THE ECOLOGY, BIOLOGY AND TAXONOMY OF *ADDISONIA EXCENTRICA* (TIBERI, 1855) (COCCULINIFORMIA: ADDISONIIDAE) FROM SOUTHERN SPAIN

ELISA ROLDÁN[1] AND ÁNGEL A. LUQUE[2]

[1]*Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, C/ Moll de Ponent, s/n, Apdo. 291, 07015 Palma de Mallorca, Spain; and* [2]*Laboratorio de Biología Marina, Departamento de Biología, Universidad Autónoma, 28049 Madrid, Spain*

Correspondence: Á.A. Luque; e-mail: angel.luque@uam.es

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ABSTRACT

This study of *Addisonia excentrica* (Tiberi, 1855) is the first on the population dynamics of any cocculiniform limpet. We conducted 2 years of monthly sampling by trawling off the coast of Malaga province, Spain (Western Mediterranean), at 47–311 m. We sampled 482 egg cases of the lesser spotted dogfish (*Scyliorhinus canicula*), in which *A. excentrica* lives and feeds, which yielded 987 specimens of *A. excentrica*. Of capsules containing *A. excentrica*, 70.3% were collected between 50 and 100 m depth, where the mean percentage of occupied capsules (incidence) was 32.8%; the greatest incidence was found between 100 and 150 m (42.1%). The maximum depth of living *A. excentrica* was 222 m. In samples collected at 50–100 m recently settled individuals (<2 mm) predominated throughout the year. Larvae and apohermaphroditic specimens with mature oocytes (>7.2 mm) were scarce, but they were also present almost all year round, suggesting continuous reproduction. A positive correlation between the relative age of the egg case and the mean length of limpets was found. The long embryonic development of dogfish and the slow decay of egg cases provides enough time for larvae or young individuals to mature within a single egg case. Once mature, the limpets leave the capsule and spawn on the nearby bottom. Continuous reproduction and aggregated egg laying areas of the dogfish may improve the chances of larvae finding the egg cases during what is presumably a short larval life, and facilitate emigration of juveniles or large specimens to neighbouring capsules. *Addisonia enodis* Simone, 1996 is considered to be a junior synonym of *A. excentrica*. The larval shell of *A. excentrica* is described; it is very similar in shape and sculpture to the larval shell of *Lepetella* species, but larger. Newly settled specimens start to discard the protoconch at a size smaller than 0.4 mm.

INTRODUCTION

The cocculiniform family Addisoniidae Dall, 1882, includes two subfamilies, Addisoniinae and Helicopeltinae Marshall, 1996. The former subfamily consists of a single genus, *Addisonia* Dall, 1882, which currently comprises three species: *A. brophyi* McLean, 1985 from the eastern Pacific, *A. excentrica* (Tiberi, 1855) from the Mediterranean and Atlantic, and *A. enodis* Simone, 1996 from the south Atlantic.

Addisonia excentrica is one of the best-known of lepetelloidean Cocculiniformia, but its biology and population dynamics are still largely unknown. It lives inside the egg cases of *Scyliorhinus canicula* (lesser spotted dogfish or small-spotted catshark) and several rays (*Raja*) species, on which it feeds (Villa, 1983; Ragozzi, 1985). *Addisonia excentrica* exhibits protandrous gonad development (Roldán & Luque, 1999). Specimens larger than 2.6 mm have ripe male sperm and the seminal tract is well developed (Warén, 1996; Roldán & Luque, 1999), and can be considered functional males. Specimens over 7.2 mm long have vitellogenic oocytes and mature sperm at the same time according to Roldán & Luque (1999) and are considered simultaneous apohermaphrodites (Ponder & Lindberg, 1997). Larval development is deduced from the larval shell to be lecithotrophic (Dantart & Luque, 1994; Roldán & Luque, 1999). The dogfish embryo developing within the capsule can coexist with *A. excentrica* specimens apparently without suffering any damage (Dantart & Luque, 1994).

The genus *Addisonia* was described by Dall (1882a), with the new species *A. paradoxa*, from the eastern Atlantic, as the type. Shortly afterwards, a second species, *Gadinia excentrica* Tiberi, 1857, was included by Dall (1882b), who suggested that the two could be synonyms. Jeffreys (1883) considered the two synonymous, under the name *A. excentrica*. However, Dautzenberg (1886) combined these species with *Gadinia lateralis* Réquien, 1848, under the name *A. lateralis*. Subsequently, Dall (1889) considered *A. paradoxa* to be just a larger variety of the European species. McLean (1985) revised the taxonomy of the genus and described a third species, *A. brophyi*, from the eastern Pacific. The only differences between *A. lateralis*, *A. paradoxa* and *A. brophyi* are in the size, shape and proportion of some radular elements. McLean (1985) retained the three species, while recognizing that it was equally reasonable to consider them as subspecies of a single, widely distributed species.

Dantart & Luque (1994) showed that *Gadinia lateralis* is a synonym of the pulmonate *Trimusculus mammilaris* (Linnaeus, 1758). Since Haszprunar (1987, 1988) had found no significant differences between the internal anatomies of *A. paradoxa* and *A. excentrica* and because the external anatomy and radula of both species were very similar, Dantart & Luque (1994) concluded that the former was a synonym of *A. excentrica* (Tiberi, 1857), but maintained *A. brophyi* as a distinct species on the basis of radular differences. Warén (1996) amended the year of description of *A. excentrica* to 1855. Simone (1996) described a new species, *A. enodis*, from the south coast of Brazil, which was distinguished by its larger size, less developed radial sculpture of its shell, relative location of the pericardial structures, shape

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of the gill, the edge of the mantle and intestine, and differences in radular morphology.

This paper presents a first contribution to the study of population dynamics of *A. excentrica*. The habitat presents obvious sampling problems, but data were obtained over 2 years as part of the daily activity of a fishing trawler from La Caleta de Vélez (Malaga).

Although the external and internal anatomy of *A. excentrica* is well known (McLean, 1985; Haszprunar, 1987; Dantart & Luque, 1994; Warén, 1996), the recent description of *A. enodis* Simone, 1996, has prompted us to review some anatomical aspects, including those relating to the gill, whose development with growth was described by Roldán & Luque (1999). Information about the larval shell, additional to that contributed by Dantart & Luque (1994) and Roldán & Luque (1999), is also presented here.

