Mass Spawning by Green Algae on Coral Reefs

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Predawn episodes of mass spawning by green algae (up to nine species in five genera on a single morning) intermittently cloud Caribbean waters. Species- and sex-specific bouts of anisogamous gamete release occurred synchronously and predictably on a given morning, with closely related species spawning at different times. Algal sexual reproduction was seasonal, but, unlike the mass-spawning behavior of other sessile marine organisms, showed no lunar or tidal cycling. The discovery of mass-spawning behavior by these algae has important implications for future studies of the reproductive ecology and speciation of a vital, yet poorly understood, component of the coral reef community.

Siphonous green algae (order Bryopsidales) currently thriving on coral reefs throughout the world are a vital component of the reef community. As large, abundant primary producers, they help anchor one of the world's most complex trophic networks by providing food and shelter to countless reef organisms (1). As competitors for space with corals and other benthic organisms (2), their shifting abundance provides an index of reef health (3). Their complex biochemistries, maintained to deter (4) or possibly attract (5) herbivores, also have noteworthy biomedical properties (6). Even in death, their discarded calcareous skeletons help build reefs (7), generate sand (8), and provide meaningful diagnostic clues to geologists and paleobiologists (9). For all their relevance to coral reef ecology, however, the basic biology of these important algae remains poorly understood; varied and complex modes of both sexual and asexual propagation (10) mask even the most basic aspects of their growth, mortality, and dispersal. In contrast to the better studied red and brown algae of temperate waters (11), field studies of reproduction by tropical green algae are rare, and beyond occasional laboratory observations of reproductive processes (12–14), the mechanism, organization, and significance of this reproduction remains virtually unexplored for natural populations.

Like many sessile marine invertebrates, sexual reproduction in Bryopsidales involves the release of anisogamous "male" and "female" gametes into the water column (15). Under these conditions, gamete concentration generally governs fertilization success, with presumably strong selection for spawning synchrony between sexes within species (16). Such synchrony may involve both the timing of gamete release by conspecifics on a given day and day-to-day patterns of synchrony within the population. Conversely, asynchronous spawning by closely related species may avoid costly hybridization [as with some corals (17)].

Recent observations of seasonal (Fig. 1A), synchronous, short-lived bouts of early morning spawning reveal that sexual reproduction by tropical green algae is an organized and pervasive phenomenon on coral reefs. An initial observation of spawning off the coast of Panama (18) by *Udotea flabellum* (at 5:53 a.m. on 26 October 1994) prompted this study. Observations of 38 additional spawns by eight species (*Caulerpa cupressoides*, *C. racemosa*, *Halimeda in-*

