

**Spatio-temporal variations in reproductive patterns and population structure of *Pasiphaea multidentata* (Decapoda: Caridea) in the Blanes canyon and adjacent margin, North-western Mediterranean Sea**

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**Abstract**

**Introduction**

The bathyal faunal communities of the NW Mediterranean slopes have been studied consistently in the last two decades, with a special focus on population structure, trophic dynamics and benthopelagic coupling of commercial deep-sea decapod crustaceans and fishes (reviewed in Sardà et al. 2004) and associated species (Cartes and Sardà, 1993; Company and Sardà, 1997, 2000; Cartes et al., 2001; Company et al., 2001, 2003, 2004). One of the major topographic features in the North-western Mediterranean slope is the presence of submarine canyons. Canyons play a major role in funnelling energy and organic matter from the shelf to bathyal and abyssal depths (Puig et al., 2000), but the implications of this enhanced organic supply in the deep-sea benthic communities is still mostly unknown. Trophic supply can follow two major pathways – vertical deposition in the water column (Billett et al., 1983; Baldwin et al., 1998; Lampitt et al., 2001) or down-slope advection on the margins (Puig et al., 2001; Bethoux et al., 2002; Canals et al., 2006) – and can be a limiting factor in the deep-

sea, being especially important in the oligotrophic Mediterranean Sea (Sardà et al., 2004). Differences in the quantity, quality and timing of organic matter input to the deep seafloor have been used to explain patterns of biomass and abundance in benthic communities (Levin et al., 1994; Gooday & Turley, 1990; Billett et al., 2001; Galéron et al., 2001; Puig et al., 2001; Gage, 2003) as well as other biological process and in particular the existence of seasonal reproduction (Tyler et al., 1994; Company et al., 2004 (MEPS)). Reproduction is a highly energetic process tightly linked to food availability and quality.

*Pasiphaea multidentata* is a deep-water caridean shrimp living between 200 and 2000 m depth in Mediterranean and Atlantic waters (Gonzalez-Gurriaran and Olaso, 1987). This species undergoes diel vertical migrations. *P. multidentata* predate on gammarids, amphipods and isopods on the benthic boundary layer and is the prey of a variety of decapod crustaceans and fishes (Cartes, 1993; Cartes et al., 1993; Bozzano et al., 1997; Baeur, 2004). The daily vertical migrations of *P. multidentata* play a major role in the transfer of energy between the pelagic and benthic layers of the North-western Mediterranean continental margin. The biological aspects of *P. multidentata* and congeneric species *P. sivado* from the NW Mediterranean Sea have been studied and related to depth of distribution (Company et al., 2001). The results showed that the shallower species, *P. sivado*, undergoes continuous reproduction and has a higher reproductive output than *P. multidentata*. In addition to its important ecological role in the ecosystem, *P. multidentata* is also an important by-catch of Mediterranean fisheries targeting the deep-water red shrimp *Aristeus antennatus*. While *A. antennatus* is one of the main commercial species in the Mediterranean, *P. multidentata* is only found in Spanish markets and occasionally in Italy, Morocco and Greece, being discarded in the other countries. It has been shown that the populations of *A. antennatus* have important spatial and temporal sex and size segregations that have been related to processes linked with energy transfer down canyon systems, food resources and intraspecific competition (Sarda et al., 1997). In this study we have described the seasonal

gametogenetic patterns of *P. multidentata* and have analysed the spatio-temporal variations in its population structure and reproduction to determine if the canyon and slope habitats have an effect in the populations of *P. multidentata* similar to that observed for *A. antennatus*.

## Material and Methods

The samples of *P. multidentata* were collected by commercial fishing bottom trawlers in 3 sites on and around the Blanes canyon: 1) the canyon head (canyon) between 364 and 585 m depth; 2) the canyon wall (wall) between 384 and 585 m depth; and 3) the adjacent continental margin (margin) between 512 and 700 m depth (Figure 1). Each site was sampled using a commercial bottom otter trawl with a 3 m mouth opening fitted with a 6 mm mesh cod-end liner. The sampling was conducted regularly on a monthly basis, covering an annual cycle between April 2003 and April 2004 (Table 1). This study is part of the project RECS II (REF, in prep?), which conducted an integral study of the Blanes canyon in relation to the exploitation of the red shrimp *Aristeus antennatus*. The samples were sorted, measured, weighted and sexed on board. Ten females of *P. multidentata* were preserved in buffered seawater formaldehyde and stored in 70% ethanol for histological analyses of gametogenesis.

The cephalothorax (CL) of all specimens was measured from the eye socket to the middle of the carapace margin. The CL size was used to plot the population structure for each sample. The specimens were sexed and the sex ratio plotted for each sample. Gender was determined under a binocular microscope based on the presence or absence *appendix masculina* on the second pair of pleopods of males. Individuals with carapace lengths smaller than 21 mm have no discernible external secondary sex structure and were classified as indeterminate, individuals with  $CL < 30$  mm are juveniles with no mature gonads and individuals with  $CL > 30$  mm CL are adults (Company et al., 2001). Sex ratio was plotted for each sample and differences tested with chi-square. Sexual maturity of females was described in relation to the gonad external morphology and using the ovary development stages defined

in Company et al., 2001: Stage I, immature females; Stage II, rest, with small and white ovary; Stage III, initiation of maturation, with ovary enlarging and more opaque; Stage IV, maturation, with ovary extending in the cephalothorax and the colour changing to red; Stage V, mature ovary occupying most of the cephalothorax, sometimes with dorsal extensions in the abdomen, a slightly red colour and visible oocytes.

