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## Spatial distribution and reproductive phenology of sexual and asexual *Mastocarpus papillatus* (Rhodophyta)

Janna L. Fierst<sup>†</sup>, Janet E. Kübler, and Steven R. Dudgeon<sup>\*</sup>

Department of Biology, California State University, Northridge, CA 91330-8303

### Abstract

Species of the genus *Mastocarpus* exhibit two distinct life cycles, a sexual alternation of generations and an obligate, asexual direct life cycle that produces only female upright fronds. In the intertidal red alga, *M. papillatus* (Kützinger) sexual fronds dominate southern populations and asexual fronds dominate northern populations along the northeast Pacific coast, a pattern of spatial separation called geographic parthenogenesis. Along the central coast of California, sexual and asexual variants occur in mixed populations, but it is not known whether they are spatially separated within the intertidal zone at a given site. We investigated reproductive phenologies and analyzed patterns of spatial distributions of sexual and asexual *M. papillatus* at three sites in this region. Sexual *M. papillatus* were aggregated lower on the shore at two sites and only reproduced during part of a year, while asexual *M. papillatus* occurred throughout the intertidal range at all sites and reproduced throughout the year. The distribution patterns of sexual and asexual *M. papillatus* are consistent with a hypothesis of shoreline topography influencing their dynamics of dispersal and colonization. Spatial and temporal partitioning may contribute to the long-term coexistence of sexual and asexual life histories in this, and other, species of *Mastocarpus*. The occurrence of geographic parthenogenesis at multiple spatial scales in *M. papillatus* provides an opportunity to gain insight into the phenomenon.

### Keywords

Apomictic; Geographic parthenogenesis; Life cycle; *Mastocarpus papillatus*; Phenology; Red algae; Sexual reproduction; Spatial division; Tidal elevation

## INTRODUCTION

Species of the genus *Mastocarpus* exhibit two distinct life cycles. The sexual life cycle consists of a heteromorphic alternation of generations whereby a diploid crust that meiotically produces spores alternates with upright, branching foliose dioecious, haploid gametophytes (West 1972; Polanshek & West 1975, 1977). The asexual life cycle, apparently derived from the more complex sexual one (Maggs 1988), consists of upright female gametophytes that are indistinguishable from sexual females but known to be diploid in at least *M. stellatus* (Maggs 1988) and produce female gametophyte offspring in the absence of fertilization (Polanshek & West 1977; Guiry & West 1983; Zupan & West 1988). Both life cycles appear to be obligate because switching between sexual and asexual modes has never been observed in culture. In the North Atlantic, *M. stellatus* ranges from Portugal to Iceland and Norway in the east and from Rhode Island to Newfoundland in the west. In the northeast Pacific, *M. papillatus* occurs from Baja California to the Gulf of Alaska (Polanshek & West 1975) and perhaps as far west as Japan (Lindstrom 2008). In both species asexual gametophytes are more frequent at northern

<sup>\*</sup>Corresponding author (steve.dudgeon@csun.edu).

<sup>†</sup>Present address: Department of Biological Sciences, Florida State University, Tallahassee, Florida 32306-1100.

latitudes, and sexual gametophytes are more frequent at southern latitudes (Guiry & West 1983; Zupan & West 1988).

The geographic separation of sexual and obligate asexual variants (i.e. geographic parthenogenesis; Lynch 1984; Bierzychudek 1985; Peck et al. 1998) is thought to be important to maintaining coexistence of sexual and asexual life cycles. As in *Mastocarpus* species, asexual variants of other taxa are often distributed more towards the poles, higher altitudes, and marginal habitats (e.g. by less favourable abiotic conditions and lower species diversity) than their sexual counterparts (Stebbins 1950, 1971; Grant 1983; Lynch 1984; Bierzychudek 1985; Haag & Ebert 2004; Kearny 2005) but in other red algae (e.g. *Bostrychia moritziana*, *Caloglossa leprieurii*) such patterns are more difficult to discern (West & Zuccarello 1999; West et al. 2001; Kamiya & West 2008). Nevertheless, these algae similarly show geographic separation of sexual and asexual life cycle variants. The latitudinal range of *M. papillatus* from Baja California (~29°N) to the Gulf of Alaska (~60°N) encompasses subarctic to warm temperate biogeographic regions. Seawater temperatures range ~20°C across this range from 3°C winter low in the north to 23°C summer high in the south, variability in photoperiod is much greater in northern than southern regions, and there is considerable variability in the intensity of upwelling and nutrient supply along the northeast Pacific coast. Sexual gametophytes are rare in most populations north of San Francisco, CA, and asexual females appear to be absent south of Point Conception (Polanshek & West 1977; Zupan & West 1988). On the broadest scale, sexual and asexual life history variants are associated with different environments but environmental variables at the geographic scale may not influence distributions of life history variants in their broad region of co-occurrence.

Sexual and asexual life history variants most commonly coexist near the middle of the geographic range along the central coast of California from San Francisco to Point Conception. In this region environmental conditions vary little compared to the whole species range and sexual and asexual variants of *M. papillatus* are abundant in the mid intertidal zone. Cool air and water temperatures characterize rocky shores in this region with low variability throughout the year.