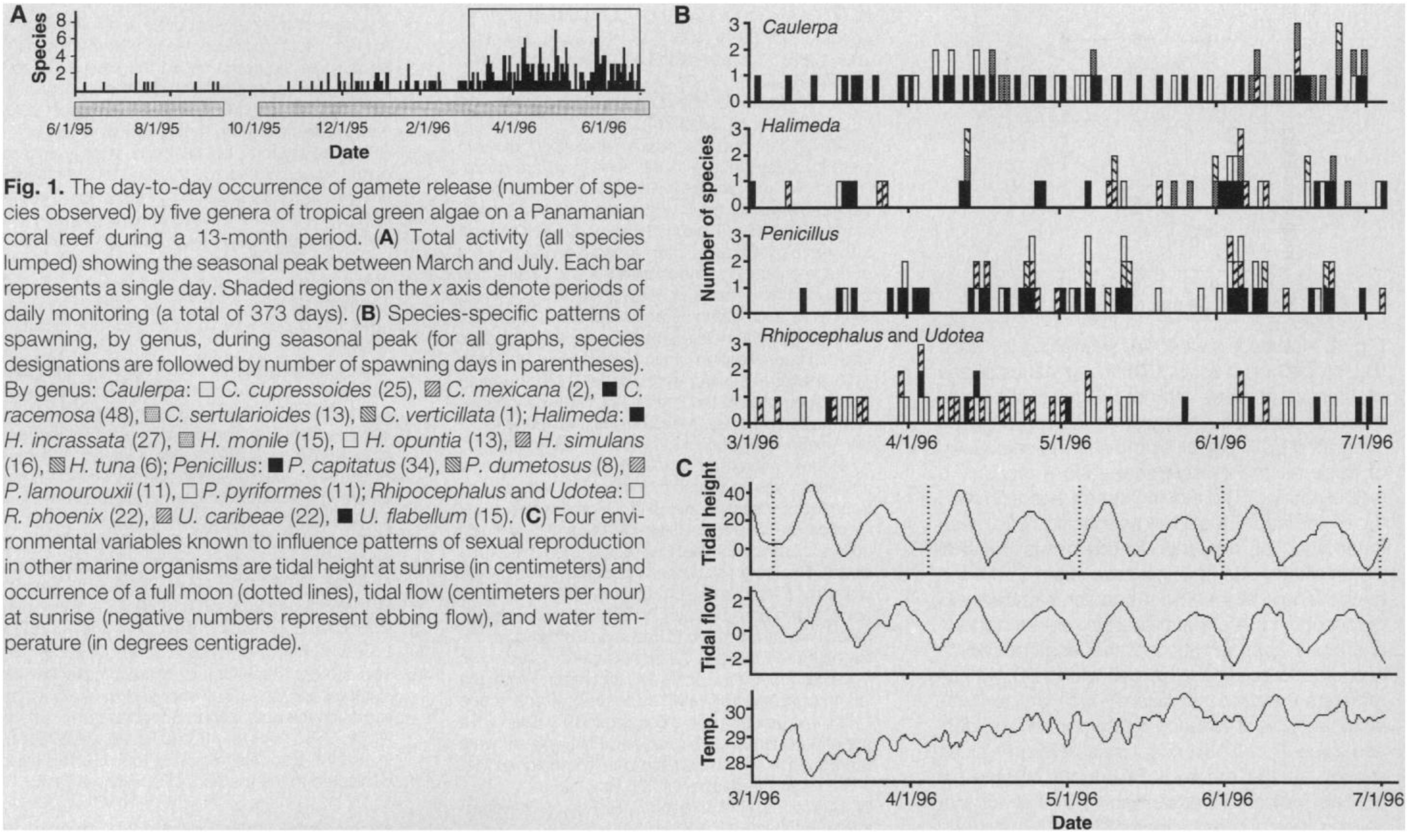

Fig. 1. The day-to-day occurrence of gamete release (number of species observed) by five genera of tropical green algae on a Panamanian coral reef during a 13-month period. (**A**) Total activity (all species lumped) showing the seasonal peak between March and July. Each bar represents a single day. Shaded regions on the *x* axis denote periods of daily monitoring (a total of 373 days). (**B**) Species-specific patterns of spawning, by genus, during seasonal peak (for all graphs, species designations are followed by number of spawning days in parentheses). By genus: *Caulerpa*: □ *C. cupressoides* (25), ▨ *C. mexicana* (2), ■ *C. racemosa* (48), ▤ *C. sertularioides* (13), ▧ *C. verticillata* (1); *Halimeda*: ■ *H. incrassata* (27), ▤ *H. monile* (15), □ *H. opuntia* (13), ▨ *H. simulans* (16), ▧ *H. tuna* (6); *Penicillus*: ■ *P. capitatus* (34), ▧ *P. dumetosus* (8), ▨ *P. lamourouxii* (11), □ *P. pyriformes* (11); *Rhipocephalus* and *Udotea*: □ *R. phoenix* (22), ▨ *U. caribeae* (22), ■ *U. flabellum* (15). (**C**) Four environmental variables known to influence patterns of sexual reproduction in other marine organisms are tidal height at sunrise (in centimeters) and occurrence of a full moon (dotted lines), tidal flow (centimeters per hour) at sunrise (negative numbers represent ebbing flow), and water temperature (in degrees centigrade).

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crassata, *H. monile*, *H. opuntia*, *Penicillus capitatus*, *P. pyriformes*, and *Udotea flabellum*) were made during 138 days of subsequent monitoring before 12 May 1995. Beginning on 6 June 1995, 25 to 50 tagged algae of 14 species (listed in Fig. 1, excluding *Caulerpa mexicana*, *C. sertulariodes*, and *C. verticillata*) were visually censused for fertility twice daily (05:00 to 07:30 and 14:00 to 16:30) except between 13 September and 30 September 1995, when no censuses were done. Extensive surveys of untagged algae were also conducted daily.

In all, 17 species of siphonous green algae showed highly organized patterns of reproductive synchrony, with tens to thousands of conspecific algal thalli becoming simultaneously fertile overnight (*19*). Fertile algae released copious green clouds or mucilaginous streams of gametes (Fig. 2) into the water column during short-lived (5 to 15 min), species-specific, periods the following morning (Fig. 3). Up to nine species within five genera spawned on the same morning, although day-to-day coordination between species appeared haphazard (Fig. 1B). During major spawns, water visibility quickly dropped to less than 1 m over large, downcurrent sections of reef, although such clouds generally dispersed within 10 to 15 min. Algae released their entire protoplasmic contents during spawning and died immediately afterward, the empty thalli often disintegrating within hours (*20*). Thus, the entire sexual episode, from the onset of fertility to death, is a remarkably transient event, lasting less than 36 hours (two-thirds of which pass in darkness).

