Published in final edited form as:

J Phycol. 2013 April 1; 49(2): 271–281. doi:10.1111/jpy.12034.

Reproductive effort of *Mastocarpus papillatus* (Rhodophyta) along the California coast¹

Stacy A. Krueger-Hadfield², Janet E. Kübler, and Steven R. Dudgeon³

Department of Biology, California State University, Northridge, California 91330-8303, Phone: (818) 677-7322, FAX: (818) 677-2034

Abstract

Species with sexual and asexual life cycles may exhibit intraspecific differences in reproductive effort. The spatial separation of sexual and asexual lineages, called geographic parthenogenesis, is common in plants, animals and algae. *Mastocarpus papillatus* is a well-documented case of geographic parthenogenesis in which sexuals dominate southern populations, asexuals dominate northern populations, while mixed populations occur throughout central California. We quantified abundances and reproductive effort of sexual and asexual fronds and tetrasporophytes at eight sites in California to test the hypotheses that (1) reduced sexual reproduction at higher latitudes and tidal heights explains the observed geographic parthenogenesis and (2) reproductive effort (spore production per blade area) declines with increasing latitude. Abundances of all phases varied site-specifically. However, there was no geographic pattern of reproductive effort of fronds. Reproductive effort of fronds was greater in 2006 than in 2007 and correlated with sea surface temperatures. Sexual fronds exhibited greater reproductive effort than did asexual fronds and sexual reproductive effort was also inversely correlated with local upwelling index. Tetrasporophytes showed greater reproductive effort in northern sites, but total supply of tetraspores per m² was greatest in the middle of the sampling range where crusts were more abundant. There was no decline of reproductive effort at higher latitudes. Geographic patterns of fecundity of life stages do not explain geographic parthenogenesis in *M. papillatus*. Site-specific differences in viability among spores or established thalli of different life cycles may explain their respective geographic distributions, as the sexual and asexual life cycles responded differently to environmental variations.

Keywords

Apomictic; geographic parthenogenesis; life cycle; *Mastocarpus papillatus*; reproductive effort; red algae; sexual reproduction

INTRODUCTION

Species with complex life cycles (i.e., alternation of generations, multiple larval or asexual stages) often display trade-offs of ecological traits that may buffer populations to environmental change and enable exploitation of a broad range of environments (Lubchenco and Cubit 1980, Valero et al. 1992, Klinger 1993, Thornber 2006). Among species with life cycles having both sexual and asexual lineages, the trade-offs of each reproductive mode illuminate the selective advantages of having both, but also point to a conflict. The

¹Date of submission: 9 May 2012; Date of Acceptance:

³Author for correspondence (steve.dudgeon@csun.edu).

²Current address- Marine Biological Association of the UK, The Laboratory, Citadel Hill, Plymouth, U.K. PL1 2PB

reproductive advantage of asexual life cycles should, in theory, enable them to out-compete sexual lineages and much research has investigated the conditions that maintain sexual reproduction in complex life cycles. Most hypotheses for the maintenance of sex emphasize the inevitably increasing mismatch through time of a suitable asexual genotype with its' environment due to environmental and/or non-adaptive genetic change. Even in slowly changing environments, subdivision of populations into demes may slow the migratory spread of asexual lineages allowing time for fitness to decline due to mutation and drift (Peck et al. 1999).

Geographic parthenogenesis is a pattern of spatially subdivided sexual and asexual lineages where asexuality predominates in peripheral environments (e.g., towards the poles, higher altitudes and low variability environments; Lynch 1984, Bierzychudek 1985, Peck et al. 1998). In Europe and North America, 36% of plant taxa are apomictic at 37°N, but >80% of taxa are apomictic above 80°N latitude (Stebbins 1967, 1971, Grant 1981, Haag and Ebert 2004, Kearny 2005). Studies of facultatively asexual plants and animals, like rotifers, *Volvox*, aphids and some cladocerans (e.g., *Daphnia*) show temporal patterns of separation of sexual and asexual reproduction (Edmondson 1955, Bell 1982, Bonner 1993, Gilbert and Thompson 1968). Geographic parthenogenesis is common among macroalgae (Zupan and West 1988, West and Zuccarello 1999, West et al. 2001, Tatarenkov et al. 2005, Kamiya and West 2008). Unlike in higher plants and animals, in many macroalgae the sexual life cycle consists of two alternating, free-living generations and the asexual variant recycles only one of those generations.

Among macroalgae, the best documented cases of geographic parthenogenesis are species in the red algal genus *Mastocarpus* (Polanshek and West 1977, Guiry and West 1983, Zupan and West 1988) in which the asexual life cycle has the appearance of the haploid female, but the ploidy of the encrusting tetrasporospyte phase (Maggs 1988, Kübler et al., in prep). In *M. stellatus* (Stackhouse) Guiry in the North Atlantic and both *M. papillatus* (C. Agardh) Kützinger and *M. jordinii* (J. Agardh) J.A. West in the Northeast Pacific, sexual and apomictic gametophytes are largely sympatric, but apomicts are more frequent at northern latitudes, whereas sexual individuals are more frequent at southern latitudes. Tetrasporophytes occur throughout the species' ranges (Polanshek and West 1977, West et al. 1978, Guiry and West 1983, Zupan and West 1988, J. Kübler and S. Dudgeon, unpubl. data). In addition to spatial separation at the geographic scale, sexual and asexual life cycles can be spatially separated along the gradient of tidal elevation at sites in central California where they both occur (Fierst et al. 2010).

Despite the widespread occurrence of geographic parthenogenesis, there is little empirical evidence to support hypotheses proposed to explain the pattern. A simple model proposed by Peck et al. (1998) to explain the coexistence of sexual and asexual life cycles suggests that a geographic pattern of parthenogenesis can result from the dispersal of individuals from areas where they are well adapted to areas where they are less well adapted. A principle assumption of the model is that reproductive output of individuals declines with increasing latitude because of a shorter growing season. We tested whether reproductive effort of *M. papillatus* varied with latitude to assess the viability of the Peck et al. (1998) dispersal model towards explaining the geographic separation of sexual and asexual lineages. The geographic range of the study spanned the region over which populations of *M. papillatus* switch from being equal mixtures of sexual and asexual individuals to predominantly asexual. We sampled reproductive efforts of both carposporophytic and tetrasporophytic phases to compare among sites in central and northern California. We also estimated the total supplies of tetraspores, sexually produced carpospores and asexually produced carpospores (hereafter, apospores to distinguish them from sexual carpospores) at eight sites from estimates of reproductive effort and abundances of tetrasporophytes and

sexual and asexual upright fronds at tidal elevations encompassing the intertidal distribution of *M. papillatus* in California. Sexual and asexual upright thalli were distinguished via culturing spores giving rise to either tetrasporophytic or gametophytic offspring (Fierst et al. 2010). Our aim was to evaluate how patterns of reproduction may bias a site towards the predominance of sexual or asexual lineages of *M. papillatus* and test the hypothesis of latitudinal variation in reproductive effort.

