Journal of the Marine Biological Association of the United Kingdom, 2010, 90(2), 291–299.
doi:10.1017/S0025315409000940

Embryonic brooding and clonal propagation in tropical eastern Pacific cupuladriid bryozoans

AARON O´DEA[1], ANDREW N. OSTROVSKY[2,3] AND FELIX RODRÍGUEZ[1]

[1]Smithsonian Tropical Research Institute, MRC 0580-01, Apartado 0843–03092 Panamá, República de Panamá, [2]Department of Invertebrate Zoology, Faculty of Biology and Soil Science, St Petersburg State University, Universitetskaja nab. 7/9, 199034, St Petersburg, Russia, [3]Department of Palaeontology, Faculty of Earth Sciences, Geography and Astronomy, Geozentrum, University of Vienna, Althanstrasse 14, A-1090, Vienna, Austria

Colonial invertebrates often mix sexual and asexual methods of propagation, and a comprehensive understanding of both is required for life history study. The asexual cloning of new colonies in cupuladriid bryozoans is much better studied than the formation of new colonies by sexual reproduction. As such, the relative investments of sexual and asexual modes of propagation remain uncertain. This preliminary study explores patterns of embryonic brooding as a measure of investment into sexual reproduction. We conduct a survey of quantity and arrangement of embryos in tropical eastern Pacific cupuladriid colonies and compare this to the frequency of cloning. Species populations show considerable variation in embryonic brooding. Patterns of brooding, both across and within species strongly support the hypothesis that as cloning increases, investment into sexual reproduction decreases. We find preliminary evidence that individual cupuladriid colonies that propagate sexually may senesce like solitary organisms, while species that regularly clone only appear to experience senescence at the level of the zooid.

Keywords: embryonic brooding, clonal propagation, tropical eastern Pacific, cupuladriid bryozoans

Submitted 3 November 2008; accepted 4 March 2009; first published online 3 August 2009

INTRODUCTION

Like many aquatic colonial invertebrates, members of the bryozoan family Cupuladriidae are able to produce new colonies by both sexual and asexual processes (Hughes & Jackson, 1980; Winston, 1988; O'Dea *et al.*, 2004). Sexual propagation occurs via the formation of a founding larva (McKinney & Jackson, 1989) while asexual or clonal propagation occurs via colony breakage, autofragmentation or budding (O'Dea *et al.*, 2008). Recruitment in some species is entirely sexual and in others it is almost entirely clonal, while the majority of species make use of the two modes of recruitment in varying proportions (Baluk & Radwanski, 1977; Winston, 1988; Thomsen & Håkansson, 1995; Håkansson & Thomsen, 2001; O'Dea *et al.*, 2004, 2008). The modes of propagation are recorded in the form of calcified skeletons of colonies (O'Dea *et al.*, 2004). It is therefore possible to examine how life histories vary amongst living populations and measure directly the evolution of reproductive life histories through geological time using fossil assemblages (Thomsen & Håkansson, 1995; Håkansson & Thomsen, 2001; O'Dea *et al.*, 2004, 2008; O'Dea, 2006).

Despite advances in our understanding of the many processes of cloning in cupuladriids (Marcus & Marcus 1962; Winston, 1988; Thomsen & Håkansson, 1995; Håkansson & Thomsen, 2001; O'Dea *et al.*, 2004, 2008; O'Dea, 2006) little is known about several aspects of sexual reproduction including embryonic brooding. This dearth of information hinders the scholarly potential of life history studies in the Cupuladriidae because it is not known if increased cloning is reciprocated by a reduced investment in sexual reproduction, as has been previously proposed (Håkansson & Thomsen, 2001).

Unlike many cheilostome bryozoans, cupuladriids do not brood embryos and larvae in specialized ovicells (McKinney & Jackson 1989) nor do they appear to possess sexually dimorphic zooids as some other free-living bryozoans do (Cook & Chimonides, 1978, 1983; Chimonides & Cook, 1981). To estimate investment in sexual reproduction it is therefore necessary to make qualitative and quantitative observations of embryos in living colonies (Jackson & Wertheimer, 1985, Herrera *et al.*, 1996).

Several important questions remain unanswered regarding sexual reproduction in cupuladriid bryozoans: (1) How much do species invest in gamete formation and sexual reproduction?; (2) Does investment in sexual reproduction decrease in species whose populations are dominated by clonal propagation?; (3) Are there patterns of embryonic brooding within cupuladriid colonies?; and (4) What is the process of embryonic brooding in colonies and how does it vary amongst taxa?

The last of these questions is addressed in a separate study (Ostrovsky *et al.*, in press) while the aim of the present study is to begin to address the first two and to make observations pertinent to the third. To do so, we make collections of live colonies from populations of four cupuladriid species from

Corresponding author:
A. O'Dea
Email: odeaa@si.edu

the tropical eastern Pacific, make observations on brooding embryos in colonies, their frequency, colour, position and morphology, and their relationship with morphology and age of the colony. We examine the relationships between all these aspects of embryonic brooding and frequency of clonal propagation. And finally, we consider these results in an evolutionary context.

MATERIALS AND METHODS

Study organisms

Cupuladriid bryozoans are common members of the soft-bottomed sand fauna in tropical and sub-tropical shelf seas (Cadée, 1975; Winston, 1988; Cook & Chimonides, 1983, 1994; O'Dea *et al.*, 2004). Larvae normally settle upon a grain of sand or other small particle (Cook, 1963, 1965; Lagaaij, 1963; O'Dea *et al.*, 2008), metamorphose and grow incrementally through the sequential iteration of auto and vibracula zooids until the colony overhangs the particle. At this point the colony becomes free-living (Cook, 1965). Vibracula zooids act as hinge and muscle for long tapered setae, which, at the colony's margin, curve outwards, and downwards to support the colony above the sediment surface. Movement of setae is used to remove depositing sediment and restrict epibiotic growth and enables colonies to 'walk' over the sediment surface and up through the sediment to the sea floor if they become buried (Cook, 1963; Lagaaij, 1963; O'Dea, in preparation).