We hypothesized that sexual and asexual life history variants display different reproductive phenologies. Our rationale for this hypothesis was that asexual gametophytes are freed from the constraint of synchronizing fertility with the opposite sex and consequently may show less seasonality in reproduction than sexual variants. We also hypothesized that sexual and asexual life history variants have different intertidal distributions on the shore. This prediction was based on previous studies of geographic parthenogenesis in other plant and animal taxa in which asexual variants differ in distributions and occur in relatively marginal habitats (Lynch 1984; Bierzychudek 1985; Haag & Ebert 2004; Kearney 2005) and an earlier study of the congener *M. stellatus* in New England in which sporophytes (representing the sexual life cycle) only occur in the low intertidal zone and asexual individuals occur higher on the shore (Dudgeon *et al.* 1999). Recently, Lindstrom (2008) showed genetically divergent lineages of *M. papillatus* occur at different tidal elevations. That pattern may represent the zonation of sexual and asexual variants that are genetically isolated from each other. We sampled fertile fronds monthly to determine their life history as a means to analyze reproductive phenologies of sexual and asexual fronds. We sampled fronds from permanent, mapped quadrats in the intertidal zone during peak reproductive periods and determined their life history in laboratory culture to analyze spatial distributions of sexual and asexual variants. We cultured carpospores for each analysis because the only way to distinguish life cycle variants of females is to grow carpospores in laboratory culture and observe the morphological fate of offspring. In the following, 'crusts' are the tetrasporophytes produced by carpospores of sexual females, and foliose 'uprights' are produced by the apospores (i.e. asexual carpospores to distinguish them from sexually produced ones) of asexual females.

## MATERIAL AND METHODS

### Study sites

We sampled three sites along the central coast of California (Fig. 1): Point Piedras Blancas (35°39'N; 121°17'W); Estero Point, Cayucos (35°26'N; 120°57'W); and Shell Beach (35°10' N; 120°41'W). These sites were chosen because they each have large intertidal populations of *M. papillatus* that are easily accessible, sexual and asexual fronds coexist in similar abundance, and the sites are representative of those along the central coast of California where overlap of the two life history variants is greatest.

Conditions at these three sites are similar, especially in the context of the wide range of conditions that occur across the species' range. Seventy-six kilometres separate the northern-most site, Piedras Blancas, from the southern-most site, Shell Beach, and Cayucos is situated in the middle, 38 km from the other sites. Light availability on any given day and photoperiod throughout the year are similar, as are patterns of variation in temperature. Annual variation in seawater temperature ranges from ~10° to 18°C at these sites and thermal variations are highly correlated across sites (P. Raimondi, unpublished data of intertidal temperatures at each site). From November to May water temperatures at these sites are usually within  $\pm 0.3^\circ\text{C}$ . When upwelling occurs between May and October, temperatures may deviate up to  $\pm 4^\circ\text{C}$  between Shell Beach (warmest) and Point Piedras Blancas (coolest), presumably due to stronger upwelling at the latter site. During periods of upwelling, Cayucos is intermediate between the other two sites in temperature.

All three sites are rock outcroppings consisting of sandstone, shale, or semiconsolidated deposits that face direct exposure to the open ocean to the southwest, and at Point Piedras Blancas, to the west as well (P. Raimondi, unpublished data). High and low tidal heights throughout the year across the 76 km of coastline deviate on average <1 cm. Despite efforts to select three very similar sites, inevitably there are differences. Two differences are hydrodynamic exposure and topography of the substrate: from homogeneous (i.e. a smoothly sloping rocky shore) to heterogeneous (i.e. large boulders and outcrops). Point Piedras Blancas experiences greater wave forces than the other two sites with Shell Beach slightly more sheltered than Cayucos. Piedras Blancas and Shell Beach are both smoothly sloping rocky shores; whereas, Cayucos is a heterogeneous rocky shore. Measurements of tidal elevation taken within 0.25 m<sup>2</sup> quadrats (average of standard deviations of  $n = 5$  measurements per quadrat from permanent quadrats at each site;  $n = 15$  at Piedras Blancas,  $n = 17$  at Cayucos,  $n = 19$  at Shell Beach) vary 7 cm, on average, at Piedras Blancas and Shell Beach ( $\pm 2$  and 1 cm standard errors, respectively) but vary 12 cm ( $\pm 2$  cm) at Cayucos. At the larger scale of between nearest neighbour quadrats, the average change in tidal elevation per meter (average slope  $\pm$  standard deviation) was  $5 \pm 3 \text{ cm m}^{-1}$  and  $8 \pm 5 \text{ cm m}^{-1}$  at Piedras Blancas and Shell Beach, respectively, but  $32 \pm 80 \text{ cm m}^{-1}$  at Cayucos. In other words, at Piedras Blancas and Shell Beach, variation in tidal elevation is low and similar at scales from 50 cm to several metres; whereas, the shore slopes much more steeply and variably from place to place on the shore at Cayucos. Piedras Blancas and Shell Beach are most similar in topography but differ most with respect to wave exposure, temperature, and upwelling intensity. Cayucos is intermediate for the set of dynamic environmental variables, but it differs strongly from the other sites in shore topography.

