The Ecological Significance of Sexual Reproduction by Tropical Green Algae

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ABSTRACT. The ability of scuba-equipped scientists to directly observe marine organisms in their natural environments has dramatically improved our understanding of marine ecological processes. Despite advances on numerous fronts, however, the basic ecology of many important groups, including the tropical green algae, remains relatively unstudied. This paper examines how aspects of sexual reproduction by these algae relate to their population dynamics and includes discussions of gamete formation, spatial dispersion of males and females, herbivory on fertile algae, the nature and timing of gamete release, fertilization success, and zygote dispersal. Further investigations into any of these topics promise to shed useful light on an ecologically important group of tropical seaweeds.

INTRODUCTION

The field of subtidal marine ecology owes much of its success to the technology of scuba. With the advent of open-circuit air delivery, scientists were no longer forced to rely on samples dredged from the depths or washed up on the shore to infer ecological processes. Organisms could be observed firsthand in their natural setting, and followed through time at a variety of depths and locations. As a result, we have learned a great deal about how marine life forms live and die beneath the waves, but there is still much to be learned.

Perhaps nowhere is this more apparent than in the study of tropical green algae. Five genera of siphonous green algae in the order Byropsidales (*Caulerpa* in the family Caulerpaceae and *Halimeda, Penicillus, Rhipocephalus,* and *Udotea* in the family Udoteacae) are particularly notable for their size, their abundance, and the range of habitats they occupy on or near coral reefs (Bold and Wynne, 1985; Littler and Littler, 1994, 2007; Hay, 1997). Prior to direct observations enabled by the application of scuba, very little was known about the ecological role of these algae.

We now know that these seaweeds are extremely important members of shallow-water, tropical marine communities. As primary producers, these algae help sustain many reef-associated herbivores (e.g., Morrison, 1988; Niam, 1988; Littler and Littler, 1994; Stachowicz and Hay, 1996; Williams and Walker, 1999; Munoz and Motta, 2000). As relatively large, structurally complex benthic flora, tropical green algae provide shelter for numerous invertebrates (e.g., Stoner, 1985; Hendler and Littman, 1986; Hay et al., 1990) while competing directly with others for space (e.g., Carpenter, 1986; Hughes et al., 1987; Littler et al., 1989; Tanner, 1995; Ceccherelli et al., 2000; Beach et al., 2003; Márquez and Diaz, 2005). Correlations between increasing green algal abundance and declining coral cover and reef biodiversity further emphasize the ecological significance of these seaweeds within coral reef communities (e.g., Porter and Meier, 1992; Hallock et

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al., 1993; Hughes, 1994a, 1994b; Ogden and Ogden, 1994; Sebens, 1994; Morand and Briand, 1996; Shulman and Robertson, 1996; Szmant, 2002; Smith et al., 2006; Nelson, 2009). Tropical green seaweeds also produce complex defensive compounds that alter the foraging of herbivorous fishes and invertebrates (e.g., Paul and Fenical, 1986; Paul, 1987; Paul and Van Alstyne, 1988; Hay and Fenical, 1992; Stachowicz and Hay, 1996) and potentially have useful biomedical properties (e.g., Fischel et al., 1995; Isassi and Alvarez-Hernandez, 1995). Even after death, the heavily calcified thalli of the Udoteacae contribute to sand production, reef building, and other important geological processes (e.g., Wefer, 1980; Drew, 1983; Flügel, 1988; Marshall and Davies, 1988; Freile et al., 1995; Braga et al., 1996; Freile and Hillis, 1997; Martin et al., 1997).

Despite the obvious relevance of tropical green seaweeds to reef-associated ecosystems, their basic biology remains rather poorly understood, particularly aspects of their reproductive life history. As with many algae, variable, often subtle modes of reproduction obscure many of the most basic aspects of their life history (Bold and Wynne, 1985; Brawley and Johnson, 1992; Lobban and Harrison, 1994); there is a paucity of careful field studies that focus on the algae themselves (Walters et al., 2002; Vroom et al., 2003; van Tussenbroek and van Dijk, 2007). Indeed, the life cycles of some of the most abundant and important groups (e.g., *Halimeda*) have yet to be followed completely in either the lab or the field (see Meinesz, 1980). Put simply, more studies are needed that examine how tropical green seaweeds live and die. This paper seeks to promote further investigations of this cycle by highlighting the potential ecological significance of different aspects of sexual reproduction by tropical green macroalgae.

