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Impact of Individual and Population-Scale Dynamics on Growth and Reproduction of Two Morphologies of Macrocystis in Central California

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IMPACT OF INDIVIDUAL AND POPULATION-SCALE DYNAMICS ON GROWTH AND REPRODUCTION OF TWO MORPHOLOGIES OF *MACROCYSTIS* IN CENTRAL CALIFORNIA

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Sarah V. Jeffries

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CALIFORNIA STATE UNIVERSITY MONTEREY BAY

The Undersigned Faculty Committee Approves the

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ABSTRACT

IMPACT OF INDIVIDUAL AND POPULATION-SCALE DYNAMICS ON GROWTH AND REPRODUCTION OF TWO MORPHOLOGIES OF *MACROCYSTIS* IN CENTRAL CALIFORNIA

By Sarah V. Jeffries

Morphological plasticity is common among seaweeds and such form alteration often results in the modification of other physiological processes, such as growth or reproduction. This study explored the consequences of morphological plasticity by comparing two of the common growth forms of the giant kelp *Macrocystis*, an ecologically important genus in nearshore temperate ecosystems. The aclonal *pyrifera* morphology of *Macrocystis* grows in deep water and reproduces via the production and release of microscopic zoospores, while the clonal *integrifolia* morphology grows in shallow water and reproduces primarily by vegetative growth of its rhizome. The effects of morphology on reproduction, biomass and growth were studied using laboratory and field surveys and experiments. Surveys of frond densities by depth found that *Macrocystis* morphology could be quantified by standardizing frond densities with the coefficient of variation. Higher coefficient of variation values indicated that fronds are significantly more clumped in deep water, indicative of the *pyrifera* morphology. The coefficient of variation also increased significantly with depth, stair-stepping between the morphologies. Secondarily, seasonal reproductive sampling showed that the *pyrifera* morphology invested more in reproductive area, resulting in higher total individual reproduction, which was also true at greater depths. The year-round reproductive potential of *Macrocystis* was observed in this study, with reproduction varying throughout the year and peaking in October. Thirdly, the *pyrifera* morphology was found to have significantly higher biomass on average, while reproductive area and total reproduction correlated positively with frond biomass. Finally, clearings in the shallow *integrifolia* bed showed that the *integrifolia* morphology was unable to regenerate removed fronds during certain times of year, calling into question the storage capabilities of the *Macrocystis* rhizome. *Macrocystis* sexual recruitment was not observed into the clearings, leaving only encroachment from bordering individuals to recolonize the disturbed space, which occurred at an extremely slow rate, resulting in potential recovery times of 30 years for the small clearings and 100 years for the large clearings. The inability of *Macrocystis* to recruit into shallow areas suggests that the *integrifolia* morphology population persists primarily through the rare recruitment of single individuals that live for long periods of time, growing vegetatively and fragmenting. The findings of this thesis suggest that these two forms, though genetically identical, are variable from one another morphologically, reproductively and physiologically and that switches in morphology have physiological and biological consequences.

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INTRODUCTION

Many seaweeds display morphological plasticity in response to variable environmental conditions. Plasticity is most commonly expressed through form (morphology) alteration due to external pressures like grazing (North, 1972; Hay, 1981; Lewis et al., 1987; Duffy and Hay, 1990), wave exposure (Sundene, 1964; Svandsen and Kain, 1971; Markham, 1972; Chapman, 1978; Druehl, 1978; Russell, 1978; Wernberg and Thomsen, 2005) and variable light quantity and quality (Cole, 1968; Lüning and Neushul, 1978; Hay, 1981; Deysher and Dean, 1986; Dring, 1992; Lobban and Harrison, 1994). Form alteration in seaweeds is important as morphological plasticity can often determine the ecological niche a species inhabits as well as the method by which other physiological processes, such as reproduction, occur (Cook, 1985; Santelices, 1990).

