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Genetic and experimental evidence for a mixed-age, mixed-origin bank of kelp microscopic stages in southern California

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Abstract. Laboratory studies have demonstrated that the microscopic stages of kelps can rapidly resume development from a delayed state. Like terrestrial seeds or aquatic resting eggs, banks of delayed kelp stages may supplement population recovery after periods of stress, playing an important role for kelp populations that experience adult sporophyte absences due to seasonal or interannual disturbances. We found that removing the microscopic stages from natural rock substratum could prevent the appearance of juvenile kelp sporophytes for three months and the establishment of a diverse kelp assemblage for over four months within a southern California kelp forest. Juveniles were observed within one month in plots where microscopic stages were left intact, which may confer an advantage for the resulting sporophytes as they attain larger sizes before later recruiting neighbors. Microsatellite diversity was high (expected heterozygosity $H_E \approx 0.9$) for juveniles and adults within our sites. Using a microsatellite-based parentage analysis for the dominant kelp, *Macrocystis pyrifera*, we estimated that a portion of the new *M. pyrifera* sporophyte recruits had originated from their parents at least seven months after their parents had disappeared. Similar delay durations have been demonstrated in recent laboratory studies. Additionally, our results suggest that zoospore dispersal distances >50 m may be supported by including additional microsatellite loci in the analysis. We propose a mixed-age and, potentially, a mixed-origin bank of *M. pyrifera* gametophytes promotes maximal genetic diversity in recovering populations and reduces population genetic subdivision and self-fertilization rates for intact populations by promoting the survival of zoospores dispersed >10 m and during inhospitable environmental conditions.

Key words: *kelp gametophyte bank; kelp genetic diversity;* Macrocystis pyrifera; *population recovery.*

Introduction

Delayed development is a strategy utilized by many organisms to regulate recovery from a bank of resistant stages after periodic large-scale disturbances that may remove much or all of their adult biomass (reviewed by Fenner and Thompson [2005]). In addition to surviving stressful conditions, some organisms (e.g., terrestrial plants, freshwater crustaceans, and marine phytoplankton) have the ability to remain in a delayed state for decades, promoting the existence of overlapping generations and the storage of intergenerational genetic diversity (reviewed by Evans and Dennehy [2005] and Honnay et al. [2008]). These banks of delayed stages increase effective population sizes and serve as a buffer against the loss of genetic diversity (Templeton and Levin 1979, Hairston and De Stasio 1988, McCue and Holtsford 1998, Honnay et al. 2008).

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Laboratory studies have shown that gametophytes and microscopic sporophytes of several kelps (order Laminariales) have the capacity to delay or slow development and then resume development after increases in nutrients and/or irradiance (Lüning 1980, Novaczek 1984, tom Dieck 1993, Kinlan et al. 2003, Ladah and Zertuche-González 2007, Carney and Edwards 2010, Carney 2011). Natural kelp populations, particularly of the giant kelp, *Macrocystis pyrifera*, experience both seasonal (i.e., predictable) and interannual (i.e., unpredictable) periods of stress that periodically result in catastrophic adult losses from some temperate coastlines in the northeastern Pacific (Dayton et al. 1984, 1992). It is hypothesized that delayed kelp stages accumulate over time on the kelp forest floor, survive these periods of adult absence, and produce adult sporophytes once conditions improve (Dayton 1985, Ladah et al. 1999). Laboratory studies have demonstrated that gametophytes of *M. pyrifera* can delay reproduction for at least seven months and consistently produce sporophytes in as few as five days once nutrients increase (Carney and Edwards 2010, Carney 2011), while microscopic sporophytes are able to

delay or slow their development for 1–2 months and then resume growth (Kinlan et al. 2003, Ladah and Zertuche-González 2007). Field investigations are still needed to determine how long these stages can survive. It is unlikely that the microscopic stages form long-lived banks fundamentally equivalent to terrestrial seed banks (i.e., survive for decades). However, even banks that are too short-lived to promote overlapping generations may still facilitate the maintenance of genetic diversity through what would otherwise be a post-disturbance population bottleneck (Zipperle et al. 2009).

During non-disturbance years when adult *M. pyrifera* are present, it is unclear whether delayed stages play a role in kelp population maintenance (e.g., Reed et al. 1997). For other organisms, even a small number of recruits contributed by delayed stages help genetically maintain existing populations (Zipperle et al. 2009) because banks of delayed stages generally have similar genetic diversity to the adult cohort (Honnay et al. 2008). Thus, when adults are present, delayed stages may help maintain genetic diversity by contributing new recruits. Field investigations are needed to test these hypotheses for kelps; the tools needed to investigate *M. pyrifera* population genetics have recently become available (see Alberto et al. 2009).

Kelp juveniles develop from haploid, unicellular, motile zoospores released by the adult sporophyte, disperse through the water column, and settle along the kelp forest floor where they germinate into haploid gametophytes, undergo reproduction and produce diploid sporophytes. *M. pyrifera* zoospore release occurs throughout the year in pulses that peak in early winter and late spring (Anderson and North 1967, Reed et al. 1996) and fertilization between gametophytes requires a zoospore settlement density >1 zoospore/mm^2 (Reed et al. 1988, Reed 1990). *M. pyrifera* is very successful at dispersing to and colonizing distant areas, due perhaps to the ability of gametophytes to delay for short periods and accumulate over time (Gaylord et al. 2006, Reed et al. 2006). However, based on assumptions of a 1-m modal dispersal distance and synchronous zoospore release from an idealized distribution of individuals, dispersal has been estimated to be more limited in kelp forest interiors (within 10 m of parent), resulting in increased genetic subdivision and self-fertilization rates ($\sim$10%; Reed et al. 1988, Graham 2003, Gaylord et al. 2006). These high rates of self fertilization could, in theory, result in deleterious effects for subsequent generations (Raimondi et al. 2004). Longer dispersal distances within a kelp forest are theoretically possible when large waves (>2 m) accompany winter storms (Reed et al. 1997, Gaylord et al. 2004). Effective long-distance dispersal (i.e., dispersal that results in recruitment) is promoted by the accumulation of delayed stages, by zoospore release occurring simultaneously from large groups of adults during storm events and periods with strong currents (Reed et al. 1988, Reed et al. 1997, Alberto et al. 2011) or, more rarely, by drifting reproductive kelp (Hernández-Carmona et al. 2006).