As with many algae, Bryopisdales reproduce both asexually and sexually. Early studies of vegetative reproduction via rhizoid extension (Hillis-Colinvaux, 1973; Walters and Smith, 1994) and, more recently, thallus fragmentation (Smith and Walters, 1999; Walters et al., 2002) support a general premise that asexual processes contribute significantly to the dynamics of green seaweed populations on and around coral reefs (e.g., Friedmann and Roth, 1977; Hillis-Colinvaux, 1980; Meinesz, 1980). At the same time, observations of seasonal pulses of highly synchronous sexual activity by green algae on Caribbean reefs (Clifton, 1997, 2008; Clifton and Clifton, 1999; van Tussenbroek et al., 2005) imply a previously underappreciated role for sexual reproduction in terms of population regulation and ecological influence. Because sexually reproducing algae die immediately following gamete release, annual peaks of reproduction by tropical green seaweeds have immediate effects on algal demography as density and cover percentage decline precipitously, often in a matter of weeks (Clifton and Clifton, 1999). While the synchronous nature of gamete release presumably boosts fertilization success during episodes of sex, how these bouts of reproduction contribute to subsequent algal recruitment and repopulation of reefs is currently unknown. The remainder of this paper examines how different aspects of sexual reproduction by green algae may contribute to their ecological significance.

GAMETE FORMATION AND SEXUAL IDENTITY

Although easily detected by a trained observer, fertility and sexual reproduction in tropical Bryopsidales is a transient, often overlooked phenomenon. It begins when, overnight, a fraction of the population (tens to thousands of thalli) changes color and/or develops external gametangia. These macroscopic features are clear and reliable indicators of an impending sexual event (Clifton and Clifton, 1999). What induces this pulse of fertility remains unknown (environmental factors such as tides, moon phase, water movement, temperature, and day length are known to organize bouts of synchronous sexual reproduction in other marine organisms; see reviews by Harrison and Wallace, 1990; Brawley and Johnson, 1992; Levinton, 1995). Within 24 or 48 hours (depending on species) the entire algal protoplasm converts to gametic products that migrate into newly developed gametangia. For all species but those in the monoecious genus *Caulerpa*, the gender of a fertile thallus (based on gametangia color and morphology; see Figure 1) becomes apparent during this time (Clifton and Clifton, 1999), facilitating measures of local sex ratios and nearest-neighbor distances prior to gamete release. For species of *Caulerpa*, macro- and microgametes concentrate in different parts of the thallus and can be identified based on color (green = microgametes, brown = macrogametes; Figure 2). These general patterns of fertility have now been verified for over 30 species in 6 genera (Clifton and Clifton, 1999; KEC, personal observation).