### Reproductive phenology

We examined temporal reproductive patterns of upright fronds of *M. papillatus* by sampling approximately every 6 weeks throughout a 1 year period in 2001 and 2002. At each site, upright fronds of *M. papillatus* were sampled from random 0.25 m<sup>2</sup> quadrats along 10–20 m transects running perpendicular to the shoreline. Reproductive maturity was classified as

nonreproductive, developing papillae, or mature papillae for the fronds borne by each discrete holdfast in each quadrat. Fully papillate, mature fronds (one or two per individual thallus, depending on frond size) were collected and transported to the lab to culture carpospores and determine life history. Twenty fronds per quadrat were sampled when possible, but at some sites in some months fewer than 20 reproductive individuals were found. In the fall, the reproductive samples from Piedras Blancas and Cayucos were numerous. For these periods, fronds from permanent transects were also used for phenology analyses.

Fronds were kept on ice in individual plastic bags, taken back to the lab, and assessed for spore release, germination, and life history. We followed the culturing procedure outlined in Zupan & West (1988) but increased the concentration of bleach used to sterilize fronds. Fronds were rinsed in filtered (GF/1 Whatman) autoclaved seawater containing 3–5% bleach for 2 min, MilliQ filtered water for 2 min, and filtered autoclaved seawater for 2 min. Fronds were air-dried for 20–30 min then papillae were excised with a sterile razor blade and incubated at 14°C under continuous illumination of 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in sterilized seawater. Carpospores released within 5 days of incubation from excised papillae were transferred to sterile Petri dishes containing 25 ml filtered (GF/1, Whatman) autoclaved seawater enriched with 20 ml l<sup>-1</sup> Provasoli's Enrichment (Provasoli 1968), 7 ml l<sup>-1</sup> Provasoli's Concentrated Antibiotic Solution (Sigma Corp.), and 250  $\mu\text{l l}^{-1}$  germanium dioxide (GeO<sub>2</sub>). Carpospores were allowed to settle and grow on glass coverslips in Petri dishes in seawater of 30 ppt salinity with temperature and illumination as above.

We defined successful release as any papilla that released spores within 5 days. Successful germination was defined as a spore that developed a thallus large enough to recognize under a dissection microscope ( $\geq 20 \mu\text{m}$ ). Life history was determined by morphology, as asexuals developed upright fronds from the basal holdfasts and sexuals developed crustose thalli. Upright projections from holdfasts were usually visible within 30–60 days of initiating spore cultures, but cultures were examined for up to 4 months at which time all could be determined.

### Spatial distribution

At each site, we established permanent transects perpendicular to the shoreline. We maintained two transects each at Piedras Blancas and Cayucos and three transects at Shell Beach. Along these transects at 3 m intervals, we positioned two non-overlapping quadrats at random horizontal distances up to 2.5 m on either side of the line, and marked three corners of each quadrat with permanent bolts, in plastic anchors, in holes drilled in the rock. For each quadrat, we estimated the percent cover of *M. papillatus* crusts by visually estimating the area covered in each 10 × 10 cm subsection and summing over the 25 subsquares in the 0.25 m<sup>2</sup> quadrat. We collected reproductive fronds for life history determination from permanent quadrats in the fall when fertility of upright fronds was greatest based on the frequency of mature papillae on fronds *in situ* and spore release rates observed in the phenology study. Carpospores from these fronds were cultured as described above. Additional randomly placed nonpermanent quadrats were sampled along these transect lines to collect percent cover data on the abundance of the crust phase.

We mapped the position of each frond by referencing the number of the 10 × 10 cm subsection in the quadrat using a standardized numbering system and the position of the frond in that 10 × 10 cm subsection by defining the squares lower left corner as the origin and estimating position of the holdfast using Cartesian coordinates (i.e. in the first quadrant). A WAAS-enabled (Wide Angle Augmentation System) Global Positioning System (Garmin eTrex series) was used to map the latitude and longitude of the lower left-hand corner of each 0.25 m<sup>2</sup> quadrat (when facing directly upshore). The previous square reference in that quadrat and Cartesian coordinates within the square were combined with this geographic position. This method allowed us to assign each holdfast coordinates to within a scale of 1 cm<sup>2</sup> and easily resample

individuals. Tidal elevations for the four corners and the middle square of each quadrat were measured with a level transit survey system.

## Data analyses

Reproductive phenology of *M. papillatus* at each site was analyzed with a *G*-test of independence of time period by carpospore release, germination, or life history.

The spatial patterns of sexual and asexual upright thalli were analyzed using several methods. First, we modelled the probability of being either an apomictic or sexual frond as a function of tidal elevation using simple logistic regression (SAS, version 9.1). This analysis tested whether life histories were distributed differently across tidal heights, separately for each site. We also analyzed spatial patterns of each type of upright and co-occurrence of sexual upright fronds and tetrasporophytic crusts using Mantel tests and spatial autocorrelation (R-Package; Legendre & Casgrain 1999) across all sites. Mantel tests estimate the association between two independent data sets based on measures of dissimilarity between samples within each data set. One Mantel test correlated a matrix of physical Euclidean distances against a similarity matrix of life history. The similarity matrix consisted of zeroes where uprights being compared were of different life histories and ones where uprights being compared were of the same life history. The resulting correlation coefficient indicated whether thalli of the same life history were likely to be closer together than were thalli of different life histories. The second Mantel test used a distance matrix of differences in percentage of crust per quadrat and the similarity matrix of life history. Percentage of crust was calculated as a distance matrix to eliminate comparisons between thalli in the same quadrat, which would necessarily have the same percentage of crust. For spatial autocorrelation, distances were divided into log-scale classes of thalli that were less than 10 cm apart, 10 cm to 1 m apart, 1 m to 10 m apart, 10 m to 100 m apart, and > 100 m apart.