MATERIALS AND METHODS

Study Species

Mastocarpus spp. in the Northeast Pacific have recently been re-defined based on genetic divergences in one nuclear (ribosomal internal transcribed spacer [ITS]) and two organellar genes (mitochondrial, cytochrome oxidase I [COI]; plastid, RUBISCO [rbcL]; Lindstrom 2008, Lindstrom et al. 2011). *M. papillatus*, previously described as ranging from Baja California to the Gulf of Alaska, was suggested to be a complex consisting of 6 or more species with much of the species diversity clustered around British Columbia (BC). Lindstrom et al. (2011) recognized the distribution of *M. papillatus* to span from Cambria (San Luis Obispo County), CA to northern Vancouver Island, B.C. Nevertheless, populations of *M. papillatus* even over this range switch from equivalent frequencies of sexual and asexual individuals (central California) to predominantly asexual individuals (north of San Mateo County, California and throughout northern California, Oregon, Washington and BC). We have confirmed using DNA microsatellite markers that all sexual individuals identified as *M. papillatus* used in this study comprise a homogeneous genetic lineage and therefore represent a single species (Kübler et al., manuscript in preparation). As expected from their obligate asexual life cycle, the direct life history uprights identified as *M. papillatus* we investigated have genetically diverged from the sexual lineage, but are allied by sharing the same microsatellite loci and most alleles at each locus. For this reason, as well as the lack of identity of tetrasporophytic phases for *Mastocarpus* spp. other than *M. papillatus* (West 1972, Polanshek and West 1977, Lindstrom et al. 2011) and *M. jadinii* (West et al. 1978) and the reproductive compatibility among all crosses of sexual *Mastocarpus* from Alaska to California (Polanshek and West 1977), we infer that our samples represent a single taxon, *M. papillatus*, consisting of a single sexual lineage and its descendant asexual lineages, which range from at least Pismo Beach (San Luis Obispo County), CA to Bodega Bay (Sonoma County), CA.

Abundance

Differences in the supplies of carpospores, apospores, or tetraspores among tidal elevations, sites or regions may be due to differences in physiological allocation to reproduction, thallus size and/or abundance. Therefore, the abundances of *M. papillatus* foliose gametophytes and crustose tetrasporophytes were estimated. The number of discrete upright-bearing holdfasts, the percentage cover of upright fronds and the percentage cover of crustose tetrasporophytes were recorded in each 0.25 m² quadrat. Data were collected along three transects at each site. The transects covered the range of sheltered to exposed habitats of *M. papillatus* on rocky shorelines. Each transect was divided into two zones, corresponding to the two common rhodophyte algae with which *M. papillatus* co-occurs in each zone: *Mazzaella* spp. in the low to mid intertidal zone, and *Endocladia muricata* in the mid to high intertidal zone. Tidal elevations were measured above mean lower low water (MLLW) at the lowest, middle and highest elevation for each transect (David White Transit Model No. 9450). In each zone, seven randomly placed 0.25 m² quadrats were sampled, additionally noting the other algal and invertebrate species present. We sampled eight sites from Bodega Bay to Pismo (Shell) Beach (from North to South; Bodega Marine Lab [−123.1 W, 38.3 N], Pescadero Point [−122.5 W, 37.2 N], Bolsa Point [−122.5 W, 37.2 N], Hopkins Marine Lab [−122.0 W, 36.6

N], Cambria [−121.1 W, 35.5 N], Cayucos [−120.9 W, 35.4 N], Montana De Oro [−120.9 W, 35.3 N], Shell Beach [−120.9 W, 35.2 N]: Locations plotted in Fig. S1, in the Supporting Information) during the late spring and early summer 2007 and during the late fall and early winter of 2007–2008. Logistic regression models the probability of an event occurring in (this case) a binary dependent variable (i.e., sexual or asexual female) as a function of continuous independent variables (i.e., latitude). We compiled all available culture data (Polanshek and West 1977, Zupan and West 1988, Fierst et al. 2010, S. Dudgeon and J. Kübler, unpubl. data) and used logistic regression to identify the region where the probability of female fronds being apomictic increased most rapidly. We sampled four sites each north and south of that region (Fig. S1).

Tetrasporophyte Reproductive Effort

Tetrasporophyte reproductive effort was estimated during their reproductive season (February to March 2007, West 1972) at the eight sites described above.

To test whether reproductive effort of tetrasporophytes varied with latitude and/or tidal elevation, tetraspore production was estimated in hand sections taken from crusts *in situ*. The highest and lowest tetrasporophytes were surveyed and the range of tidal heights divided into 6 equidistant elevation stations. At each station, five distinct crusts were sampled from haphazard locations from ~0.5 to 10 m horizontal distance apart. The exact tidal elevation above MLLW was recorded for each individual. Razor blade sections were mounted on glass slides in the field and fixed in successive steps of 10%, 30% and 70% seawater in Karo syrup with 2–3 drops of phenol per ml in each treatment (Schneider and Searles 1991). Slides were transported to the lab for microscopic analysis. The density of tetrasporocytes (1 tetrasporocyte yields 4 tetraspores) was recorded in a single focal plane viewed in each sample at 400X magnification on an inverted microscope (Zeiss Axiovert 100).

Tetraspore viability was tested by culturing tetraspores released in the lab. Tetrasporophyte-encrusted rocks were collected at low, middle and high tidal heights using a hammer and chisel. Rocks were bagged with a seawater-dampened paper towel, transported to the lab on ice, and stored at 4°C until processed (within 5 d of collection). Tetraspores are released in clumps following re-immersion of thalli in seawater (West 1972). Fertile sections of tetrasporophytes were scraped with sterile razor blades and the tetrasporic clumps disrupted with sterile, plastic pestles in 1.5 mL microcentrifuge tubes. Liberated tetraspores were placed in plastic 100 × 15 mm petri dishes and allowed to settle onto round 25 mm coverslips in autoclaved seawater enriched with 20 mL · L⁻¹ Provasoli's Enrichment (PES, Provasoli 1968), 7 mL · L⁻¹ Provasoli's Concentrated Antibiotic Solution (Sigma Corp.) and 250 µL · L⁻¹ germanium dioxide (GeO₂) at 14°C under continuous illumination of 50 µmol photons · m⁻² · s⁻¹. Culture medium was changed weekly unless visible fouling was observed in which case the cultures were immediately cleaned. Viability was assessed as the development of a basal disc (holdfast) within 30 d of release.