The prevalence of cloning amongst cupuladriids is correlated with a suit of morphological variables. Decreased cloning is observed in species whose colonies are small, heavily calcified and domed shaped, while conversely, elevated cloning is observed in species that produce large, thinly calcified and flat colonies (O'Dea *et al.*, 2004). Thus, colony morphology may be used as an adaptive strategy to control levels of clonal reproduction. In support of this idea, several species possess highly specialized morphologies that increase cloning by either enhancing the likelihood of fragmentation (e.g. peripheral fragmentation) or are designed specifically to undergo colony fission (e.g. colony budding and autofragmentation) (Marcus & Marcus, 1962; Håkansson & Thomsen, 2001; O'Dea *et al.*, 2008).

Collections

Colonies of cupuladriids were collected from near Las Perlas Archipelago, in the Gulf of Panama, in the tropical eastern Pacific using dredge-sampling methods from the RV 'Urraca' (Figure 1). In total, 27 dredges were made ten of which contained living cupuladriid colonies at a variety of depths (Table 1). All dredge material was washed on board through a 2 mm sieve. Four species of two genera of cupuladriid bryozoans were collected. During their examination, living colonies were kept in open seawater tanks with water sourced from the Gulf of Panama.

Morphologies and demography of colonies

All living colonies were sorted into species and their median diameters measured. Diameter was used to calculate colony surface area by modelling the colony on a flat circle (πr^2).

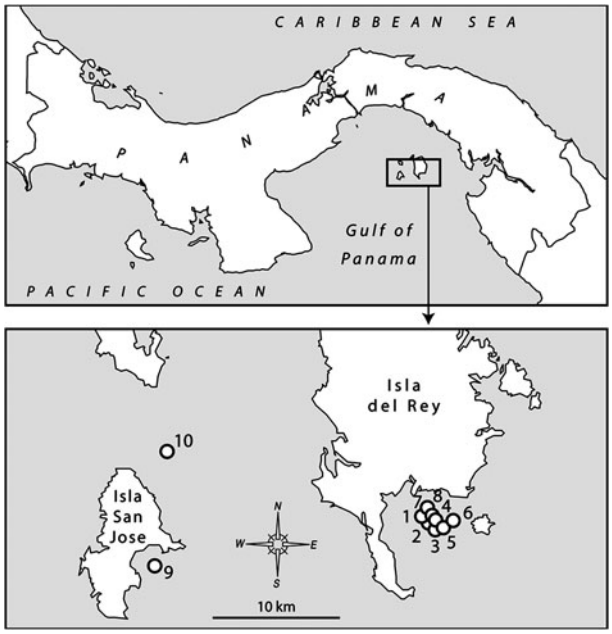

Fig. 1. Area of study in the Gulf of Panama, tropical eastern Pacific. Numbers relate to samples presented in Table 1.

Diameter is linearly related to the age of the colony (O'Dea & Jackson, 2002) while area is linearly related to number of zooids. No Pacific species produces highly domed colonies (O'Dea *et al.*, 2004) and thus the flat circle model is a reliable estimate of colony surface area.

Prevalence of embryos

Identified colonies were first separated into clonal or sexual as described in O'Dea *et al.* (2004, 2008) and the percentage of sexual colonies for each species' population estimated. Colonies were then separated into those with brooding embryos (termed 'fertile') and those with no visible brooding embryos. We found that in all species, early and medium-sized oocytes tended to be of a yellow colour and therefore liable to be concealed by the yellow or brown cuticle of the zooidal frontal wall and sometimes heavy algal epibiotic growth. It was therefore only possible to ensure that large mature oocytes and embryos brooded in internal sacs were counted. In *Discoporella marcusorum* the coloration of the frontal cuticle is so intense that the oocytes and embryos were best observed through the semitransparent calcified basal wall of the colony where their presence could easily be detected.

The numbers of visible embryos in each 'fertile' colony were counted. For each species, colony area data were used to determine the mean number of embryos per mm^2.

RESULTS

Species occurrences and abundances

A total of 1040 living cupuladriid colonies were collected from the ten dredges (Table 1). Four species were collected: *Cupuladria exfragminis, Discoporella marcusorum*, *D. cookae* (Herrera *et al.*, 2006, 2008) and *Discoporella* sp. nov. P1. The most abundant species' were *D. marcusorum* and

Table 1. Location of collections of four species of tropical eastern Pacific cupuladriid colonies with the abundance of living colonies and number and percentage of colonies observed with embryos in each site for each species and the entire cupuladriid assemblage.

Sample	Longitude	Latitude	Depth (m)	*Cupuladria exfragminis*			*Discoporella cookae*			*Discoporella marcusorum*			*Discoporella* sp. nov. P1			Total number colonies
				No. colonies	No. fertile	% fertile	No. colonies	No. fertile	% fertile	No. colonies	No. fertile	% fertile	No. colonies	No. fertile	% fertile	
1	−78.8845	8.2810	16.8	0	0	–	82	6	7.3	0	0	–	0	0	–	82
2	−78.8821	8.2781	17.1	0	0	–	29	2	6.9	0	0	–	0	0	–	29
3	−78.8788	8.2764	18.2	0	0	–	19	0	0	0	0	–	0	0	–	19
4	−78.8758	8.2825	16.6	0	0	–	2	0	0	0	0	–	0	0	–	2
5	−78.8728	8.2796	18.5	0	0	–	5	0	0	0	0	–	0	0	–	5
6	−78.8684	8.2813	18.5	0	0	–	2	0	0	0	0	–	0	0	–	2
7	−78.8789	8.2849	15.9	0	0	–	215	38	17.7	0	0	–	0	0	–	215
8	−78.8780	8.2837	16.1	0	0	–	84	14	16.7	0	0	–	0	0	–	84
9	−79.0846	8.2487	10.0	0	0	–	5	0	0	0	0	–	0	0	–	5
10	−79.0775	8.3035	26.0	74	4	5.41	0	0	–	521	177	34.0	2	2	100	597
			Total	74	4	5.41	443	60	13.5	521	177	34.0	2	2	100	1040
% abundance in sampled population				7.11			42.60			50.10			0.19			

D. cookae. Fewer colonies of *C. exfragminis* were found while *Discoporella* sp. nov. P1 was rare (Table 1).

Morphologies and demography of colonies

Mean colony diameters and mean colony areas of species and the percentage of sexually-produced colonies in populations are presented in Table 2. Data are consistent with results found by O'Dea *et al.* (2004). For example, species with large colonies produce the lowest percentage of sexually-produced colonies.