## RESULTS

### Reproductive phenology

Sexual development of mature papillae showed the same strong seasonal pattern at all three sites (Fig. 2). Fronds were typically smooth and nonpapillate at all three sites late in winter and remained so at Cayucos into the spring. Papillae began development during spring, and by the middle of May about 20% of females at Shell Beach and Piedras Blancas had mature papillae. The frequency of papillate females declined slightly at these sites and remained low throughout the summer, similar to the frequency at Cayucos. Frequency of papillate females rose sharply in the fall, and during October 97% and 80% of females at Piedras Blancas and Cayucos, respectively, had mature cystocarps. The increase in females with mature papillae was later and less dramatic at Shell Beach reaching only 45% of the female population by late November. By the end of December at all three sites the frequency of papillate females declined to its winter minimum.

Spore release depended on period of the year (open bars vs all other shadings on Fig. 3) for all three sites (Piedras Blancas  $G_{\text{adj}} = 23.19$ ,  $\nu = 7$ ,  $P < 0.001$ ; Cayucos  $G_{\text{adj}} = 38.38$ ,  $\nu = 7$ ,  $P < 0.001$ ; Shell Beach  $G_{\text{adj}} = 61.71$ ,  $\nu = 7$ ,  $P < 0.001$ ). Rates of spore release for Shell Beach were lower than those for either Cayucos or Piedras Blancas, and in six of eight periods < 40% of papillate fronds from Shell Beach released spores. Of those spores that were released, the probability of germination in the lab varied significantly between periods (Fig. 3, comparing light grey vs dark grey plus black bars; Piedras Blancas  $G_{\text{adj}} = 32.16$ ,  $\nu = 7$ ,  $P < 0.001$ ; Cayucos  $G_{\text{adj}} = 132.97$ ,  $\nu = 7$ ,  $P < 0.001$ ; Shell Beach  $G_{\text{adj}} = 25.26$ ,  $\nu = 6$ ,  $P < 0.001$ ). More than 70% of released spores germinated in samples from Shell Beach between 1 October and 14 May of the following year and from Piedras Blancas throughout the year, except 15 May–30 June.

Rates of spore germination from Cayucos exceeded 60% in four of the eight sampling periods, with three of those windows occurring during fall and winter (1 October–31 December and 15 February–31 March). Apart from the 15 May–30 June window during spring, spores from Cayucos germinated less than 50% of the time during the spring and summer.

The timing of reproduction by the two life histories (black vs dark grey bars in Fig. 3) differed significantly with respect to the eight periods of the year at Cayucos ( $G_{\text{adj}}=16.09$ ,  $\nu = 7$ ,  $P = 0.01$ ) and Piedras Blancas ( $G_{\text{adj}}=43.14$ ,  $\nu = 7$ ,  $P < 0.001$ ) but not at Shell Beach ( $G_{\text{adj}} = 5.62$ ,  $\nu = 6$ ,  $P = 0.25$ ). During one period (15 August–30 September) at Shell Beach, no fronds successfully reproduced (analysis for that site contains one less degree of freedom.) At Cayucos, apomictic fronds reproduced all year except between 15 August and 30 September. In contrast, sexual fronds reproduced only during the latter half of the year (15 May–31 December). Sexual fronds were proportionally most abundant between 1 July and 30 September. At Piedras Blancas, sexual reproduction occurred between 15 August and 14 May, and sexual fronds were proportionally most abundant between 15 November and 14 February. Apomictic reproduction occurred throughout the year at Piedras Blancas. In contrast to the other sites, reproduction by both life histories was seasonal at Shell Beach, with virtually no reproduction occurring late in the summer. From 1 October through 30 June apomictic fronds were reproductive and comprised most of the reproductive individuals from the first half of the year (1 January–30 June). The window for sexual reproduction was distributed similarly but slightly broader, being absent only between 15 August and 30 September. Only sexual fronds reproduced in midsummer (1 July–14 August), and sexual fronds were slightly more common in late November and December.