Carpoporophyte Reproductive Effort

Reproductive effort of sexual and apomictic upright *M. papillatus* thalli was estimated by sampling during the sexual reproductive season, October 2006 to early January 2007 (Fierst et al. 2010). Subsequent collections were made during the 2007–2008 season. Sexual females produce carpospores primarily during the late fall and winter, whereas apomictic females can produce spores throughout the year (Maggs 1988, Fierst et al. 2010). Upright fronds were sampled at the eight sites as described above.

To test whether reproductive effort varied with latitude or tidal elevation, the following were estimated: the production of spores per papilla, the density of papillae per frond area, the projected area of the frond and spore fate (sexual vs. apomictic) from 30 papillate females collected at each site along a 30 m long transect perpendicular to the shoreline covering the range of tidal elevation of *M. papillatus* at each site. The highest and lowest *M. papillatus* were recorded with the transit level survey system and the difference in tidal elevation was divided into 6 equidistant height stations with all upright thalli collected from each of 5 discrete holdfasts (an individual holdfast assumed to be a genet) within ± 1 cm expected tidal elevation at each station, haphazardly located from ~0.5 to 10 m apart horizontally. The exact tidal elevation above MLLW was recorded for each putative genet. Fronds were transported to the lab on ice in individual plastic bags containing seawater-dampened paper towels. They were stored at 4°C until processed (within 5 d of collection).

In the lab, the projected area of three randomly selected upright fronds from each individual holdfast were measured in digital images (Nikon D200) using NIH Image J (Rasband 2007). The densities of papillae were estimated by counting papillae under three, 12 mm diameter, round coverslips placed haphazardly in the papillate region of each thallus. Spore counts were obtained from excised papillae, disrupted in 1 mL of seawater. Ten μL of the spore-seawater mix were examined microscopically. Spores were counted in ten random fields of view at 100 $\times$ magnification on the inverted microscope (Zeiss Axiovert 100). The average of the ten counts of spores were multiplied by 1000. This value yielded the average numbers of spores per papilla. Reproductive effort per unit area of thallus was estimated from the average spore density per papilla multiplied by the average number of papillae per quadrat divided by the quadrat area (cm^2). Reproductive effort per frond was estimated by multiplying the value for reproductive effort per unit area of the thallus by the projected area of the frond.

The culturing methods followed those outlined in Zupan and West (1988) with an increased concentration of bleach to sterilize the fronds (Fierst et al. 2010). Upright fronds were rinsed in autoclaved seawater containing 3–5% bleach for 2 min, MilliQ filtered water for 2 min, autoclaved seawater for 2 min, and then air-dried for 20–30 min. Papillae were excised using sterile razor blades, placed onto round 25 mm coverslips in plastic 100 $\times$ 15 mm petri dishes and incubated at 14°C under continuous illumination of 50 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in autoclaved seawater (Precision Instruments Model 818 Low Temperature Illuminated Incubator). Spores released onto coverslips formed a pink halo around the papilla and were transferred to glass baby food jars containing 30 mL antibiotic- and nutrient-enriched autoclaved seawater (PES, Provasoli 1968) and cultured under the conditions described above.

The definition of successful release was any papilla that released spores within 5 d of excision. Successful germination was characterized by a spore, which developed to a size recognizable under a stereomicroscope ($>20 \mu\text{m}$). The life history (sexual or apomictic) was determined by appearance. Sexual spores developed into purplish-red crustose thalli, whereas apomictic spores developed into upright foliose thalli from a basal holdfast (Zupan and West 1988, S. Krueger-Hadfield, pers. obs.). These distinguishing characteristics were typically visible within 30–60 d of culturing. Ambiguous cultures were kept until the life history was apparent, or up to 4 months.

Spore Supply

Tetraspore supply over the range of habitats of *M. papillatus* was estimated by converting the number of tetraspores per μm^2 (in a single focal plane at 400 $\times$ magnification) to cm^2 (the area of the quadrat used in sampling abundances) and the resulting value multiplied by the percentage cover of crusts. The exact tidal elevation above MLLW for each individual

collected for tetrasporophyte reproductive effort estimation was classified into the low, mid and high tidal elevations recorded for the abundance data.

Carpospore and apospore supply were estimated over the range of habitats of *M. papillatus* by multiplying the percentage of reproductive fronds per individual holdfast, the number of fronds per holdfast, the average density of upright-bearing holdfasts at discrete tidal elevations and the average reproductive effort per frond within the same tidal elevation. Tidal elevations were classified into low, mid and high elevations as described above.

Data Analysis

All data analyses were performed using the Statistical Analysis System (SAS, version 9.1.3 or 9.2). The abundances of *Mastocarpus papillatus* uprights and crustose tetrasporophytes were analyzed separately as 5-factor mixed model ANOVA's with region, site nested within region, tidal elevation, season and transect as independent variables. All independent variables except site were considered fixed, orthogonal factors, the latter a random factor nested within region. The summary of the sources of variation of this model is shown in Supplementary Information (Table S1, in the Supporting Information). However, sources of variation in the model that were highly non-significant ($p > 0.20$) were post hoc pooled with the appropriate error term (Underwood 1997). The remaining, less complex, ANOVA models for other analyses in this study were treated similarly with respect to highly non-significant sources of variation. The densities of upright-bearing holdfasts and all spore supplies were $\log_e(x+1)$ transformed and the percentage cover of uprights and tetrasporophytic crusts were arcsine square root transformed to improve normality and heteroscedasticity. Variance components in these, and all analyses with random effects in the models, were estimated using the restricted maximum likelihood method.

We estimated the probability that an upright individual chosen at random from a population was asexual (determined from carpospores cultures) using logistic regression with tidal elevation as the independent variable and the sexual or asexual life history as the binary response variable.

Reproductive effort data contained many 0's (plants that failed to reproduce) along with many highly fecund individuals. We hypothesized that such patterns reflected different processes related to (1) the incidence of infertility and (2) fecundity of fertile individuals. Data were analyzed with different methods to address these two separate questions: (1) does sterility increase with increasing tidal elevation and (2) among reproductive individuals, does reproductive effort vary among regions, sites or intertidal habitat?