The gametogenetic studies were conducted in 5 to 10 females from each site sampled in spring, summer, autumn and winter. The ovaries were dissected out, dehydrated in graded alcohols, cleared with Clear<sup>®</sup> liquid and embedded in paraffin wax. Paraffin sections were cut at 7  $\mu$ m and stained with Haematoxylin and Eosin. All oocytes sectioned through the nucleus (n= 50 to 120 per individual) were measured (feret diameter) using SigmaScan Pro5. The measurements were grouped in 50  $\mu$ m size classes and the percentage frequency of oocytes-sizes calculated for each size-class in each individual. The pooled oocyte-size data (mean  $\pm$  standard deviation) was plotted for each sample (site and month).

## **Results**

### *Population structure*

The population structure of *P. multidentata* was compared amongst populations in the three sampling sites throughout the year (Figure 2). The samples collected in spring in the margin showed the presence of 3 cohorts: a first peak of small indeterminate individuals (CL < 20 mm) marking the recruitment season, a second peak of juveniles (CL = 20-30 mm) and a third smaller group of large mature males and females (CL > 30 mm). The wall and canyon site follow a similar pattern with the exception of indeterminate individuals, which could be a consequence of the small sample size. In summer, the populations of the 3 sites have a similar structure, with two main cohorts: a first one composed by juveniles (CL = 20-30 mm) and a second one of mature males and females (CL > 30 mm). In autumn, the populations at the 3 sites are composed by a group of juveniles coming from the previous

recruitment season and a group of large adults with maturing gonads. In late winter, the populations are mainly composed by juveniles and young adults.

### *Sex ratio*

The sex ratio showed significant differences from the 1:1 expected ratio in the populations from all sites and seasons (Chi Square,  $\chi^2$ ,  $df=1$ ,  $\alpha<0.001$ ) except for the samples from spring/canyon and summer/margin ( $\alpha<0.005$ ) and spring/wall ( $\alpha<0.01$ ). Females dominated the populations at all times and there were no significant differences in the proportion female/male between sites (Figure 3).

### *Ovary maturation cycle*

Figure 4 shows the percentage of females with ovaries at different development stages in the 3 sites, grouped by season. The pattern is similar in the three sites. Over 40% of the female population had ovaries in the rest stage (II) throughout the year at all sites. Gonads in the resting and early developmental stages (II and III) are found in spring. The maturation of ovaries (stage III) is initiated in summer. Mature gonads (stages IV and V) are found in summer and autumn, and in winter most females are again at the resting ovary stage. The populations from the margin and canyon head have the highest proportion of mature females in summer (Figure 4A,C), while the population from the canyon wall shows a slight delay in the maturation of females, with a higher proportion of females in stages IV and V in autumn (Figure 4B).

### *Gametogenesis*

The ovaries of *P. multidentata* are paired organs overlaying the digestive gland. The immature and resting ovary is small and white. As the ovary matures, the two lobes extend dorsally towards the abdomen and become more opaque and red in colour. In fully mature females, the ovaries occupy most

of the dorsal cephalothorax and can extend as two dorsal filaments down to the 4<sup>th</sup> segment of the abdomen. These ovaries are red in colour and the oocytes are densely packed and visible to the naked eye. The histological sections showed previtellogenic oocytes characterised by their large nucleus/cytoplasm ratio and basophilic cytoplasm that stains purple with haematoxylin (Figure 5A,B). During development, the growing previtellogenic oocytes migrate from the periphery of the gonad towards the centre (Figure 5A). The previtellogenic oocytes grow to approximately 180-200  $\mu\text{m}$  in diameter before undergoing vitellogenesis. The onset of vitellogenesis is characterised by the presence of lipidic granules in the cytoplasm and a change to acidophila, resulting in the oocytes staining pink with eosin (Figure 5C,D). The vitellogenic oocytes have a small nucleus/cytoplasm ratio and are surrounded by a mono-layer of follicular cells (Figure 5D). The maximum oocyte size measured at each site was 1418  $\mu\text{m}$  for a female sampled on the canyon, 1281  $\mu\text{m}$  for a female sampled on the wall site and 1263  $\mu\text{m}$  for a female sampled on the margin.