### Spatial distribution

Spatial distributions of sexual and asexual *M. papillatus* varied in a site-specific fashion. Three patterns stand out among these differences. First, the intertidal distribution at Piedras Blancas is shifted to a much lower range of tidal elevations [−0.2 to +0.7 m mean lower low water (MLLW)] compared to the similar ranges observed at Cayucos and Shell Beach (approximately +0.6 to +1.6 m MLLW). Second, censuses of individuals at Piedras Blancas and Shell Beach (when both life history types are reproductive) showed that 43% and 38%, respectively, were sexual. In contrast, only 19% of individuals at Cayucos were sexual. Third, sexual and asexual upright fronds are spatially separated in the same fashion at two sites, Piedras Blancas and Shell Beach but not at Cayucos, where sexual fronds are uncommon, and there is great variation of tidal height over very short distances. At both Piedras Blancas and Shell Beach, the probability of being apomictic increased significantly with increasing tidal elevation (Piedras Blancas, logistic regression deviance,  $G = 8.606$ ,  $\nu = 1$ ,  $P = 0.003$ ; Shell Beach,  $G = 16.935$ ,  $\nu = 1$ ,  $P < 0.001$ ; Fig. 4). This similarity of pattern is consistent despite the 80 cm downward shift of the species range at Piedras Blancas relative to that at Shell Beach. At Cayucos, asexual individuals occurred commonly throughout all tidal heights; whereas, sexual fronds occurred infrequently ( $n = 12$  for Cayucos relative to  $n = 34$  and  $n = 23$  at Piedras Blancas and Shell Beach, respectively) and were distributed over the intertidal range ( $G = 1.33$ ,  $\nu = 1$ ,  $P = 0.126$ ).

The distribution of *M. papillatus* crusts also showed similar patterns at two sites, Piedras Blancas and Shell Beach, and a slightly different pattern at Cayucos (Fig. 5). The distribution of crustose tetrasporophytes was shifted to lower tidal elevations at Piedras Blancas relative to the other two sites. At both Shell Beach and Piedras Blancas, tetrasporophytes were generally rare, especially at the upper and lower limits of their intertidal range, and most abundant towards the lower end of the middle portion of their range. In contrast at Cayucos, tetrasporophytes were distributed in the opposite fashion, being most abundant at the upper and lower limits of the range and less abundant in the middle. Interestingly, tetrasporophytes

were much more abundant at this site, where sexual gametophytes were least abundant, than at either of the other two sites.

Fronds were likely to be of the same life history if short distances separated them or they resided in quadrats with similar proportions of diploid crust. The significant negative correlation coefficients of both tests indicated an inverse relationship between life history similarity and physical distance (Mantel  $r = -0.039$ ,  $P = 0.01$ ), and between life history similarity and the difference between proportions of diploid crusts sampled in paired quadrats ( $r = -0.070$ ,  $P = 0.009$ ). Both the proportion of sexual fronds and percent cover of crusts decreased with tidal elevation at Piedras Blancas and Shell Beach (cf. Figs 4, 5), supporting the results of the Mantel test; however, the curious pattern at Cayucos was inconsistent and may have contributed to the low correlation coefficient.

Spatial autocorrelation analysis across all sites showed a significant positive relationship for the second distance class (10 cm to 1 m distances) and a significant negative relationship for the fourth distance class (10 to 100 m distance; Fig. 6). A lack of significance at the smallest distance class even when the magnitudes of Moran's I values were high stemmed from a small sample size (order of magnitude less than other distance classes) in this distance and the lack of concordance of the sign of the association between sites. The negative autocorrelation value for nearby fronds at Cayucos probably reflects the heterogeneous shoreline topography of Cayucos that resulted in individuals a short Euclidean distance apart being at very different tidal elevations. Consequently, nearby fronds at Cayucos were likely to be in different microhabitats and of different life history; whereas, nearby fronds at Shell Beach and Piedras Blancas were in the same microhabitat and likely to be of the same life history.

## DISCUSSION

### Spatial distributions of variant gametophytes

Sexual and asexual gametophytes of *Mastocarpus papillatus* showed different intertidal distributions and reproductive phenologies where they are sympatric along the central California coast. These results are consistent with a hypothesis of microgeographic parthenogenesis (i.e. spatial separation of sexual and asexual variants among microhabitats on a single shore). In addition to the well-established geographic parthenogenesis over a latitudinal scale, sexual and asexual variants were separated at local spatial scales, within and between sites.

We observed microgeographic parthenogenesis at two sites, Piedras Blancas and Shell Beach. At both sites, sexual gametophytes were more common at lower tidal elevations and fronds were more likely to be apomictic towards the upper limit at each site. At both of these sites, tetrasporophytic crusts were uncommon and distributed more abundantly lower on the shore. It is worth emphasizing that the two sites showing similar spatial patterns were the sites that differed most in wave exposure, temperature, and hence presumably upwelled nutrients but were most similar in shoreline topography.

We suggest that shoreline topography is primarily important in influencing patterns of life history separation through its influence on hydrodynamics, spore dispersal, and microhabitat variation, all of which affect patterns of algal colonization (in this case by conspecific sexual and asexual variants) in the intertidal zone (Johnson 1994; Brawley & Johnson 1991; McNair *et al.* 1997; Johnson & Brawley 1998; Dudgeon *et al.* 2001; Dudgeon & Petraitis 2005). Recruitment of either gametophyte or tetrasporophyte phases of *Mastocarpus* is infrequent (Dudgeon *et al.* 1999), and reproductive traits likely restrict successful recruitment to near the parents. Both carpo- and tetra-spores are released in a gelatinous matrix (S. Dudgeon, personal observation), the latter released from crusts at the substrate along with the upper perithallial

filaments. Dispersal of gelatinous masses in boundary layer flow conditions is probably extremely limited (see Denny & Shibata 1989; McNair *et al.* 1997). We suggest that limited dispersal generates clumped patterns of increasing life history similarity with decreasing distance (Keeling & Rand 1995; Carrillo *et al.* 2002). The coincidence of the tetrasporophyte and sexual gametophyte distributions, Mantel test results, and spatial autocorrelation analyses suggest that microgeographic parthenogenesis may be maintained by the dynamics of very localized dispersal and recruitment of spores up and down the shore.