If banks of microscopic stages are primarily composed of delayed gametophytes (instead of delayed microscopic sporophytes), self-fertilization rates would be reduced in natural kelp forests. Adult sporophytes exhibit more or less continuous zoospore release and currents within a kelp forest vary over time (reviewed by Gaylord et al. [2004]), resulting in overlapping settlement clouds of zoospores from individuals from different directions and different distances away. Delayed development in these gametophytes results in the accumulation of delayed stages from multiple parents in a given area, presumably in densities sufficient for fertilization (reviewed by Gaylord et al. [2006]). When suitable conditions induce synchronous resumption of development, these gametophytes will be able to fertilize one another. Thus, gametophytes may have the opportunity to mate with conspecifics of different ages and different parents, resulting in reduced population genetic structure across space and time. A bank of delayed sporophytes could have the same benefits, although only after at least one generation and the effect may be diluted by the severe reduction in numbers of individuals (i.e., possible genotypes) that occur between each life-stage transition (Chapman 1984, Schiel and Foster 2006).

Contrary to organisms for which the ability to delay development is inversely related to the ability to disperse through space (e.g., Venable and Lawlor 1980), delayed development and dispersal can covary when the survival of dispersed propagules is variable (Cohen and Levin 1987). This may be the case for kelps because the vast majority of their released zoospores never result in settled gametophytes (Schiel and Foster 2006). In this study we performed field experiments to assess the contribution of delayed microscopic stages to recruitment density of several kelp species, and to the genetic diversity of a *M. pyrifera* kelp population within a natural kelp forest. We also estimated the delay duration of microscopic stages of *M. pyrifera* and the dispersal distance of *M. pyrifera* zoospores using a genetic-based parentage analysis.

Methods

Field sites

Field experiments were performed in the Point Loma kelp forest, San Diego, California, USA (32°42′42.53″ N, 117°15′39.26″ W), one of the largest and most well-studied kelp forests in the world. Following zoospore dispersal estimates by Reed et al. (1988) and Graham (2003), we marked three circular 314-m^2 benthic sites (10 m radius) at least 50 m apart from their centers at a depth of 12–14 m using scuba in August 2006 (Fig. 1). Site 1 was located on the outer edge of the kelp forest while site 2 and 3 were located closer to the inner, shoreline edge. Sites were marked with a 100-cm^2 PVC plate with attached surface buoy line installed at each site center. Latitude and longitude coordinates were

recorded for each buoy on the surface so the sites could be relocated. Within each site, every reproductive adult *Macrocystis pyrifera* (Linnaeus) C. Agardh was tagged and their location mapped in October 2006 (68 total adults: 33 in site 1, 18 in site 2, and 17 in site 3; see Fig. 1; Plate 1). All sporophytes without sporophylls were removed (approximately <4 stipes [Neushul 1963]) and kept clear throughout the study, except in treatment plots (see *Methods: Effect of delayed stage removal on kelp recruitment*). Tagged individuals were relocated on 24 January and 2 April 2007. Absence was noted when only parts of the holdfast remained as regrowth from hapteran tissue is not observed in this population.

Effect of delayed stage removal on kelp recruitment

To determine the contribution of delayed stages to kelp recruitment, we compared sporophyte recruitment among substrates with and without microscopic stages. We removed all macroalgae (≥5 mm in length) by hand from three 1-m^2 plots at each site and then secured a 0.25-m^2 water-tight sterilization tent to the substratum in the center of each plot. Each tent was injected with 1 L of household bleach where it remained for at least 24 h (methods described by Edwards [1999, 2000]) (see Plate 1). In a previous experiment, pieces of rock from both sterilized and control plots were collected immediately after sterilization and seeded with zoospores in the laboratory, and no differences were observed in zoospore settlement and recruitment of sporophytes (M. Edwards, *unpublished data*). At each site, macroalgae (≥5 mm in length) were removed by hand from three additional 1-m^2 hand-cleared but unsterilized plots, and three additional 1-m^2 plots were left unmanipulated (see Plate 1). Treatment plots were installed during two independent trials in August 2006 (Fall, Trial 1) and March 2007 (Spring, Trial 2). During these times natural zoospore release is seasonally low, thus settlement into the treatment plots could be minimized just prior to manipulation. For trial 2, treatment plots were moved to different haphazardly chosen locations within each of the three 314-m^2 sites. Latitude and longitude coordinates were attained for each tagged reproductive adult and each 1-m^2 treatment plot in each site (314 m^2).