#### *Oocyte-size distributions*

The overall distribution of oocyte sizes throughout the year was similar for the females sampled at the three sites (Figure 6A-D). In winter, the ovaries were in an early stage of development, with most oocytes between 100-300  $\mu\text{m}$  diameter, corresponding to previtellogenic and early vitellogenic stages. The largest oocytes ranged between 430 and 640  $\mu\text{m}$ , except in one individual from the canyon, which had a few vitellogenic oocytes reaching 1400  $\mu\text{m}$  (Figure 6A). These could be non-spawned oocytes in the process of re-absorption. The ovaries of females sampled in spring had a slightly bimodal oocyte-size distribution, with a first peak in the 200  $\mu\text{m}$  size-class corresponding to previtellogenic oocytes and a second peak of early vitellogenic oocytes at around 400  $\mu\text{m}$  (Figure 6B). The largest oocytes ranged between 540 and 590  $\mu\text{m}$ . In summer, the diagram of oocytes-sizes followed a bimodal distribution, again with a peak of previtellogenic oocytes around 200  $\mu\text{m}$  and a second extended mode of larger vitellogenic oocytes between 600 and 1000  $\mu\text{m}$  in diameter (Figure 6C). The largest oocytes ranged

between 1040 and 1280  $\mu\text{m}$ . Finally, the females sampled in autumn presented a marked bimodal distribution of oocytes sizes, with a first peak of previtellogenic oocytes and a second peak of large vitellogenic oocytes ranging between 600 and 1400  $\mu\text{m}$  (Figure 6D). The largest oocyte sizes ranged between 1260 and 1420  $\mu\text{m}$ . The overall largest oocyte sizes were found in females from the canyon population in all seasons excluding spring. However, the analysis of variance for maximum oocyte size with season and site was only significant for season ( $F= 22.031$ ,  $df= 3$ ,  $P< 0.001$ ), with maximum size increasing from winter (February sample) to autumn (September sample) and no significant differences in mean largest oocyte size amongst populations ( $F= 1,357$ ,  $df= 2$ ,  $P= 0.264$ ). The mean size of vitellogenic oocytes – after excluding the individual with oocytes  $> 650 \mu\text{m}$  sampled in winter in the canyon – increased between February and September, with no significant differences between sites (Figure 7).

## Discussion

The data presented in this study indicate that there were no major differences in the population structure and reproductive biology of *Pasiphaea multidentata* at the 3 study sites in the Blanes canyon and adjacent margin in the NW Mediterranean. The population structure followed similar trends in the 3 sites, with non-reproductive individuals ( $CL < 30 \text{ mm}$ ) present in the populations collected in spring and summer and large mature individuals ( $CL > 30 \text{ mm}$ ) dominating in autumn and winter. This coincides with the population structure described by Company et al. (2001) for a population of *P. multidentata* from the Barcelona margin. The populations of *P. multidentata* were dominated by females at the three sites and all seasons. The depth of maximum abundance for *P. multidentata* is 550 m, with a depth distribution range between 128 and 2261 m. It has been shown that large individuals of *P. multidentata* ( $CL > 30 \text{ mm}$ ) stay close to the seafloor during day and night, undergoing only short diel migrations (Company et al., 2001; Aguzzi et al., 2007), while females are commonly found deeper in the population's distribution range (Cartes and Sarda, 1992; Cartes et al., 1993). This could explain

the higher proportion of females sampled at all sites and also observed by Company et al. (2001) in the Barcelona population. The dominance of females has also been described for the deep-water red shrimp *Aristeus antennatus*, which is the main crustacean target for Mediterranean fisheries to which *P. multidentata* is associated. Studies on spatio-temporal distributions of *A. antennatus* have shown that this species in the NW Mediterranean has important sex and size distribution segregations, with a higher proportion of males and juveniles between 1000-2200 m depth (Sarda and Cartes, 1993) and a higher proportion of females above 1000 m depth. *A. antennatus* has a seasonal reproductive pattern, with maturation of gonads in spring and summer (Demestre and Fortuño, 1992) and it has been shown that the proportion of reproductive females increases on the middle slope (~650 m) in spring and summer, while between late summer and early winter the population spreads over entering shallower waters in the canyons (Sarda et al., 1994; Sarda et al., 1997). Although *P. multidentata* has also a seasonal reproductive cycle, with mature individuals in winter (Company et al., 2001), the present results do not show a spatio-temporal variation in population structure similar to that observed for *A. antennatus*. While *A. antennatus* is a dominant benthic species on the upper and middle slope, *P. multidentata* is bathypelagic with extensive diel vertical migrations, mainly on the small and medium size individuals, while the large specimens stay close to the bottom even at night (Cartes et al., 1993). Because *A. antennatus* has a strong benthic character, the spatio-temporal variations in its population distribution can be related to seafloor characteristics, such as seasonal variations in food availability and intra-specific competition for limited resources (Sarda et al., 1997). In the case of *P. multidentata*, its benthopelagic behaviour may explain the lack of differences in population structure between sites, as the species is less linked to seafloor processes.