Seaweeds propagate through sexual or asexual (vegetative) reproduction, or a combination of both strategies (Dring, 1992; Lobban and Harrison, 1994). Sexual reproduction involves the meiotic formation of haploid male and female gametes followed by fertilization (syngamy) to form a diploid zygote that develops into an adult sporophyte (North, 1971; Santelices, 1990; Lobban and Harrison, 1994). Sexually-produced individuals tend to be solitary and free-standing with offspring that disperse away from their parents (Harper, 1977), and have the potential for higher genetic variability, which can later increase the probability of an individual's survival during periods of high environmental stress (North, 1971; Santelices, 1990; Collado-Vides, 2002). However, sexual reproduction requires a greater expenditure of resources than asexual reproduction, with greater risks of reproductive failure (Clayton, 1981; Russell, 1986; Santelices, 1990; Vernet and Harper, 1990). Due to the high risks associated with

sexual reproduction, some species utilize vegetative reproduction as a means to propagate and potentially escape the limitations of sexual reproduction or as a refuge from harsh environmental conditions that make sexual reproductive success less likely (Deysher and Dean, 1986; Graham, 1996; Billingham et al., 2003; Tatarenkov et al., 2004).

Unlike offspring of sexual reproduction, vegetatively-produced individuals are not products of syngamy and are therefore genetically identical to their parent (Harper, 1977; Stebbins and Hill, 1980; Cook, 1985; Santelices, 2004). In many cases, the vegetative parent creates rhizomes that are horizontally elongated to acquire more space, from which offspring directly arise (Cook, 1985; Santelices, 2004). These aspects of vegetative propagation enable species utilizing rhizomes to recovery quickly post-disturbance; creation of offspring does not require the investment risks of sexual reproduction and a single individual could potentially colonize a large amount of space by itself (Fahrig et al., 1994; Santelices, 2004; Wright and Davis, 2006). In seaweeds, vegetative species are typically perennial and tend to form thick stands of shoots that are connected by rhizomes, with offspring arising directly from the parent (Cook, 1985; Santelices, 2004). However, due to the fact that offspring are genetically identical to their parent, vegetatively-produced populations experience a loss of genetic variability and are more likely to be impacted by environmental changes or disasters such as disease (Stebbins and Hill, 1990; Santelices, 1990; Collado-Vides, 2002). Though a population's persistence in a particular area is heavily influenced by its ability to successfully reproduce, the method by which it propagates is often determined by its morphological

form. Such is the case with the kelp genus *Macrocystis*, whose reproductive mode is affected by the morphology of its holdfast (Cook, 1985; Santelices, 1990).

Macrocystis pyrifera is a large temperate kelp that is globally distributed and is both economically and ecologically important (Dayton, 1985; Graham et al., 2007). Along the west coast of North America, *Macrocystis* ranges from southern Alaska to Baja California (Nicholson, 1979; Graham et al., 2007). Until its recent synonymization (Demes et al., 2009), the genus *Macrocystis* was considered to be divided into four species, three of which (*M. pyrifera, M. integrifolia, M. angustifolia*) were primarily based on differences in holdfast morphology (Fig. 1; Setchell, 1932; North, 1971; North, 1972; Lobban, 1978; Brostoff 1988). This history of multiple recognized species was likely due to the prevalence of two very different growth forms within the genus *Macrocystis*: aclonal and clonal. The aclonal (solitary) form (henceforth referred to by its historic name *M. pyrifera*) is described as having a conical, mounding holdfast and, in California, is most often found in water deeper than 3 m (Setchell, 1932; Abbott and Hollenberg, 1976; Graham et al., 2007). The clonal form (henceforth referred to by its historic name *M. integrifolia*) is described as having a rhizome-like holdfast from which shoots grow directly and, in California, is always found in water shallower than 3 m (Setchell, 1932; Neushul, 1971; Abbott and Hollenberg, 1976; Graham et al., 2007). *Macrocystis angustifolia* was a morphological intermediate between *M. pyrifera* and *M. integrifolia*, exhibiting a holdfast that was a combination of the mounding and rhizomatous growth forms (Brostoff, 1988; Westermeier et al., 2007; Demes et al., 2009). Interestingly, the method of reproduction varies between *M. pyrifera* and *M. integrifolia*. *M. pyrifera* only reproduces sexually, producing numerous reproductive