More closely related species often spawned on the same morning (Fig. 1B) but did so at different times (Fig. 3), presumably reducing the mixing of gametes with the greatest potential for hybridization (*21*). Lower ambient light levels and water temperatures typically delayed spawning, increasing the range of spawning times observed during the study (*22*). Gametes remained motile for 40 to 60 min after release, although gamete movement stopped after fusion, with the resultant, negatively buoyant zygote quickly sinking (*23*).

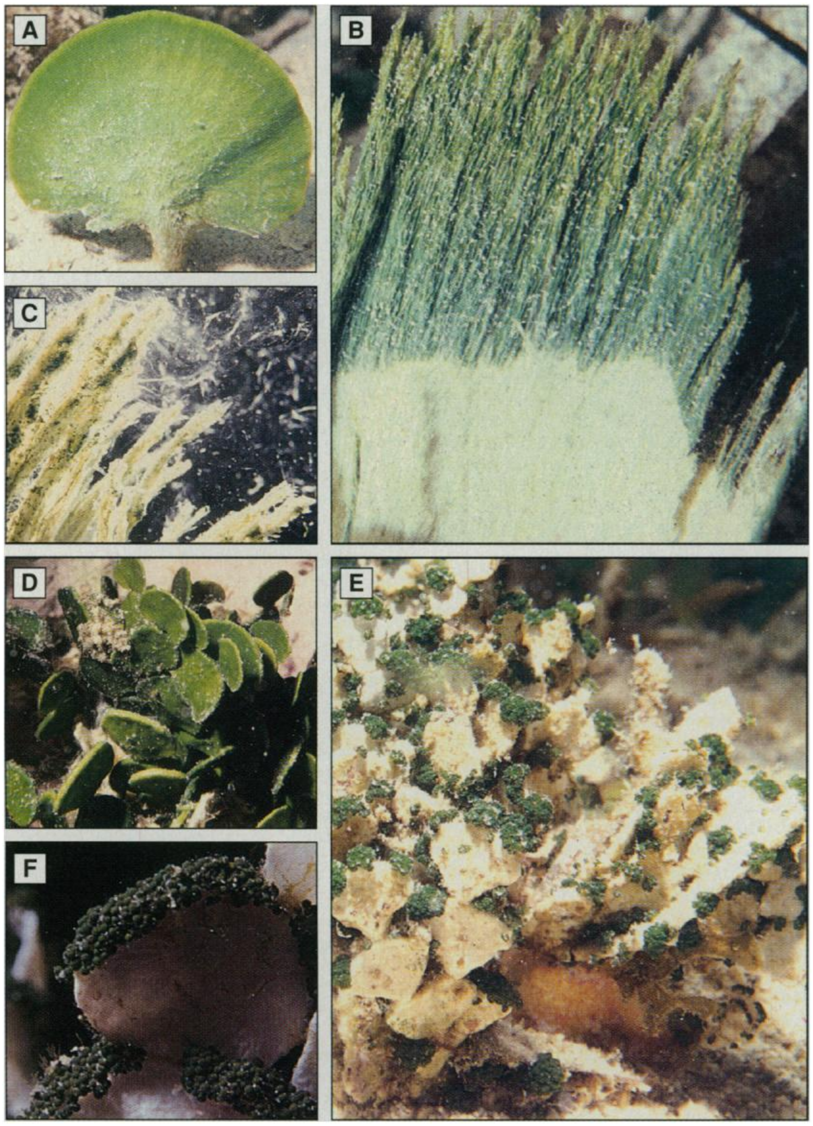

Fig. 2. Photographs of green algae before and during gamete release. (A) through (C) *Udotea caribeae*. (**A**) A typical green thallus of nonfertile plant. (**B**) A close-up of green gametangia of fertile female is shown. This condition developed overnight as the entire protoplasmic contents migrated from the thallus below (now white). (**C**) An extreme close-up of gamete release shows individual gametes (green spots) visible within mucilaginous streams. (D) through (F) *Halimeda*. (**D**) Nonfertile *Halimeda tuna*. (**E**) Fertile *H. simulans*, with clustered gametangia along blade margins; (**F**) A close-up of gametangial clusters on a fertile plant.

