

Asexual propagation in the marine bryozoan *Cupuladria exfragminis*

Aaron O’Dea

Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Panama, Republic of Panama
Scripps Institute of Oceanography, La Jolla, CA 92093-00244, USA

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Abstract

Colonies of the free-living bryozoan *Cupuladria exfragminis* [Herrera-Cubilla, A., Dick, M.H., Sanner, J., 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.] from the Gulf of Panama are observed to separate into viable fragments without external force. The process, termed autofragmentation, involves the development of distinctive morphologies designed to assist in colony division. Culturing *C. exfragminis* shows that fragments that are a consequence of autofragmentation are significantly better able to regenerate than fragments originating from mechanical fragmentation. However, the relative importance of auto- versus mechanical fragmentation in natural populations remains unknown. Autofragmentation in *C. exfragminis* is found to be stimulated by cooling waters, and analysis of growth in natural populations demonstrates that colonies undergo periodic fragmentation coincident with lowered water temperatures during seasonal upwelling in the Gulf of Panama. These observations suggest that *C. exfragminis* uses the drop in temperature that occurs during upwelling as a cue to indicate times of increased food availability which may assist in the successful regeneration of fragments. These results are discussed in the context of the morphological, ecological and evolutionary significance of autofragmentation as a method of asexual propagation in cupuladriids.

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1. Introduction

In addition to sexual reproduction, most colonial organisms have the ability to create new colonies asexually, for which the colonial form is particularly well suited (Highsmith, 1982; Jackson, 1985; Håkansson and Thomsen, 2001). Many bryozoans, corals and ascidians are for example composed of tens to millions of genetically identical, iterated modules that can often survive, grow and reproduce independently or in small groups if they separate from the parent colony. As such,

establishing new individual colonies without the use of sex can be achieved relatively simply by either budding extensions that separate to form new colonies or by the division of the colony into two or more parts that can then each regenerate (e.g. Marcus and Marcus, 1962; Highsmith, 1982; McKinney, 1983; Hughes and Cancino, 1985; Jackson, 1985; Jackson and Hughes, 1985; Jackson and Coates, 1986; Cheetham et al., 2001; O’Dea et al., 2004; Turon, 2005). These processes are widespread in the marine realm and are an extremely important method of maintaining populations in a large number of colonial taxa (Walker and Bull, 1983; Wulff, 1991; Acosta et al., 2001; Bastidas et al., 2004; Slobodov and Marfenin, 2004). The relative importance that colonial organisms

E-mail address: odeaa@si.edu.

place upon asexual and sexual propagation varies considerably, often between closely related taxa (McKinney, 1983; Cheetham et al., 2001; Håkansson and Thomsen, 2001; O'Dea et al., 2004), and is a central part of a species' life history and evolutionary outcome (Highsmith, 1982; Jackson and Hughes, 1985; Jackson and Coates, 1986; Cheetham et al., 2001; O'Dea et al., 2004).

The bryozoan family Cupuladriidae is comprised of species that make new colonies through exclusively sexual means, others that propagate almost entirely asexually, as well as species that are intermediate with a

mix of sexually and asexually produced colonies (Håkansson and Thomsen, 2001; O'Dea et al., 2004). The mode by which cupuladriid colonies have formed, either sexually or asexually, is clearly preserved in their calcified skeletons (Fig. 1), which in turn form a rich fossil record.

It is therefore possible to measure the relative abundance of sexual and asexually produced colonies in a population, a species, and a fossil assemblage. As a consequence, cupuladriid bryozoans offer a unique system with which to study the evolution of reproductive life history variation. Currently, however, our