blades called sporophylls at its base just above the holdfast. These sporophylls contain sori that produce and release microscopic zoospores. In addition, *M. pyrifera* can be continuously reproductive (Neushul, 1963; Buschmann, 2006), though its reproductive effort likely varies seasonally. A defining physical characteristic of the *pyrifera* morphology is that the primary dichotomy/meristem occurs above the holdfast (Neushul, 1971). This positioning of the meristem is important as sporophylls grow above the primary dichotomy and its position above the holdfast allows for more propagule dispersal due to their height in the water column (North, 1972). On the other hand, *M. integrifolia* rarely produces sporophylls, and propagates primarily via vegetative growth of the rhizomatous holdfast, with new fronds arising directly from the holdfast (Setchell, 1932; Scagel, 1948; Abbott and Hollenberg, 1976). Unlike the *pyrifera* form, the primary dichotomy for the *integrifolia* morphology is contained within the rhizome (Neushul, 1971). Damage to the meristem can often lead to lack of recovery of the plant (North, 1971; Dayton et al., 1992) and the protected meristem of the *integrifolia* morphology likely allows for rapid re-growth of fronds after a disturbance to the rhizome.

These two growth forms of *Macrocystis* exist in close proximity in some regions of California (e.g. Carmel Bay, Point Piños, Cambria, Bodega Bay; pers. obs.; Fig. 2), and thus may be members of the same interfertile population (Mackenzie, 1993; Lewis and Neushul, 1994; Westermeier et al., 2007; Macaya and Zuccarrello, 2010) with the potential for plasticity in growth form depending on environmental conditions (North, 1972; Demes et al., 2009). The depth segregation of these growth forms suggests the existence of a gradient of *M. integrifolia* to *M. pyrifera* from intertidal to deep subtidal depths (Nicholson, 1979; Graham et al., 2007; Demes et al., 2009). Morphological

gradients have been well-documented in terrestrial systems (Harper, 1977), but are under-studied in the subtidal marine environment. This deficit results in a lack of understanding about the environmental causes of morphological variation, and the greater effects of changing morphology on the organisms themselves as well as on their associated communities.

In order to fill this void, I focused on the relationship between individual and population-scale dynamics within *Macrocystis* in relation to the two different growth forms of this species. I consider individual-scale dynamics to be driven by investment; whether an individual invests its resources (derived from biomass) into vegetative growth or reproductive potential affects the longevity of that individual and its relative contribution to the persistence of the larger population (Reed, 1987; Pfister, 1991; Graham, 2002; Santelices, 2004; Demes and Graham, 2011). On the other hand, population-scale dynamics are driven by recovery and recruitment; how large-scale impacts such as disturbance affect the persistence of an entire population (Harper, 1977). Specifically, I addressed the following questions regarding the consequences of morphological gradients and plasticity in *Macrocystis*: 1) Does increasing water depth correlate with a switch from clonal to aclonal morphologies? 2) Does morphology correlate with sexual reproductive investment and output? 3) Does individual biomass correlate with morphology and sexual reproductive investment and output? 4) What mechanisms does the *integrifolia* morphology use to recover after biomass loss?

To address the first question, I hypothesized that the frequency of the clonal growth form decreased as depth increased, and that morphology changes in a step-wise pattern with depth. Second, I hypothesized that reproductive output and investment

increased with increasing water depth and that the sexual reproductive variables were higher in the *pyrifera* morphology. Third, I hypothesized that the morphologies differed significantly in regards to their biomass; a *pyrifera* individual would have significantly more biomass than an *integrifolia* individual and I also hypothesized that individuals with higher biomass would have higher reproductive output and investment. Finally, I hypothesized that, when disturbed, the *integrifolia* morphology would recolonize primarily through regrowth of lost vegetative fronds rather than encroachment from bordering rhizomes or recruitment of new individuals. Answers to these questions will provide novel information about the consequences of morphological shifts in *Macrocystis*.