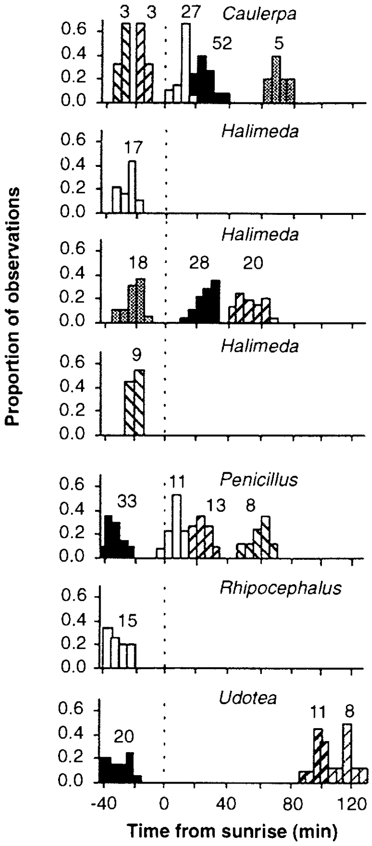

Fig. 3. Species-specific histograms of the onset of spawning relative to sunrise (dotted line). Species designations are as in Fig. 1, sample sizes for each species are given above bars, and times of spawning initiation are means for days with multiple observations. Apparently overlapping spawning times for *C. cupressoides* and *C. racemosa* reflect spawns on different days (mean difference between species for four, same-day spawns = 25 min, t = 5.107, $P <$ 0.001). Species of *Halimeda* are separated by clade (*21*). The separate plotting of male (heavier hatching) and female (lighter hatching) gamete release for *U. caribeae* highlights the earlier spawning of males (*27*).

Unlike animals, the gametes of both sexes in siphonous green algae are motile. In the genus *Halimeda*, strongly phototactic macrogametes may cover relatively long (50 to 100 cm) distances in a matter of minutes (*24*). Such motility may substantially alter the kinetics of fertilization during broadcast spawns and explain why, in species within the four dioecious genera (*25*) [where color identifies parental sex (*26*)], males consistently began releasing gametes several minutes before females (*27*).

Although the diel-timing of spawning was easily predicted once fertile algae were detected on a reef, and algal sexual activity was broadly seasonal (*28*), the environmental or biological factors triggering the onset of sexual reproduction remain unknown. The extent of fertility within the population varied unpredictably both within and between species (*29*), and its occurrence showed no apparent relation to lunar, tidal, or water temperature conditions (Fig. 1, B and C). In contrast, mass-spawning corals and gorgonians show extreme levels of within-population synchrony, with highly predictable patterns of annual and lunar cycling (*16*). The extent to which these differences relate to aspects of life history, such as rates of mortality and clonal reproduction, awaits further study.

Ten years have passed since the announcement of mass spawning by corals (*30*) galvanized scientists worldwide and heralded a new era of reef studies. The discovery of highly organized patterns of spawning by green algae on coral reefs offers similar promise. Future studies of fertilization dynamics, reproductive isolation, life history, the biochemical shifts associated with the sexual process, and the environmental triggers associated with this reproduction seem likely to provide many new insights into the ecological and evolutionary processes occurring within this diverse biome.