We used logistic regression to test whether the probability of crustose tetrasporophytes bearing tetraspores varied with tidal elevation. In the case of upright fronds, we first tested whether the incidence of sterility between years was the same from site to site using a 3-way log linear analysis. Then, we tested whether sterility was associated with tidal zone and/or life history (sexual or asexual) with a 3-way log linear analysis. For this analysis, the specific question addressed was whether the distribution of sterile fronds across tidal zones is homogeneous among sexual and asexual life histories. Log linear analyses were performed using the deviance of the G-statistic between reduced and saturated models without and with, respectively, the term of interest. Testing proceeded from highest to lowest order terms and those with non-significant G-values were dropped from the model, whereas those with significant G-values were retained.

Among thalli that produced reproductive spores, we tested for differences in reproductive effort of thalli between northern and southern regions, among sites within regions and among years (upright thalli only) initially using ANCOVA with tidal elevation as the

covariate. ANCOVAs proceeded by testing the separate slopes model followed by the homogeneity of slopes model with respect to each main effect. The lack of significant covariate terms in these analyses resulted in the covariate being dropped from the analyses, which were then run as ANOVAs. Response variables were log transformed prior to analysis. Reproductive effort data of sexual and asexual upright fronds were analyzed twice: once using all spore-bearing fronds without regard to their life history (n=395) and then using a subsample (n=153; 75-asexual, 78-sexual) of spore-bearing fronds with known life history determined by culturing offspring.

The total supplies of spores (carpospores, apospores and tetraspores) during peak phenologies of upright and crustose thalli were compared among regions, sites and tidal zones (and between years for upright thalli) using 3- (or 4- in the case of upright fronds) factor mixed model ANOVAs. Site was considered a random, nested factor the others fixed and orthogonal. The spore supply response variables were log transformed prior to analysis. As with the reproductive effort data, carpo-/apospore supply data were analyzed both with and without regard to life history of the individuals. The analysis without regard to life history included all individuals sampled from the field and provides the best estimate of carpospore production during autumn for comparison among sites and regions. The analysis with regard to life history is based on the sub-sample of 153 known individuals from spore cultures. This analysis estimates the proportion of total spores that are sexually or asexually produced in the autumns of 2006 and 2007 at each site. The analysis of proportions of each life history at each site was done using PROC RELIABILITY for binomially distributed data in SAS.

RESULTS

Abundance of *M. papillatus*

Densities of *M. papillatus* individuals differed between northern and southern regions across a gradient of wave exposure in both lower and upper ranges of its tidal distribution ($F_{\text{region} \times \text{exposure} \times \text{zone}(4,620)} = 2.60, p=0.03$). At both tidal ranges in the southern region and the lower tidal range in the north, densities were comparable ranging between 2 to 5 plants per 0.25 m² and varied little across exposure. However, the density of plants increased in the upper zone in the north in the more exposed and more sheltered habitats (Fig. 1). Differences among sites and between spring and autumn among sites were also significant sources of variation explaining ~20 and 7.5%, respectively, of the variance in plant density. Similar trends were observed in percent cover of upright plants, but percentage cover data showed greater sensitivity to spring/autumn changes among sites (data not shown).

The percentage cover by crustose tetrasporophytes showed a similar pattern of abundance characterized by less variation in percentage cover across tidal zones and exposures in the south compared to that in the north ($F_{\text{region} \times \text{exposure} \times \text{zone}(2,602)} = 3.21, p=0.04$). Average percentage cover ranged from 2 to 7% with the maximum occurring in sheltered habitats of the lower intertidal zone of northern shores and the minimum occurring in exposed lower intertidal habitats of northern shores (Fig. 2). Similarly to upright fronds, differences among sites accounted for ~20% of the variation observed in percentage cover of crustose tetrasporophytes. Small, but significant (~5 to 7% of the variance in cover), changes in percentage cover of tetrasporophytes between spring and autumn sampling probably reflect the recruitment, growth and mortality dynamics of epiphytes (e.g., barnacles, ephemeral algae) and the overhanging algal canopy.

Reproductive Effort

Tetrasporophytes—Although abundances of tetrasporophytes were similar between lower and upper tidal zones, the probability of producing tetraspores during winter in these respective zones was not. The probability of a tetrasporophyte bearing tetraspores declined steadily from ca. 0.7 at +0.5 m MLLW to 0.2 near their upper limit (+1.6 m MLLW; $G=19.23$, $df=1$, $p<0.0001$, $n=240$; Fig. 3.). Among those that did produce tetraspores in the field during winter, the reproductive effort of tetrasporophytes was similar among sites within a region and significantly greater in the north (back-transformed average = 44 tetraspores per field of view at 400X magnification) compared to the south (back-transformed average = 31 tetraspores per field of view at 400X magnification; $F_{\text{region}(1,111)} = 3.98$, $p=0.04$).

Differences in abundance of tetrasporophytes among sites and tidal elevations, as well as reproductive effort between regions, resulted in wide variation in estimates of the total supply of tetraspores per m^2 among sites ($F_{\text{site}(\text{region})(6,97)} = 7.19$, $p<0.01$), but not overall between between north and south ($F_{\text{region}(1,6)} = 0.88$, $p=0.38$). Tetraspore supply was greatest at Pescadero Point and similarly high at sites from Monterey in the north to Cayucos in the south (Fig. 4). Tetraspore supply was least at Montana De Oro near the southern end of our sampling range. Approximately 59% of the variance in the supply of tetraspores per m^2 occurred among sites, 11% was associated with differences in supply between zones across the sites, and the remaining 30% represented variation from plot to plot.

Tetraspore viability was poor across all latitudes and tidal elevations, but there was greater viability at southern sites ($G=3.51$, $df=1$, $p<0.04$, Table 1). Of the tetraspores that developed a holdfast ($n=7$), six were from three of the four southern sites (Montana De Oro, Cayucos and Cambria), whereas only 1 tetrasporophyte produced viable tetraspores from one northern site (Bolsa Point). Viable tetraspores were from crusts that occurred low on the shore, with the exception of one crust collected high on the shore at Cayucos.

Carpoporophytes

Upright fronds chosen at random were more likely to be asexual when sampled from higher tidal elevations (Fig. 5; $G=12.44$, $df=1$, $p<0.01$, $N=173$). Probabilities of a randomly sampled frond being asexual ranged from ~0.25 near 0 m MLLW to ~0.8 above +2.25 m MLLW.