MATERIALS AND METHODS

Morphological changes of Macrocystis *with depth*

To test the hypotheses that the clonal *Macrocystis* growth form decreased in frequency with increasing water depth and that morphology changed with depth in a stepwise pattern, a morphological survey was designed. In September 2014, a subtidal survey was conducted in Stillwater Cove, Pebble Beach, California using *Macrocystis* frond density as a quantitative proxy for holdfast morphology. A 50 m transect line was laid out from a water depth of 0 m to 2 m (below MLLW) using a dive computer to determine water depth to the nearest 0.3 m (Fig. 3). Ten perpendicular transects were placed at five m intervals along the 50 m transect; the length of the perpendicular transects varied from six m to 11 m in order for each transect to contain at least one *Macrocystis* individual. A 25 x 25 cm three-sided quadrat was used to make measurements of *Macrocystis* frond density continuously 25 times from the leading end

of each perpendicular transect. Gross holdfast morphology (rhizomatous or mounding) and depth measurements were also noted for each of the ten transects. Additionally, mounding *pyrifera* individuals were surveyed using the continuous quadrat method at several depths beyond the deep end of the 50 m transect in order to provide an endpoint to compare the distribution of the fronds of shallow individuals to deeper "true" *pyrifera* populations (Fig. 3).

The variance in frond density was related to the distribution of the fronds. The variance was higher for the mounding form as many fronds emanate from the apex of the solitary holdfast and there tends to be open space between individuals; whereas the rhizomatous form had lower variance as fronds were more evenly distributed along the length of the rhizome, and rhizomes frequently overlap (methods adapted from Greig-Smith, 1964). However, because variances are inflated with higher means, the standardized coefficient of variation (CV, the square root of the variance divided by the mean) was also utilized (Zar, 1984; Gotelli and Ellison, 2013). Rather than simply visually observing the changes in growth form along a spectrum, these calculations allowed for a less subjective way to quantify the gradient of morphological change with depth. Analyses compared average frond density (per 0.25 m^2) by depth and morphology using a linear regression and two-sample t-test respectively. Variance in frond density was compared by morphology using a two-sample t-test. Finally, frond CV was compared by morphology and across depths (MLLW) using a two-sample t-test and a linear regression respectively.

Reproductive changes of Macrocystis *with morphology*

To test the hypotheses that sexual reproductive output and investment are greater in deeper water, vary seasonally throughout the year, and are higher for the *pyrifera* morphology, quarterly field surveys were conducted from July 2013 to April 2014. On each sampling date, a 50 m transect tape was placed from a water depth of approximately 1 m to 3 m MLLW (Fig. 3). This transect encompassed both the *M. pyrifera* and *M. integrifolia* morphologies. A 1 m swath was surveyed along both sides of the transect tape; all visually reproductive individuals within this swath were sampled. *Macrocystis* sori were relatively easy to observe underwater as they appeared a milky white (Neushul, 1963), so it was feasible to identify and collect reproductive samples while diving. A haphazardly placed 25 x 25 cm quadrat was used to collect reproductive material from that individual to be analyzed in the laboratory. This quadrat size was selected as it represented the approximate maximum *pyrifera* holdfast size observed in the sampling area (Fig. 3). The use of a quadrat served to standardize the amount of reproductive material that was collected for a *M. pyrifera* individual, and as a proxy for an "individual" for the *M. integrifolia* morphology as differentiating independent "individuals" of this morphology can be difficult.

Reproductive samples were brought back to the lab for processing. The samples were purged of any visibly non-reproductive material, weighed for total sporophyll wet weight, and the total number of sporophylls in each sample was counted. Each reproductive sporophyll was measured for area and wet weight and then the sori were excised and separately measured for area and wet weight. Finally, three sporophylls with visible sori were randomly chosen and a single hole-punch sample (area of ~20mm^2) was

taken from the center of each of the three sori and cultured in sterile seawater in an incubator set to conditions roughly mimicking average ambient conditions for deep, reproductive individuals (12º C, 40 µM photons m^{-2} s^{-1}, 14:10 light:dark photoperiod). The punches were left in the petri dishes for 24 hours before being removed, at which time settled zoospore output counts were made (10 fields of view at 400x magnification) and averaged to represent sexual reproductive output (zoospores/mm^2; adapted from Amsler and Neushul, 1989 and Kinlan et al., 2003). These cultures were monitored weekly and the presence of microscopic sporophytes was noted as they indicated zoospore viability and therefore the individuals' ability to successfully reproduce. Petri dishes were disposed of when microscopic sporophytes were observed or after three months.