We counted the number of juvenile kelps (≥5 mm in length) present in the center 0.25 m^2 of each treatment plot approximately every four weeks (August 2006–January 2007 for Trial 1 and March–October 2007 for Trial 2). Kelp sporophytes are not identifiable to species until they are 2–10 cm long, which requires between 10–45 days after gametophyte fertilization (Dayton et al. 1984). Juveniles approximately 2–10 cm long were identified as *M. pyrifera*, *Pterygophora californica*, or *Laminaria farlowii*. These species were monitored because they are the dominant kelps in San Diego. Juveniles less than 2 cm long were recorded as unidentifiable juvenile kelp. During spring 2007 (Trial 2), we also recorded the number of juvenile *Desmarestia ligulata*, an opportunistic brown alga that recruits rapidly in high densities from a bank of delayed stages during early spring (Edwards 2000). Comparisons were made between sterilized and hand-cleared plots to assess the contribution of pre-existing microscopic stages, and between hand-cleared and control plots to assess the effect of removing adult macroalgae.

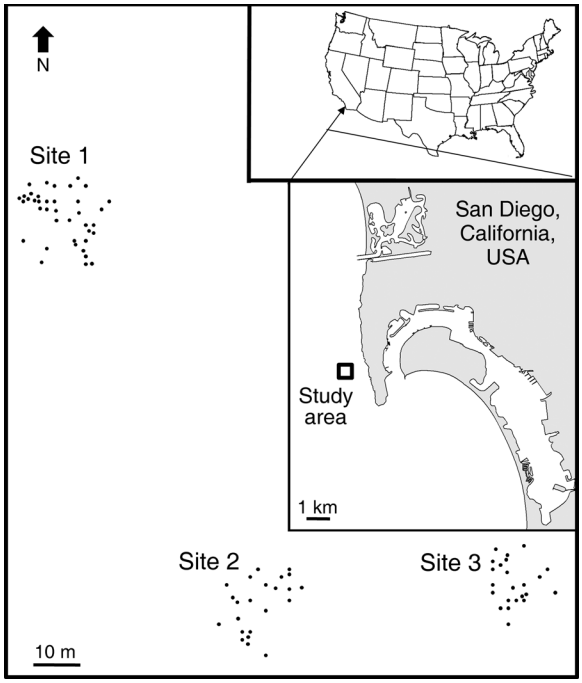

FIG. 1. Map of the location of three field sites in the Point Loma kelp forest, San Diego, California, USA. The locations of tagged reproductive adults and 1-m^2 treatment plots are shown for each site and are differentiated further in Fig. 4.

A distribution-free permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was used to compare macroscopic abundance of unidentified juvenile kelp, *M. pyrifera*, *P. californica*, *L. farlowii*, and *D. ligulata* through time among the three treatments and three sites (treatment was considered a fixed factor; sampling week and site were random factors). The analysis used Bray-Curtis distances calculated from the abundance matrix of the five types of macroalgae. Zero-adjusted Bray-Curtis distances were used because they are the most appropriate for species abundance data sets that include samples where none of the species are present for similar biological reasons (Clarke et al. 2006). Because some species were much more abundant than others, data from 2006 and 2007 were fourth-root and square-root transformed, respectively (Anderson 2001) and a subset of 999 unique permutations (alpha = 0.05) was used as a default setting (PERMANOVA PLUS routine of PRIMER v.6; Anderson et al. 2008). Post-hoc similarity percentage (SIMPER) analysis was then used on the untransformed data to identify the species that contributed most to differences among

sampling weeks, among treatments and among sites (Clarke and Warwick 2001).

Microsatellite amplification of Macrocystis pyrifera

Adult and juvenile *M. pyrifera* from the three sites were used for genetic diversity and parentage analyses (see *Methods: Longevity and dispersal of delayed stages in* Macrocystis pyrifera). We collected a single growing tip (youngest and cleanest tissue) from every tagged reproductive adult in the three 314-m^2 sites during October 2006. Between April and October 2007, we collected the first 10 juveniles identified as *M. pyrifera* in each treatment plot. All tissue samples were preserved in silica drying gel. DNA was extracted from each sample using the DNeasy Plant Mini Kit (Qiagen). PCR reactions for 10 microsatellite (SSR) primers were multiplexed according to temperature requirements and amplified simultaneously (Appendix A). Genetic diversity was compared among (1) *M. pyrifera* juveniles that recruited from the bank of delayed stages and newly settled stages (hand-cleared plots), (2) juveniles that recruited from only newly settled stages (sterilized plots), and (3) the adults within each study site (see Appendix B for more details on methodology).

Longevity and dispersal of delayed stages in Macrocystis pyrifera

We used parentage analysis to identify parent–juvenile pairs from the *M. pyrifera* adults and juveniles that were sampled within our three sites. From these pairs, we obtained a coarse estimate of delay durations for *M. pyrifera* microscopic stages and realized dispersal distances for *M. pyrifera* zoospores based on geographic location and date of disappearance/collection of these individuals. Recruits in each plot from Trial 2 were matched with their putative parents from the adult surveys. Parentage assignments were made using CERVUS 3.0.3 (Kalinowski et al. 2007), which uses simulated data sets to calculate expected distributions of likelihood ratios under a specific confidence threshold (80% or 95%). The log-likelihood score for each parent–juvenile pair is defined as the natural logarithm of the ratio between the likelihood that the candidate parent is the true parent and the likelihood that the candidate is not the true parent. CERVUS also allows for the possibility of selfing (juvenile assigned one parent twice) and assigns confidence levels for this situation. The likelihood approach assumes loci are independent and conform to Hardy-Weinberg expected genotype frequencies, but is robust against deviations in one or two loci. When deviations from Hardy-Weinberg genotype frequencies are detected at many or all loci, confidence in the parentage assignments should be interpreted cautiously (Dakin and Avise 2004). An excess of homozygotes is typically interpreted as the presence of null alleles which, at high frequencies ($\geq$0.2), can inflate the probability of excluding a true parent (Dakin and Avise 2004). High null allele frequencies were inferred for three of our 10 loci (see *Results: Microsatellite characteristics*). Homozygote excess may alternatively be due to non-random mating or subpopulation structure. However, in the absence of known parent–offspring pairs with which to test for repeated homozygote mismatches, these possibilities cannot be distinguished. To account for these other factors, we compared the results of two parentage analyses: one using the seven loci with low null allele frequencies ($\leq$0.17), and one using all 10 loci. Although parent–juvenile pairs were reported using $\geq$80% confidence, conclusions are based only on pairs identified with $\geq$95% confidence due to departures from Hardy-Weinberg genotype frequencies.