Frequency histograms of colony size (area) show a multimodal distribution in the sampled population of *C. exfragminis* (Figure 2A), corroborating the multimodal demographic distribution discovered by O'Dea (2006) for the same species in the same region. The sampled population of *D. cookae* shows a bimodal size distribution (Figure 2B), while *D. marcusorum* is unimodal (Figure 2C).

Prevalence of embryos

In each of the four species at least one colony was found to be fertile. However, the percentage of fertile colonies varied considerably among species, ranging from 5% to 100% (Table 1). The frequency of embryos in colonies also varied greatly among species (Figure 3A, B). The maximum number of embryos observed in colonies of both *D. marcusorum* and *D. cookae* was around 60 (Table 2) but *D. marcusorum* had, on average, significantly greater numbers of embryos per colony ($t = 7.139$, $\mathrm{df} = 962$, $P < 0.001$). This was also true for the density of embryos per mm^2 of colony ($t = 7.645$, $\mathrm{df} = 962$, $P < 0.001$), despite *D. marcusorum* having smaller zooids (Herrera-Cubilla *et al.*, 2008). *Cupuladria exfragminis* had on average small numbers of embryos per colony (Table 2). Only two colonies of *Discoporella* sp. nov. P1 were found, and although both were fertile, the sample size was considered too small to conduct accurate statistical analyses.

Plots of colony area against number of embryos observed in colonies of *C. exfragminis*, *D. marcusorum* and *D. cookae* show the demography of embryo brooding in the three populations at the time of sampling. The size of the smallest fertile colony was similar in both *Discoporella* species at approximately 10 mm^2 (Figure 3E, F). In general, the most embryo-rich colonies occurred amongst the largest colonies in both *D. cookae* and *D. marcusorum*, but *D. marcusorum* showed a decline in the numbers of embryos in the very largest colonies (Figure 3E, F).

Figure 3G–I show how embryo density varied with colony size. Embryo density in the population of *D. cookae* revealed a very different pattern than absolute frequency, with density tending to decrease as colonies get larger (Figure 3H). In *D. marcusorum* density tracked absolute number, although the fall-off in embryo brooding in larger colonies is slightly exaggerated (Figure 3I).

Brooding and clonal propagation

Colonies of species that predominantly propagate clonally (*C. exfragminis* and *D. cookae*) were found to have fewer embryos than predominantly sexually-reproducing species (*D. marcusorum*) and all had many less than *Discoporella* sp. nov. P1 which has never been observed to clone (Figure 4A, B; Table 2). This pattern appears to be valid

Table 2. Colony morphometrics, frequency and density of embryos, and percentage of clonal propagation in three species of tropical eastern Pacific cupuladriids. SD, standard deviation. * Note that only two colonies of *Discoporella* nov. sp. P1 were collected meaning data on morphologies are unlikely to be representative, but ** was corroborated by O'Dea *et al.* (2008) who also found 100% sexual colonies with a much larger sample size.

	Cupuladria exfragminis			***Discoporella cookae***			***Discoporella marcusorum***			***Discoporella* nov. sp. P1***		
	Sexual	**Asexual**	**All**	**Sexual**	**Asexual**	**All**	**Sexual**	**Asexual**	**All**	**Sexual**	**Asexual**	**All**
Number colonies	2	72	74	71	364	435	456	65	521	2	–	2
Mean colony diameter (mm)	9.08	10.02	9.99	6.95	5.04	5.36	4.96	4.29	4.88	7.75	–	7.75
(SD)	(3.07)	(4.07)	(4.03)	(1.24)	(1.89)	(1.93)	(1.47)	(0.84)	(1.42)	(1.48)	–	(1.48)
Mean colony area (mm^2)	68.43	91.63	91.00	39.18	22.78	25.45	21.02	14.99	20.26	48.04	–	48.04
(SD)	(43.8)	(67.79)	(67.16)	(13.07)	(15.15)	(16.01)	(12.32)	(6.31)	(11.91)	(18.08)	–	(18.08)
Maximum number of embryos observed	13.00	5.00	13.00	50.00	55.00	55.00	57.00	50.00	57.00	22.00	–	22.00
Mean number of embryos	6.50	0.10	0.27	1.73	1.32	1.39	5.70	1.66	5.20	14.50	–	14.50
(SD)	(9.19)	(0.61)	(1.62)	(7.09)	(5.81)	(6.03)	(10.01)	(6.85)	(9.76)	(10.61)	–	(10.61)
Mean density of embryos mm^{-2}	0.065	0.00	0.002	0.04	0.04	0.04	0.18	0.06	0.16	0.37	–	0.37
(SD)	(0.092)	(0)	(0.02)	(0.15)	(0.15)	(0.15)	(0.31)	(0.2)	(0.3)	(0.36)	–	(0.36)
Colony area with maximum number of embryos (mm^2)	99.40	187.48	99.40	61.51	64.33	64.33	32.67	37.39	32.67	35.26	–	35.26
% sexual colonies*			2.7			16.3			87.5			100**

even if the poorly-sampled *Discoporella* sp. nov. P1 is removed.