The spatial dispersion of fertile male or female thalli presumably plays an important role in fertilization success. Proximity to other reproductive individuals coupled with the synchrony of release generally influences gamete concentration in broadcast-spawning organisms, and gamete concentration influences the likelihood that gametes of opposite sex will encounter one another following release (Lotterhos and Levitan, 2010). The spatial dispersion of males and females may be especially important for dioeceous, sand-dwelling species that occur in a broad range of densities (from isolated individuals to large "meadows" with densities of hundreds of individuals/m^2; e.g., *Halimeda incrassata*, *H. monile*, *H. simulans*, *Penicillus* spp., *Rhypocephalus phoenix*, and *Udotea* spp.). For these species, small, same-sex clusters of two or three individuals (within 5 cm of one another; KEC, unpublished data) are commonly encountered. It remains to be known whether these represent genetically identical thalli derived from vegetative reproduction (ramets), in which case the synchronous release of gametes would boost gamete concentrations and reduce the likelihood of gamete limitation (sensu Levitan, 1993), or whether they are genetically distinct thalli (genets) derived from sexual reproduction and recruitment into the population, in which case synchronous reproduction would increase gamete competition for fertilization. Gender-specific dispersion is, at first glance, less relevant for monoeceous species such as *Caulerpa*. However, differential patterns of sexual allocation to micro- and macrogamete production coupled with the overall spatial distribution of reproductive thalli could still play an
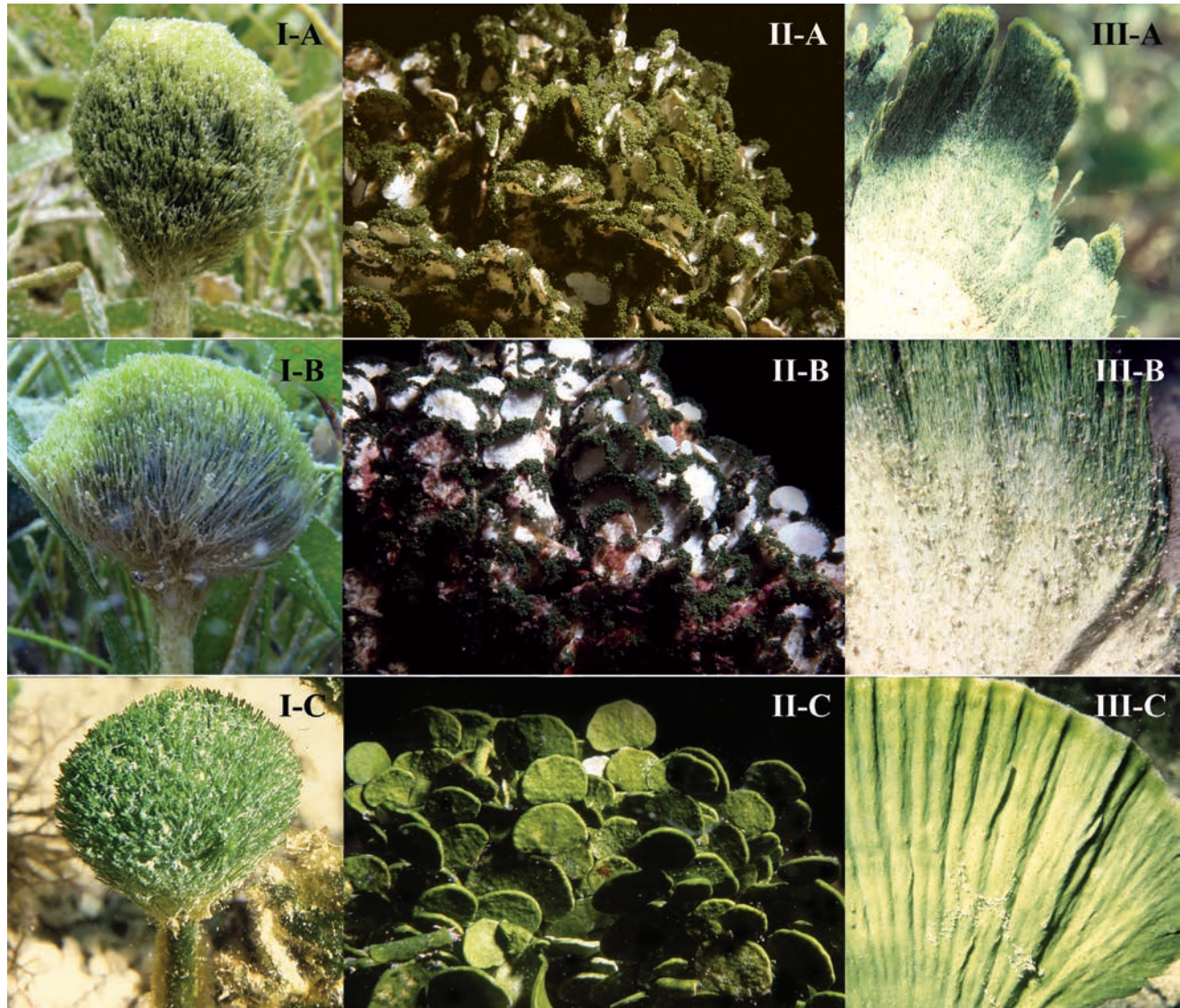
FIGURE 1. Examples of fertility—evident by the presence of gametangia and color change, as well as gender identity—in dioecious species of siphonous green seaweeds. Species by column: (I) *Penicillus capitatus* (note the white stipe and lighter color of the capitulum in fertile seaweeds); (II) *Halimeda tuna* (external gametangia and white blades denote fertile condition); (III) *Udotea caribea* (note white blade and newly developed gametangia along terminal blade edge). Gender by row: (A) fertile males (lighter green color for all species and rounded tip morphology of gametangia for *U. caribea*); (B) fertile females (darker green/bluish color for all species and spiked tip morphology of gametangia for *U. caribea*); (C) nonfertile thalli for all three species. Photos by Kenneth Clifton.

important ecological role in determining the likelihood of successful zygote formation.

SEXUAL REPRODUCTION AND HERBIVORY

A diverse array of crustaceans, echinoderms, mollusks, and fishes consume siphonous green algae as part of their regular diet. These seaweeds counter with an evolved combination of chemical and physical defenses that deter rates of feeding by different herbivores to varying degrees (e.g., see Hay and Fenical, 1992; Williams and Walker, 1999; Paul et al., 2001; Molis et al., 2008; and references therein). To date, studies of herbivory on Bryopsidales have focused almost exclusively on the consumption on nonreproductive thalli.