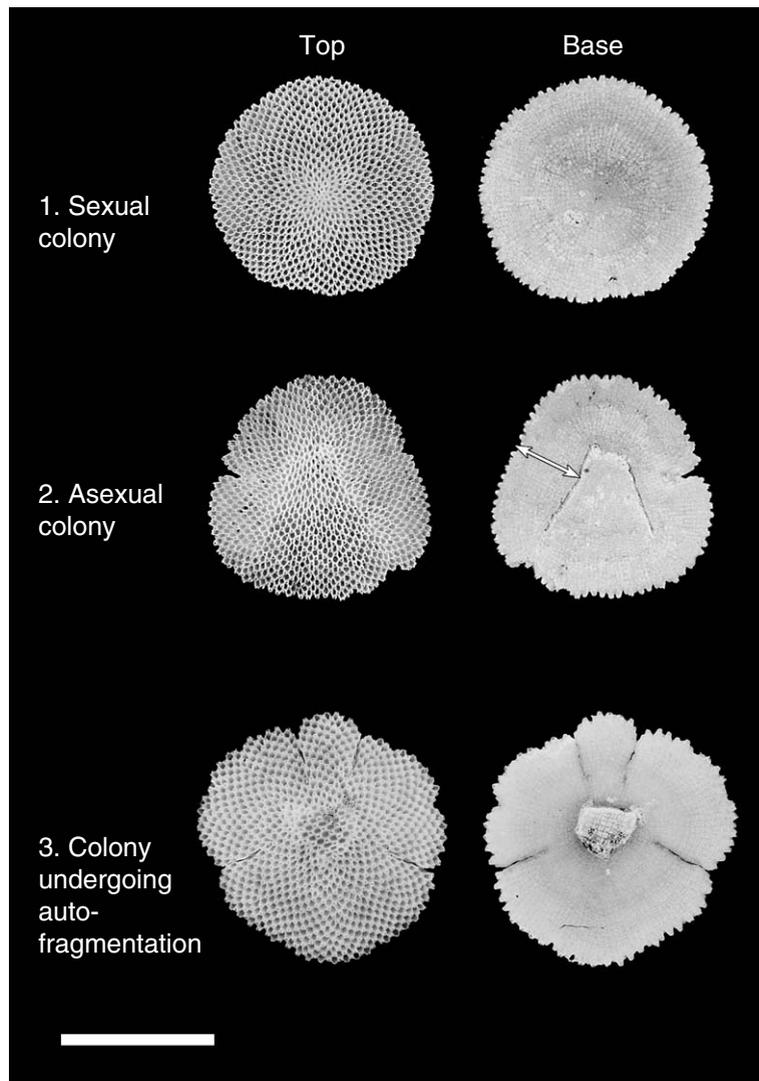


Fig. 1. Propagation of new colonies in *Cupuladria exfragminis*. 1. Sexually produced colony, showing ancestral region and radially-symmetrical growth. 2. Asexually produced colony, showing morphological asymmetry, lines of fragmentation and regenerative growth from original fragment, with arrow illustrating method used to measure amount of regenerative growth since fragmentation. 3. Colony preparing to autofragment with characteristic notches demarcating radial uncalcified lines along which the colony will fragment into four parts. Scale bar=5 mm.

understanding of the nature of asexual propagation in cupuladriids is limited. This paper addresses this by exploring asexual propagation in a single cupuladriid species.

1.1. Life histories of cupuladriid bryozoans

The Cupuladriidae originated in the Palaeocene and by the Miocene had become abundant constituents of tropical shelf waters as they are today (Cook and Chimonides, 1983, 1994). Like most cheilostome bryozoans, cupuladriids form colonies that are composed of iterated zooids united by a calcified skeleton, each zooid genetically identical to the others (McKinney and Jackson, 1989). Colonies of all species are based on the same basic morphological plan, featuring unilaminar radial budding of zooids that creates small disc or dome shaped colonies (e.g. Fig. 1). As far as is known, all cupuladriids also share the same basic life habit, living unattached (free living) to substrate in their adult stage and resting free on, or in, the sea floor sediment. Very similar morphologies and life habits are shared by the unrelated cheilostome families Lunulitidae (Cook, 1965) and Mamilloporidae (Cheetham and Jackson, 2000).

Each normal feeding zooid (autozooid) of a cupuladriid colony is associated with a single modified zooid called a vibraculum that possesses an extended movable seta designed to clean the colony surface of deposited sediment and allows the colony to move through and over the sea floor (Cook, 1963; Lagaaj, 1963).

The range of strategies used to propagate new individuals varies considerably between cupuladriid species. For example, within the relatively small area of the southwestern Caribbean coast of Panama, one can find species that propagate entirely sexually through the production of larvae, some that propagate almost all of their colonies asexually via fragmentation and regeneration, and others that have a mix of asexual and sexually produced colonies (O'Dea et al., 2004).

Variation in reproductive life history strategy is tightly correlated with colony morphology. Species that commonly propagate asexually have morphologies that promote colony fragmentation such as lightly calcified, flat and large colonies (Marcus and Marcus, 1962; Baluk and Radwanski, 1984; Winston, 1988; Thomsen and Håkansson, 1995; Håkansson and Thomsen, 2001; O'Dea et al., 2004). Species that commonly use sexual reproduction on the other hand are usually small, highly domed and heavily calcified in order to protect the colony from breakage (O'Dea et al., 2004). Disposition to fragmentation is taken to a more complex morphological level in two Recent and one fossil species whose

colonies sprout new colonies from marginal growth in a process called colonial budding (Marcus and Marcus, 1962; Håkansson and Thomsen, 2001; Herrera-Cubilla et al., 2006).