Vertical separation of sexual and asexual gametophytes was not observed at Cayucos. This site was intermediate to the other sites for environmental variables but differed most in shoreline topography. Crustose tetrasporophytes were not distributed in a monotonically declining fashion with increasing tidal elevation at this site. Tetrasporophyte abundance was highest at the lower and upper ends of their tidal distribution. Sexual gametophytes were distributed accordingly nearby these tetrasporophytes, hence, occurred interspersed with asexual gametophytes throughout the intertidal range of *M. papillatus*. We suggest that at Cayucos, too, the lack of spatial separation of life cycle variants was influenced by the topography of the shoreline that created microhabitat patches and affected hydrodynamic patterns, thereby influencing dispersal and colonization patterns of sexual and asexual variants in the intertidal zone.

The lack of microgeographic parthenogenesis at Cayucos raises several questions about coexistence of variant life histories. For instance, does the lack of spatial separation between variant gametophyte life histories contribute to the low incidence of sexual gametophytes at this site? Cayucos showed the greatest abundance of tetrasporophytes but a much lower frequency of sexual gametophytes compared to the other two sites where sexual and asexual gametophytes are spatially separated. The interspersed sexual and asexual females at Cayucos may increase the incidence of competition between them, and co-occurrence of variant life cycles might involve niche partitioning between the heteromorphic gametophyte and sporophyte phases. Alternatively, there may be differential productivity or selection between the variants at this site that is less evident at Piedras Blancas or Shell Beach.

Differing proportions of life history variants among shores, such as we have observed between Cayucos and the other two sites, occurs with gametophyte and sporophyte life history stages of isomorphic florideophytes (Fierst *et al.* 2005). Sexual and asexual gametophytes of *M. papillatus* are likewise isomorphic, and the pattern between Cayucos and adjacent shores may reflect similar processes operating to those that spatially separate other isomorphic algae (e.g. competition; Hughes & Otto 1999).

### **Reproductive phenologies and spatial distributions of life cycle variants**

Although the timing of development of reproductive papillae on fronds was similar among sites, sexual and asexual fronds differed in the timing of successfully producing offspring in cultures from two of the three sites. Reproductive phenologies of sexual and asexual variants were most similar at Shell Beach where the variants are most spatially separated. Shell Beach was the only site where nutrient limitation stress was evident in the 'bleaching green' of all rhodophytes on the shore in summer (J. Kübler & S. Dudgeon, personal observation). Neither life history type produced many offspring during this period. Both sexual and asexual gametophytes reproduced beginning in fall and continuing into spring with sexual gametophytes showing more marked seasonality, biased towards fall and early winter.

Piedras Blancas also showed spatial separation of sexual and asexual variants. At this site, the reproductive season of sexual fronds was completely nested within that of asexual gametophytes. Sexual reproduction occurred primarily in fall and winter; whereas, asexual reproduction occurred year-round.

At Cayucos, where gametophytic variants overlapped in spatial distributions, reproductive phenologies of sexual and asexual fronds diverged the most. Sexual fronds reproduced in spring and summer when asexual fronds reproduced least, they overlapped during fall, and asexual fronds also reproduced in winter and early spring when sexual fronds did not.

Patterns at these sites highlight the potential adaptive significance of reproductive phenologies and the influence of location on this life history trait. Narrower windows and more marked peaks of sexual reproduction presumably reflect the dependence of successful fertilization on synchronized maturation of carpogonia and release of spermatia. In contrast, the broader temporal window for reproduction by asexual gametophytes is consistent with release from the constraint of synchronized mating. Continuously reproducing asexual populations would exhibit greater population growth rates than discrete generation populations for a given reproductive rate (all else being equal). Environmental constraints on reproduction, such as may occur during summer at Shell Beach, may nullify this advantage by imposing the same demographic schedule on both sexual and asexual life cycles.

Since the life history of gametophytes is identified by culturing carpospores, the life histories of fronds that neither released nor germinated spores are unknown. Hence, we can not distinguish periods of poor reproductive success in the lab due to failure of one or both of the life history variants. Our sampling design allowed only 1 week for fronds to succeed in releasing spores. Our rationale was to determine reproductive status under recent natural conditions. However, cystocarps of either life cycle under uniform laboratory conditions can produce spores over many months in culture (West & Crump 1974, 1975). Nevertheless, the patterns of reproduction across these three shores suggest that temporal partitioning may facilitate coexistence of sexual and asexual life cycle variants, particularly in locations (e.g. Cayucos) where spatial separation is absent (see Armstrong & McGehee 1980 for a general treatment).