MATERIAL AND METHODS

All specimens of *Addisonia excentrica* studied were located inside 593 egg capsules of the lesser spotted dogfish (*Scyliorhinus canicula*) collected from February 2000 to December 2001 at depths between 47 and 311 m off the coast of the province of Malaga (Spain, Western Mediterranean) by the trawler *Algornaga*. Sampling was performed within the area located between El Placer de las Bóvedas (36°24′00″N, 04°59′00″W) and Playa de Burriana (36°43′58″N, 03°52′01″W). Daily trawling effort was similar throughout the sampling period; the trawler worked from 6 a.m. to 6 p.m. from Monday to Friday, with two or three hour-long trawls per day depending on depth and distance from the port (La Caleta de Vélez). Changes in fishing practice (trawling for shrimp in deeper waters) and poor weather may have affected catches of dogfish egg cases during some months. Trawling did not take place during closed fishing seasons (June and July, 2000; May, 2001) or bad weather. The egg cases of *S. canicula* were chosen for study because they were much more abundant in the area than those of *Raja* species. Eggs just trawled were preserved in 70% ethanol, and labelled with locality data (GPS coordinates) and depth.

During the first 6 months of sampling, 234 egg cases from the entire range of depths were studied. After this, the study concentrated on the interval between 50 and 100 m, since this was the range with greatest abundance of egg cases and was sufficiently narrow for the environmental conditions to be considered reasonably constant.

In total, 482 egg cases from these depths were examined over the 2 years of sampling, corresponding to a rate of about 30 per month. Fewer egg cases were studied in five of the months when captures were scarce, and none were obtained during a further 5 months (June and July 2000; March, April and May 2001) due to changes in fishing tactics of the boat and closed fishing seasons.

In total, 987 specimens of *A. excentrica* were studied under the stereomicroscope. Fifteen specimens of shell length between 0.4 to 4.1 mm, from 11 of which the radula was extracted, were examined under scanning electron microscope (SEM, Philips XL20 and Philips XL30). Eleven larvae were also studied under SEM. Soft parts were critical-point dried before coating with gold.

Gonad development was studied from February 2000 to February 2001, using histological sections of the gonad of three adult specimens (more than 7.2 mm length, according to Roldán & Luque, 1999) per month, except for September and October 2000, when suitable adult specimens were not found. Specimens were embedded in Paraplast and cut in 7-μm sections, then stained with Mayer's haematoxylin–eosin. Ovary maturity grade was defined according to Roldán & Luque (1999). The largest diameter of oocytes with observable nucleolus was measured with an ocular micrometer in 20 histological sections per specimen at a magnification of 200 times.

Statistics were performed using STATISTICA and SPSS 11 Windows work packages.

RESULTS

Population dynamics

Of all egg cases 70.3% were collected from depths between 50 and 100 m, and 75.0% of the cases collected in this bathymetric range contained *Addisonia excentrica*. The highest percentage occupation (incidence) (42.1%) was recorded between 100 and 150 m (Fig. 1). The greatest depth at which an egg case was collected was 311 m, but the maximum depth at which a living specimen of *A. excentrica* was collected was 222 m.

A total of 851 specimens was found within egg cases collected between 50 and 100 m; 13 of these had only the larval shell and 36 retained the larval shell after the teloconch had partially developed. The mean percentage of egg cases occupied by *A. excentrica* was 32.8% (SD = ±0.01), very similar to that recorded in the first 6 months for the 50–100 m interval (33.0%). The greatest incidence (53.3%) was recorded in April 2000, the only month when the number of occupied egg cases exceeded the number of empty cases, although the difference was not statistically significant ($\chi^2 = 0.436$, $P < 0.509$). In October 2000 the percentages were equal, and almost equal in February 2001. The lowest percentage was recorded in July 2001 (23.3%).

Due to the impossibility of determining the time the egg cases of the dogfish had spent on the seabed since they were deposited, a relative age for the cases was established. The first categories were defined by the degree of development and maturity of the dogfish embryo. In hatched egg cases, the consistency (based on thickness, degree of flexibility, and number and severity of perforations) and transparency of the wall were used to define the degree of deterioration, which is assumed to be related to the time it has been in the water. Eight relative ages of egg cases were defined: (0) only yolk, embryo not differentiated; (1) embryo in initial stages of differentiation, visible to naked eye, egg case without respiratory slits; (2) embryo with external gill, smaller or same size as vitelline sac (≤3 cm), egg case with respiratory fissure or slits; (3) embryo with external gill, larger than vitelline sac (≥5 cm), egg case with respiratory slits; (4) embryo with internal gill; (5) embryo nearly mature, with pigmentation; (6) egg case with hatching

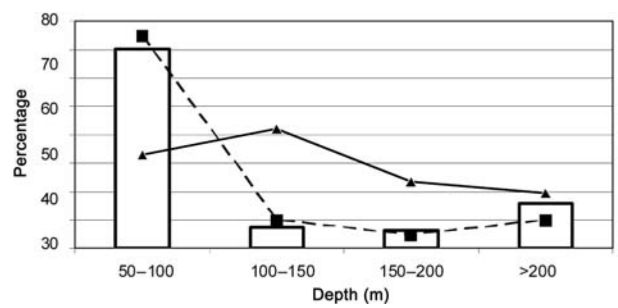

Figure 1. Distribution of egg cases of *Scyliorhinus canicula* by depth and percentage of occupation (incidence) of *Addisonia excentrica*. Histogram, percentage of egg cases; square, percentage of egg cases with *A. excentrica*; triangle, incidence of *A. excentrica* (number of egg cases with *A. excentrica*/number of egg cases at depth interval, as percentage). $n = 234$ (number of egg cases collected during first 6 months, when egg cases from all depths were considered).

slit; transparency, thickness and flexibility similar to cases containing an embryo; (7) egg case translucent, but with thinner and less flexible walls, with perforations; (8) egg case opaque, wall thin and easily deformed, with perforations and missing pieces. The degree of coverage of the egg case with epibionts was not correlated with the stage of development of the embryo [Spearman's $R = -0.012$, $n = 115$, $t(n-2)$, $P = 0.903$], so this was not considered useful as an indicator of the age of the egg cases.