Within each of the three species that are able to clone (*C. exfragminis, D. cookae* and *D. marcusorum*), clonally-produced colonies were found to have fewer embryos per colony and lower embryo densities than sexually-produced colonies (Table 2). This tendency was significant for *C. exfragminis* ($t = 7.194$, df = 72, $P < 0.001$ and $t = 7.317$, df = 72, $P < 0.001$ respectively) and *D. marcusorum* ($t = 3.150$, df = 519, $P < 0.01$ and $t = 3.068$, df = 519, $P < 0.01$ respectively), but non-significant for *D. cookae* ($t = 0.525$, df = 433, $P = 0.600$ and $t = 0.3454$, df = 433, $P = 0.730$ respectively).

Observations on arrangement of embryos within colonies

The position of brooding embryos in colonies appeared to vary considerably between species. The following tendencies were observed. In the few fertile colonies of *C. exfragminis* that were collected, embryos tended to be scattered throughout the colony somewhat randomly (Figure 3A), while in *D. marcusorum* embryos were predominantly located in zooids at the peripheral margins of colonies (Figure 3C). In contrast, embryos in *D. cookae* were most often observed in zooids in the central parts of colonies (Figure 3B). This was true in both cloned and sexually-produced colonies of this species.

DISCUSSION

This study aimed to better understand patterns of embryonic brooding within and between cupuladriid species, with particular focus on the relationship between frequency of embryo brooding and cloning. Although the study was restricted to a small number of species, we observed striking differences in brooding biology within and between species populations that may help clarify the nature of reproductive life history variation among cupuladriid bryozoans.

Embryo frequency and clonal propagation

Cupuladriids do not possess specialized brood chambers (ovicells) unlike many other cheilostome taxa (Ostrovsky *et al.*, in press). In those ovicellate cheilostomes that have so far been

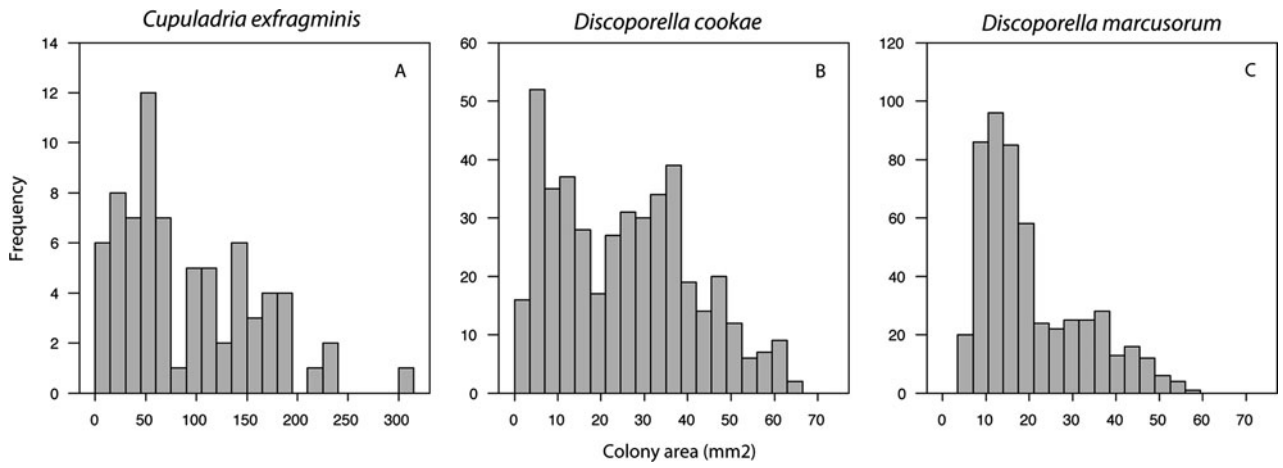

Fig. 2. Frequency histograms of colonies of estimated colony area (mm^2) in three species of tropical eastern Pacific cupuladriids.