The gross morphology and microscopic examination of ovaries confirmed the seasonal reproductive cycle of *P. multidentata* from the NW Mediterranean, previously reported by Company et al. (2001) from gross morphology analyses in a population on the slope off Barcelona. The patterns are similar in



the Blanes and Barcelona populations, with a clear active reproductive period during the late summer and autumn months. Ovary maturation starts in late spring and the highest proportion of females with well developed ovaries are found in summer. The patterns were also similar in the 3 study sites, but the population sampled on the canyon wall seems to have a slight delay in the maturation of females as the highest proportion of females with well developed ovaries (stage V) was found in the autumn samples instead of summer (WHY?). The seasonal reproductive trend is clearly shown in the histological analysis of oocyte development. The gametogenesis is typical of caridean shrimp. The oogenic processes in decapods comprises a proliferative phase where oogonia are produced by mitotic divisions in the germinal epithelium and a differentiative phase where a sexual response triggers the onset of gametogenesis and the development of oogonia into previtellogenic oocytes by meiotic divisions (Adiyodi and Subramoninan, 1983; Baeur, 2004). A pool of previtellogenic oocytes was present in the ovaries of *P. multidentata* at all times in all sites. In spring, the distribution of oocyte-sizes becomes slightly bimodal, indicating the start of vitellogenesis, which starts at oocytes sizes of around 180  $\mu\text{m}$  and is characterised microscopically by the presence of yolk granules in the cytoplasm. The developing oocytes are surrounded by a mono-layer of accessory cells involved in the transfer of nutrients into the growing cells. The proportion and size of vitellogenic oocytes in *P. multidentata* increases from spring to autumn, with the largest oocytes observed measuring between 1200-1400  $\mu\text{m}$ . The large size of mature vitellogenic oocytes ( $> 1400 \mu\text{m}$ ) corresponds to the egg size (1870  $\mu\text{m}$  in length) previously reported from ovigerous females (Company et al., 2001). This egg size is indicative of lecithotrophic larval development with a reduction in the number of larval stages (Baeur, 2004). Abbreviated larval development provides a higher larval survival probability and is typical of deep-sea caridean shrimp (Baeur, 2004). Deep-sea larvae are exposed to fluctuations in food supply and high predation risk during their planktonic phase in the water column. Large, advanced larvae have the necessary reserves to develop to juvenile through a smaller number of instars, decreasing the time spent in the water column and therefore decreasing mortality risk (King and Butler, 1985; Clarke and Gore, 1992;

Ramirez-Llodra, 2002; Baur, 2004). We do not know the larval and recruitment stages of *P. multidentata*, and in general very little is known about larval ecology of deep-sea species (Young, 2003). Future studies focusing on these first stages of the life cycle of deep-sea species will provide essential information to understand the maintenance of a population and dispersal potential. This would be especially valuable when analysing commercial and associated species with the aim to develop management options.

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## Legends

**Figure 1.** Map showing the bathymetry of the area around the Blanes canyon and the location of the sampling sites.

**Figure 2.** Population structure of *Pasiphaea multidentata* from the Blanes canyon area in Spring, summer, autumn and winter. Black squares, margin; Dark grey triangles, canyon wall; Grey circles, canyon head; m w, c = sample size at the margin, wall and canyon sites.

**Figure 3.** Sex ratio of *Pasiphaea multidentata* from 3 sites in 4 seasons. A, margin; B, canyon wall; C, canyon head. Plain bars, females; Dashed bars, males.

**Figure 4.** Percentage frequency of each ovary maturation stage of *Pasiphaea multidentata* in 3 sites and four seasons. A, margin; B, canyon wall; C, canyon head. SPR, spring; SUM, summer; AUT, autumn; WIN, winter. Light grey, resting stage (II); Dashed grey, initiation of maturation (III); Dark grey, maturing ovaries (IV); Black, mature ovaries (V).

**Figure 5.** Histological sections of ovaries from *Pasiphaea multidentata*. A, section showing a new cohort of previtellogenic oocytes developing amongst vitellogenic oocytes; B, section of a developing ovary with previtellogenic and early vitellogenic oocytes; C, section showing early vitellogenic oocytes; D, section showing vitellogenic oocytes surrounded by accessory cells. ac, accessory cells; gvo, growing (early) vitellogenic oocytes; lg, lipidic granule; pvo, previtellogenic oocyte, vo, vitellogenic oocyte.

**Figure 6.** Oocyte size distribution of *Pasiphaea multidentata* from 3 sites (margin, wall and canyon) and 4 seasons. A, winter; B, spring; C, summer; D, autumn.

**Figure 7.** Mean size of vitellogenic oocytes of *P. multidentata* in the 3 sites and 4 seasons. Black square, margin; Dark grey triangle, canyon wall; Grey circle, canyon head.

**Table 1.** Sampling sites, date and depth. M, margin (41.6°N 2.8°E); W, wall (41.5°N 3.0°E); C, canyon (41.6°N 2.8°E).

<b>Date</b>	<b>Site</b>	<b>Depth (m)</b>
<b>Spring</b>		
14/04/2003	M	600
	W	585
	C	585
29/05/2004	M	667
	W	603
	C	NS
<b>Summer</b>		
20/08/2003	M	700
	W	520
	C	364
<b>Autumn</b>		
30/09/2003	M	631
	W	567
	C	502
17/12/2003	M	512-530
	W	402-512
	C	475-512
<b>Winter</b>		
03/02/2004	M	512
	W	384-510
	C	512
10/03/2004	M	640
	W	567
	C	585
<b>Spring</b>		
21/04/2004	M	695
	W	585
	C	NS