This paper investigates the process of asexual propagation in *Cupuladria exfragminis* (Herrera-Cubilla et al., 2006). First, in response to chance observations made by F. Rodríguez that a small number of colonies fragmented without external mechanical force, we make observations on autofragmentation and regeneration in living colonies of *C. exfragminis*. Second, the relative ability of colonies to regenerate following auto- vs. mechanical fragmentation is investigated. Third, the demography of fragmentation in a natural population of *C. exfragminis* is examined. And fourth, controlled culture is used to investigate environmental stimuli that *C. exfragminis* may use as a cue to fragment.

1.2. Study location

The benthos of the Gulf of Panama in the Tropical eastern Pacific (Fig. 2) is composed mostly of shelf deposits of sand and mud. Low energy mangroves dominate the coast while patch reefs sparsely edge the many islands (Fig. 2). From January to April, south-westerly trade winds pass over the Isthmus of Panama pushing strong coastal upwelling in the Gulf away from the coast driving strong coastal upwelling (D'Croz and Robertson, 1997). Surface water temperatures in the region can drop from a non upwelling temperature of around 28 °C to as low as 18 °C in a matter of a week (D'Croz and Robertson, 1997) and upwelled deep waters significantly

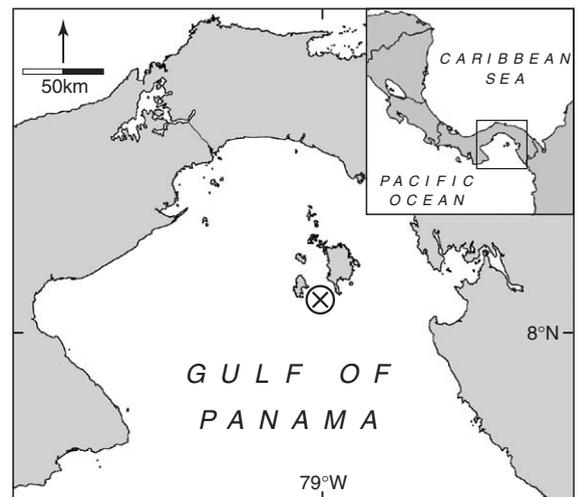


Fig. 2. Location of study site in the Gulf of Panama, tropical eastern Pacific.

increase nutrient levels in surface waters, driving high planktonic primary productivity.

2. Materials and methods

2.1. Study species

C. exfragminis (Herrera-Cubilla et al., 2006) (Fig. 1) is the most commonly occurring and abundant cupuladriid species along the Pacific coast of southern Central America (O'Dea et al., 2004; Herrera-Cubilla et al., 2006), and appears to be widely distributed along the whole of the eastern Pacific coast of Tropical America. It is easily distinguished from other Central American *Cupuladria* by its flat and thinly calcified colonies, relatively small zooids and the largest ratio of vibracular length to autozooid length of all cupuladriid species in Tropical America (Herrera-Cubilla et al., 2006). When alive, its colonies possess a characteristic dark brown colour.

C. exfragminis is the eastern Pacific sister species of the Atlantic *C. biporosa* (Dick et al., 2003; Herrera-Cubilla et al., 2006). The two probably diverged due to the formation of the Isthmus of Panama (Dick et al., 2003) which began to affect marine connections between the Pacific and Caribbean around 15 million years ago (Coates et al., 2005).

2.2. Collections

Over two thousand *C. exfragminis* colonies were collected by making eight dredge samples in sand and mud in the southern area of the Pearl Islands, Gulf of Panama, in the tropical eastern Pacific (Fig. 2). Dredge samples ranged in depth from 12 to 46 m. Bottom conditions where colonies occurred ranged from low energy silt to higher energy sand. Dredge samples were immediately washed through a 3 mm sieve and all cupuladriids retained. Nearly 2000 of the colonies found were alive, the rest were collected dead. All dead colonies were well preserved indicating a recent (Holocene) death rather than being reworked fossil colonies. Dead colonies were dried and stored while living colonies were kept alive in running sea-water aquaria at Naos Marine Laboratory, Smithsonian Tropical Research Institute, Panama.

2.3. Observations on autofragmentation

Eighty of the largest *C. exfragminis* colonies were kept individually separate in 1 × 1.5 cm boxes within plastic ice-cube trays. Each box contained a teaspoon of sediment and the trays were suspended in aquaria in

open-flowing sea water. This system ensured that colonies were protected from the physical processes that could cause them to fragment, such as currents or the actions of predators or grazers. As such, the fragmentation of any colony could be unambiguously attributed to the colony self fragmenting (autofragmentation). Colonies were observed three times each week over a six week period.

2.4. Colony regeneration

To investigate if colonies that autofragment are better prepared to regenerate following fragmentation than colonies that fragment simply as a result of unexpected, external physical forces (mechanical fragmentation), the difference in the rate of regenerative growth between colonies that had autofragmented and colonies that were deliberately broken by hand was tested.