Parentage simulations were run for 10 000 simulated offspring. The proportion of candidate parents included in the analysis was unknown. However, based on the average adult densities observed in the present study (0.07 $\pm$ 0.03 individuals/m^2 [mean $\pm$ SD]) and the potential for zoospores to disperse 100 m (Reed et al. 2006, Alberto et al. 2010), we calculated that the 65 adults used for parentage analysis in this study constituted at least 3% of the candidate parents. This estimate is conservative considering the effective population sizes reported for nearby populations (Alberto et al. 2010) and the variability in previous adult density estimates for Point Loma (Dayton et al. 1984, 1992). In order to capture our uncertainty in this simulation parameter, we compared analyses using our calculated percentage of candidate parents between 3.25%, 6.5%, 13% (corresponding to 2000, 1000, and 500 candidate parents). We set the proportion of loci mistyped, the error rate in the likelihood equations, and the error rate for all loci as 1% (Alberto et al. 2010). Due to the large number of candidate parents estimated and the fact that each adult *M. pyrifera* releases male and female gametophytes, we conducted single parent (maternity) simulations using the above parameters. Maternity assignments ($\geq$80% confidence) were then determined for each collected *M. pyrifera* juvenile from the pool of all sampled adults. Finally, a second maternity analysis was conducted using any parents identified in the first analysis as known parents in order to assign a complete parent–parent–juvenile trio from the pool of all sampled adults.

After the parentage assignments were completed, we calculated the time elapsed from when the identified parent was observed to be absent (census performed on 24 January and 2 April 2007) and the time when its assigned offspring was collected (between 27 April and 10 October 2007). The date the parent was observed to be absent was used as a conservative estimate of when its zoospores settled. We then inferred the delay duration based on previous data that newly settled *M. pyrifera* gametophytes in southern California produce sporophytes in 30–35 days (Deysher and Dean 1986), which grow large enough to be identified to species (2–10 cm) in another 10–45 days (Dayton et al. 1984). Thus, to estimate the potential range of delay durations for each

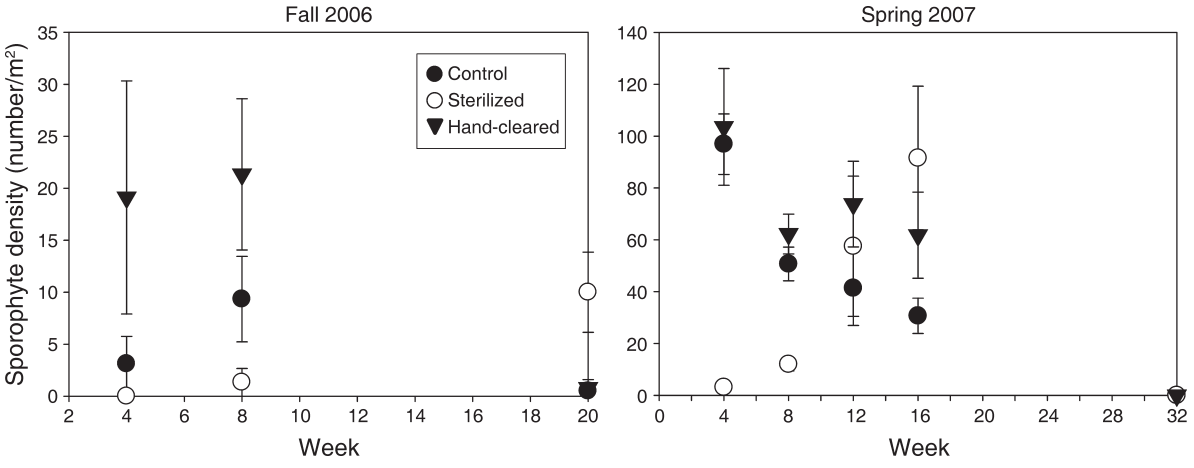

FIG. 2. Recruitment of unidentified juvenile kelp sporophytes too small to identify to species (<2 cm) into treatment plots during Trial 1 (September 2006–January 2007) and Trial 2 (April–October 2007). Sites were averaged. Note the differences in scale on axes. Error bars represent ±SE.

individual, we subtracted 40 days (minimum estimate) or 80 days (maximum estimate) from the time elapsed between parental absence and juvenile collection. Geographic distance was quantified between each parent and its assigned juvenile and used as a proxy for zoospore dispersal distance. Because storm events may increase zoospore release and dispersal distances (Reed et al. 1988, 1997, Gaylord et al. 2004), we quantified wave intensity as a qualitative correlate during the period of the study. Ocean wave data were obtained for southern California between 1 October 2006 and 30 April 2007 from the National Oceanic Atmosphere Administration's Weather Buoy Data web page (Mission Bay weather buoy 46231, 32°748′ N, 117°370′ W; data *available online*).[6] Wave intensity was estimated by calculating horizontal orbital displacements at the water's surface using linear wave theory (i.e., linear combinations of significant wave height and dominant wave period [Denny 1988]).