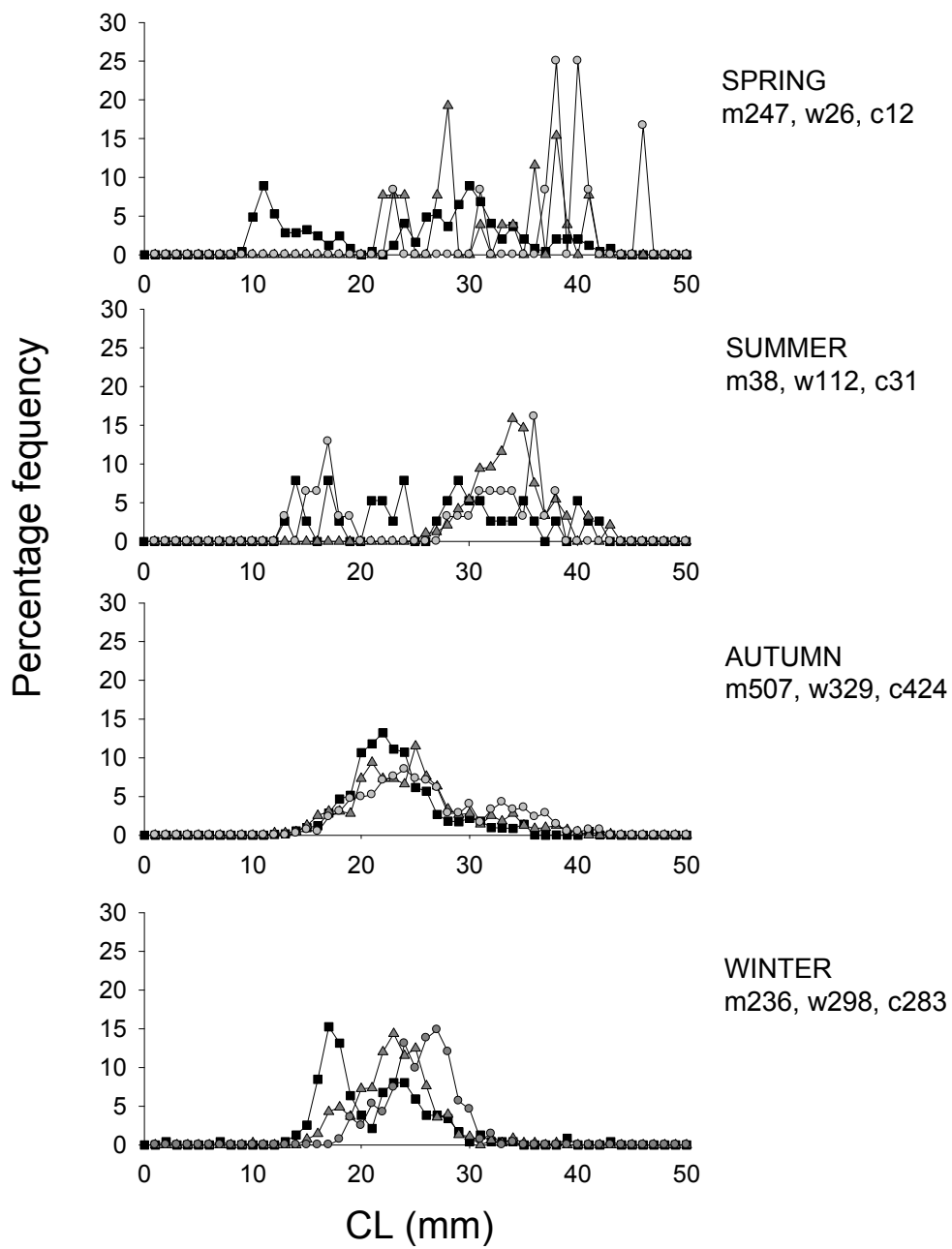
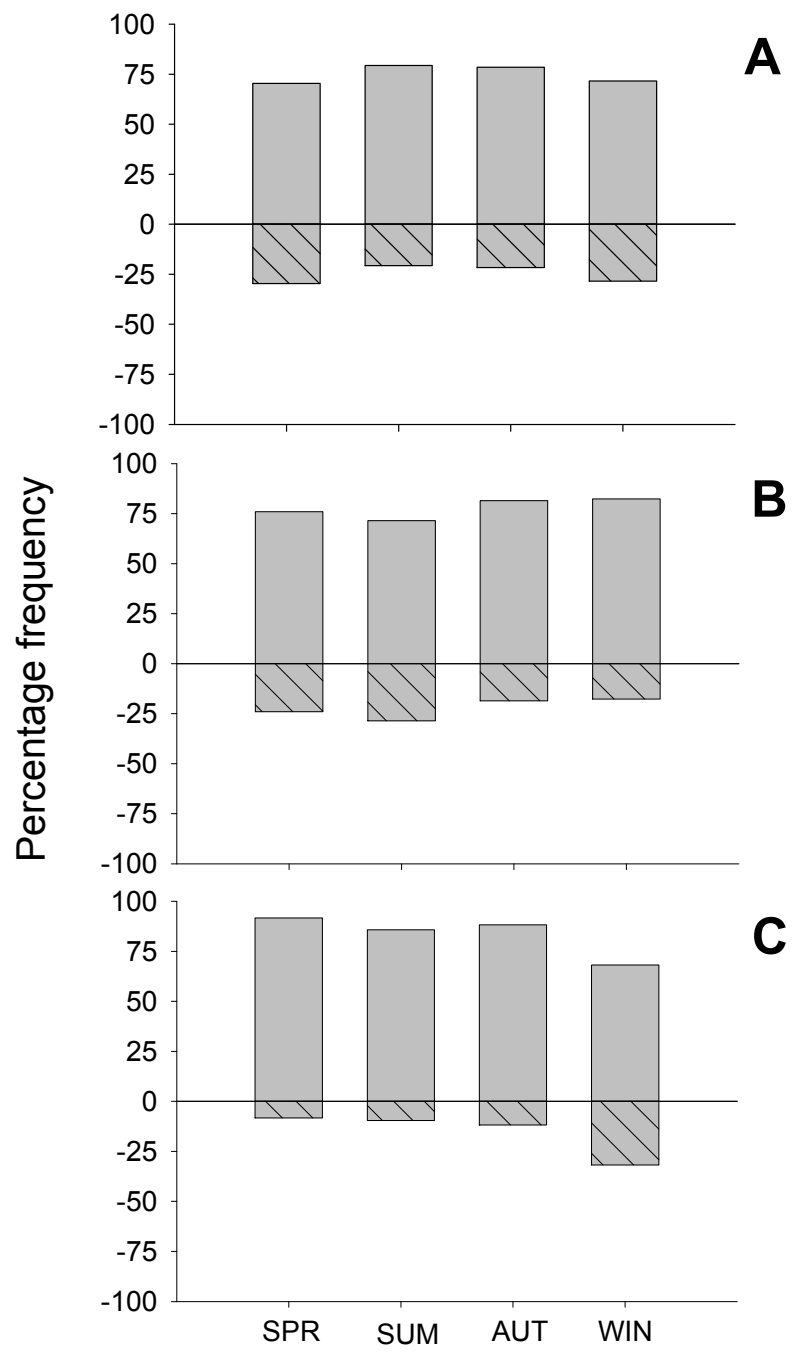