### Comparing geographic and within-shore patterns of parthenogenesis

Patterns of geographic parthenogenesis are characterized by asexual organisms having larger ranges, extending to higher latitudes and elevations, occupying recently disturbed habitats, and dominating consistently marginal abiotic habitats that vary little, if at all, like xeric, arid, or glaciated areas (Glesener & Tilman 1978; Bierzychudek 1985). The within-shore distribution of sexual and asexual *M. papillatus* paralleled the large-scale pattern of geographic parthenogenesis in this species in several respects. Asexual thalli were distributed across a wider range of tidal elevations than were sexual thalli and their distributions extended to higher tidal elevations. Higher tidal elevations are drier, and for many species, more stressful and less productive habitats. Sexual fronds that were aggregated lower on the shore occurred in more biotically variable and diverse habitats, more comparable to those of lower latitudes.

Comparisons between tidal elevation and latitude are limited, however, by differences in the forces which can act at different scales. To the extent that environmental gradients exist at both small and large spatial scales, studies of geographic parthenogenesis at small scales are especially useful. Small-scale studies are more tractable, the ability to replicate environmental gradients at small scales at multiple locations increases the experimental power of tests of causes underlying patterns of parthenogenesis, and therefore may provide insight into general mechanisms of geographic parthenogenesis and coexistence of sexual and asexual variants.

*Mastocarpus papillatus* separates distributions of sexual and asexual life histories in space and/or in timing of reproduction. Such partitioning may contribute to the long-term coexistence of sexual and asexual life histories in a single species. At the scale of a shoreline, the cause of spatial separation of sexual and asexual life history variants is consistent with a hypothesis of shoreline topography influencing distributions of microhabitats and dispersal and colonization

dynamics. The roles of microhabitat variation, dispersal, and colonization dynamics in geographic parthenogenesis of *M. papillatus* remains to be tested. The occurrence of geographic parthenogenesis at multiple scales in *M. papillatus* provides an unusual opportunity to gain insight into the phenomenon.

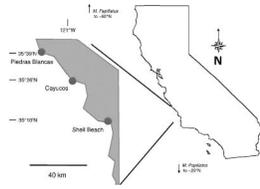
## Acknowledgments

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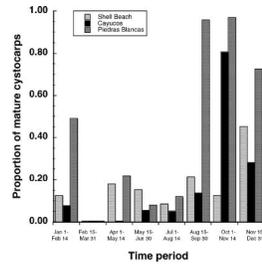
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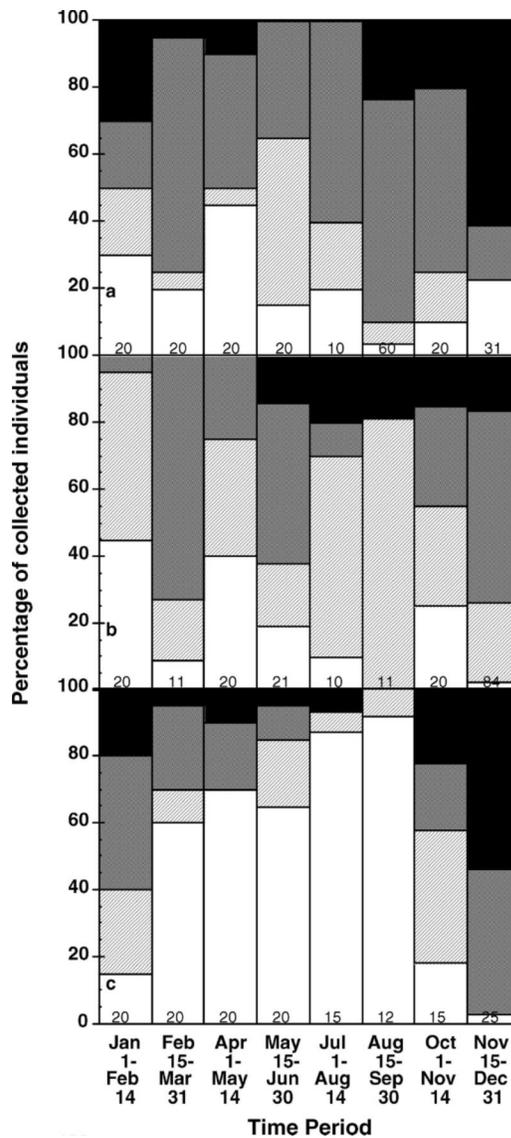
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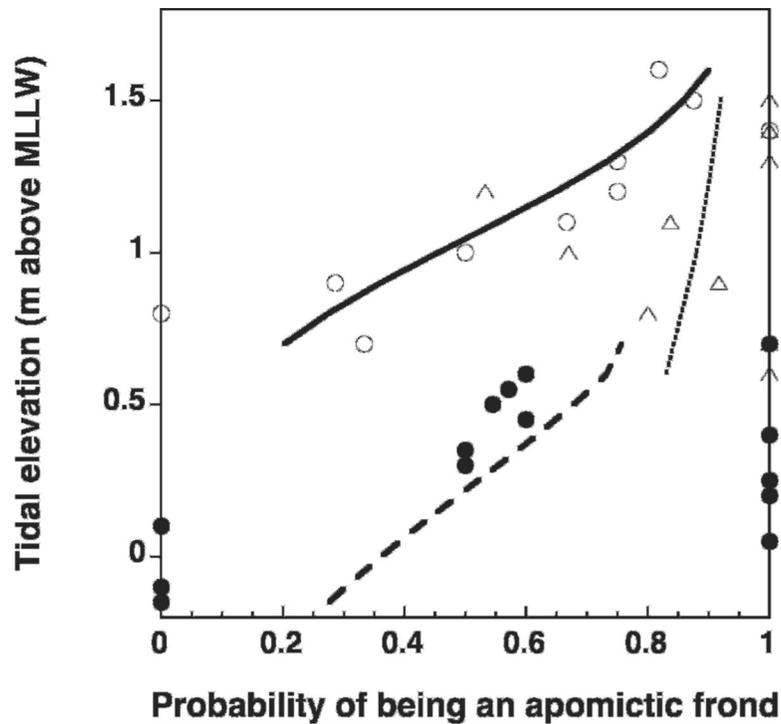
**Fig. 1.** Map of the California Coast emphasizing the central coast section (inset) with locations of sites (circles). Arrows at top and bottom indicate that latitudinal range of *M. papillatus* distribution extends northwards to the Gulf of Alaska (~60°N) and southward to Baja California (~29°N).