Linear regressions were used to test for differences across depths in reproductive investment and reproductive output. Reproductive investment variables were: total soral area, average soral weight, and average soral percent cover (soral area/sporophyll area). Reproductive output variables were: average zoospore output and total reproductive output. Total reproductive output per "individual" (25 x 25 cm quadrat) was calculated by multiplying average zoospore output (per mm^2) by total soral area (mm^2). Two-way ANOVAs were used to compare the effects of morphology and month on all reproduction variables. All reproductive data were 4^{th} root transformed for the ANOVAs due to high variability within the morphologies.

Impacts of biomass on reproduction of Macrocystis

To test the hypotheses that individual biomass was higher in the *pyrifera* morphology and reproductive output and investment were higher in individuals with

more biomass, a field survey was conducted wherein 15 entire *Macrocystis* individuals were collected: eight *M. integrifolia* and seven *M. pyrifera.* Gross morphology and depth were noted before the collection of each sample. As described above, 25 x 25 cm three-sided quadrats were used to act as a proxy for an *M. integrifolia* "individual" due to the difficulty in differentiating true individuals of this morphology. These samples were brought back to the lab and measured for total biomass, holdfast biomass, frond biomass, frond number. Two reproductive investment variables (total sporophyll biomass and total soral area) and two reproductive output variables (average zoospore output and total reproductive output) were also measured. Zoospore output was calculated through 24 hour cultures, as described above. Total reproductive output was calculated by multiplying total soral area by average zoospore output.

In order to test for morphological and component (holdfast vs. fronds) differences in biomass, a two-way ANOVA was used. A significant interaction term indicated that one group is driving significance (e.g. *pyrifera* holdfast), rather than an entire factor (e.g. morphology). Sources of significant differences in morphology and component biomass were identified using a Tukey HSD post hoc comparisons test. If there was a significant difference in biomass between the components, the component which accounted for a majority of the total individual biomass was compared with the reproductive investment and output variables using linear regressions in order to test for biomass impacts on sexual reproduction. Biomass and reproductive data were 4^{th} root transformed due to high variability within the morphologies.

Impact of disturbance on recovery of the clonal growth form

A field-based biomass removal experiment was used to test the relative importance of several methods of disturbance recovery in the *integrifolia* morphology: vegetative growth of the rhizome, regrowth of removed fronds from an intact rhizome, and sexual recruitment. This experiment addressed the hypothesis that the clonal *integrifolia* morphology will recover from disturbance primarily though regrowth of fronds from intact rhizomes rather than through vegetative elongation of the rhizome or sexual recruitment, and secondarily it was hypothesized that smaller clearings would recover faster than larger clearings. Fifteen permanent circular plots were established in the *M. integrifolia* bed at a depth of approximately 1 m below MLLW in September 2013. The plots were either fully-cleared (completely cleared of all *Macrocystis* tissue), partially cleared (all *Macrocystis* fronds removed, leaving rhizomes intact) or control (no *Macrocystis* tissue removed). Three plots with diameters of 1.5 m and 3 m were created for each clearing type, resulting in six clearings for each treatment. These sizes were selected to be roughly equivalent to their water depth (or twice that for the larger clearings). This ensured that the light and water conditions in the center of the clearings varied from the conditions outside the clearings. Along with three control plots, all clearings were centrally marked with a tagged galvanized nail allowing them to be found and tracked over time. Eight frond density measurements using haphazardly placed three-sided 15 x 15 cm quadrats were obtained before clearing to ensure that all clearing locations had similar *Macrocystis* densities and therefore could be compared (one-way ANOVA; $F_{14,120}=0.66$, $p=0.80$).