The potential for chemical and physical defenses to herbivory to be altered by a shift from vegetative to reproductive
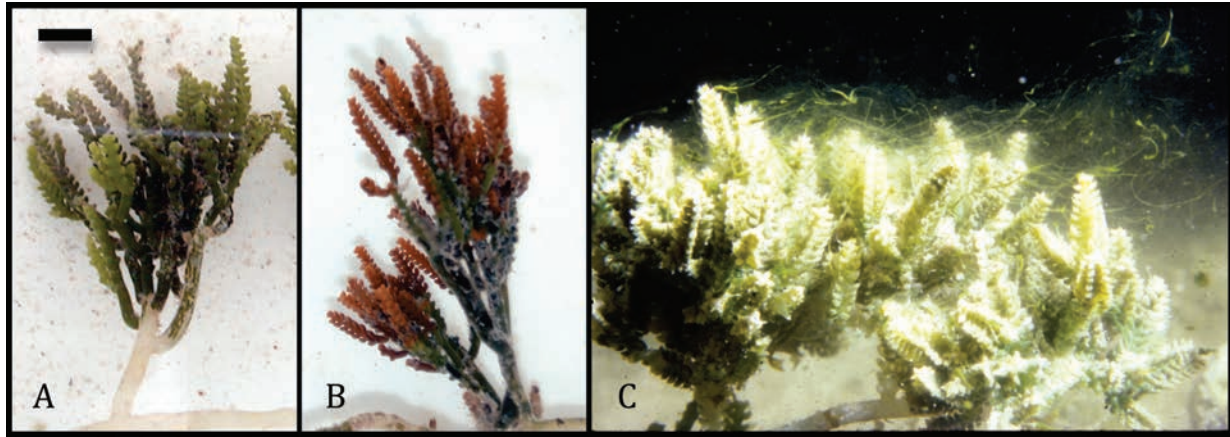
FIGURE 2. Progression of fertility in the monoeceous green alga *Caulerpa cupressoides*. (A) Cellular contents migrate from rhizoids into unspecialized gametangia at terminal ends of blades approximately 48 hours prior to gamete release. (B) Roughly 24 hours later, segregation of macrogametes (brown, terminal areas of blade) and microgametes (greener, basal sections) occurs, leading to a two-colored appearance. (C) The discharge of both micro- and macrogametes occurs from separate small tubular orifices that develop 1–3 hours prior to gamete release. Scale bar = 1 cm (all three images). Photos by Kenneth Clifton.

state seems high. Uncalcified gametangia, the structures that house gametes prior to their release, develop during the initial stages of fertility. Thus, they are potentially exposed to herbivores that would normally be deterred by the presence of $CaCO_3$ for one or more night/day cycles, depending on the species of algae. The chemical defenses of these seaweeds may be similarly altered, as the conversion of the entire thallus from vegetative protoplasm to gametes occurs during a similar time span. While the phenomenon has not been investigated sufficiently, observations of heavy infestations of the sacoglossan sea slug *Elysia tuca* on fertile specimens of sand-dwelling *Halimeda incrassata* and *H. monile* (hundreds of slugs on a single reproductive thallus versus a normal load of one or fewer slugs on a nonreproductive thallus; KEC, pers. obs.) indicate that some species of herbivores respond directly to the expression of sexual reproduction by green algae. Severe herbivory on the gametangia of fertile specimens of *Udotea*, *Penicillus*, and *Rhypocephalus* have also been observed (Figure 3). If a shift to fertility does attract a disproportionate level of herbivory, it may represent an additional cost of reproduction to the seaweed; the phenomenon certainly merits further study.

GAMETE CHARACTERISTICS

The flagellated, anisogamous gametes produced by siphonous green algae are known from a variety of studies (e.g., Goldstein and Morrall, 1970; Meinesz, 1972, 1980; Kajimura, 1977; Hillis-Colinvaux, 1980; Enomoto and Ohba, 1987; Clifton and Clifton, 1999). The size of biflagellated male microgametes is relatively consistent across taxa, whereas female macrogametes