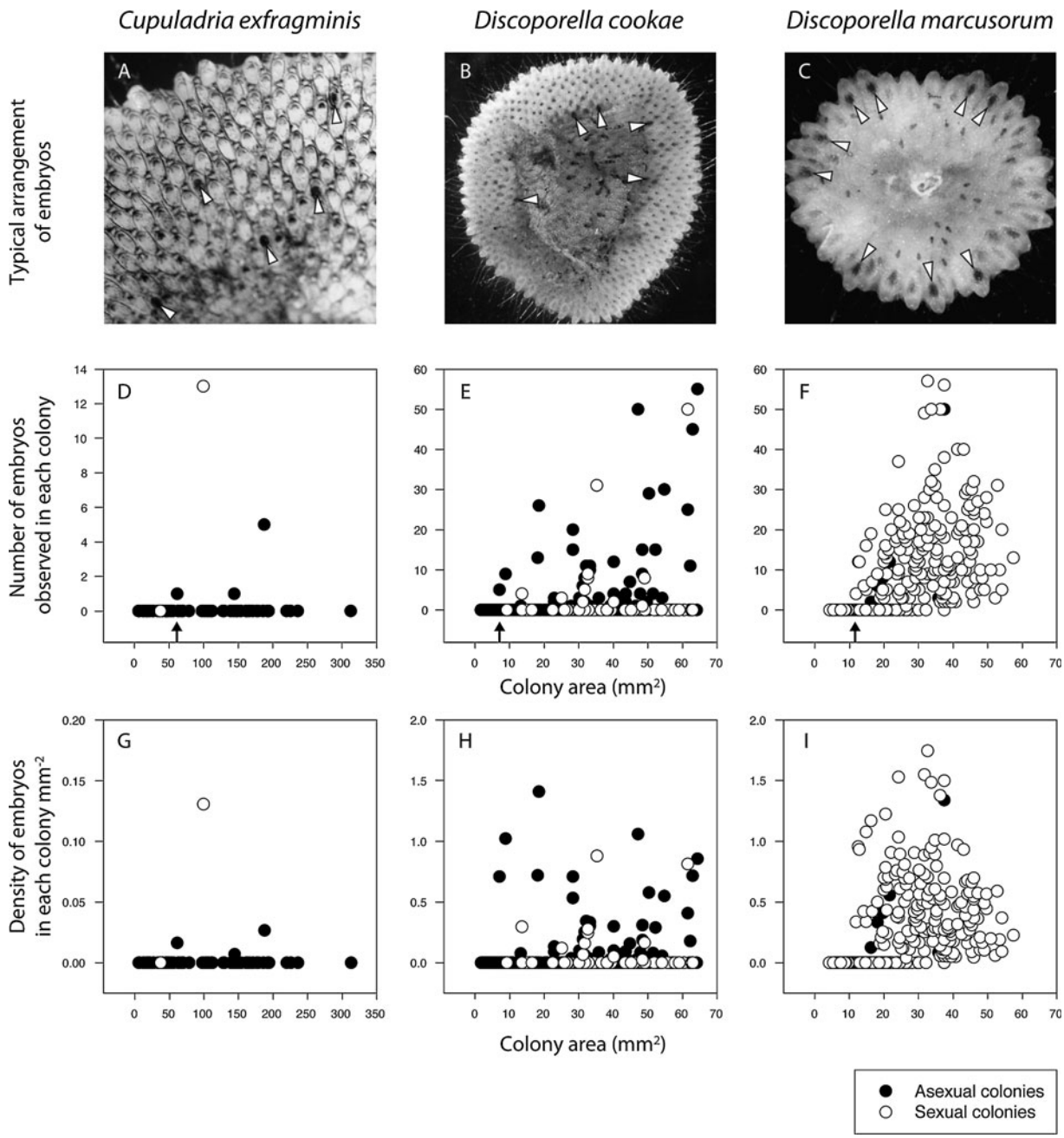

Fig. 3. Embryo arrangement (top), number (middle) and density (bottom) in colonies of *Cupuladria exfragminis* (left), *Discoporella cookae* (centre) and *Discoporella marcusorum* (right). (A–C) Photographs with arrows labelling brooding embryos within colonies that illustrate the typical arrangement of embryos in each species (see text for details); (D–F) colony area versus total number of embryos observed within individual colonies. Arrows indicate size of smallest fertile colony; (G–I) colony area versus density of embryos (number of embryos per mm^2) observed within individual colonies.

examined, a negative relationship tends to exist between the amount of cloning and the frequency of ovicells suggesting a direct trade-off between investment in clonal and sexual modes of propagation. In a study of encrusting reef corals, Jackson & Wertheimer (1985) showed that species that readily clone produce, in general, fewer embryos. Likewise, by collating data from fossil and Recent erect, encrusting and free-living bryozoans over millions of years Thomsen & Håkansson (1995) and Håkansson & Thomsen (2001) showed a strong negative relationship between frequency of clonal propagation and the numbers of ovicells. They suggested that as phyletic lineages invest more heavily in cloning through geological time they invest proportionally less energies into sexual reproduction. Essentially, they thought that energy for sexual reproduction is diverted into vegetative growth and enhancement of cloning by fragmentation. Indeed, there is good evidence that among bryozoan species there is often a clear trade-off in the relative investment into vegetative growth and sexual reproduction (Herrera *et al.*, 1996).

Our embryo survey data from four species of cupuladriids support this hypothesis, suggesting a negative relationship between frequency of embryos and the proportion of cloned colonies. *Cupuladria exfragminis* was found to brood the lowest overall number of embryos and is a species that propagates almost entirely clonally, has thinly-calcified colonies and

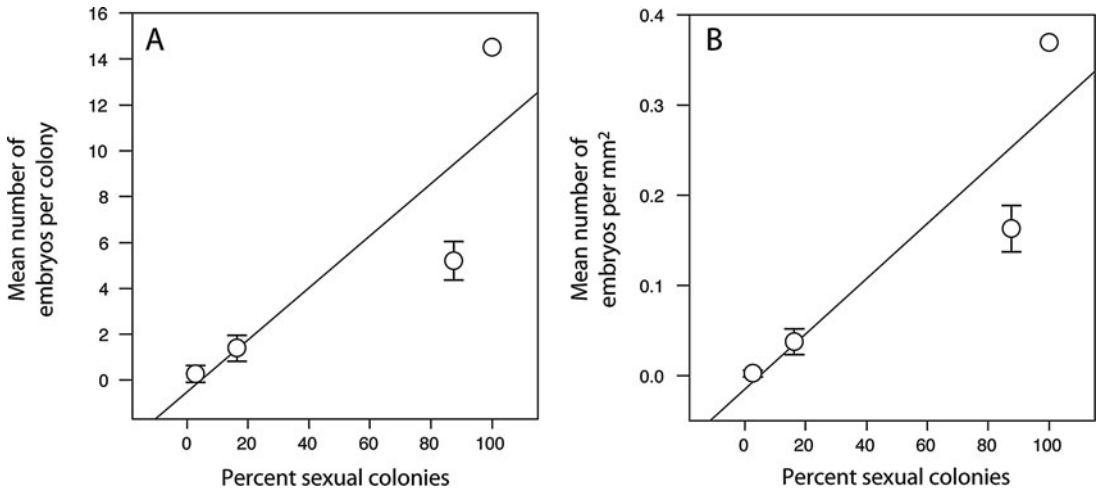

Fig. 4. Relationship of per cent of sexually-produced colonies and mean number (A) and mean density (B) of embryos in colonies of four species of tropical eastern Pacific cupuladriids. Error bars represent 95% confidence intervals. Fitted lines are based on the least squares method.

undergoes autofragmentation (O'Dea, 2006). The maximum mean number of embryos and maximum embryo densities were observed in colonies of *Discoporella* sp. nov. P1, despite only two colonies collected and analysed. This species also propagates entirely sexually and produces a thickly calcified skeleton to prevent fragmentation (O'Dea *et al.*, 2004, 2008). Within these two extremes of approach to propagation, the other two species analysed also fit this pattern: *D. cookae* has lower mean number of embryos and lower mean embryo densities than *D. marcusorum* and produces thinner, less calcified colonies and propagates considerably more of its colonies clonally.