The frequency of sterile upright fronds was greater in 2007 than 2006 at all sites except Cayucos. Odds ratios indicate that in 2007 fronds at all sites were at least 2–3 times more likely to be sterile (usually more), but fronds at Cayucos were a little more than even odds to be sterile in 2006 ($G = 39.9$, $df=8$, $p<0.0001$, $N=463$; Table 2). Sexual and asexual fronds were homogeneously distributed with respect to their frequencies of empty cystocarps among tidal elevations (~11%; $G=1.15$, $df=2$, $p=0.56$, $N=173$).

The reproductive effort of all carposporophyte fronds (including those of unknown life history) expressed per cm^2 of blade area differed principally between years with carpospores production in 2006 greatly exceeding that in 2007 ($F_{\text{year}(1,379)} = 38.60$, $p<0.01$). However, 5% of the variance in carpospore production was explained by sites differing in the pattern of carpospore production between years ($F_{\text{year*site}(\text{region})(13,379)} = 2.41$, $p<0.01$). Shell Beach and Cayucos, the first- and third-most southern sites showed less variation in carpospore production per unit area in contrast to all other sites, where reductions between years were large (Figure 6A). No significant difference in carpospore production of all carpospore-producing plants was observed overall among sites across years. Ninety-five percent of the variance in carpospore production per unit frond area occurred among replicate plots and

reflects the importance of microhabitat variation. Of the 153 individuals of known life history sampled in 2006 and 2007, fecundity of sexual individuals was greater than that of asexuals ($F_{1,149}=4.13$, $p=0.04$). However, sexual and asexual fronds showed similar carpospore production in 2007 that was much lower per unit frond area than in 2006, particularly in sexual individuals (Fig. 6B). Carpospore production on a per frond basis also varied differently between years among sites, but different frond sizes among both sites and tidal elevations obscured overall differences among sites or between years (data not shown).

The total supply of carpospores per m^2 varied greatly between years ($F_{1,15}=18.8$, $p<0.001$) and among sites ($F_{6,6}=10.5$, $p=0.006$), especially in 2006 (Fig. 7). Sites accounted for 44.6% of the variance in the supply of carpospores. In 2006, supplies of carpospores ranged from less than 10^7 per $0.25m^2$ at both Bodega Marine Lab and Montana De Oro, to greater than 10^8 per $0.25m^2$ at Cambria and Bolsa Point. Supplies of carpospores were much more similar in 2007 among sites when plants at all sites were less fecund. The estimated proportions of sexual and asexual spores differed considerably among sites and among years at each site (Fig. 8). The contribution by sexual fronds to the total supply of carpospores increased considerably in 2007 compared to 2006 at all but the three most northern sites and, indeed, declined to near zero at the two northern-most sites. The proportion of asexual spores was greatest at Hopkins Marine Lab (0.78) and Cayucos (1.0) in 2006, but declined to <0.5 at both sites in 2007.

DISCUSSION

A principal aim of this study was to test the hypothesis that the reproductive output of *M. papillatus* declined with increasing latitude, around the switchpoint along the California coast northward of which asexual plants predominate. We found no evidence to support this hypothesis. Neither reproductive efforts, nor total supplies of spores produced by upright or crustose sporophytes displayed a monotonic decline from maximum values at the southern-most site. On the contrary, tetrasporophytes displayed the opposite pattern of greater reproductive effort per unit area of crust in the northern region. Greater abundance of tetrasporophytes in the middle of our sampling range contributed to the total supply of tetraspores being highest from Pescadero to Cayucos. Reproductive efforts and total supplies of spores of upright fronds also were greatest in the middle of our sampling range where the transition to largely asexual reproduction occurs (Zupan and West 1988, J. Kübler and S. Dudgeon, unpubl. data).

The pattern of reproductive effort did not support the model of Peck et al. (1998) to explain the geographic separation of sexual and asexual lineages. Geographic parthenogenesis of *M. papillatus* along the California coast cannot be explained by a simple model of varying fecundity and dispersal. Patterns of post-settlement mortality among sexual and asexual upright fronds and a second free-living generation in the life history of *M. papillatus*, not considered by Peck et al. (1998), could underlie the pattern of geographic parthenogenesis independent of any association with migratory status or fertility of individuals at different latitudes. We hypothesize that stronger selection against sexual lineages leads to numerical dominance by asexual individuals in northern populations.

Our results provide insight into the effects of population structure and temporal variability in abundance and reproductive output on shifting the balance of life cycle ratios from site to site and with environmental conditions

Population Structure

The relative abundances of sexual and asexual gametophytes and sporophytes can strongly influence spatial patterns of parthenogenesis within and among sites. Our data confirm the

pattern of spatial separation of life history variants across tidal elevations within a shore reported by Fierst et al. (2010), in which sexual fronds tend to occur lower on the shore than asexual fronds. Fronds sampled towards the upper limit of the distribution of *M. papillatus* are ~12 times more likely to be asexual than those sampled near the lower limit. Whereas the data of Fierst et al. (2010) consisted of many samples from three sites, the present data represent an equivalent overall sampling intensity spread across eight geographically widespread sites (~300 km) indicating that 'microgeographic parthenogenesis' with respect to tidal elevation is a general pattern.

This local spatial separation of sexual and asexual upright fronds could reflect the distribution of crustose tetrasporophytes (that give rise to sexual uprights) more than the dynamics of upright fronds. We observed an increased frequency of sterile tetrasporophytes with increasing tidal elevation. Of the tetraspores that were viable in laboratory culture, 83% were collected from crusts low on the shore. In contrast, tidal elevation had no effect on the production of carpospores, or apospores. Spatial correlation analyses between distributions of tetrasporophytes and sexual upright thalli suggest very limited dispersal and recruitment of tetraspores (Fierst et al 2010). Together, these results imply that a very low supply of viable tetraspores to habitats high on the shore limits the distribution and abundance of sexual gametophytes there.

Across all sites, gametophytic plants were common throughout the intertidal range of *M. papillatus* averaging 16–24 genets per m² (with many fronds per genet). On a percentage cover basis, upright plants were twice as abundant as crustose tetrasporophytes (8 vs. 4%). Abundances of both generations varied in space and over time, but their respective patterns were often concordant.