occur in two forms, depending on species. *Halimeda*, *Caulerpa*, and one species of *Udotea* (*flabellum*) produce biflagellated, phototactic macrogametes that morphologically resemble microgametes but are 2–34 times larger (Clifton and Clifton, 1999) and possess an obvious eye-spot. Relative gamete size may play an important role in fertilization dynamics in terms of both gamete encounter rates (Levitan, 2006) and gamete behavior. Although negatively buoyant, biflagellated macrogametes can swim relatively long (1–5 m) distances toward light (Clifton, 1997) prior to fertilization. As in other green algae (e.g., Togashi et al., 1998), zygotes of these species show negative phototaxis immediately after fertilization (KEC, unpublished). In contrast, *Penicillus*, *Rhipocephalus*, and three species of *Udotea* (*abbottiorum*, *caribaea*, and *cyathiformes*) produce large (100 μm diameter) stephanokont gametes (Figure 4) with flagella arrayed along a membranous, sheetlike tail (Clifton and Clifton, 1999). To date, these large, macroscopically visible gametes (Figure 5) have not been well studied (Meinesz, 1980; Littler and Littler, 1990); however, observations of freshly released material indicate that these gametes are relatively immotile. Under calm conditions they quickly sink, tail up, to the bottom, where flagellar motion drives water past the gamete. This may increase encounter rates with microgametes. Upon fusion, the membranous tail quickly (30–90 s) stops moving and is absorbed into the zygote. Both negative phototaxis and negative buoyancy presumably limit the distance a zygote disperses, however the extent of this limitation and the degree to which it influences local population dynamics following zygote development and recruitment awaits further study.

Given that siphonous green algae convert their entire protoplasm into gametes, and in keeping with a simple model of quantity versus quality, the number of gametes produced by an alga
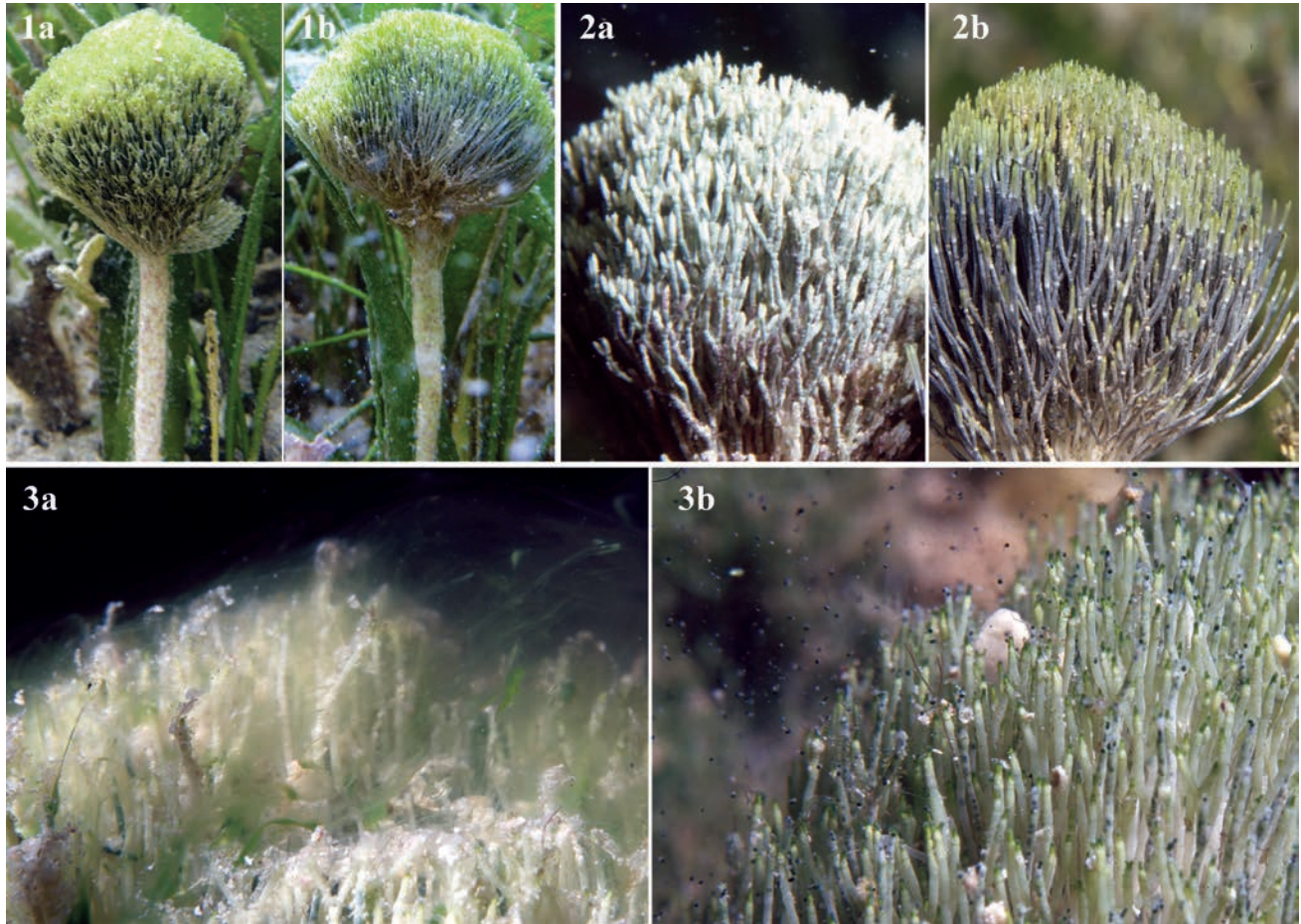
FIGURE 3. Details of protoplasm migration, gametangia development, and gamete release for male and female *Penicillus lamourouxii*. (1) Fertile male (a) and female (b) thalli showing diagnostic white stipe caused by protoplasmic migration. The darker, blue/green coloration of the female and the lighter green color of the capitulum are both reliable indicators of fertility and gender. (2) Closer view of the top section of a fertile male (a) and female (b) 12 hours prior to gamete release. Note the light green, uncalcified extension of siphonous tubes and clearly evident sexually dimorphic colorations. (3) Gamete release from male (a) and female (b) seaweeds. Individual macrogametes can be seen wafting away from the female. Photos by Kenneth Clifton.
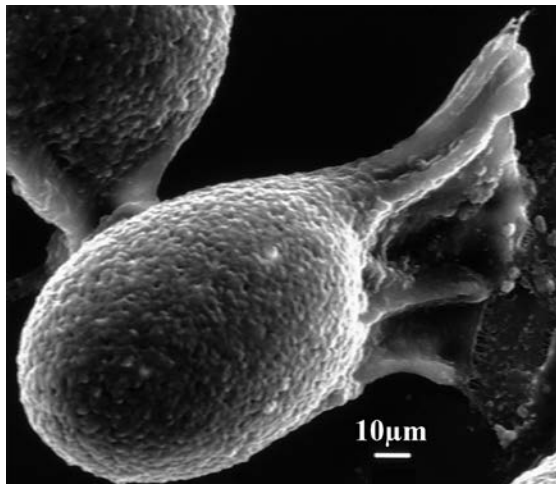