RESULTS

Effect of delayed stage removal on kelp recruitment

The differences in juvenile kelp recruitment abundance between treatments depended on sampling week (Fig. 2, Appendix C: Table C1; week × treatment, $F_{4,48} > 4.9$, $P = 0.001$). Two months after sterilization, abundance in plots where the bank of delayed stages was left intact was significantly higher than in plots where it was removed (Fig. 2). Differences in abundance were reduced between treatments during the following weeks. Kelp recruitment was generally lower during fall 2006 than spring 2007 (Figs. 2 and 3), likely due to the relatively higher concentrations of seawater nitrate made available by spring upwelling in 2007 (Carney and Edwards 2010) and the higher number of zoospores released during the winter and spring in general (Anderson and North 1967, Reed et al. 1996). Juvenile kelp abundance in hand-cleared plots was more similar to control plots than to sterilized plots (SIMPER dissimilarity 2006, 37 ± 1 [mean ± SD] and 79 ± 2.5, respectively; 2007, 9.9 ± 1.2 and 25 ± 1.2, respectively; Appendix C: Table C2). Average dissimilarity in kelp abundance between consecutive weeks and between treatments was primarily contributed to by unidentified (<2 cm) juvenile kelp, which reached higher densities than the other categories of macroalgae sampled (Table C2; SIMPER analysis dissimilarity contributed by unidentified juveniles between weeks in 2006, 63–79%; weeks in 2007, 34–53%; between control and hand-cleared plots in 2006, 50%; in 2007, 31%; between sterilized and hand-cleared plots in 2006, 85%; in 2007, 42%). Individual kelp species abundances were reduced for more than four months after sterilization in the plots where the microscopic stages were removed (Fig. 3). In 2007, additional dissimilarity between weeks was contributed by high initial densities of *D. ligulata* where microscopic stages were intact (52 ± 33 recruits/m^2 [mean ± SE]; Table C2; 24–37%). Kelp abundance was significantly different among sites (Table C1; 2006 site × treatment, $F_{4,48} = 2.5$, $P = 0.045$; 2007 site, $F_{2,48} = 7.79$, $P = 0.001$), as expected for spatially variable kelp forests (Dayton et al. 1984).

Microsatellite characteristics of Macrocystis pyrifera

High levels of genetic diversity were observed for the 162 *M. pyrifera* individuals sampled (including adults and juveniles; heterozygosity across 10 loci = 0.9 ± 0.01 [mean ± SE]; Appendix D). Departures from Hardy-Weinberg genotype frequencies were detected for seven of the 10 loci analyzed ($P < 0.05$) and high frequencies of null alleles (≥0.20) were detected at three of the loci (Appendix D). Thus, separate analyses of genetic diversity were performed using all 10 loci and the seven