There were significant differences in the abundance of the egg cases of different relative ages among the months sampled (Kruskal–Wallis, $\chi^2 = 39.102$; $P = 0.001$). Each sampling year was homogeneous and there were no differences between corresponding months (Kruskal–Wallis, 2000, $H = 12.198$, $P = 0.094$; Kruskal–Wallis, 2001, $H = 11.013$, $P = 0.201$), although there was a significant difference between both years (Kolmogorov–Smirnov, $\mathcal{Z} = 1.939$; $P = 0.001$). In 2000 the older egg cases (categories 5, 6, 7) were more abundant (Fig. 2), while in 2001 the youngest egg cases (categories 0, 1, 2) and those of category 5 were the most common. In both years, egg cases of category 5 were the most abundant and those of categories 3 and 4 were the rarest, with similar values (Kruskal–Wallis $H = 51.740$, $P = 0.000$). The highest incidence of *A. excentrica* was observed in egg cases of category 3 in 2000, and of category 6 in 2001. No egg cases of category 8 were found between 50 and 100 m.

Egg cases of relative ages 0 and 1 were colonized by *A. excentrica* despite the absence of respiratory slits. One example was found in each category: one small specimen (0.42 mm long) in March 2000 and another (0.64 mm long) in May 2000.

The population size of *A. excentrica* within a single egg case varied from 1 to 113 individuals (one egg case in April 2000) (Fig. 3), although in 55.5% of the months sampled there were no egg cases with more than 10 specimens per egg case (mean = 4.519; SD = ± 10.260). No significant monthly differences were found between the numbers of specimens per egg case (Kruskal–Wallis: $\chi^2 = 20.551$, $P = 0.247$).

The shell length of the specimens ranged between 0.26 and 10.13 mm. The 0–1 mm size class was the most abundant in 50% of the months sampled (Fig. 4). In February 2000 and 2001, those of 0–2 mm made up 50% of the sample, and 57.0% in December 2000. In September 2000, the 3–4 mm size class was the most abundant (33.3%), although 0–1 mm specimens appeared at a not much lower frequency (22.2%). In November of 2000, 79.4% of the specimens occurred in three size classes (0–1, 1–2 and 2–3 mm) at very similar percentages. Individuals from 1 to 2 mm predominated in June, August and September 2001. The percentage of the 0–1 mm class was the highest in April 2000 (85%) due to the single egg case containing 113 specimens, but even excluding this exceptional case this class continued to be predominant (62%) and the distribution of the other classes scarcely changed in this month. The only months with clearly bimodal distributions were August 2000 and July 2001. In August 2000 most specimens were *c.* 1 mm long, although there was another peak of abundance of those measuring 5 mm. In July 2001, the most abundant size class was 8–9 mm (23%), although 47.4% of specimens measured between 0 and 3 mm.

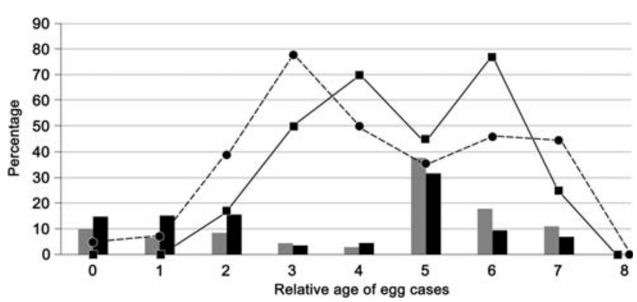

Figure 2. Percentage of different age categories (see Results) of egg cases of *Scyliorhinus canicula* collected during entire study, at 50–100 m depth. Grey, year 2000; black, year 2001; spot, incidence of *Addisonia excentrica* in 2000, square, incidence of *A. excentrica* in 2001. $n = 482$. Kolmogorov–Smirnov $\mathcal{Z} = 1.939$; $P = 0.001$.

The gonad of all specimens larger than 7.2 mm had mature sperm and vitellogenic oocytes (gonad maturity grade 5 or over and simultaneous apohermaphroditic, according to Roldán & Luque, 1999) throughout the year. Specimens longer than 9 mm showed more than 50% of vitellogenic oocytes and thus they were considered fully mature. The median largest diameter of vitellogenic oocytes was 81.25 μm (SD = ± 2.478).

The egg cases were often inhabited by specimens of different size (Fig. 6O). Of the studied egg cases, 36% contained specimens longer than 7.2 mm (simultaneous apohermaphroditic), but only 1.6% (eight egg cases) contained one (and no more) mature individual of at least 9 mm (February, March, April, May and September 2000, and June, July and November 2001). The number of specimens within each of these eight egg cases was low (a maximum of eight, usually smaller than 2 mm). Seven of these (87.5%) contained a potentially reproductive population, with at least one individual over 2 mm long (functional male).

A small number of larvae (16) was found throughout the 2 years of sampling in almost all months, except May, July, September and December, with a maximum of five larvae in April 2000. The juvenile specimens retaining the larval shell were also scarce (26), with a maximum of 11 specimens in the same month and year; none were found in February, July, September and November. Larvae and juveniles that retained the protoconch were rarer in 2001 (three larvae and three juveniles) than in 2000 (13 larvae and 23 juveniles).

There was a positive correlation between the relative age of the egg case and the mean length of specimens (Spearman's $R = 0.333$; $t(n-2) = 4.047$; $P \approx 0.000$; number of egg cases = 43, number of specimens = 142). Larvae were more numerous in egg cases of age 3 (Fig. 5). Sexually immature specimens (<2 mm long) predominated in egg cases of all ages, but they were exclusive in egg cases of ages 0 and 1 and more abundant in capsules of ages 4 and 6. Males (2–7.2 mm) appeared within egg cases aged 2–7, being less abundant in egg cases of age 7. Apohermaphroditic specimens (>7.2 mm) were found inside older egg cases (≥age 4), tending to increase in the oldest egg cases. Mature apohermaphrodites (>9 mm) only appeared in egg cases of ages 5–7.

Anatomy

The radial sculpture is only distinguishable under the stereomicroscope in shells larger than 9 mm. The concentric growth rings were always more or less apparent (Fig. 6A).