Fifty two of the colonies from the previous observations (Section 2.3) autofragmented in a 23 day period (27th July 2004 to the 19th August). In response, 52 whole colonies of *C. exfragminis* from the same population were broken, each into two pieces. The largest fragment from each auto- and each mechanically fragmented colony was then used to monitor post-fragmentation growth rate differences between auto and mechanically fragmented colonies. Each colony was kept in individually marked boxes within a single aquarium.

Each month for five months the rate of regenerative growth in each fragment was measured as the maximum distance of new growth from the line of fragmentation (e.g. Fig. 1). Growth rate for each colony at each month was then calculated as $\mu\text{m day}^{-1}$. The difference in growth rate between auto and mechanically fragmented colonies was analysed using one-way analysis of variance (ANOVA) (MINITAB) for each month and for the whole five months.

2.5. Demography of fragmentation and regeneration

One of the dredge collections retrieved 785 living colonies of *C. exfragminis*. Each of these colonies was first separated into sexually- and asexually-produced colonies using colony morphology (see Fig. 1). Seven hundred and sixty four colonies (97.3%) had originated from asexual propagation by fragmentation while only 21 (2.7%) were sexually produced. This is higher than the mean value of 75% of asexually produced colonies found for the same species by O'Dea et al. (2004) along the whole Pacific coast of Panama (at this time *C. exfragminis* was identified as preliminary species *C. 5 biporosa*). For each of the asexually produced colonies, the maximum

distance from the ultimate line of fragmentation to the growing edge was measured (e.g. Fig. 1). The demography of asexual growth in a single population of *C. exfragminis* could then be examined by constructing a size frequency histogram of the amount of regenerative growth since fragmentation.

2.6. Autofragmentation and temperature

As a result of observations on the timing of autofragmentation it became apparent that autofragmentation in *C. exfragminis* may be controlled by environmental cues such as the drop in temperature that occurs during upwelling periods along coastal regions of the tropical eastern Pacific. To investigate the effects of temperature upon rates of autofragmentation, 330 of the largest, whole and living colonies from the dredge collections that remained after the preceding experiments were chosen. Two closed-water aquaria were arranged and maintained at 28 °C. One hundred and sixty five of the colonies were randomly placed into individual boxes in each aquaria using random number tables.

The first aquarium was maintained at 28 °C (± 0.5 °C) throughout the experiment, while the second was lowered to a temperature of 20 °C (± 0.5 °C) which equals a strong upwelling temperature in the Gulf of Panama. To prevent temperature shock to the colonies, water temperature was lowered slowly from 28 °C to 20 °C, over a one week period. Aquaria water was replaced three times each week to replenish food levels. Water was fully oxygenated throughout. For a total of 32 days, colonies were inspected twice daily for fragmentation.

3. Results

3.1. Observations on autofragmentation

Out of the 80 colonies of *C. exfragminis*, 38 were observed to autofragment over a six week period. The total number of fragments produced was 58 and ranged from two to five per colony. For the first three months of the experiment no colonies fragmented; all of the 38 colonies that fragmented did so in a four day period that were associated with an unplanned drop in water temperature of around 9 °C that occurred when the water heating system failed.

Within one week of fragmentation, all colonies that had fragmented had begun regenerative growth along the fractured margins. Recently fragmented colonies were observed to be active, apparently more so than entire colonies, and were observed to move away from strong light.

3.2. Colony regeneration

In the first month following fragmentation, the mean rate of growth in *C. exfragminis* was significantly less in mechanically fragmented than autofragmented colonies (Table 1; Fig. 3). By the second, and all subsequent months, the difference between the two modes of fragmentation was not significantly different (Table 1). Nonetheless, using the total amount of growth over the whole study period, mean growth rate was significantly faster in autofragmented colonies than mechanically fragmented colonies (Table 1).

In both auto- and mechanically fragmented colonies, mean rate of marginal growth after fragmentation was around five or six times faster in the first month than each of the subsequent four months (Fig. 3). Aside from the initial post-fragmented growth spurt, the rate of growth of *C. exfragminis* colonies was found to be slow (Table 1) compared to the rates of growth measured in other types of Bryozoa (e.g. McKinney and Jackson, 1989; O'Dea and Okamura, 1999), but the data corroborate previously estimated growth rates of cupuladriids (O'Dea and Jackson, 2002).