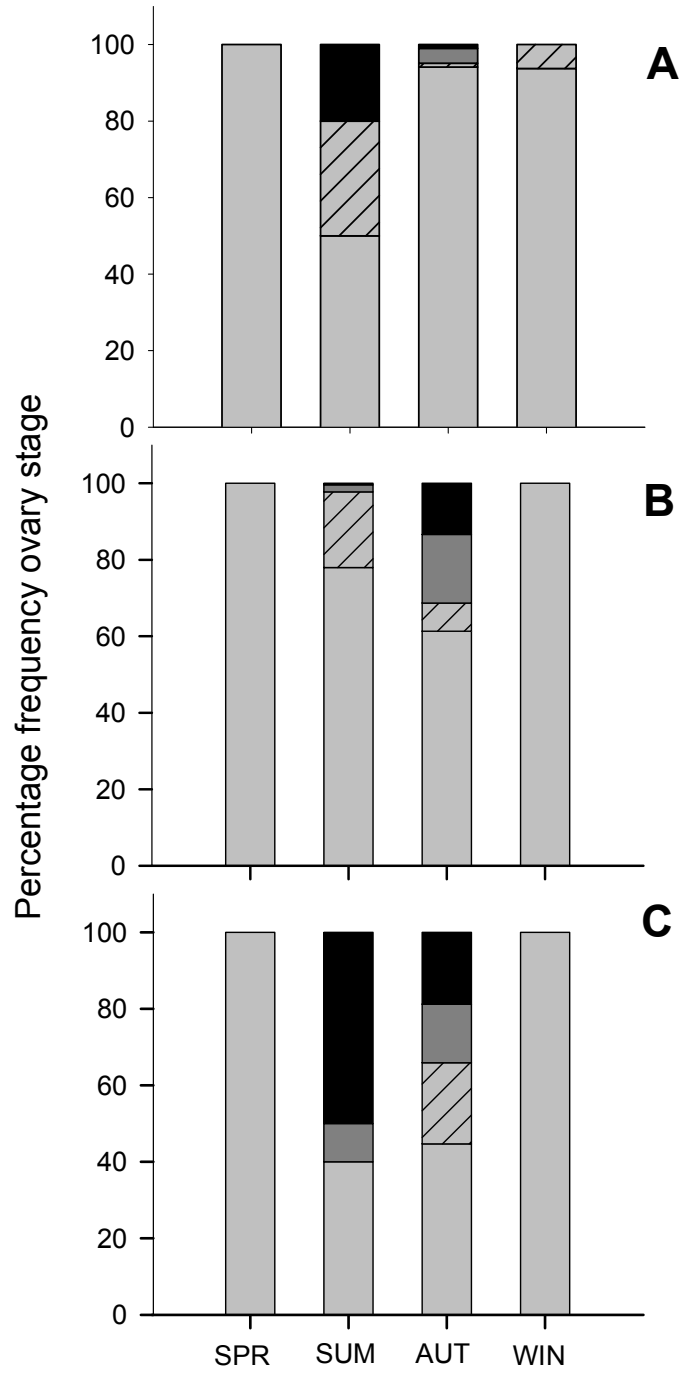