[6] http://www.ndbc.noaa.gov

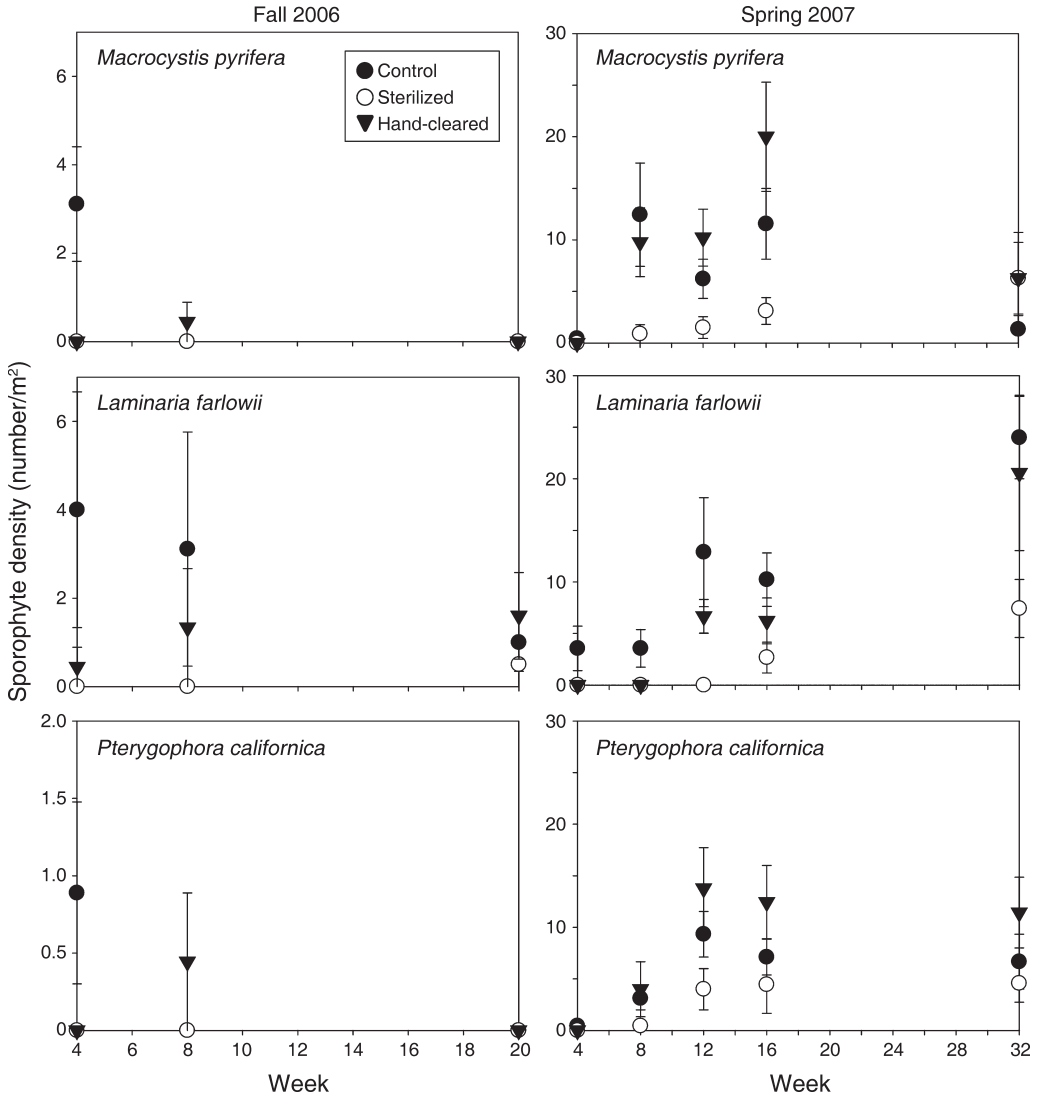

FIG. 3. Densities of juvenile sporophytes (>2 cm) for the three most conspicuous kelp species in treatment plots during Trial 1 (September 2006–January 2007) and Trial 2 (April–October 2007). Sites were averaged. Note the differences in scale on axes. Error bars represent ±SE.

with low null allele frequencies. Our interpretations were based on seven loci.

Longevity and dispersal distance of delayed stages in Macrocystis pyrifera

The seven loci with low null allele frequencies (0.05 ± 0.02 [mean ± SE]; Appendix D: Table D11) used for parentage analysis were characterized by high average polymorphic information content (0.89 ± 0.01; Table D1), high average number of alleles (22 ± 1.9; Table D1), and a high combined probability of parental exclusion (0.999). Collectively, these indicate a high degree of power for parentage analyses (see Table 3 in Bolormaa et al. 2008). After excluding individuals that amplified for only one to three loci each, 97 juveniles (46 from site 1, 34 from site 2, and 17 from site 3) and 64 adults (33 from site 1, 16 from site 2, and 15 from site 3) were analyzed. Juveniles from both sterilized and hand cleared treatment plots were included in the parentage analysis because juvenile sporophytes were observed in four of the nine sterilization plots within four weeks after sterilization during spring 2007 (representing 2% of juveniles counted in sterilization plots). Either a small portion of juveniles that recruited into sterilized plots survived the sterilization process as delayed microscopic stages or detached zygotes (fertilized eggs) dispersed into the plots after sterilization. Zygote dispersal has been suggested for *D. ligulata* (Edwards 2000), which were among those juveniles recruiting into our sterilization plots. Thus, a very small portion of juveniles in the sterilization plots were potentially offspring of tagged adult *M. pyrifera*. In fact, 3% of juveniles included in the

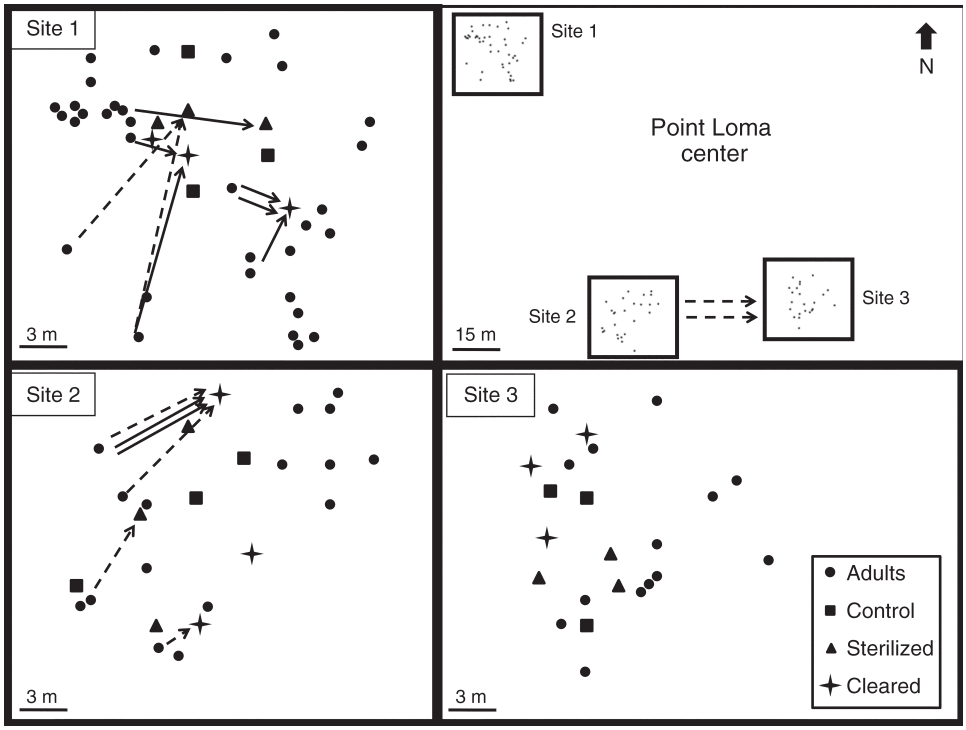

FIG. 4. Parent–offspring pairs identified in the center of the Point Loma kelp forest using seven loci at 95% confidence (solid arrows) and 80% confidence (dashed arrows). Dispersal is shown between sites (top right) and within each site (top left, bottom left, and bottom right).

parentage analyses were collected from sterilization plots and assigned to parents that had disappeared before sterilization. However, because this percentage was small, we are confident that juveniles from sterilized and hand-cleared plots represented different treatments in the analyses of genetic diversity.