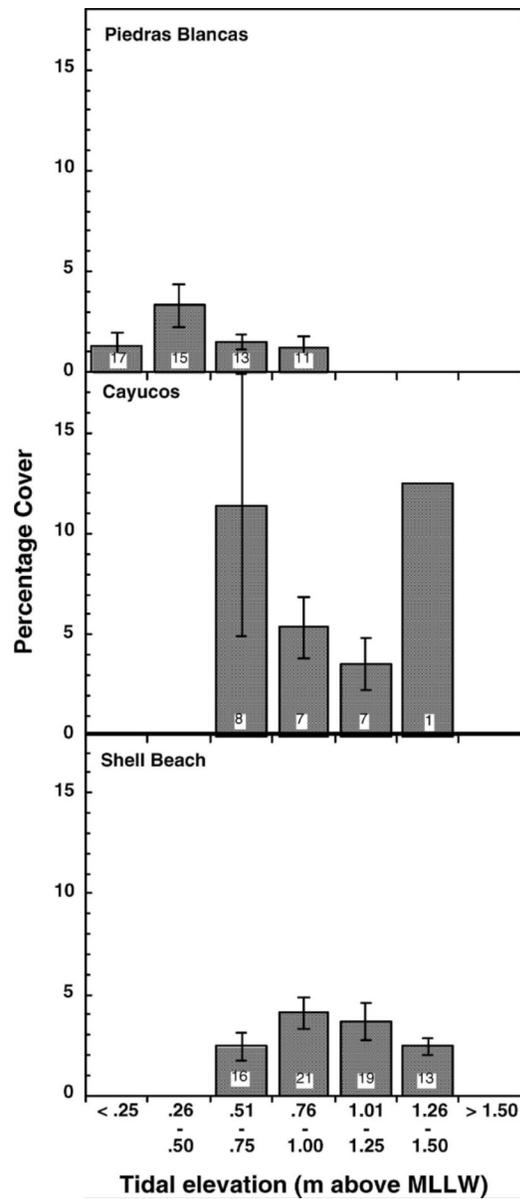
**Fig. 2.** Proportions of female fronds bearing mature cystocarps during eight periods in a year, at each of three sites, Shell Beach (hatched bars), Cayucos (solid bars), and Piedras Blancas (stippled bars). Sample sizes are as indicated in Fig. 3.



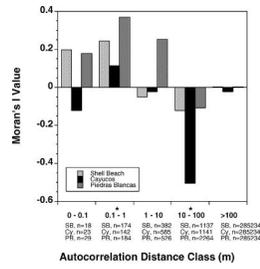
**Fig. 3.** Percentage of collected individuals that were sexual (solid bars), asexual [stippled (dark grey) bars], or of undetermined life history either because spores did not germinate (hatched bars), or none were released (open bars) during eight periods in 1 year. Data were based on the results of carpospore cultures from Piedras Blancas (a), Cayucos (b), or Shell Beach (c). Sample sizes at each site and time are indicated.



**Fig. 4.** Logistic regression showing the probability of a frond sampled at random being apomictic as a function of tidal elevation at Shell Beach (open circles, solid line), Cayucos (triangles, dotted line), and Piedras Blancas (solid circles, dashed line). Regression lines represent the best fit line to the data estimated using maximum likelihood. Data symbols represent the frequency of apomictic fronds sampled as a proportion of the total at each tidal elevation. Total samples were  $n = 60$ ,  $72$ , and  $79$  for Shell Beach, Cayucos, and Piedras Blancas, respectively.



**Fig. 5.** Percent cover by diploid tetrasporophytic crusts as a function of tidal elevation at Piedras Blancas (top), Cayucos (middle), and Shell Beach (bottom). Tidal elevation was determined for the corners and the middle of each quadrat. Sample sizes for each tidal range at each site are shown at the base of each column.



**Fig. 6.** Moran's I value for each autocorrelation distance class, for three sites on the coast of California. Distance ranges used and respective sample sizes are shown along the abscissa. Asterisks indicate statistically significant comparisons for a distance class resulting from concordance across all three sites.