In 1-mm long specimens the first digitiform branchial leaves appear in the lower right area of the pallial cavity (Fig. 6D, E). The number of branchial leaves increases significantly with shell length (Roldán & Luque, 1999) and they gradually acquire the typical triangular shape seen in the adults (Fig. 6F–H), until they occupy the entire right side of the pallial cavity. The shape of the branchial leaves varies along the anteroposterior axis of the specimen: the anteriormost leaves are broader at the base and not as lengthened in the distal area, whereas in those towards the posterior the leaf bases are narrower and the distal end more lengthened. At the base of the gill (in the medial area) there is a fold that begins

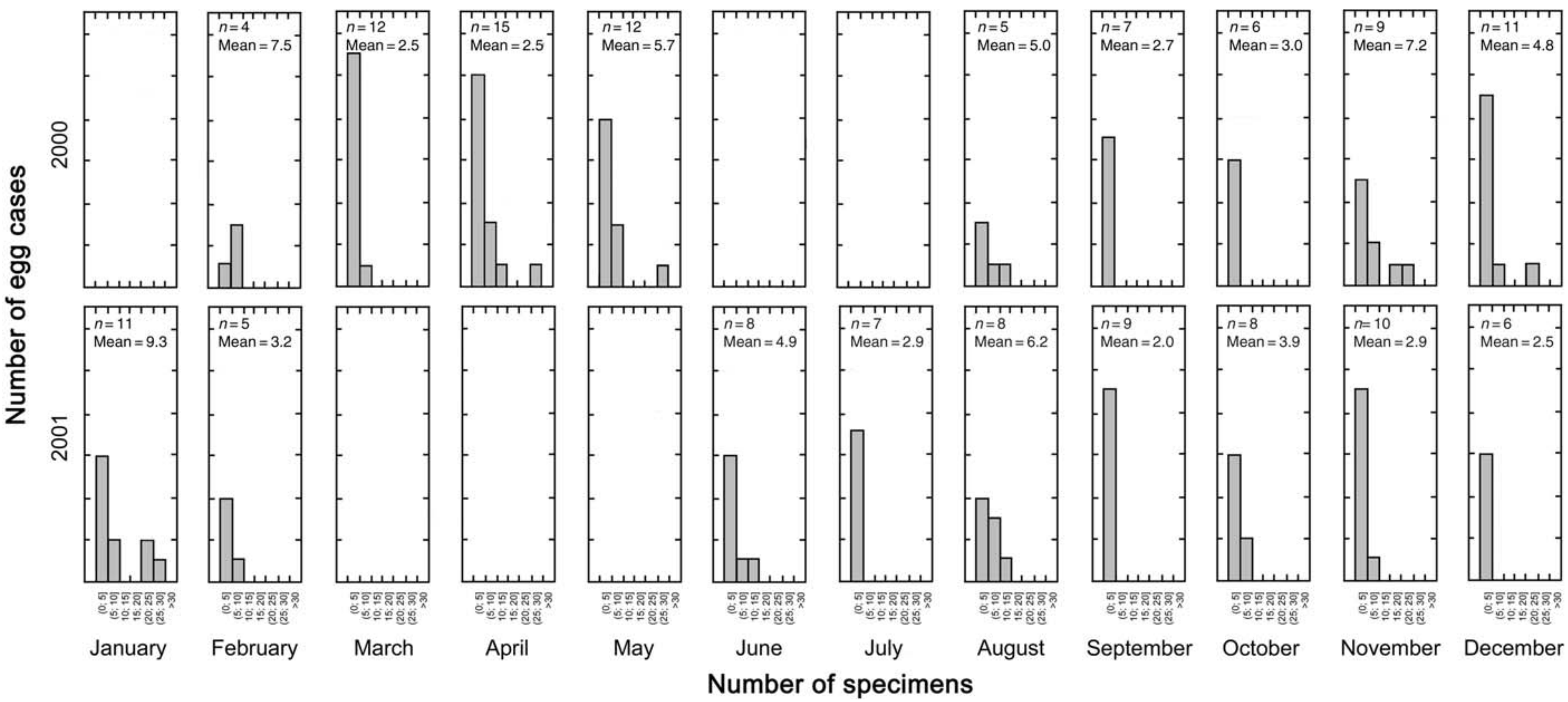

Figure 3. Population size of *Addisonia excentrica* (specimens per egg case collected during entire study, at 50–100 m depth). The outlying value of an egg case with 113 specimens (April 2000) has been excluded for clarity. Abbreviations: *n*, number of egg cases; mean, mean number of specimens per egg case. Total number of egg cases = 153.