Figure 2. Ramirez-Llodra et al.



**Figure 3.** Ramirez-Llodra et al.





**Figure 4.** Ramirez-Llodra et al.

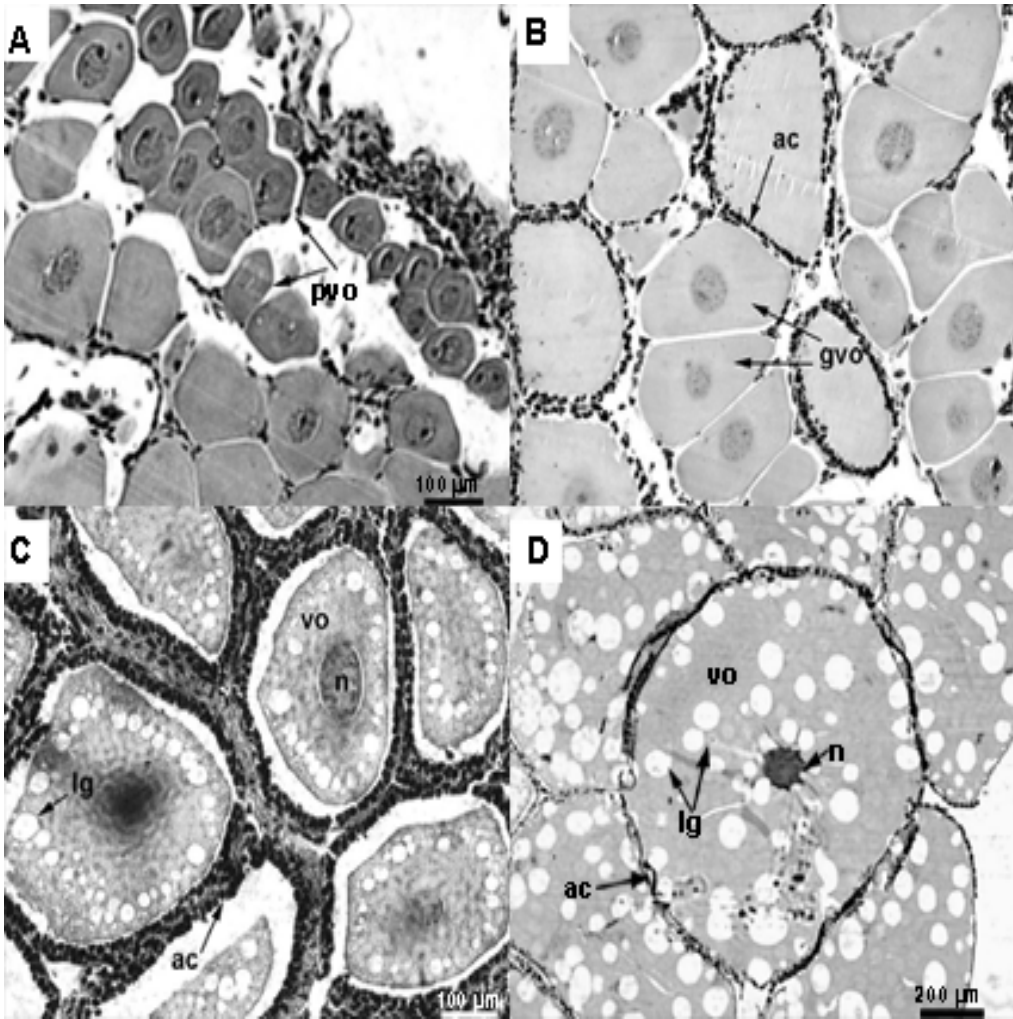