3.3. Demography of fragmentation

The maximum amount of regenerative growth in colonies of *C. exfragminis* from a single dredge sample from the Gulf of Panama was found to be 11.6 mm (Fig. 4). The overall mean amount of regenerative growth in the dredge sample was 4.75 mm (± 1.90), the median was 4.77 mm and the mode 3.6 mm. Colonies with large amounts of regenerative growth always originated from a small fragment. Large colonies were made up of both large fragments with a small amount of regenerative growth and small fragments with a large amount of regenerative growth. Maximum colony size in *C. exfragminis* may

Table 1

Mean rate of growth in colonies of *Cupuladria exfragminis* following auto- and mechanical fragmentation with results of one-way ANOVAs

Month	Mean growth rate ($\mu\text{m day}^{-1}$) (SD)		ANOVA	
	Autofragmentation ($n=52$)	Mechanical fragmentation ($n=52$)	F	P
1	22.49 (14.87)	15.48 (11.32)	7.32	0.008 **
2	4.51 (5.11)	4.26 (4.70)	0.07	0.790 ns
3	1.88 (1.71)	2.45 (3.41)	1.19	0.278 ns
4	3.19 2.24	3.90 3.35	1.64	0.204 ns
5	2.15 (2.42)	2.84 (3.21)	1.50	0.223 ns
All 5 months	7.54 (4.53)	5.62 (3.13)	6.33	0.013 *

SD=standard deviation. * $p < 0.05$, ** $p < 0.01$, ns=non significant.

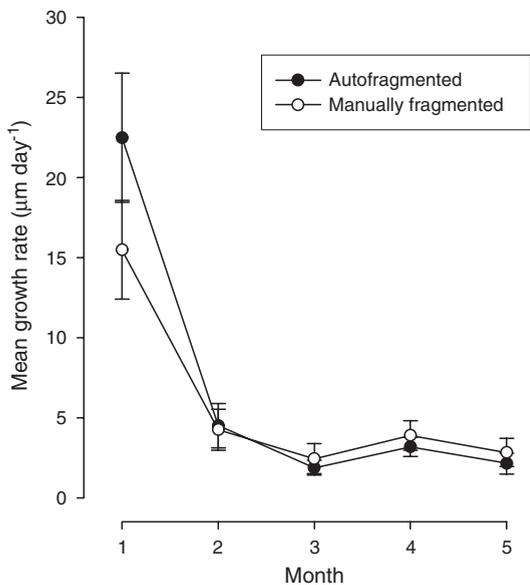


Fig. 3. Comparison of mean rate of marginal growth in colonies of *Cupuladria exfragminis* for five months after auto- (black) and mechanical (white) fragmentation. $N=52$ for each treatment. Error bars represent 95% confidence limits.

therefore be constrained by a physical limit of mechanical or auto-fragmentation rather than an ontogenetic limit such as determinate growth. Because auto-fragmentation appears to take place in large colonies whether they result from a small or a large fragment suggests that amount of regene-

rative growth does not overtly control maximum colony size. Thus, growth of a single genet could potentially be perpetual.

Frequency histogram of the amount of regenerative growth since fragmentation uncovers a multimodal distribution (Fig. 4). Assuming that growth rate between colonies did not vary significantly within the population, each peak represents a cohort of colonies that had fragmented at the same time. One can reasonably assume that growth was comparable among colonies because previous estimates from other bryozoans suggest that growth rate does not significantly vary between colonies of the same species (O'Dea and Okamura, 1999). Moreover, the colonies from this single dredge are likely to be highly genetically related given the high rate of asexual propagation. Thus, the multi-modal distribution suggests that colonies within this population of *C. exfragminis* are fragmenting periodically, and in unison. Distance between cohorts is uniform throughout the range at around 2 mm (Fig. 4).

Frequency histogram also demonstrates that the population was inadequately sampled. Colonies with a very small amount of regenerative growth since fragmentation are missing from the sample (Fig. 4). This is due to the fact that by sieving samples at 3 mm immediately after dredge collection, small colonies with a small amount of regenerative growth were lost. Additionally, fresh regenerative growth is often overlooked in large colonies because newly fragmented large colonies often have an