The geographic pattern of parthenogenesis emerges from the aggregate effects of local, habitat-specific patterns of recruitment and mortality of sexual and asexual upright and crustose thalli at different tidal heights and exposures. Abundances of upright plants, and of crusts, were similar between northern and southern regions, but those abundances are distributed differently among sites and habitats. In the 4 northern sites, upright fronds were most dense in habitats that are typically occupied by asexuals. Frond densities were greater at higher tidal heights and at hydrodynamically sheltered sites. Frond densities were lowest in low intertidal habitats of more exposed shores. Likewise, abundance of crustose tetrasporophytes varied more at northern sites, especially in the lower intertidal zone. Tetrasporophytes only rarely occurred on exposed shores and were 6–7 times more abundant on sheltered shores. Plant densities in the south were similar across tidal heights and exposures. Equivalent densities among habitats would result in equivalent numbers of sexual and asexual plants, all else being equal.

Temporal variation in demographic parameters caused changes in abundances of upright and crustose thalli; seasonal changes explained 9–12% of the variation in abundances (summed variance components of sources containing season). We suggest that this variability combined with interannual variability drives variation in the strength of the geographic parthenogenetic pattern and the latitude of the transition to asexuality.

Although various forms of asexual reproduction co-occurring with sexual reproduction are known in all three macroalgal groups (Churchill and Moeller 1972, Hawkes (1990), Graham and Wilcox (2000), West et al. (2001), Toste et al. 2003, Hwang et al. 2005, Oppliger et al. 2007), their respective distributions in space or time are less often reported. Among studies that do report distributions, the geographic separation of sexual and asexual variants is common (West and Zuccarello 1999, West et al. 2001 [and references therein], Tatarenkov et al. (2005), Kamiya and West 2008). Separation of life cycle variants is not necessarily

along a latitudinal gradient (as in classical geographical parthenogenesis models; Lynch 1984, Bierzychudek 1985, Peck et al. 1998). Several reports involve species distributed along east-west running coastlines as, for instance, the South Australia coast (West et al. 2001). Among heteromorphic algae, adaptive trade-offs between crustose and upright phases, are often suggested associated with susceptibility to disturbance versus slowed growth and reduced competitive ability, enabling them to exploit different habitats (Littler and Littler 1980, Lubchenco and Cubit 1980, Slocum 1980, Steneck and Dethier 1994). Among isomorphic algae, it is hypothesized, but largely untested, that separation of either sexual and asexual variants, or haploid and diploid isomorphs, reflects niche partitioning to reduce the intensity of intraspecific competition (Peck et al. 1998, Hughes and Otto 1999). Explicit experimental tests of this hypothesis would provide needed insight into the processes underlying patterns of geographic parthenogenesis.

Temporal variation in reproductive output

Between-year differences in reproductive effort and the total supplies of spores produced by sexual and asexual plants were among the largest effects observed in this study. Carpospore output per cm² of frond area in 2006 was several times that in 2007 at all but one site (Cayucos). Likewise, the total supply of carpospores (which, essentially, is the product of effort multiplied by abundance) at each site was several times greater in 2006 than 2007, but only at sites in the center of our sampling range (Bolsa Point, Hopkins Marine Lab and Cambria). That pattern may reflect reduced abundances of fronds at the northern and southern edges of our sampling range. The between-year difference was consistent between sexual and asexual thalli, but the decline of reproductive effort in 2007 was greater for sexual than for asexual plants. In that lower fecundity year, sexual thalli showed greater geographic variation in the supply of carpospores produced compared to that of asexual thalli. In addition to reduced effort among fertile thalli, the incidence of sterile fronds was greater in 2007 than 2006.

The geographic scale over which reproductive output differed between years suggests regional oceanographic effects in central and northern California influenced populations of *M. papillatus* along the coast. We collected sea surface temperature (SST °C) and upwelling index (m · day⁻¹) data for 2006 and 2007 *post hoc* to determine any relationships between oceanic conditions and reproductive output of *M. papillatus*. SST data (Geostationary-orbiting Operational Environmental Spacecraft-GOES) represented the 0.5° longitude × 0.5° latitude cell containing each site. Upwelling index (Seawinds instrument aboard QuikSCAT satellite) represented the average of the two contiguous 0.25° × 0.25° offshore cells (adjacent north-south cells) nearest the site (NOAA CoastWatch Program, West Coast Regional Node).

SST data in 2006, when reproductive outputs were greater, were 1 to nearly 4°C warmer in all corresponding months than in 2007, which resulted in a positive correlation between spore production and SST for both sexual and asexual thalli (sexual; $r=0.478$, $p<0.0001$, asexual; $r=0.311$, $p=0.005$). Cooler SST in 2007 were associated with slightly greater rates of upwelling in most of the corresponding months that year. Spore production of sexual thalli was weakly, but significantly, negatively correlated with upwelling indices ($r=-0.207$, $p=0.05$), however, spore production of asexual thalli was independent of upwelling intensity ($r=-0.012$, $p=0.91$). The stronger relationships between spore production and SST compared to those between spore production and upwelling may reflect either of two factors: (1) an artifact caused by the scale of sampling whereby SST data for geographic cells include the locations of site, whereas upwelling data are from the nearest cells offshore to each site, or (2) real biological differences in the relative strengths of relationships between spore production and temperature, or nutrient supply.

Given biological differences, it is interesting that reproductive output of sexual thalli declined more between a warmer year of average upwelling intensity (2006) and a cooler year with moderately stronger upwelling (2007) than did that of asexual thalli. These observations are consistent with the expected greater sensitivity of sexual lineages to environmental change relative to that of asexual lineages (Lynch 1984). The inability of asexual lineages to adaptively track environmental change, through recombining genotypes, selects for clonal lineages that perform at least adequately across a range of environments (i.e., jacks of all trades). This effect is hypothesized to select for general-purpose genotypes among asexual lineages that contribute to the widespread distribution and collective ecological success of asexual lineages (Lynch 1984). Although any single asexual female is typically obligately so, the ecological success of species with complex life cycles, like *M. papillatus*, may emerge from an apparent ‘facultative’ mode of reproduction operating at the population level. Asexual lineages contribute more to ecological success of the species when suitably stable environments prevail, whereas sexual lineages contribute more during periods of environmental change as observed in numerous truly facultatively asexual species (e.g., diatoms, cladocerans, etc.). Therefore, patterns of geographic parthenogenesis should fluctuate in strength, with greater or lesser spatial separation, and location of the transition zone depending on environmental variation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This paper represents partial fulfillment of the requirements for the Master’s degree of S. A. Krueger-Hadfield. We are grateful to the following individuals or groups for support of our work: B. Krueger and T. Krueger for extensive field assistance, Don Canestro at the Kenneth S. Norris Rancho Marino Reserve in Cambria and the staffs at the Hopkins Marine Lab and Bodega Marine Lab. Oceanographic data were provided by the NOAA CoastWatch Program West Coast Regional Node. This research was supported by a Sigma Xi Grant-in-aid-of-research (2006), CSUN Graduate Studies, Research and International Programs Thesis Support Grant (2006–2007), the Women’s Environmental Council Scholarship (2006–2007), the Sally Casanova Pre-Doctoral Scholarship from The California State University Pre-Doctoral Program (2006–2007) and a Marine Technology Society Scholarship (2007–2008) to S. A. Krueger-Hadfield, and by grants from the National Institutes of Health (NIH-5SO6GM48680) and the CSUN Sponsored Programs Office to J. E. Kübler and S. R. Dudgeon. Support for the writing of this manuscript was provided by grants from the National Science Foundation (DEB-1020480, DEB-1059916). This is contribution number 181 from the CSUN Marine Biology Group.