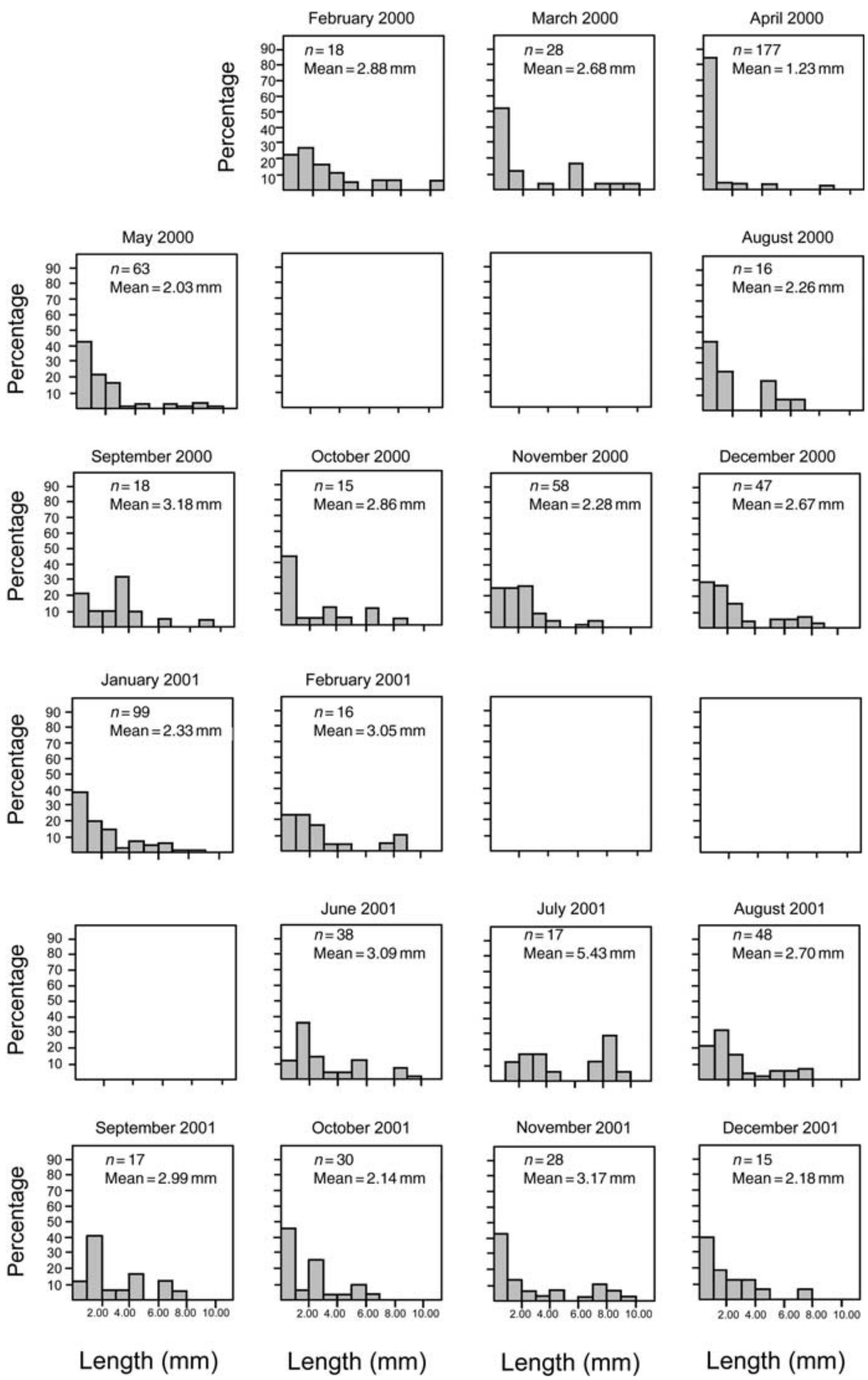

Figure 4. Monthly size distribution of *Addisonia excentrica* in 2000 and 2001 (mean length in mm). Total number of studied specimens = 748.

just behind the seminal canal and extends two-thirds of the length of the body (Fig. 6B).

The edge of the mantle is covered by papillae each with a distal tuft of cilia (Fig. 6I, J). A projection was observed on both the right and left sides of the mantle edge, as described for *A. enodis* by Simone (1996). Lateral pallial tentacles, like those described by Warén (1996), were not observed in any of the adult specimens.

Dorsal examination of specimens reveals (by transparency) the shell muscle, pericardial area (including auricle and ventricle), rectum, gonad and digestive gland (Fig. 6C). The distribution of these organs is as described in previous studies (McLean, 1985; Haszprunar, 1987; Dantart & Luque, 1994; Warén, 1996). Examination of the radula of 11 specimens of lengths between 0.7 and 4.1 mm yielded no new data, and confirmed that there are no ontogenetic changes in radular morphology (Fig. 6K–N).

Larval shell

As noted earlier, 16 larvae and 26 juveniles that retained their larval shell after their teleoconch had partially developed were

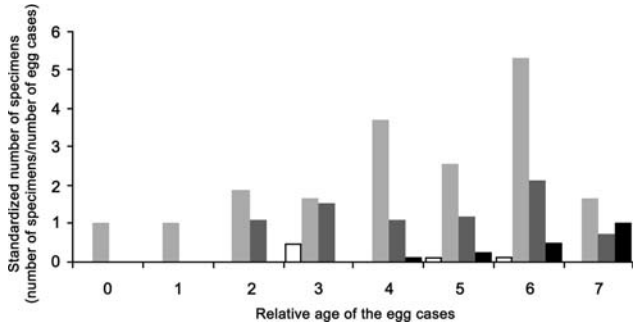

Figure 5. Relation between relative age of the egg cases and maturity of *Addisonia excentrica* specimens. White, larval specimens; grey, immature specimens (<2 mm); dark grey, male specimens (2–7.2 mm); black, mature simultaneous apohermaphroditic specimens (>7.2 mm). Number of egg cases = 144; number of specimens = 686.

found (Fig. 7A–I). One of these juveniles (0.1 mm) with only slight development of the teleoconch still had its operculum (Fig. 7B), which was absent in juveniles with a more developed teleoconch. Juveniles start to discard the larval shell at a size smaller than 0.4 mm, although a single specimen 1.76 mm long and 1.56 mm wide was found with the protoconch still attached (Fig. 7G, H), but larger specimens lacked the protoconch. The mean length of the larval shell was 0.31 mm (SD = ± 0.031), the mean width 0.21 mm (SD= ± 0.030) and the mean height 0.20 mm (SD= ± 0.022).

The study of the external anatomy and the radulae of these juveniles supports their identification as *A. excentrica*, and confirms that the larvae found together with the juveniles belong to this same species (Figs 6K, 7E–I).

DISCUSSION

Population dynamics

This is the first study of the population dynamics of a cocculiniform species. *Addisonia excentrica* is one of the best-known cocculiniforms, but there has not been any comparable study of such a large number of specimens and egg cases over a long period and within a narrow geographical area. Dantart & Luque (1994) described some biological aspects based on observations of 167 individuals from different locations (Catalonia, Malaga and Bissagos archipelago). Other studies considered fewer specimens (Warén, 1996), except for that of Gubbioli & Nofroni (1986), who examined 250 egg cases of *Raja clavata*.

Scyliorhinus canicula inhabits a wide bathymetric range, from 30 to 400 m, in the Mediterranean (Whitehead *et al*., 1986). Like other oviparous chondrichthyans (e.g. *Raja* species) its nursery areas are typically in shallower water than adult habitats (Wheeler, 1978; Ellis *et al*., 2005). Mature and laying females have been reported from 100 to 400 m in the Cantabrian Sea, being more abundant in the deepest levels (Rodríguez-Cabello, Velasco & Olaso, 1998), and between 44 and 523 m in the Alborán Sea, where more than 70% were concentrated within the ranges 0–100 and 200–400 m (J. Rey, personal communication). The results of the current study agree with the latter data, with most of the egg cases (70.3%) found between 50 and 100 m and the deepest collected at 311 m.

Addisonia excentrica was more abundant at depths between 50 and 100 m, which falls within the range (50–200 m) reported by Dantart & Luque (1994), although incidence was highest between 100 and 150 m. The incidence has been reported as *c*. 14% in egg cases of *R. clavata* (Gubbioli & Nofroni, 1986) and 50% in those of *S. canicula* (Dantart & Luque, 1994). The mean incidence for the 2 years sampled (32.8%) fell within this range, with a maximum (53.3%, April 2000) similar to the value obtained by Dantart & Luque (1994). Our results suggest that depth influences the distribution of *A. excentrica*, but a more detailed comparison is not possible, since previous studies have not reported the depth of collection.