FIGURE 4. Scanning electron microscope image of stephanokont macrogamete from *Rhipocephalus phoenix*. Flagellae that line the edge of the membranous tail are not visible, probably due to loss during sample preparation. Photo by Kenneth Clifton.
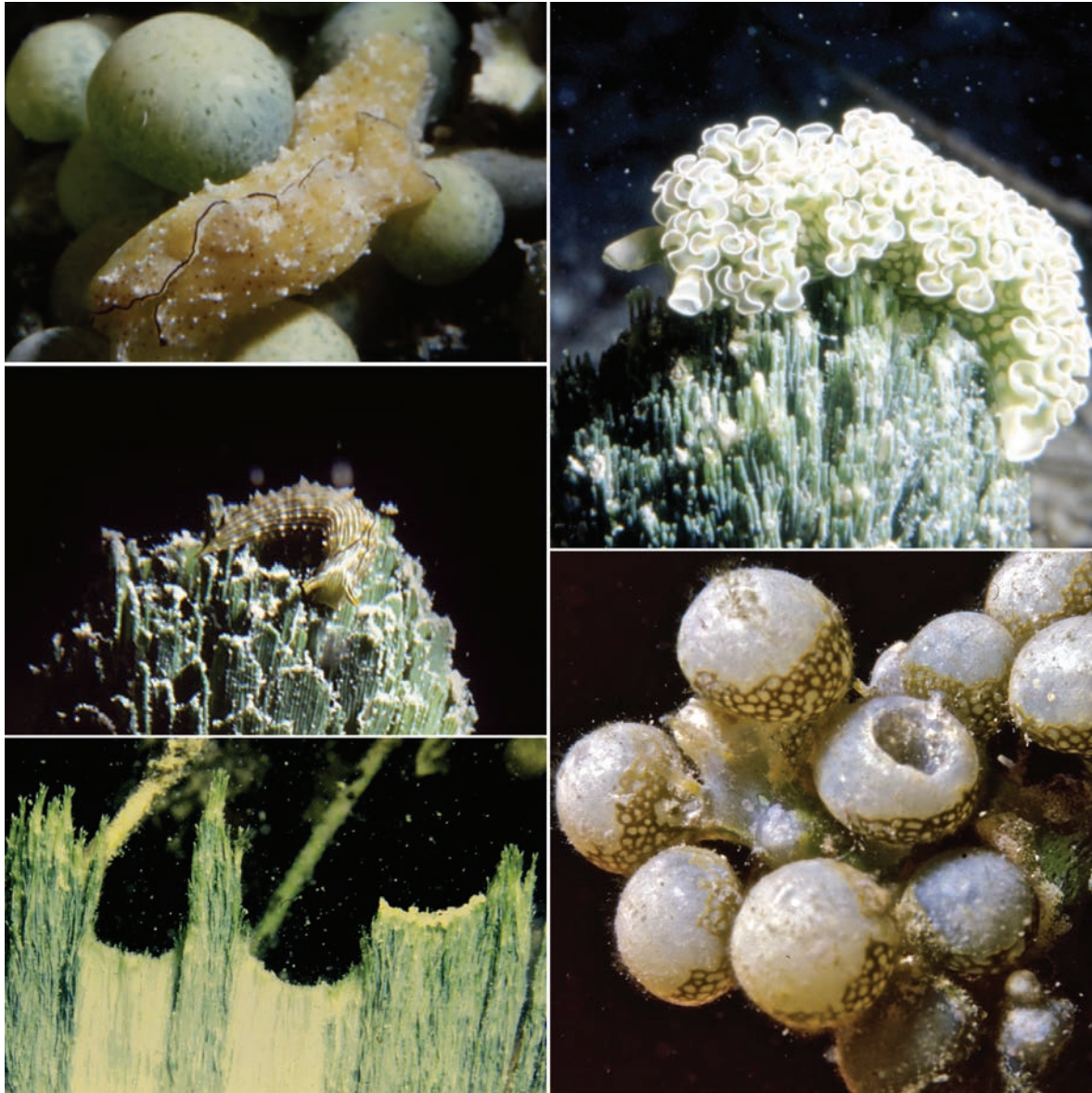
FIGURE 5. Examples of herbivores known to consume siphonous green algae and herbivory on fertile seaweeds. Clockwise from top left: the saccoglossan sea slug *Elysia subornata* on nonfertile *Caulerpa racemosa*; *E. crispata* on nonfertile *Penicillus lamourouxii*; a close up view of fertile *C. racemosa* showing unspecialized gametangia along blade bases and an apparent bite wound (source unknown) to one blade; parrotfish bite wounds to gametangia of female *Udotea caribaea*; and *E. subornata* on nonfertile *Rhipocephalus phoenix*. Photos by Kenneth Clifton.