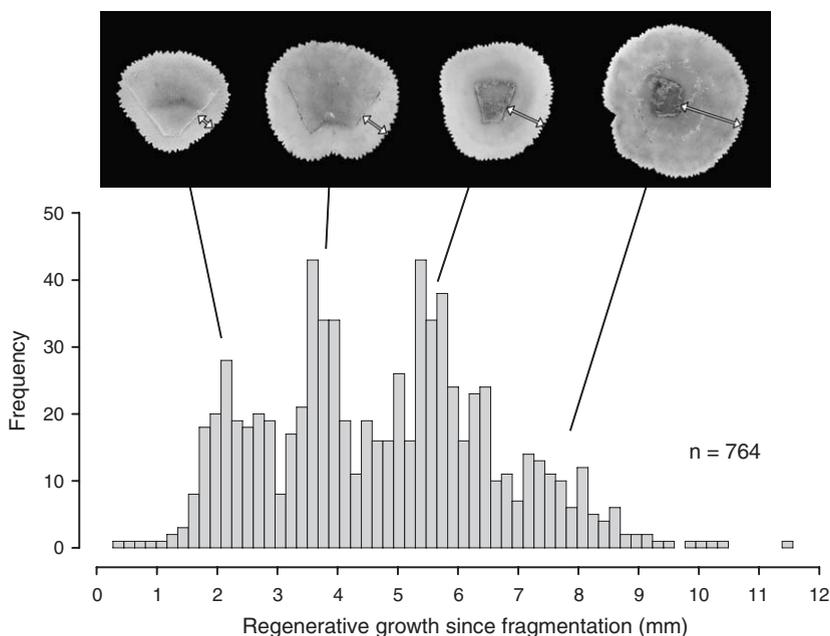


Fig. 4. Frequency histogram of regenerative growth since fragmentation in a population of *Cupuladria exfragminis* from the Gulf of Panama. Photographs of example colonies from each cohort showing method of measuring amount of regenerative growth since fragmentation.

older line of fragmentation and new regenerative growth is more difficult to see than older fragmentation which is much more obviously demarcated meaning it is measured more often. As such, a certain amount of noise will be expected in the frequency distribution of amount of regenerative growth.

3.4. Fragmentation and temperature

Twenty one of the 165 (12.7%) colonies in the low water temperature aquaria autofragmented while none of the 165 (0%) colonies in the warm water aquaria fragmented during the experiment. Those colonies that autofragmented in the low temperature aquaria were all large with notched margins (e.g. Fig 1).

The first colony to fragment did so ten days into the experiment and the ultimate 21 days later. From all fragmented colonies, 54 fragments were produced. The number of fragments produced by each colony ranged from two to five and the mean per colony was 2.57.

4. Discussion

4.1. Autofragmentation in *C. exfragminis*

Large colonies of *C. exfragminis* were observed to spontaneously fragment into two or more pieces without any external force. The term autofragmentation, used here to describe this process, is typically used to describe asexual propagation in aquatic plants where segments naturally separate from the parent plant (e.g. Madsen and Smith, 1997). It is applied here because the morphology of fragments is similar to those produced during mechanical fragmentation (see Marcus and Marcus, 1962; Baluk and Radwanski, 1984; Winston, 1988; Håkansson and Thomsen, 2001; O'Dea et al., 2004). Autofragmentation shares many similarities with colony fission that commonly occurs in cnidarians such as soft corals (McFadden, 1991) and Sea Anemones (Geller et al., 2005), and with autotomy that occurs in some gorgonian corals (Walker and Bull, 1983). Additionally, as will be described shortly, autofragmentation in cupuladriids, although morphologically distinct, is similar in process to colonial budding that has been described in other cupuladriid species (Marcus and Marcus, 1962; Håkansson and Thomsen, 2001).

The physiological processes of autofragmentation at the zooidal level were not examined in this study and so it remains unclear as to exactly how colonies separate into fragments. Nonetheless, it is possible to see in a large colony that lateral interzooidal calcification is first reduced and then stopped at points along colony margins

from where the colony will fragment. As the colonies continue to grow, these locations with no calcification form characteristic deep notches in the margin of the colony (e.g. Fig. 1).

Other cupuladriid species separate viable colony parts under a process called colonial budding (Marcus and Marcus, 1962). Colonies of *Discoporella umbellata* from the South American Atlantic (Marcus and Marcus, 1962; Håkansson and Thomsen, 2001) and *D. marcusae* from the eastern Pacific (Herrera-Cubilla et al., 2006) both produce lobes of growth from the colonies margins that are connected to the 'parent' colony by only a few zooids. A calcified connection is maintained between the bud and the colony so the bud requires a small amount of mechanical force to separate. As such, this form cannot be precisely labelled as self-separation even though such morphology predisposes the buds to breakage. However, in a possibly more advanced form, the Miocene cupuladriid *Reussirella haidingeri* reduced the connection between its buds and parent colony to a single zooid and made the connection between them uncalcified (Håkansson and Thomsen, 2001). As the bud developed, this connection could be lost, presumably by the colony itself, and the bud could separate from the 'parent' colony as and when desired (Håkansson and Thomsen, 2001).

The evolutionary relationship between autofragmentation and colonial budding remains unknown. However, observations made in this study illustrate how colonial budding may have evolved from autofragmentation. During autofragmentation, colonies stop marginal growth to produce a notched margin (e.g. Fig. 1). If this cessation of growth covered a much wider marginal region, these notches would extend laterally towards each other and the part of the colony that continued marginal growth would then produce what would essentially be a 'bud.'

