) (Sipuncula; Sipunculidea). *Invertebrate Reproduction & Development* 50, 173–180.

McMillan (1968) *British shells.* London: Frederick Warne & Co. Ltd., 196 pp.

Morton B. and Harper E.M. (2009) Drilling predation upon *Ditrupa arietina* (Polychaeta: Serpulidae) from the Mid-Atlantic Açores, Portugal. *Açoreana Suplemento* 6, 157–165.

Morton B. and Salvador A. (2009) The biology of the zoning subtidal polychaetes *Ditrupa arietina* (Serpulidae) in the Açores, Portugal, with a description of the life history of its tube. *Açoreana Suplemento* 6, 145–156.

Murina V.V. (1975) Ways of evolution and phylogeny of the phylum Sipuncula. *Zoologicheskii Zhurnal* 54, 1747–1758.

Murina G.V.V., Pancucci-Papadopoulou M.A. and Zenetos A. (1999) The phylum Sipuncula in the eastern Mediterranean: composition, ecology, zoogeography. *Journal of the Marine Biological Association of the United Kingdom* 79, 821–830.

O'Connor M.I., Bruno J.F., Gaines S.D., Halpern B.S., Lester S.E., Kinlan B.P. and Weiss J.M. (2007) Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proceedings of the National Academy of Sciences of the United States of America* 104, 1266–1271.

Pancucci-Papadopoulou M.A., Murina V.V. and Zenetos A. (1999) *The phylum Sipuncula in the Mediterranean Sea*. Athens: National Center for Marine Research, 109 pp.

Papazacharias A., Koukouras A., Kevrekidis T. and Voultsiadou E. (1998) Infra – and circalittoral soft substratum macrofaunal assemblages of Kavala Gulf (Aegean Sea). *International Review of Hydrobiology* 83, 461–477.

Picard J. (1965) Recherches qualitatives sur les biocoenoses marines des substrats meubles dragables de la région marseillaise. *Bulletin des Recherches et Travaux de la Station Marine d'Endoume* 52, 1–160.

Pilger J.F. (1987) Reproductive biology and development of *Themiste lageniformis*, a parthenogenic sipunculan. *Bulletin of Marine Science* 41, 59–67.

Por F.D. (1975) Boring species of Aspidosiphon (Sipuncula) on the coasts of Israel. In Rice M.E. and Todorović M. (eds) *Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura*. Belgrade: Naucno Delo Press, pp. 301–304.

Rice M.E. (1967) A comparative study of the development of *Phascolosoma agassizii*, *Golfingia pugettensis*, and *Themiste pyroides* with a discussion of developmental patterns in the Sipuncula. *Ophelia* 4, 143–171.

Rice M.E. (1970) Asexual reproduction in a Sipunculan worm. *Science* 167, 1618–1620.

Rice M.E. (1975a) Sipuncula. In Giese A.C. and Pearse J.S. (eds) *Reproduction of marine invertebrates. Volume II: entoprocts and lesser coelomates*. New York: Academic Press, pp. 67–125.

Rice M.E. (1975b) Observation of the development of six species of Caribbean Sipuncula with a review of development in the phylum. In Rice M.E. and Todorović M. (eds) *Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura*. Belgrade: Naucno Delo Press, pp. 141–160.

Rice M.E. (1976) Larval development and metamorphosis in Sipuncula. *American Zoologist* 16, 563–571.

Rice M.E. (1981) Larvae adrift: patterns and problems in life histories of sipunculans. *American Zoologist* 21, 605–619.

Rice M.E. (1988). Observations on development and metamorphosis of *Siphonosoma cumanense* with comparative remarks on Sipunculus nudus (Sipuncula, Sipunculidae). *Bulletin of Marine Science* 42, 1–15.

Rice M.E. (1989) Comparative observations of gametes, fertilization, and maturation in sipunculans. In Ryl J.S. and Tyler P.A. (eds) *Reproduction, genetics and distributions of marine invertebrates. 23rd European marine biology symposium*. Fredensborg: Olsen & Olsen, pp. 167–182.

Rice M.E., Reichardt H.F., Piraino J. and Young C.M. (2012) Reproduction, development, growth, and the length of larval life of *Phascolosoma turnerae*, a wood-dwelling deep-sea sipunculan. *Invertebrate Biology* 131, 204–215.

Saiz-Salinas J.I. (1986) Los gusanos sipuncúlidos (Sipuncula) de los fondos litorales y circalitorales de las costas de la Península Ibérica, Islas Baleares, Canarias y mares adyacentes. *Monografías del Instituto Español de Oceanografía* 1, 84.

Saiz-Salinas J.I. (1993) Sipuncula. In Ramos M.A. *et al.* (eds) *Fauna Ibérica. Volume 4*. Museo Nacional de Ciencias Naturales. Madrid: CSIC, 200 pp.

Saiz-Salinas J.I. and Villafranca-Urchegui L. (1990) Sipuncula from the Alboran Sea and Ibero-Moroccan Bay. *Journal of Natural History* 24, 1143–1177.

Sardá R., Pinedo S. and Martin D. (1999) Seasonal dynamics of macro-infaunal key species inhabiting shallow soft-bottoms in the Bay of Blanes (NW Mediterranean). *Acta Oecologica* 20, 315–326.

Scheltema R.S. and Hall J.R. (1975) The dispersal of pelagosphaera larvae by ocean currents and the geographical distribution of sipunculans. In Rice M.E. and Todorović M. (eds) *Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura*. Volume 1. Belgrade: Naucno Delo Press, pp. 103–116.

Schembri P.J. and Jaccarini V. (1978) Some aspects of the ecology of the echiuran worm *Bonellia viridis* and associated infauna. *Marine Biology* 47, 55–61.

Schulze A. and Rice M.E. (2009) *Nephasoma pellucidum*: a model species for sipunculan development? *Smithsonian Contributions to the Marine Sciences* 38, 209–217.

Somaschini A., Martini N., Gravina M.F., Belluscio A., Corsi F. and Ardizzone G. D. (1998) Characterization and cartography of some Mediterranean soft-bottom benthic communities (Ligurian Sea, Italy). *Scientia Marina* 62, 27–36.

Troncoso J.S. and Urgorri V. (1992) Asociación de *Tellimya phascolionis* (Dautzenberg *et* Fischer, 1925) (Bivalvia, Montacutidae) con el sipun-cúlido *Phascolion strombi* (Montagu, 1804) en la Ría de Ares y Betanzos (Galicia, NO de España). *Boletín de la Real Sociedad Española de Historia Natural (Sección Biológica)* 88, 189–194.

Vera-García F., García-Cascales J.R. and Hernández-Guillén Z. (2008) *Atlas de irradiación solar y temperatura ambiente de la Comunidad Autónoma de la Región de Murcia*. Universidad Politécnica de Cartagena y Agencia de Gestión de la Energía de la Región de Murcia (ARGEM). Cartagena. 136.

Wanninger A., Koop D., Bromham L., Noonan E. and Degnan B.M. (2005) Nervous and muscle system development in *Phascolion strombus* (Sipuncula). *Development, Genes and Evolution* 215, 509–518.

and

Ying X.P., Dahms H.U., Liu X.M., Wu H.X., Zhang Y.P., Chen C. and Yang W.X. (2009) Development of germ cells and reproductive biology in the sipunculid *Phascolosoma esculenta*. *Aquaculture Research* 40, 305–314.

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