should be related to gamete size. Preliminary investigations support this contention. Samples of total gamete release by a single thallus into known volumes of seawater reveal that, as expected, larger seaweeds release a greater number of gametes, and further that females produce fewer gametes than males for a given thallus size (Figure 6). There appears to be no relationship between gamete size and thallus size.

THE TIMING OF GAMETE RELEASE

The reproductive synchrony of siphonous green algae can be evaluated on several temporal scales. On the diel scale, in all species studied to date, both micro- and macrogametes are released directly into the water column during a single, brief (typically 5–15 min), highly synchronous pulse of early morning
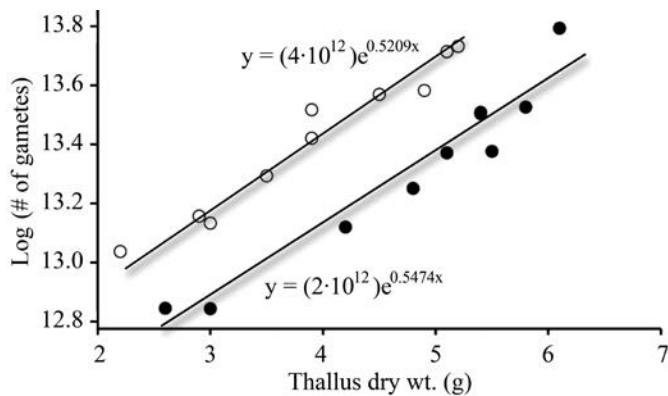
FIGURE 6. Number of gametes released versus thallus size for 10 male (microgametes, open circles) and 10 female (macrogametes, filled circles) *Udotea flabellum*. The effect of sex on the relationship between gamete number and thallus size was significant (ANCOVA; $p = 0.035$).

reproduction (Clifton, 1997). The alga dies immediately following gamete release (holocarpy). The timing of release appears to be organized around changing light levels associated with the onset of daylight, though water temperature also plays a role (Clifton, 1997). The duration of gamete release varies between species, with larger, epilithic species tending to release their gametes more rapidly (Clifton and Clifton, 1999). With many thalli reproducing synchronously, dense gamete clouds may extend down-current for tens of meters, though these generally dissipate within 5–10 minutes even under the calmest conditions. The precise timing of release relative to sunrise remains consistent for a given species across seasons and years. Up to nine species from five genera have been observed to release gametes on the same morning; however, each species has a specific, narrow time of release (Clifton and Clifton, 1999). Some species show overlapping times of gamete release, though more closely related species (Hillis et al., 1998; Vroom et al., 1998; Kooistra et al., 1999) reproduce at different times (Clifton, 1997). This may reduce hybridization if gamete viability is short lived relative to the time between release by different species.