The number of individuals per egg case found in this study varied from 1 to 113 (highest in a single egg case with numerous young specimens), with a mean of 4.5. Dantart & Luque (1994) found between two and six individuals, with a maximum of 26 young specimens, and Gubbioli & Nofroni (1986) reported a maximum of three to four specimens per egg case in *R. clavata*.

In the Mediterranean, female *S. canicula* constantly produce egg cases, with slight seasonal fluctuations. The percentage of females with eggs within the oviducts is relatively high in winter, increases in spring and reaches a maximum in summer, before declining slightly in autumn (Capapé, 1977; Capapé *et al*., 2008). The egg-laying season in British Atlantic waters also extends throughout the year (Sumpter & Dodd, 1979; Ellis & Shackley, 1997). The thornback ray (*Raja clavata*), whose egg cases are also inhabited by *A. excentrica*, lays egg cases almost throughout the year, except in April and August (Capapé *et al*., 2007).

The egg cases of *S. canicula* are closed when the embryo is small. As the embryo grows it develops external branchial filaments. According to Ballard, Mellinger & Lechenault (1993), beyond an embryo size of *c*. 40 mm, the mucopolysaccharides that cover the openings in the corners of the egg case dissolve and allow water to enter the case. However, we found functional openings in cases with embryos smaller than 30 mm. The duration of the period of development is variable and highly influenced by temperature, being longer at lower temperatures. In the Mediterranean it is between 180 and 271 days (Capapé, 1977), for which reason it is difficult to determine the actual time that an egg case has been in the water, even when the embryo is still inside. *Raja clavata* also has a long intracapsular development (112–144 days; Ellis & Shackley, 1995).

The egg cases of *S. canicula* have very effective anti-fouling protection. The process by which they become covered with macroepibionts is so slow that it outperforms commercial treatments (Thomason, Davenport & Rogerson, 1994; Thomason, Marrs & Davenport, 1996), and should ensure minimum coverage during embryonic development. However, in the egg cases examined there was no correlation between embryonic development and the coverage of epibionts. This was not due to a lack of coverage, because cases at all stages of embryonic-development exhibited epibionts. For this reason, we utilized relative age classes defined by the degree of embryonic development and state of preservation of the egg case.

The differences in abundances of the age classes of egg cases in the two sampled years may reflect variations in the reproductive success of *S. canicula*. However, stage 5 may encompass a longer period of time than the others, which would account for its greater abundance in both years.

The pattern of colonization of the egg cases by *A. excentrica* is one of the most enigmatic aspects of its ecology. Villa (1983) and McLean (1985) considered that they probably penetrate in the larval stage. Dantart & Luque (1994) suggested that young specimens could also enter through the egg case ventilation slits, close to where they are generally situated within the egg case, which agrees with our observations. However, the lecithotrophic development of the larva deduced from the protoconch (Dantart & Luque, 1994; Roldán & Luque, 1999) indicates that only a short time is available for the location of a scattered and not very abundant food-substrate. The