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15. The production of anisogamous gametes from various species of *Caulerpa*, *Halimeda*, and *Udotea* known from earlier work (*12, 13*) was confirmed microscopically for all species during the present study.
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18. Most observations of gamete release were made on WB-12, a large patch reef near the Smithsonian Tropical Research Institute's field station off Punta San Blas, Panama, although spawns by species of *Caulerpa*, *Halimeda*, and *Penicillus* were also seen on nearby reefs [House, PT-12, Tian tupo, Ulagsukun-C, and WB-20; for locations, see D. R. Robertson, *Copeia* **1987**, 637 (1987)].
19. Fertility (the conversion of protoplasmic contents into gametes with subsequent migration into gametangia) occurred within a 12-hour period, overnight, and can be detected, in situ, in all five genera, on the basis of color change and the appearance of gametangia [previously known for *Halimeda* (*13*)]. No evidence of spawning outside the morning observation period was ever detected.
20. This pattern of fertility and death, known as holocarpy, is diagnostic of the order (*10*). All traces of *Caulerpa*, *Penicillus*, and *Rhipocephalus* were generally gone from the reef within 24 hours. Remnants of the collapsed thalli of *Halimeda* and *Udotea caribeae* persisted 24 to 48 hours after spawn, and the blade *U. flabellum* often persisted for weeks.
21. Placement of the five *Halimeda* species within three cladistic sections (*13*) was recently validated by molecular techniques [L. Hillis and J. Engman, *J. Phycol.* **32**, 21 (1996)]. Gametes lose motility, in vivo, 45 to 60 min after release.
22. Colder water delayed spawning by ~8 min/°C for *C. racemosa*, the most actively reproductive species in the study (Regression; $R^2 = 0.43$; $P = 10^{-4}$; $n = 37$). This trend was present in all genera (analysis of covariance for 10 spp. with >5 observations; $F(1,126) = 7.98$; $P < 0.005$). Increasing light levels apparently initiate the process of gamete release (*14*) and dark mornings caused by heavy overcast, rain, or turbid water typically delayed gamete release on the reef by 5 to 15 min.
23. I observed gamete behavior on uncovered microscope slides at 100× and 400×, using gametes released into aquaria (10 gallons) or plastic buckets (5 gallons).
24. Eyespots are a diagnostic feature of many bryopsidalian macrogametes (*13*). Although the expression of phototaxis within the group is not well studied, *Halimeda* macrogametes were observed to swim rapidly toward a light source in both the laboratory and the field.
25. Species of *Halimeda* (*11*), *Penicillus* (*12*), *Udotea* (*12*), and *Rhipocephalus* (confirmed, this study) are dioecious; *Caulerpa* is monoecious [M. Goldstein and S. Morall, *Ann. N.Y. Acad. Sci.* **175**, 660 (1970)].
26. For dioecious species, the light green color of fertile "males" is easily distinguished, in situ, from the brownish, dark green [*Halimeda* (*13*)], or gray (*Udotea*, *Rhipocephalus*, *Penicillus*) colors of females. These color differences before gamete release allowed the detection of sex-specific patterns of spawning.
27. Gamete release by male algae began 1 to 19 min before female algae began releasing gametes during all 56 pairwise comparisons between sexes. Mean difference (min) ± 1 SE and sample size, by species: *Halimeda incrassata* (6.2 ± 1.0, 9); *H. monile* (2.8 ± 0.5, 5); *H. opuntia* (4.2 ± 0.4, 5); *H. simulans* (7.0 ± 0.7, 5); *H. tuna* (3.5 ± 0.9, 4); *Penicillus capitatus* (4.0 ± 0.7, 9); *P. dumetosus* (19.5 ± 0.5, 2); *P. lamourouxii* (4.0 ± 0.6, 3); *P. pyriformes* (4.3 ± 2.9, 3); *Rhipocephalus phoenix* (2.7 ± 0.9, 4); *Udotea flabellum* (5.0 ± 1.4, 5); *U. caribeae* (6.0 ± 0.3, 7).
28. Of the spawns observed between 5 June 1995 and 3 July 1996, 83% took place between March and July (48% of the sampling period). In the previous year, between 24 October 1994 and 1 May 1995, 90% of the 39 observed spawns took place after 1 March 1995 (50% of the sampling period). Panama's dry season (January through April) is characterized by strong trade winds and greater levels of solar irradiance [J. D. Cubit *et al.*, *Coral Reefs* **8**, 59 (1989)]. Overall levels of algal reproduction were higher in 1996 than in 1995 (for example, compare the occurrence of spawning during June 1995 and June 1996, Fig. 1A). The eight species observed spawning before 5 June 1995 (*18*) spawned at a rate of 0.3 spawn per day between 26 October 1994 and 3 July 1995 (172 days of observation). The same eight species spawned at more than twice that rate (0.7 spawn per day) between the same dates a year later (251 days of observation).
29. The number of thalli that released gametes on a given morning was estimated qualitatively. Within a species, only 3 to 5% of the population typically became simultaneously fertile (several hundred plants), although larger spawns (15 to 20%) did occur. Levels of fertility in one species appeared unrelated to the activity of other species. The abundance of more actively spawning species (for example, *Caulerpa racemosa*, 39 spawns during the 125-day period between 1 March and 3 July 1996) declined noticeably during the study but was not quantified.
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31. I thank the people of Kuna Yala and the Republic of Panama for permitting this work; L. Clifton, V. Francesco, P. Nachtrieb, M. Rodruigez, and M. Schlaepfer for their predawn efforts; E. Clifton, P. Raimondi, and D. Reed for their comments; and the Smithsonian Tropical Research Institute (STRI) Marine Environmental Sensing Program for water-temperature data. Supported by STRI, Smithsonian's Scholarly Studies program (SS1234-530A), and the University of California Toxics Program.

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