Cheetham *et al.* (2001) also observed an increase in clonal reproduction over several millions of years in some Caribbean populations of *Metrarabdotos* but unlike the inference of Thomsen & Håkansson (1995) and Håkansson & Thomsen (2001), the authors concluded that the trends were purely an ecophenotypical response to the occurrence of upwelling that could support clonal propagation through increased vegetative growth of colonies. This raises the question of whether life history variation in embryo frequency and prevalence of cloning is controlled to a greater extent by environmental variations (ecophenotypically) or by the genotype.

It is clear that in cupuladriids frequency of cloning is controlled to a great extent by the genotype. This is demonstrated by the variety of distinct morphologies that are likely to have evolved in cupuladriids to either enhance or prevent fragmentation and clonal recruitment (O'Dea *et al.*, 2008). As a result, strategies of life history in cupuladriids must be under selective pressure, and it is therefore necessary to consider the evolutionary benefits of one over the other at any particular time or environment (see O'Dea *et al.*, 2008). Nonetheless, there does appear to be limited evidence that both the amount of cloning and the relative investment into embryonic brooding is ecophenotypically variable within species populations. For example, autofragmentation in *C. exfragminis* occurs most frequently during times of increased planktonic productivity during upwelling in the Gulf of Panama that results in a seasonal pattern of clonal recruitment (O'Dea, 2006). Similarly, populations of *C. biporosa* have greater prevalence of cloning in regions of higher nutrients along the Caribbean coast of Panama (O'Dea *et al.*, 2004). Future study is however required to gauge the plasticity of investment into clonal and aclonal reproduction within species, and appreciate its potential importance in the evolution of cupuladriid life histories.

The relationship between the frequency of embryonic brooding and clonal propagation observed between cupuladriid species is mirrored by variation that occurs within species. Our data show that clonally-produced colonies have on average fewer embryos than sexually produced colonies. This could be explained in a number of ways. Firstly, it is known that immediately following fragmentation colonies increase marginal growth rate four-fold, probably in order to rapidly attain a 'normal' discoidal shape and repair fractured zooids (O'Dea, 2006). Nutrients that are used for reparative growth are therefore unavailable for gamete formation and/or embryo brooding, and brooding frequency may consequently be reduced. Secondly, cloned colonies may have a different tempo of sexual reproduction than sexually-produced colonies, and our single sampling session failed to observe alternative seasonal investments in sex within clonal colonies. Thirdly, the process of fragmentation could alter colony morphology to such an extent that the ability of a colony to brood embryos is itself compromised. This may occur if fragmentation hinders the movement of energies around the colony that are needed to support embryonic brooding, or if oocytes are destroyed during fragmentation. And finally, because the zooids that comprise clones are likely to be old, having originated from the fragment of another colony, senescence at the zooidal level may reduce the ability of the colony as a whole to brood (Palumbi & Jackson, 1983).

Arrangement of embryos within colonies

Comparative observation of the arrangement of embryos in two species (*Discoporella cookae* and *D. marcusorum*) suggests that the location of oocytes in a colony is also related to the frequency of clonal propagation. The predominantly sexually-reproducing of the two species (*D. marcusorum*) tends to brood its embryos at the periphery of a colony while the predominantly clonal species (*D. cookae*) tends to brood in the central portion of the colony. If fragmentation

is more likely to occur it may be advantageous for a colony to ensure that assets such as embryos are located in those regions less likely to be damaged. The central zone in colonies of *D. cookae* may be safer compared to marginal areas that may be more susceptible to fragmentation. Indeed, most embryos in cloned colonies of *D. cookae* were observed in the very central part of the colony, which normally remains intact during any subsequent fragmentation either because of greater calcification of older zooids or the preferential breakage along lines of previous fracture (O'Dea, 2006). Additionally, the peripheries of regenerated fragments are always devoid of embryos. *Discoporella marcusorum*, on the other hand can take advantage of the lower risk of fragmentation and brood its embryos in the periphery of colonies. Brooding in the peripheral zone of colonies should be more advantageous given that zooids at the periphery are often more efficient feeders (Cook, 1977; Cook & Chimonides, 1978; McKinney & Jackson, 1989; Okamura *et al.*, 2001).

Similar patterns are observed in a number of other free-living bryozoan groups, which are unrelated to the cupuladriids, suggesting a universal explanation. In members of the genus *Selenaria* reproductive zooids are located at the peripheral margins of colonies and members of this genus have never been observed to clone via fragmentation. Colonies of *Selenaria* therefore reach sexual maturity only when the colony reaches maximum size and this trait may only be feasible because the colony is not at risk of fragmentation. In *D. marcusorum* the ontogeny of sexual reproduction is not as strict as in *Selenaria* because brooding zooids were also observed in very small colonies (Figure 3), however low rates of fragmentation may permit the potentially advantageous delay in brooding until colonies reach a certain size.

Both *Otionella tuberosa* and *Petasosella moderna* regularly clone by fragmentation and have their reproductive zooids scattered across the colony (Cook & Chimonides, 1985). This is similar to the situation seen in *D. cookae* and may be beneficial if peripherally brooded embryos are more prone to damage during fragmentation. Despite these parallel patterns, the location of reproductive zooids and its association with clonal propagation has yet to be assessed in most free-living bryozoans, but one exception to this general pattern is *Otionellina squamosa* that propagates clonally but also has peripheral zones of reproductive zooids (Cook & Chimonides, 1984; Ostrovsky *et al.*, in press).