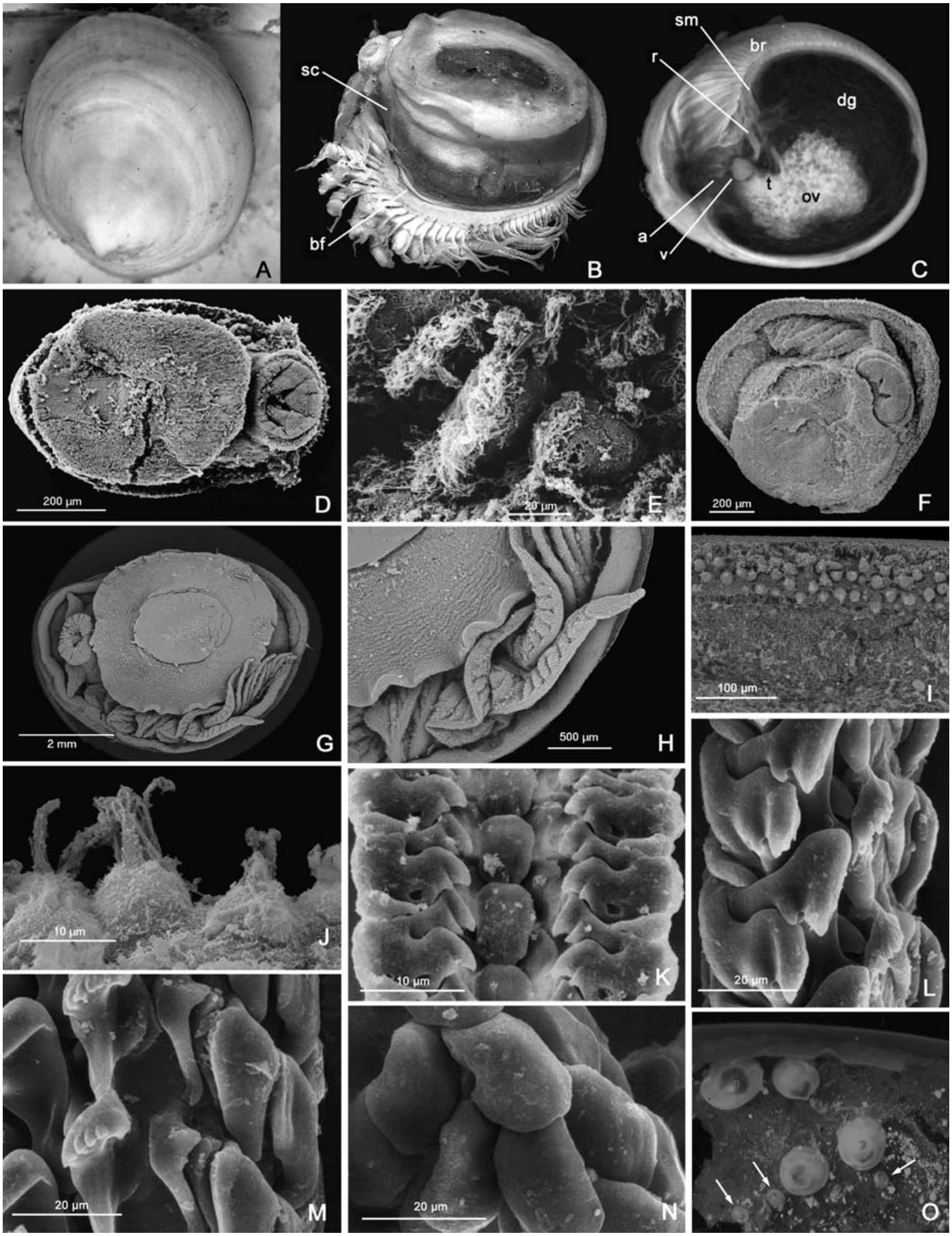

Figure 6. *Addisonia excentrica*. **A.** Dorsal view of a 10.12 mm (shell length) specimen attached to inner surface of an egg case of *Scyliorhinus canicula*. **B, C.** Ventral and dorsal view (without shell) of same specimen. **D.** Specimen of 1.02 mm (shell length) with three small branchial leaves. **E.** Detail of branchial leaves of this specimen (small laminae without a definite shape). **F.** Specimen of 1.64 mm (shell length), with nine branchial leaves with a shape similar to that of the completely formed gill. **G.** Specimen of 8.32 mm (shell length), with completely formed gill. **H.** Detail of branchial leaves of specimen in **G. I.** Papillae on edge of mantle (specimen of 8.3 mm shell length). **J.** Detail of some mantle edge papillae showing distal ciliary tuft (specimen of 1.52 mm). **K.** Radula of a 0.7 mm (shell length) specimen. **L**–**N.** Radula of a 4.1 mm (shell length) specimen. **O.** Partial inner view of a dogfish egg case showing four medium-sized and three small specimens (arrows). Abbreviations: a, auricle; bf, branchial fold; br, branchia; dg, digestive gland; ov, ovary; r, rectum; sc, seminal canal; sm, shell muscle; t, testicle; v, ventricle.

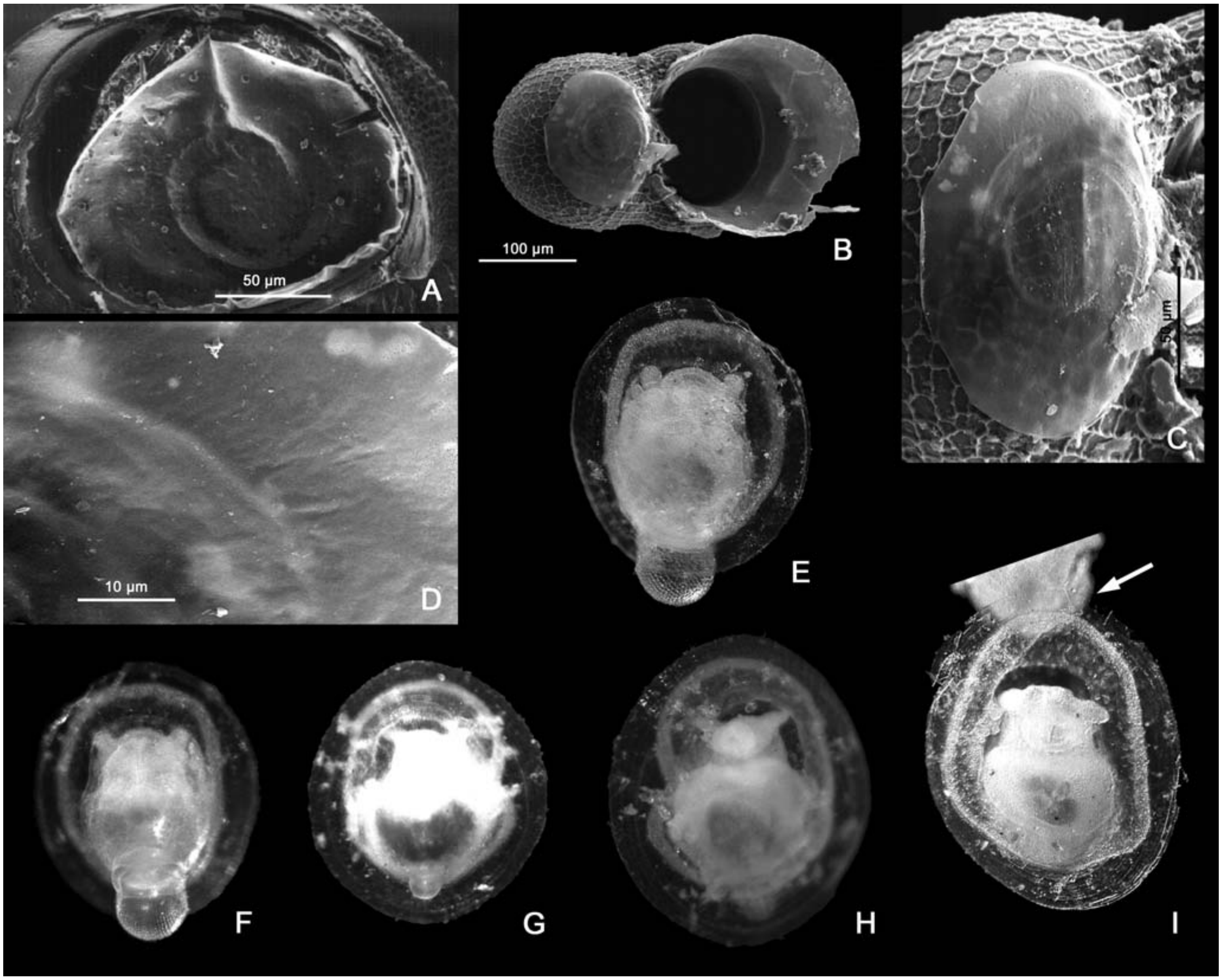

Figure 7. *Addisonia excentrica*. **A.** External view of operculum. **B.** Juvenile beginning to form teleoconch and retaining operculum. **C.** Internal view of operculum. **D.** Detail of internal wrinkle of operculum. **E–I.** Juveniles retaining protoconch. **E, F.** Ventral and dorsal views of a 1 mm (shell length) specimen. **G, H.** Dorsal and ventral views of a 1.76 mm (shell length) specimen. **I.** Ventral view of a 0.9 mm (shell length) specimen; the larval shell has detached and appears near the cephalic area of the animal (arrow).

protoconchs of many other cocculiniform species that also feed on scattered and scarce food substrates suggest that a short lecithotrophic development may be a common feature. The requirement of a hard substrate for fixing the egg cases of both dogfish and rays could determine places where they may accumulate. Ellis *et al*. (2005) detected certain sites where the largest catches of dogfish egg cases were associated with corals, bryozoans, hydroids and sponges. These specific laying areas, where egg cases could be replenished year after year, could facilitate colonization by the larvae of *A. excentrica*. This would also permit the emigration of juveniles to neighbouring egg cases and even of large specimens in the case of older egg cases (ages 6, 7 and 8), in which the perforations would facilitate their exit. The presence of specimens of different size within the same egg case (Fig. 6O) may indicate either successive larval settlements or immigration events.