Green algae may show a broad seasonal peak of sexual reproduction that shifts with latitude (Clifton, 2008), but unlike many broadcast-reproducing invertebrates they exhibit no obvious lunar or tidal cycling (Clifton, 1997). During seasonal peaks of reproductive activity, populations of green algae undergo iterative bouts of sexual reproduction with varying degrees of intensity. Only a subset of a given population releases gametes on a particular morning (generally about 5%, though major bouts of sexual reproduction involving more than 45% of the population can occur). Episodes of sexual reproduction typically occur on numerous reefs within a given geographic region on the same morning, including on reefs several kilometers apart.

During the seasonal peak of activity, bouts of sex occur on roughly two-thirds of mornings; this frequency varies somewhat interannually (e.g., bouts of gamete release occurred on 42%, 80%, and 68% of days during the seasonal reproductive peaks in 1995, 1996, and 1997, respectively). When sexual reproduction is observed on a given morning, several species are often involved. On a single Panamanian patch reef in 1996, 16 species of green algae collectively underwent 233 bouts of gamete release between March and July, with 97 additional events occurring between March and mid-May the following year. A recent comparison of algal sexual reproduction in St. Croix and Panama indicates that similar levels of activity occur throughout the Caribbean (Clifton, 2008).

Given the holocarpic life history of these seaweeds, iterative bouts of sexual reproduction have a rapid and dramatic impact on adult algal distribution and abundance (Clifton and Clifton, 1999). Algal cover can drop dramatically, freeing up space for future colonization, perhaps by the same species, or more likely by other members of the benthic community. Our understanding of the nature of algal population dynamics and their effect on the overall organization and function of coral reefs remains in its infancy. Similarly, little is known of how the postreproductive disintegration and dissolution of these calcified seaweeds contributes to rates of $CaCO_3$ input into reef sediments.

ZYGOTE FORMATION AND DISPERSAL

Very little is currently known about the early life history of siphonous green seaweeds. Although mature algae are easily maintained in aquaria (e.g., Hillis-Colinvaux, 1980; Drew and Abel, 1988; Ohba et al., 1992), zygote development in the laboratory has been described for only *Caulerpa serrulata* (Price, 1992) and *Halimeda tuna* (Meinesz, 1972). After five months the latter produced an alga quite different from the parents, and the complete life cycle of the genus remains unresolved. Meinesz (1980) reported adult-like algae from the zygotes of *Flabellia petiolata* (formerly *Udotea*) after seven months, and Friedmann and Roth (1977) describe an "*espera*" (non-adult-like) state of *Penicillus capitatus* that arose after months of culturing. It is unclear whether these nonadult morphs represent a natural stage of development or simply an artifact of in vivo conditions.

These laboratory observations should encourage further study of zygote development, particularly under natural conditions. Such data are fundamental to an understanding of how algal recruitment and rates of growth from zygote to adult thallus influence population dynamics. The relatively slow rates of development reported from the lab coupled with field observations of delayed recovery of populations following peaks of sexual activity suggest that algal recruitment occurs several months after fertilization, with a possible cryptic stage of life history occurring before the production of an adult form. Perhaps this allows green algae to persist through unfavorable seasonal periods of temperature, salinity, or light in a manner

analogous to terrestrial seed banks (Hoffmann and Santelices, 1991). Seasonality in Panama (Cubit et al., 1989), where most of the longer-term data on temporal patterns of green algal sexual reproduction have been obtained, is known to be ecologically significant for herbivorous reef fishes and their algal foods (e.g., Robertson, 1990; Clifton, 1995).

CONCLUSION

Numerous studies have implied a significant ecological role for siphonous green algae within tropical marine communities. To date, however, the basic biology of these algae remains poorly understood. The presence of complex life-history strategies and remarkable interspecific variation in the manner in which different algae reproduce are partly to blame for this dearth of information, yet this very same complexity and variation offer marine ecologists unparalleled opportunities to explore the ways in which factors such as timing and location of reproduction, patterns of gamete size and behavior, and fertilization success and zygote development ultimately influence where and how abundantly these algae occur. Investigations of the origins and consequences of algal reproduction remain a critical step toward improved understanding of the biological mechanisms that underlie their ecological significance.

ACKNOWLEDGMENTS

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