All species that regularly use fragmentation to propagate are predisposed to fragmentation by mechanical means because of their weakly calcified colonies and/or colonial growth (Håkansson and Thomsen, 2001; O'Dea et al., 2004). Both autofragmentation and colonial budding may have evolved from colonies requiring happenstance to fragment if it became advantageous for the 'parent' colony to have more control over the timing of fragmentation, as has been shown for other colonial organisms (e.g. Highsmith, 1982; Lee and Noble, 1990). It may be important for example that a cupuladriid fragment or bud is retained until it is sufficiently large to maintain itself independently to escape smothering by sedimentation, although no data currently exists demonstrating a relationship between survivability and fragment size. It may also be that having control over the timing of fragmentation allows the 'parent' colony to ensure that separation coincides with a beneficial

biotic or abiotic factor that encourages regenerative growth. Support for this idea is provided here as *C. exfragminis* appears to use the lowered temperatures that occur during upwelling as a cue to autofragment in coincidence with the high food levels caused by upwelling, thereby increasing the chances for fragments to regenerate fully (see next section).

4.2. Stimulation of autofragmentation

Lowered temperatures similar to those experienced during upwelling times in the Gulf of Panama stimulated autofragmentation under culture conditions. This only occurred in large colonies that were prepared for fragmentation with heavily-notched colony margins. Demographic structure of growth in a single population from the Gulf of Panama shows a strong trend for periodic fragmentation, and the strongest cause of periodic changes in the region is intense seasonal upwelling. Comparing the amount of growth between cohorts of fragmentation to previous estimates of growth rates in cupuladriids (O'Dea and Jackson, 2002) strongly suggests that the periodic fragmentation observed here is annual. There is therefore strong evidence to suggest that seasonal upwelling stimulates *C. exfragminis* to autofragment. Colonies may use the lowered temperatures as a cue to indicate times of higher food levels that occur during upwelling times. Higher food levels have been observed to stimulate asexual propagation in a Seastar (Sköld et al., 2002) where it was believed to assist in the regeneration of new individuals. Similarly, higher food levels could help cupuladriid fragments regenerate, particularly in providing energies to defend against deleterious processes (see next section).

An association between food levels and rates of asexual propagation in cupuladriids can be best explained by understanding that new individuals propagate basically because of colony vegetative growth (Håkansson and Thomsen, 2001), and that growth in bryozoans increases with increasing food levels (Winston, 1976; Jebram, 1977; O'Dea and Okamura, 1999). Gross patterns in the prevalence of asexual propagation between cupuladriids from the Caribbean and eastern Pacific mirror these patterns with higher rates of asexual propagation by fragmentation occurring in areas of higher food availability (O'Dea et al., 2004).

4.3. Colony regeneration

Regenerative growth after fragmentation in colonies of *C. exfragminis* that had autofragmented was

significantly faster than in colonies that were mechanically fragmented. This could be because colonies of *C. exfragminis* prepare for fragmentation by reserving energies, thereby enabling more vigorous regenerative growth. Another explanation is that mechanical fragmentation damages colony structure more than autofragmentation. This could lead to a lower initial growth rate or an increased likelihood that adverse events, such as infection, take place. In support of the latter, autofragmented colonies are characterised by their clean lines of fracture that often cut along zooid margins while zooids are often split open when mechanically fragmented, leaving polypide tissues exposed.

Regenerative growth in *C. exfragminis* is substantially faster in the first month following fragmentation irrespective of whether the colony auto or mechanically fragmented. This in part supports the suggestion by Håkansson and Thomsen (2001) that fragmented colonies try to grow to a standard round disc shape with entire marginal growth as quickly as possible. However, although the initial growth spurt measured here is sufficient to generate the first two or three rows of zooids, it is not enough to create a complete circular colony shape. The growth spurt can be better explained either as a means of protecting against infection of recently opened internal tissues, or mechanically in that the presence of a set of radially distributed marginal vibracula around the whole periphery of a fragment assists in movement through sediment. The latter may be particularly important for small fragments that could potentially be easily buried during times of high sedimentation rate.

4.4. Prevalence of autofragmentation

Despite the experimental data presented here, the prevalence of autofragmentation in natural populations of *C. exfragminis* remains unknown. Understanding the potential causes of mechanical fragmentation and estimating rates of autofragmentation may enlighten the relative importance of the two.