References

- Bell, G. The Masterpiece of Nature. University of California Press; Berkeley, CA: 1982. p. 635
- Bierzychudek P. Patterns in plant parthenogenesis. *Experientia*. 1985; 41:1255–64.
- Bonner, JT. Life Cycles. Princeton University Press; Princeton, NJ: 1993. p. 224
- Churchill AC, Moeller HW. Seasonal patterns of reproduction in New York populations of *Codium fragile* (Sur.) Hariot Subsp *tomentosoides* (van Goor) Silva. *J Phycol*. 1972; 8:147–52.
- Edmondson WT. Seasonal life history of *Daphnia* in an arctic lake. *Ecology*. 1955; 36:439–55.
- Fierst J, ter Horst C, Kübler JE, Dudgeon S. Fertilization success can drive patterns of phase dominance in complex life histories. *J Phycol*. 2005; 41:238–49.
- Fierst J, Kübler JE, Dudgeon S. Spatial distribution and reproductive phenology of sexual and asexual *Mastocarpus papillatus* (Rhodophyta). *Phycologia*. 2010; 49:274–82. [PubMed: 20802792]
- Gilbert JJ, Thompson GA. Alpha tocopherol control of sexuality and polymorphism in the rotifer, *Asplancha*. *Science*. 1968; 159:734–38. [PubMed: 5638176]
- Graham, L.; Wilcox, L. Algae. 1. Prentice Hall Publishers; Englewood Cliffs, NJ: 2000. p. 700
- Grant, V. Plant Speciation. Columbia University Press; New York, NY: 1981. p. 563

- Guiry MD, West JA. Life history and hybridization studies on *Gigartina stellata* and *Petrocelis cruenta* (Rhodophyta) in the North Atlantic. *J Phycol.* 1983; 19:474–94.
- Haag CR, Ebert D. A new hypothesis to explain geographic parthenogenesis. *Ann Zool Fenn.* 2004; 41:539–44.
- Hawkes, MW. Reproductive Strategies. In: Cole, KM.; Sheath, RG., editors. *Biology of the Red Algae*. Cambridge University Press; Cambridge, U.K: 1990. p. 455–76.
- Hwang I, Kim HS, Lee WJ. Polymorphism in the brown alga *Dictyota dichotoma* (Dictyotales, Phaeophyceae) from Korea. *Mar Biol.* 2005; 147:999–1015.
- Hughes JS, Otto SP. Ecology and the evolution of biphasic life cycles. *Am Nat.* 1999; 154:306–20. [PubMed: 10506546]
- Kamiya M, West JA. Origin or apomictic red algae: outcrossing studies of different strains in *Caloglossa monosticha* (Ceramiales, Rhodophyta). *J Phycol.* 2008; 44:977–84.
- Kearny M. Hybridization, glaciation and geographical parthenogenesis. *Trends Ecol Evol.* 2005; 20:495–502. [PubMed: 16701426]
- Klinger T. The persistence of haplodiploidy in algae. *Trends Ecol Evol.* 1993; 8:256–58. [PubMed: 21236162]
- Lindstrom SC. Cryptic diversity and phylogenetic relationships within the *Mastocarpus papillatus* species complex (Rhodophyta, Phylloporaceae). *J Phycol.* 2008; 44:1300–08.
- Lindstrom SC, Hughey JR, Martone PT. New, resurrected and redefined species of *Mastocarpus* (Phylloporaceae, Rhodophyta) from the northeast Pacific. *Phycologia.* 2011; 50:661–83.
- Littler MM, Littler DS. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *Am Nat.* 1980; 116:25–44.
- Lubchenco J & Cubit J. Heteromorphic life histories of certain marine algae as an adaptation to variation in herbivory. *Ecology.* 1980; 61:676–87.
- Lynch M. Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. *Q Rev Biol.* 1984; 59:257–90.
- Maggs CA. Intraspecific life history variability in the Florideophycidae (Rhodophyta). *Bot Mar.* 1988; 31:465–90.
- Oppliger LV, Correa J, Peters AF. Parthenogenesis in the brown alga *Lessonia nigrescens* (Laminariales, Phaeophyceae) from Central Chile. *J Phycol.* 2007; 43:1295–1301.
- Peck JR, Yearsley JM, Waxman D. Explaining the geographic distributions of sexual and asexual populations. *Nature.* 1998; 391:889–92.
- Peck JR, Yearsley JM, Guillame B. The maintenance of sexual reproduction in a structured population. *Proc R Soc Biol Sci Ser B.* 1999; 266:1857–63.
- Polanshek AR, West JA. Culture and hybridization studies on *Gigartina papillata* (Rhodophyta). *J Phycol.* 1977; 13:141–149.
- Provasoli, L. Media and prospects for the cultivation of marine algae. In: Watanabe, A.; Hattori, A., editors. *Cultures and collections of algae; Proceedings of the United-States Japan Conference, Hakone; Kyoto, Japan.* Japanese Society of Plant Physiologists; 1968. p. 63–75.
- Rasband, WS. Image J. US National Institutes of Health; Bethesda, MD, USA: 2007. <http://rsb.info.nih.gov/ij/1997-2007>
- Schneider, CW.; Searles, RB. *Seaweeds of the Southeastern United States*. Duke University Press; Durham, NC: 1991. p. 569
- Slocum C. J. Differential susceptibility to grazers in two phases of an intertidal alga: advantages of heteromorphic generations. *J Exp Mar Biol Ecol.* 1980; 46:99–110.
- Stebbins, GL. *Variation and Evolution in Plants*. Columbia Univ. Press; New York, NY: 1967. p. 643
- Stebbins, GL. *Chromosomal Evolution in Higher Plants*. Arnold Publishers, Ltd; London: 1971. p. 216
- Steneck RS, Dethier MN. A functional group approach to the structure of algal-dominated communities. *Oikos.* 1994; 69:476–98.
- Tatarenkov A, Bergstrom L, Jönsson RB, Serrao EA, Kautsky L, Johanneson K. Intriguing asexual life in marginal populations of the brown seaweed *Fucus vesiculosus*. *Mol Ecol.* 2005; 14:647–51. [PubMed: 15660953]