By the time treatments were installed in March 2007, 68% of the tagged adult *M. pyrifera* were absent; 96% were absent by April 2007. The same 11 absent adults were assigned as parents to one or more juvenile *M. pyrifera* with ≥80% confidence for all candidate parent pool size (CP) estimates (Fig. 4). Since results varied little among CP estimates tested, we focused our interpretations on the pairs identified using 1000 CP. Eight parent–juvenile pairs were identified with ≥95% and eight with ≥80% confidence (Fig. 4). Juveniles were assigned parents from the same site as well as different sites (Fig. 4). Second parents were not identified with ≥95% confidence, thus self-fertilization was not detected within our data set.

The range of times elapsed between when a parent was first observed to be absent and when the assigned juvenile offspring was collected were between 2 and 8.5 months with ≥95% and between 1 and 5 months with ≥80% confidence (Fig. 5a). From this, we conservatively estimated a range of maximum delay durations for some juveniles of at least 5.8–7 months. Parents may have been removed up to two months earlier than estimated and it is not possible to determine when zoospore release actually occurred before they were removed. Eight and six juveniles were from parents in the same site within 12 m (≥95% and ≥80% confidence, respectively) and two were from parents from different sites within 40–60 m away (Fig. 5b; ≥80% confidence).

The same 13 parent–juvenile pairs were identified using 10 loci, regardless of CP estimate, nine of which were also identified using seven loci. Although more parent–juvenile pairs were identified with 95% confidence when 10 loci were used, the range of dispersal distances were identical to analyses using seven loci. However maximum delay durations of only 4.5 months were estimated using 10 loci. For all confidence levels, 97 pairs (including juveniles paired to two parents] were identified by both sets of loci and 45 of these were identical. Similar to seven loci, second parents were not assigned using 10 loci and self-fertilization was not detected.

DISCUSSION

Removing settled kelp microscopic stages from the substratum delayed the recruitment of juvenile kelp and the recovery of the dominant kelp assemblage for more than two months during fall 2006 and spring 2007. In contrast, recruitment occurred within one month where the microscopic stages remained intact. Recruitment episodes occurring between four-week sample dates may

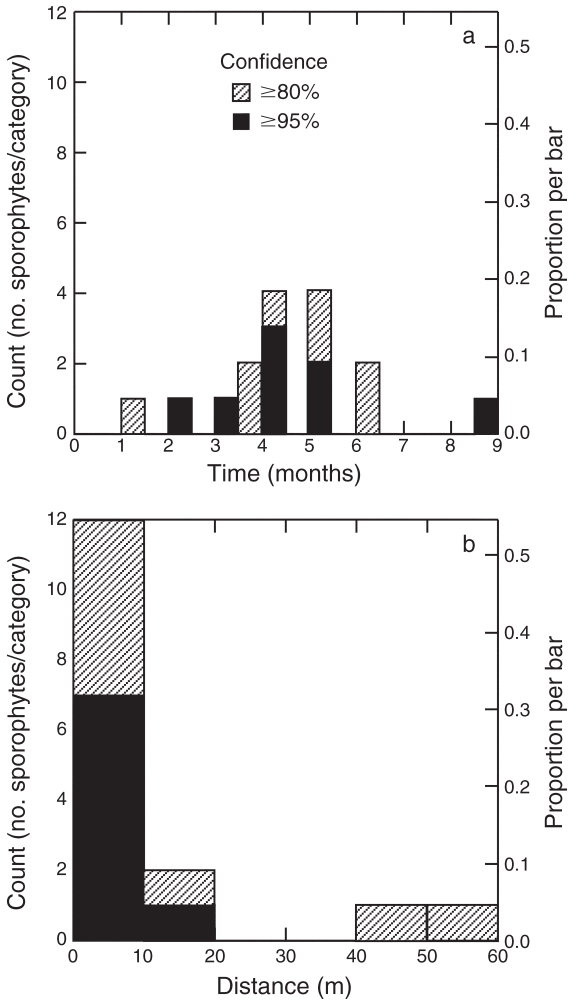

FIG. 5. Results of parentage analysis at 80% and 95% confidence levels for juvenile *M. pyrifera* using seven loci: (a) minimum time elapsed between when assigned parent was first observed to be absent and when juvenile sporophyte (assigned offspring) was collected and (b) the distribution of geographic distances between adult–juvenile pairs identified by parentage analysis.

have been missed, thus, we may have underestimated recruitment abundance. Rapid recruitment results in sporophytes that reach larger sizes earlier than later recruiting neighbors, potentially conveying a competitive advantage for faster development. It is well established that the species to recruit first in a patch in a kelp forest will initially dominate (Dayton et al. 1984), similar to other types of biological communities.

Our discussion focuses on results calculated from the more robust parentage assignments with ≥95% confidence. Immediate recovery where microscopic stages were intact may have been promoted by gametophytes that had been released as zoospores during the low nutrient concentrations of the previous fall and winter, and not to slow-growing microscopic sporophytes. Several lines of evidence support this theory. First, the parentage analysis suggests that a portion of the *M. pyrifera* sporophyte recruits collected arose from stages that had delayed for at least 2–7 months, durations that have been observed for gametophytes in the laboratory (Carney 2011). Second, laboratory studies have shown that kelp gametophytes consistently delay development and then resume development rapidly after increases in nutrients (Carney and Edwards 2010, Carney 2011). Third, increases in nitrate concentrations were observed in the experimental sites starting in early March 2007 (Carney and Edwards 2010) and simultaneous increases in irradiance likely occurred due to canopy loss associated with winter storms (e.g., 96% tagged adults were lost during winter). Thus, we hypothesize that a gametophyte bank of mixed age contributed new *M. pyrifera* juveniles to the population, even when adults were present. However, a precise definition for "mixed age" cannot be inferred. Gametophytes may persist for many months, but it is unlikely that these gametophyte banks contain a larger degree of overlapping generations than exists in the adult cohort as adults can survive 5–7 years (Dayton et al. 1984).