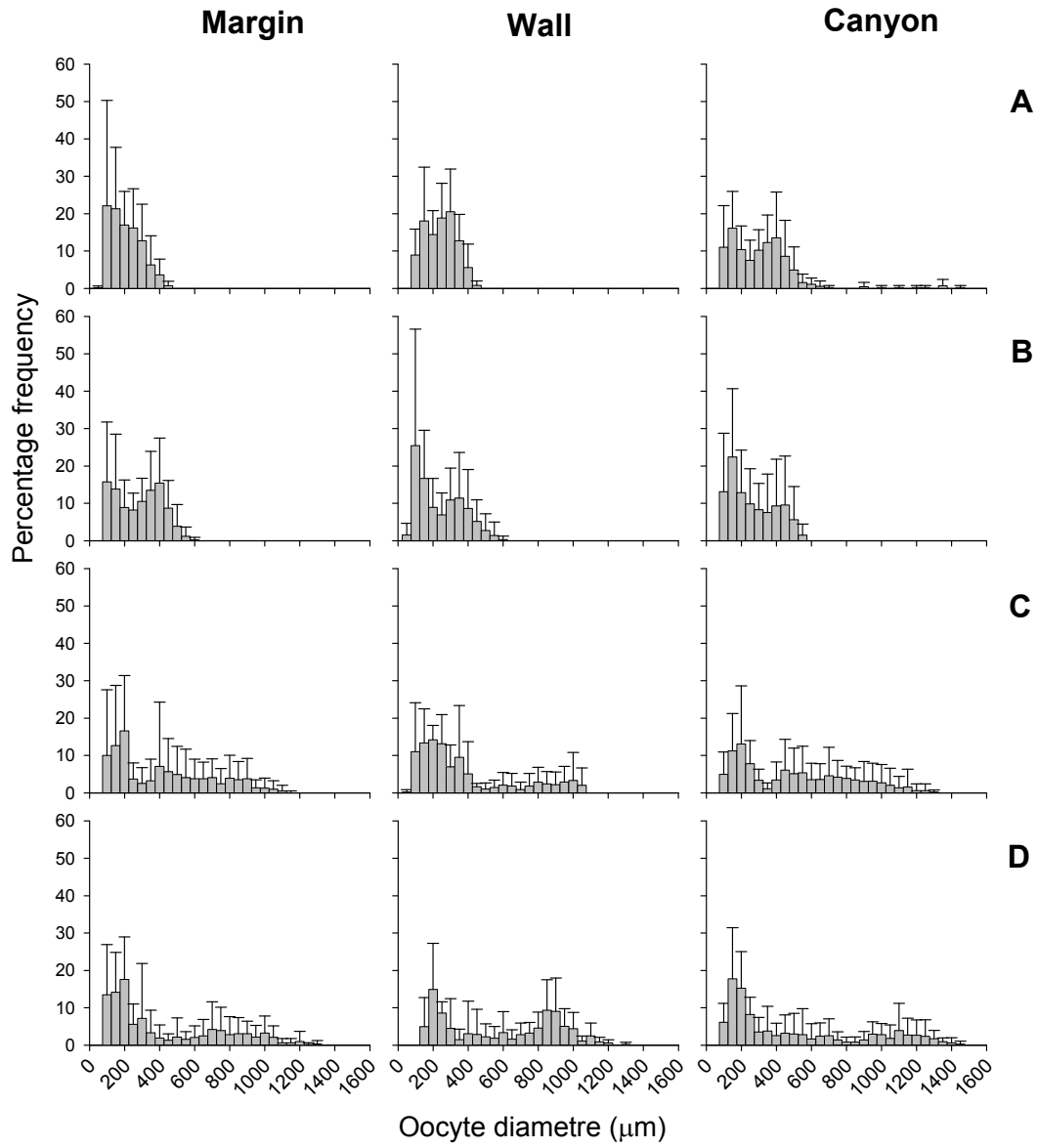
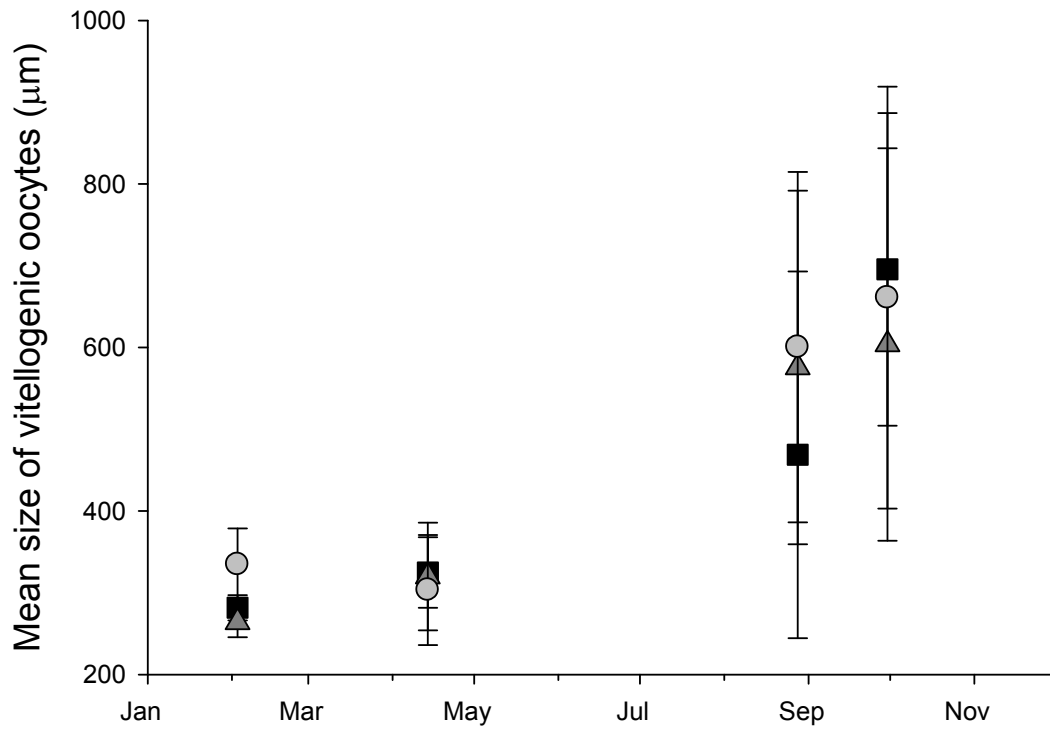


Figure 5. Ramirez-Llodra et al.



**Figure 6.** Ramirez-Llodra et al.



**Figure 7.** Ramirez-Llodra et al.