Although fragmentation by wave action is a central means of asexual propagation in many reef corals (Dollar, 1982; Highsmith, 1982; Coffroth and Lasker, 1998), most cupuladriids inhabit muddy and sandy habitats deeper than 20 m (Cadée, 1975; Cook and Chimonides, 1994; O'Dea et al., 2004), and as such there is strong rationale to disregard such a process as important in the fragmentation of most cupuladriid populations (but see Winston, 1988). Indeed, confirmation that *C. exfragminis* has the ability to autofragment may illuminate a long-standing dilemma caused by records of populations with high levels of fragmented and regenerated colonies living in very low energy environments

(Darteville, 1935; Cadée, 1975). It is now not necessary to invoke processes such as wave or current action to explain asexual propagation, although it is highly unlikely that all cupuladriid species have the ability to autofragment.

Other processes of mechanical fragmentation include (1) direct predation, (2) indirect predation or grazing of animals or plants living on or in cupuladriid colonies, or (3) inadvertent fragmentation. Crabs have been observed to graze upon the epibionts that live on the surfaces of cupuladriid colonies (Greeley, 1967), and crabs are often associated with cupuladriids in trawl collections. Preliminary observations by F. Rodriguez have shown that this behaviour can result in the breakage of small groups of zooids from colony margins while the colony is handled by the crabs' claws. Cupuladriids have also been found in the gut contents of holothurians (Silén, 1942; Lagaaij, 1963) and echinoderms (Silén, 1942) which may also be an important factor in the fragmentation of mechanically weak colonies if grazing pressure is high. Cupuladriids and Holothurians are often found in high abundance together, but it remains unknown if the holothurians are ingesting the colonies for direct use as food or to graze colony epibionts, nor to what extent fragmentation occurs during the process of ingestion. Baluk and Radwanski (1977) used evidence of high crab and holothurian densities to explain high rates of fragmentation in a middle Miocene population of *Cupuladria canariensis* (Busk, 1859). Bottom feeding organisms disrupting the sediment may also cause inadvertent mechanical fragmentation of cupuladriid colonies.

Although it is clear that external mechanical forces created by direct and indirect predation could play an important role in the fragmentation of cupuladriid colonies there remains a dearth of empirical data to fully elucidate the impacts of biotic interactions upon the reproductive life histories of cupuladriid species.

Nonetheless, this study demonstrates that autofragmentation of *C. exfragminis* can occur quite readily. Indeed, most large colonies of *C. exfragminis* produce marginal notches that are characteristic of colonies approaching autofragmentation (Fig. 1), suggesting that autofragmentation could be an eventual part of the natural ontogenetic growth of colonies. But, some colonies, especially those that are sexually produced develop without showing morphological signs of autofragmentation. The final moment of autofragmentation appears to be controlled by an abiotic or biotic stimulus because the colonies that autofragmented in this study did so more or less in unison over a relatively short period of time. Further studies should investigate why

some colonies autofragment while others do not, the potential causes of autofragmentation under culture conditions and the prevalence of autofragmentation in natural communities.

Our understanding of the frequency of different modes of reproduction in cupuladriids is potentially limited by poor sampling because it is currently unclear how prevalent small, sexually produced colonies are within populations. This is extremely important because a sampled population may appear to comprise 100% asexually-produced living colonies, not because the population has ceased to put energy into gamete formation and reproduce asexually, or that asexuality is the preferred mode of propagation for that species, but simply that all small, sexually produced colonies die at an early age and are therefore not being included in the sample (see Cheetham et al., 2001). To understand fully the prevalence of sexuality in cupuladriid assemblages future studies should collect data from whole assemblages, including those smaller than 3 mm that would have been lost in this study.

4.5. Model of propagation in *Cupuladria exfragminis*

From the data presented here a model of propagation in *C. exfragminis* is proposed. The majority of colonies of *C. exfragminis* are produced asexually, although it is currently difficult to assess exactly how much sexual reproduction occurs in *C. exfragminis* because very small colonies were not included in this analysis. Either sex very rarely occurs, or all sexually produced larvae fail to create colonies larger than a couple of millimetres. Calcification of all colonies is low and colonies are therefore prone to mechanical fragmentation (by actions such as epibiotic grazing by crabs). If the colony is not fragmented by mechanical processes, morphological traits develop as the colony grows that prepare the colony for autofragmentation. Lateral interzooidal calcification ceases at two to five regular spaced points along the colony margin creating uncalcified radial lines as the colony continues to expand in size, which eventually leads to deep marginal notches. *C. exfragminis* uses the lower temperatures that occur during times of upwelling in the Gulf of Panama as a cue to indicate times of the coincident increase in food availability, and the colony autofragments by dividing its colony along the uncalcified radial lines. Initial regeneration of fragments following fragmentation occurs at an unusually rapid rate to ensure survival of the colony. This rate is faster if the colony autofragmented than mechanically fragmented, probably as a result of preparations made by the colony prior to fragmentation.

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