Although the large-scale random sampling employed here, along with the disturbance of benthic habitats by intense trawling in the study area, decrease the ability to detect patchiness in the distribution of *A. excentrica* (Thrush & Dayton, 2002), we presume that both limpets and dogfish egg cases have aggregated distributions. This agrees with observations we have made on other lepetelloidean species (*Lepetella* species) and their food substrate (empty tubes of the polychaete *Hyalinoecia*, which also show a patchy distribution).

The mechanism by which young specimens enter egg cases that lack openings remains unknown. Larvae or very small specimens may be able to penetrate the mucopolysaccharide layer that closes the respiratory and posterior hatching slits of the egg cases. However, only young specimens (<2 mm long) were found inside egg cases of age 0 and 1 with no functional respiratory slits, whereas larvae were found mainly within egg cases of stage 3 in which the slits were just opened or even in older cases (stages 5–6). Villa (1985) and Dantart & Luque (1994) also reported adult specimens living within empty cases or those almost completely lacking yolk that were not yet fissured or perforated.

The population of *A. excentrica* exhibited scarcely any variation throughout the year in this study. It was mainly made up of small (<2 mm) recently settled specimens, representing more than 50% of the individuals in all months except September 2000 and July 2001. Older egg cases contained larger specimens, but mature apohermaphroditic individuals were uncommon throughout the sampling period, appearing only in 1.6% of the older egg cases (stages 5–7). Adult specimens have been found outside egg cases (Dantart & Luque, 1994), but their abundance may be understimated by sampling methods. The long embryonic developmental period of both dogfish and thornback ray, together with the apparently slow decay process of their egg cases, provides enough time for maturation of apohermaphroditic individuals within a single egg case. Apohermaphroditic specimens with mature oocytes, larvae and high percentages of recently settled individuals were found throughout most of the year, suggesting that *A. excentrica* has continuous reproduction. The host species *S. canicula* and *R. clavata* also show continuous or almost continuous reproduction, so larvae of *A. excentrica* have permanent access to their required food-substrate.

Nothing is known about spawning in *A. excentrica*. Neither spawn nor brooding limpets were found in any egg case and there is, as yet, no evidence for brooding in other Lepetelloidea or Cocculinoidea (Huys *et al*., 2002). The known facts lead us to suggest that once larvae or young individuals colonize a egg case, they remain inside until they reach maturity. Once mature they leave the case, which probably does not represent a suitable food supply and/or shelter as it ages and decays. Spawning may then occur on the nearby bottom, enabling larvae to find new egg cases during the presumably short larval life.

The close dependence of *A. excentrica* on egg cases of dogfish and thornback ray also has implications for conservation. These two species are fished intensively in the Mediterranean and European Atlantic (both as target species and bycatch), and in recent years their populations have been reduced in some areas. *Raja clavata* has been considered a Lower Risk/Near Threatened species since 2000 in the IUCN Red List (IUCN, 2009) and has been highlighted by the OSPAR Commission (2008) to receive greater protection in the North Sea. The conservation status of *S. canicula* seems to be better (status of Least Concern), since there is no evidence to indicate that the global population has declined significantly, although localized depletions have been reported in some areas (Cavanagh & Gibson, 2007; Gibson *et al*., 2008). There are no management programmes for sustainable fisheries of either species. The impact of trawling in disturbing suitable laying areas and removing egg cases (and limpets) is unknown, but may be high in intensively trawled areas such as that where this study was carried out, and should be added to the direct impact of fisheries.

Taxonomy

The description of *A. enodis* Simone, 1996, was based on a single specimen and differences from previously described species were poorly evaluated. No account was taken of the suggested synonymy of *A. paradoxa* and *A. excentrica* (Dantart & Luque, 1994; Warén, 1996). The size of the holotype of *A. enodis* (16.5× 14.0 × 9.3 mm) is within the range of both *A. excentrica* (maximum 17.0 × 14.0 × 8.0 mm) and *A. paradoxa* (20.3 × 16.0 × 10.5 mm; specimen from North Carolina recorded by McLean, 1985). The poorly developed radial sculpture reported by Simone (1996) is not a useful character in diagnosis, since it only appears vaguely in some large specimens of *A. excentrica* (Dantart & Luque, 1994; maximum size studied by these authors 12.0 × 9.5 × 4.5 mm). In the present study, a faint radial sculpture was observed under the stereomicroscope only in the largest specimens (>9 mm). Regarding external anatomy, the shape of the gill of *A. enodis* is very similar to that of *A. excentrica*. The fold in the medial zone of the gill and the projections of the mantle edge are also present in the specimens of *A. excentrica* examined in the present study. Moreover, the branchial leaves with lengthened distal arms illustrated in the original description of *A. enodis* are similar to those of *A. excentrica* (McLean, 1985; Haszprunar, 1987; Dantart & Luque, 1994; see Figs 6, 7), as is the position of the pericardial structures (Haszprunar, 1987; Fig. 6). Examination of the SEM photographs by Simone (1996) suggests that the supposed differences in the radula, with the shorter central tooth with a central depression and three lateral teeth clearly separated into three pieces in *A. enodis*, could be artifacts. Alternatively, since these observations were based on a single specimen, they could be a malformation or individual peculiarity. At least, the shape of the radula is much more like that of *A. excentrica* than that of the remaining congeneric species *A. brophyi* (McLean, 1985). Therefore, *A. enodis* Simone, 1996, is here considered to be a junior synonym of *A. excentrica* (Tiberi, 1855).

The study of the external anatomy of the juvenile specimens that retained their larval shell led to their identification as *A. excentrica*, thus confirming the previous descriptions of Dantart & Luque (1994) and Roldán & Luque (1999). These authors did not find protoconchs in specimens larger than 0.6 mm. However, in this study individuals up to 1.76 mm long retained the larval shell joined to the teleoconch. Larval shells of *Lepetella* species, as described by Warén (1991) are smaller, but have similar shape and homogeneous reticulate sculpture.

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