Do colonies or zooids senesce?

Counting absolute number of embryos allows the exploration of the reproductive effort of the population as a whole whereas measuring the densities of embryos within colonies better reflects the ability of a unit area of bryozoan colony or a species population to reproduce sexually. Combining results from these two approaches reveals that patterns of reproductive investment amongst the species studied are highly variable (Figure 3D–I), and helps to reveal a species' life history strategy.

In *D. cookae*, a clear decline in embryo density occurs as colonies increase in size (Figure 3H) demonstrating that the increase in total number of embryos as colony size increases seen in Figure 3E is simply a product of larger colonies having more space to brood, and that in fact the most densely-brooding colonies are small. This may be in part because zooids of old fragments seem to be unable to support brooding in *D. cookae*. It is often observed in many cupuladriid species that zooids located in the central regions of colonies may die off or completely seal shut frontal openings by secondary calcification. This senescence of individual zooids may be unavoidable (Palumbi & Jackson, 1983; Jackson & Hughes, 1985) or it may have functional significance, particularly to assist in the formation of expellent currents (Silén & Harmelin, 1974; Okamura *et al.*, 2001) or perhaps more importantly in free-living species, to strengthen colonies.

In *D. marcusorum*, which on the whole reproduces sexually, the very oldest colonies also show a decline in embryonic density, although the pattern is unlike that seen in *D. cookae* (Figure 3H, I). One explanation is that colonies themselves are senescing and as they reach a determinate size/age (Herrera-Cubilla *et al.*, 2008) reproductive ability or investment decreases, as occurs in solitary organisms. Although modular organisms are generally thought to persist by modular turnover (Jackson & Hughes, 1985) many cupuladriids, including *D. marcusorum*, are perhaps more similar in their life histories to solitary organisms than other colonial invertebrates. They are free-living, reproduce sexually and have determinate growth and one can apply the same selective pressures that account for such processes in solitary organisms, such as increased predation, accident and disease probability to explain why reproductive effort per unit size should reduce ontogenetically (Kirkwood, 1977).

Discoporella cookae, which propagates mostly clonally, cannot be treated in the same way because it has indeterminate growth with fragments playing an essential role in the founding of new colonies. Given its capacity to clone through vegetative growth, senescence in *D. cookae* is probably restricted to the level of the zooid, meaning clones are potentially able to propagate eternally as is the case in most bryozoans (Palumbi & Jackson, 1983). Testing this could involve comparing the capacity to brood of newly budded zooids from old clones with those from new clones of species that normally reproduce sexually. Reduced capacity to brood in old clone zooids could suggest senescence.

Summary

This preliminary study explored the reproductive life histories in free-living cupuladriid bryozoans by conducting surveys on embryos in four species. The data, although limited in scope, highlight several important aspects of cupuladriid reproductive biology:

1. Frequency of embryos is highly variable both within and between species.
2. Investment in embryonic brooding appears to be negatively correlated with the incidence of clonal propagation amongst species.
3. Cloned colonies brood fewer embryos than sexually-produced colonies of the same species. This could be due to the diversion of energies away from gamete formation to growth, morphological disruption that physically limits brooding ability, or zooid senescence.
4. The arrangement of brooding embryos within colonies is variable and distinct amongst species, and may depend upon whether the species produces most of its colonies sexually or if it tends to clone.

5. Colonial senescence may cause the ontogenetic reduction of embryo density in species that preferentially propagate sexually. The life histories of such species may be more easily understood if they are considered as solitary rather than colonial organisms.
6. Future work is required to expand and clarify these data and hypotheses. More species need to be analysed, and it is crucial that spatial and temporal replication be incorporated to elucidate seasonal variations in brooding and the effects of different environments upon embryonic brooding.

ACKNOWLEDGEMENTS

Jeremy Jackson assisted with many aspects of the production of this paper. Rachel Collin kindly provided laboratory space, microscope and camera, and also helped collect material. Andrés Gómez, Etelyn González, Graciela Quijano, Marissa Quintero, Aileen Terrero and the crew of the RV 'Urraca' helped collect material. Amalia Herrera made comments to the manuscript. Anonymous referees gave very helpful comments on the manuscript.

The Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT) funded the project. A. O'Dea was supported by the Tupper fellowship programme at STRI and National Science Foundation grant EAR03-45471. F. Rodríguez was supported by the Smithsonian Tropical Research Institute. A. Ostrovsky would like to thank Austrian Science Fund (FWF, grant P19337-B17) and Russian Foundation for Basic Research (RFBR, grant 07-04-00928a) for financial support. We would also like to thank Eldredge Bermingham and Harilaos Lessios from STRI for additional funds.

REFERENCES

Baluk W. and Radwanski A. (1977) The colony regeneration and life habitat of free-living bryozoans, *Cupuladria canariensis* (Busk) and *C. haidingeri* (Reuss) from the Korytnica Clays (Middle Miocene): Holy Cross Mountains, Poland. *Acta Geologica Polonica* 27, 143–156.

Cadée G.C. (1975) Lunulitiform bryozoa from the Guyana shelf. *Netherlands Journal of Sea Research* 9, 320–343.

Cheetham A.H., Jackson J.B.C. and Sanner J. (2001) Evolutionary significance of sexual and asexual modes of propagation in Neogene species of the bryozoan *Metrarabdotos* in tropical America. *Journal of Paleontology* 75, 564–577.

Chimonides P.J. and Cook P.L. (1981) Observations on living colonies of *Selenaria* (Bryozoa, Cheilostomata). II. *Cahiers de Biologie Marine* 22, 207–219.

Cook P.L. (1963) Observations on live lunulitiform zoaria of Polyzoa. *Cahiers de Biologie Marine* 4, 407–413.

Cook P.L. (1965) Notes on the Cupuladriidae (Polyzoa, Anasca). *Bulletin of the British Museum (Natural History) Zoology* 13, 151–187.