- Thornber C. Functional properties of the isomorphic biphasic algal life cycle. *Integr Comp Biol.* 2006; 46:605–14. [PubMed: 21672771]
- Toste MF, Parente MI, Neto AI. Life history of *Colpomenia sinuosa* (Scytosiphonaceae, Phaeophyceae) in the Azores. *J Phycol.* 2003; 39:1268–74.
- Underwood, AJ. *Experiments in Ecology: Their design and analysis using Analysis of Variance.* Cambridge University Press; Cambridge, U. K: 1997. p. 524
- Valero M, Richerd S, Perrot V, Destombe C. Evolution of alternation of haploid and diploid phases in life cycles. *Trends Ecol Evol.* 1992; 7:25–9. [PubMed: 21235940]
- West JA. The life history of *Petrocelis franciscana*. *Br Phyc J.* 1972; 7:299–308.
- West JA, Zuccarello GC. Biogeography of sexual and asexual populations in *Bostrychia moritziana* (Rhodomelaceae, Rhodophyta). *Phycol Res.* 1999; 47:115–23.
- West JA, Polanshek AR, Shevlin DE. Field and culture studies on *Gigartina agardhii* (Rhodophyta). *J Phycol.* 1978; 14:416–26.
- West JA, Zuccarello GC, Kamiya M. Reproductive patterns of *Caloglossa* species (Delesseriaceae, Rhodophyta) from Australia and New Zealand: multiple origins of asexuality in *C. lepriurii* Literature review on apomixis, mixed-phase, bisexuality and sexual compatibility. *Phycol Res.* 2001; 49:183–200.
- Zupan JR, West JA. Geographic variation in the life history of *Mastocarpus papillatus* (Rhodophyta). *J Phycol.* 1988; 24:223–29.

Fig. 1. Average density of holdfasts bearing upright fronds of *M. papillatus* in habitats of different hydrodynamic exposure. Stippled bars represent lower-mid intertidal habitats and hatched bars represent upper-mid intertidal habitats. Top and bottom plots show the averages of the four northern and southern sites, respectively. Values represent averages $\pm$ 95% confidence intervals of $n=7$ quadrats. Pie diagram shows variance components for random factors in the model as percentage of total variance. Exploded pie sections indicate significant random effects.

Fig. 2. Average percentage cover of tetrasporophytes of *M. papillatus* in habitats of different hydrodynamic exposure. Stippled bars represent lower-mid intertidal habitats and hatched bars represent upper-mid intertidal habitats. Top and bottom plots show the averages of the four northern and southern sites, respectively. Values represent averages $\pm$ 95% confidence intervals of $n=7$ quadrats. Pie diagram shows variance components for random factors in the model as percentage of total variance. Exploded pie sections indicate significant random effects.

Fig. 3.

Logistic regression showing probability of tetrasporophytes bearing tetraspores as a function of tidal elevation during their reproductive season. Solid symbols represent northern sites, open symbols represent southern sites. Circles – BML and CA; Squares – PP and CY; Diamonds – BP and MO; Triangles – HML and SB. Data points represent proportion of crusts that bore tetraspores in each vertical 10 cm bin between 0.0 m and +2.0 m MLLW.

Fig. 4. Estimate of total supply of tetraspores per m² at eight sites in central and northern California during winter 2007. From left to right, sites are arranged from north to south. Columns represent averages $\pm$ 95% confidence intervals of n 10 samples per site. Codes to designate sites as in Figure S1.

Fig. 5.

Logistic regression showing probability of randomly sampled fronds being asexual as a function of tidal elevation. Data points represent proportion of fronds sampled that were asexual in each vertical 10 cm bin between 0.0 m and +2.4 m MLLW.

Fig. 6.

Reproductive effort of upright fronds of *M. papillatus* expressed as the number of carpospores per cm^2 of blade area. (A) Reproductive effort of all upright fronds without regard to life cycle at eight sites in central and northern California in 2006 (hatched bars) and 2007 (solid bars). Columns represent averages $\pm$ 95% confidence intervals of $n = 24$ in 2006 and $m = 16$ in 2007. Codes to designate sites as in Figure S1. From left to right, sites are arranged from north to south. (B) Reproductive effort of a sub-sample of 153 individuals of known life history of the original 463 individuals sampled. Columns represent averages $\pm$ 95% confidence intervals of asexual (hatched bars) and sexual (solid bars) of $n=75$ asexual and $m=78$ sexual fronds sampled equivalently across all sites in 2006 and 2007.

Fig. 7. Estimate of total supply of carpospores per 0.25 m² without regard to life cycle at eight in sites in central and northern California in 2006 (hatched bars) and 2007 (solid bars). From left to right, sites are arranged from north to south. Columns represent averages $\pm$ 95% confidence intervals of n = 24 in 2006 and m = 17 in 2007. Codes to designate sites as in Figure S1.

Fig. 8.

Proportion of carpospores produced at each site that are either of sexual (solid bars) or asexual (hatched bars) life cycles in 2006 (top plot) and 2007 (bottom plot) at eight sites in central and northern California. From left to right, sites are arranged from north to south. Codes to designate sites as in Figure S1.

Table 1

Germination success of tetraspores in laboratory culture from freshly-collected tetrasporophytes in field populations in central and northern California.

Region	Germination	Non-germination	Column totals
South	6	30	36
North	1	35	36
Row totals	7	65	72

Frequency of sterile females (papillae lacking carpospores) of *M. papillatus* across eight sites (BML=Bodega Marine Lab; PP=Pescadero Point; BP=Bolsa Point; HML=Hopkins Marine Lab; CA=Cambria; CY=Cayucos; MO=Montana De Oro; SB=Shell Beach) in northern and southern regions along the coast of California in 2006 and 2007.

Table 2

Region	Site	2006		2007	
		Spores Absent	Spores Present	Spores Absent	Spores Present
North	BML	2	28	9	16
	PP	2	28	8	22
	BP	1	29	3	27
	HML	0	30	7	21
South	CA	1	29	5	24
	CY	6	24	8	22
	MO	4	26	5	16
	SB	0	30	7	23