Even if the bank of delayed *M. pyrifera* stages does not contain overlapping generations, it likely reduces the loss of genetic diversity that would otherwise occur via drift between the adult and juvenile sporophyte stages within a generation. The majority of kelp zoospores released do not survive to become settled gametophytes (7–14 orders of magnitude are lost) and from these, very few become adult sporophytes (Chapman 1984, Schiel and Foster 2006). Both microscopic life stage transitions represent bottlenecks where genetic diversity can be reduced. However, even short delay durations allows zoospores released during poor environmental conditions to persist and contribute future sporophytes when conditions are better, potentially reducing the bottleneck occurring at this stage. We did detect a high amount of genetic diversity within the Point Loma kelp forest (expected heterozygosity $H_E \approx 0.9$) but a lack of genetic differentiation among *M. pyrifera* adults, juveniles from hand-cleared plots and juveniles from sterilized plots. This lack of differentiation may support that the majority of *M. pyrifera* recruits come from newly settled stages when adults are present (reported by Reed et al. 1997). Even so, examples of elevated genetic diversity in cyst or seed banks are rare, only occurring when delayed stages persist much longer than adults (Templeton and Levin 1979, Hairston and De Stasio 1988, McCue and Holtsford 1998, Honnay et al. 2008).

Although our data support the existence of a "mixed-age" gametophyte bank, evidence that the bank of delayed stages was also of "mixed origin" was only supported with ≥80% confidence. Two sporophyte recruits in site 3 were detected to originate from parents in site 2, 40–60 m away; distances that may be associated with storm driven waves (>2 m) that occurred during the winter months (Reed et al. 1988, 1997, Gaylord et al. 2004) or by strong current velocities

PLATE 1. Subtidal field sites in the Point Loma kelp forest, San Diego, USA. (Top left) Adult *Macrocystis pyrifera* sporophyte with numbered tag on holdfast; (top right) L. Carney injecting bleach into a sterilization tent affixed to the rocky substratum; (lower left) sterilized plot after tent was removed; (lower right) vegetation within a control plot. Photo credits: top left, Stacie Fejtek; other three, Levi Lewis.

(Gaylord et al. 2006). Long dispersal distances occurring within the interior of the kelp forest would be consistent with an inverse correlation between genetic subdivision and dispersal ability (Bohonak 1999). Stronger evidence for longer dispersal distances may have been possible by including more loci with low null allele frequencies in the analyses. Additionally, since the number of potential parents increases quadratically with distance from offspring, it is unlikely that both parents would have been identified without greatly increased adult sample sizes. Although there is a large amount of evidence that *M. pyrifera* zoospores can disperse >100 m into open space from patches of kelp (Gaylord et al. 2004, 2006, Reed et al. 2004, Alberto et al. 2010), distances >40 m have never been observed directly *within* a large kelp forest. It is promising that we observed these longer dispersal distances within a kelp forest from juveniles on a very limited amount of substrate surface sampled, even if only two examples were observed. More work is needed to challenge the hypothesis that large *M. pyrifera* populations are genetically subdivided and experience high self-fertilization rates (Graham 2003, Gaylord et al. 2006). We did not detect self-fertilization events for the individuals we sampled in the Point Loma *M. pyrifera* population, perhaps due to the small percentage of the population we were able to sample. However, very low genetic differentiation for highly variable microsatellites suggests that self-fertilization is balanced by gene flow throughout the Point Loma kelp forest. The movement of zoospores both within and among localized patches of kelp is likely to exceed the minimum rates of exchange necessary for significant population subdivision (Wright 1931).

M. pyrifera may have the ability to disperse across both time and space as do other organisms with variable propagule survival, such as the seeds of terrestrial plants (Cohen and Levin 1987). In fact, for *M. pyrifera*, the presence of a mixed-age gametophyte bank may promote the success of long-distance dispersal because even short delay periods could increase the probability that cross-fertilization will occur between gametophytes originating from multiple zoospore pulses that vary across time and space (Gaylord et al. 2006). Our work supports the hypothesis that kelp recruitment is partially

regulated by the presence of delayed gametophytes in a mixed-age gametophyte bank. More work is needed to determine the extent to which the bank is of mixed origin. Recruitment from this bank appears to be in response to an increase in resources (nutrients and/or irradiance) and may be important for regulating the recovery of a genetically diverse population after interannual events such as ENSO that cause widespread adult mortality. Although perhaps not as numerically important as newly settled stages from nearby adults, delayed gametophytes may promote rapid recruitment during the early spring that confers a competitive advantage for the resulting sporophytes as they grow larger.

Last, we propose that the combination of potential zoospore dispersal >40 m and the ability of the resulting gametophytes to delay for many months would enable *M. pyrifera* gametophytes to mate with conspecifics of different ages and of different origins. Consequently, delayed gametophytes may contribute to the genetic maintenance of intact populations and reduce genetic subdivision within large *M. pyrifera* forests.

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Supplemental Material

Appendix A

PCR reaction conditions and analysis (*Ecological Archives* E094-178-A1).

Appendix B

Methodology for measuring genetic diversity of *Macrocystis pyrifera* (*Ecological Archives* E094-178-A2).

Appendix C

Kelp recruitment results (*Ecological Archives* E094-178-A3).

Appendix D

Genetic diversity in *Macrocystis pyrifera* (*Ecological Archives* E094-178-A4).