Cook P.L. (1977) Colony-wide water currents in living Bryozoa. *Cahiers de Biologie Marine* 18, 31–47.

Cook P.L. and Chimonides P.J. (1978) Observations on living colonies of *Selenaria* (Bryozoa, Cheilostomata) I. *Cahiers de Biologie Marine* 19, 147–158.

Cook P.L. and Chimonides P.J. (1983) A short history of the lunulite Bryozoa. *Bulletin of Marine Science* 33, 566–581.

Cook P.L. and Chimonides P.J. (1984) Recent and fossil Lunulitidae (Bryozoa, Cheilostomata), 1. The genus *Otionella* from New Zealand. *Journal of Natural History*, 18, 227–254.

Cook P.L. and Chimonides P.J. (1985) Recent and fossil Lunulitidae (Bryozoa, Cheilostomata), 4. American and Australian species of *Otionella*. *Journal of Natural History* 19, 575–603.

Cook P.L. and Chimonides P.J. (1994) Notes on the family Cupuladriidae (Bryozoa), and on *Cupuladria remota* sp. n. from the Marquesas Islands. *Zoologica Scripta* 23, 251–268.

Håkansson E. and Thomsen E. (2001) Asexual propagation in cheilostome Bryozoa: evolutionary trends in a major group of colonial animals. In Jackson J.B.C., Lidgard S. and McKinney F.K. (eds) *Evolutionary patterns: growth, form and tempo in the fossil record*. Chicago and London: University of Chicago Press, pp. 326–347.

Herrera A., Jackson J.B.C., Hughes D.J., Jara J. and Ramos H. (1996) Life-history variation in three coexisting cheilostome bryozoan species of the genus *Stylopoma* in Panama. *Marine Biology* 126, 461–469.

Herrera-Cubilla A., Dick M.H., Sanner J. and Jackson J.B.C. (2006) Neogene Cupuladriidae of tropical America. I: Taxonomy of Recent *Cupuladria* from opposite sides of the Isthmus of Panama. *Journal of Paleontology* 80, 245–263.

Herrera-Cubilla A., Dick M.H., Sanner J. and Jackson J.B.C. (2008) Neogene Cupuladriidae of tropical America. II: Taxonomy of Recent *Discoporella* from opposite sides of the Isthmus of Panama. *Journal of Paleontology* 82, 279–298.

Hughes T.P. and Jackson J.B.C. (1980) Do corals lie about their age? Some demographic consequences of partial mortality, fission and fusion. *Science* 209, 713–715.

Jackson J.B.C. and Hughes T.P. (1985) Adaptive strategies of coral-reef invertebrates. *American Scientists* 73, 265–274.

Jackson J.B.C. and Wertheimer S.P. (1985) Patterns of reproduction in five common species of Jamaican reef-associated bryozoans. In Nielsen C. and Larwood G.P. (eds) *Bryozoa: Ordovician to Recent*. Dredensborg, Denmark: Olsen and Olsen, pp. 161–168.

Kirkwood T.B.L. (1977) Evolution of aging. *Nature* 270, 301–304.

Lagaaij R. (1963) *Cupuladria canariensis* (Busk)—portrait of a bryozoan. *Palaeontology* 6, 172–217.

Marcus E. and Marcus E. (1962) On some lunulitiform Bryozoa. *Universidade de São Paulo Boletins da Faculdade de Philosophia, Sciéncias e Letras, Zoologia* 3, 111–353.

McKinney F.K. and Jackson J.B.C. (1989) *Bryozoan evolution*. Boston: Unwin Hyman.

O'Dea A. (2006) Asexual propagation in the marine bryozoan *Cupuladria exfragminis*. *Journal of Experimental Marine Biology and Ecology* 335, 312–322.

O'Dea A. and Jackson J.B.C. (2002) Bryozoan growth mirrors contrasting seasonal regimes across the Isthmus of Panama. *Palaeogeography Palaeoclimatology Palaeoecology* 185, 77–94.

O'Dea A., Herrera-Cubilla A., Fortunato H. and Jackson J.B.C. (2004) Life history variation in cupuladriid bryozoans from either side of the Isthmus of Panama. *Marine Ecology Progress Series* 280, 145–161.

O'Dea A., Jackson J.B.C., Taylor P.D. and Rodríguez F. (2008) Modes of reproduction in Recent and fossil cupuladriid bryozoans. *Palaeontology* 51, 847–864.

Okamura B., Harmelin J-G. and Jackson J.B.C. (2001) Refuges revisited. Enemies versus flow and feeding as determinants of sessile animal

distribution and form. In Jackson J.B.C., Lidgard S. and McKinney F.K. (eds) *Evolutionary patterns: growth, form, and tempo in the fossil record*. Chicago and London: University of Chicago Press, pp. 61–93.

Ostrovsky A.N., O'Dea A. and Rodriguez F. (2009) Comparative anatomy of internal incubational sacs in cupuladriid bryozoans and the evolution of brooding in free-living cheilostomes. *Journal of Morphology* (in press).

Palumbi S.R. and Jackson J.B.C. (1983) Ageing in modular organisms: ecology of zooid senescence in *Steginoporella* sp. (Bryozoa: Cheilostomata). *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 164, 267–278.

Silén L. and Harmelin J-G. (1974) Observations on living Diastoporidae (Bryozoa, Cyclostomata), with special regard to polymorphism. *Acta Zoologica* 55, 81–96.

Thomsen E. and Håkansson E. (1995) Sexual versus asexual dispersal in clonal animals—examples from cheilostome bryozoans. *Paleobiology* 21, 496–508.

and

Winston J.E. (1988) Life histories of free-living bryozoans. *National Geographic Research* 4, 528–539.

Correspondence should be addressed to:
A. O'Dea
Smithsonian Tropical Research Institute
MRC 0580-01, Apartado 0843–03092
Panamá, República de Panamá
email: odeaa@si.edu