Frond density was measured in partial and control plots using a three-sided 15 x 15 cm quadrat. Ten quadrats were sampled (counting all fronds 20cm or longer) on each sampling date by haphazardly distributing the quadrats around the center of each clearing. To quantify regrowth of bordering individuals into fully cleared plots (henceforth referred to as vegetative encroachment), tape measures with lengths corresponding to the radii of the plots (0.75 m and 1.5 m, for the 1.5 m and 3 m plots respectively) were used to quantify the amount of encroaching rhizome by comparing the current size at time of measurement with the initial size of each plot. These tape measures were hooked around the center marker, and then spun around the circumference of the plot. Any point where the actual radius of the plot was less than the length of the tape measure indicated a point of encroachment. This encroachment distance was recorded at ten systematically selected headings around the circumference of the plots, with a different random starting point during each sampling period (Fig. 4). Finally, sexual recruitment was counted in all treatments (full, partial, control) on each sampling date. As young *Macrocystis* recruits can be easily confused with recruits of other kelp species, this study counted recruits only if they were documented on one sampling date and then were found again at a later date and identified as *Macrocystis*. This necessary constraint limited potential observations of recruitment to those individuals that survived and developed enough to be positively identified as *Macrocystis*, reducing the likelihood of making such observations. Recruitment was measured *in situ* in order to understand the role of sexual recruitment on the colonization of available space. All plots were cleared of *Macrocystis* tissue (except controls) and subsequently sampled on the same day throughout the study period.

As clearings were tracked over time and compared between sizes, analysis of covariance (ANCOVA) tests were used for each treatment. For partial and control plots, frond densities were compared over time and between treatments. Percent of original full clearing remaining was compared over time and between clearing sizes. Encroachment patterns in full clearings were mapped using the polar plot add-on for Microsoft Excel and Image J photo analysis software. Finally, recruitment was compared between clearing treatments, sizes, and with the controls.

RESULTS

Morphological changes of Macrocystis *with depth*

In order to test the hypothesis that the clonal *integrifolia* morphology decreases in frequency as water depth increases, a survey using frond distribution as a proxy for morphology was conducted in September 2014. A significant decreasing trend in average frond density with depth was found (Fig. 5); fronds were significantly denser in shallower transects than deeper transects (linear regression: frond density= -0.56*depth+4.02, $F_{1,6}$=7.20, p=0.04, r^2=0.59). However, average frond density (per 0.25 m^2) did not significantly differ between the morphologies (Fig. 6), though this is likely due to the large variation in frond number within the *integrifolia* morphology (t-test: $t_{1,17}$=1.703, p=0.11). Given the similarity in average overall frond density between the morphologies, calculations of frond density variance were made to see if fronds were distributed differently between the morphologies. The variance in frond density along each transect revealed a highly non-significant difference between the morphologies (Fig. 7; t-test: $t_{1,17}$= -0.392, p=0.70), suggesting that the spread of fronds along each transect was similar. However when variances were compared with total number of fronds on each

transect (Fig. 8), there was a significant increasing trend (linear regression: variance=0.44*frond number=0.55, $F_{1,18}$=22.24, p<0.001, r^2=0.57) indicating that variance was inflated by high frond numbers and may not be the best way to compare morphologies when using frond distribution as a proxy.

Coefficient of variation (CV) values indicated the distribution of vegetative fronds along each transect but are not inflated by high means. A strong association of morphology with depth was found using frond density CV values (Fig. 9). Coefficient of variation increased with water depth, with low CV *integrifolia* transects occurring in shallower water, and higher CV *pyrifera* transects occurring in deeper water (linear regression: CV=0.81*depth+2.26, $F_{1,6}$=35.48, p<0.01, r^2=0.88). Additionally, deeper *pyrifera* control transects followed the depth relationship observed with shallower transects (Fig. 9). The *pyrifera* morphology was found to have a significantly higher average CV value (Fig. 10), reflecting the more clumped frond distribution in the *pyrifera* zone than the *integrifolia* zone (t-test: $t_{1,17}$= -4.68, p=0.01).

These results indicated that the morphologies can be quantitatively differentiated using the coefficient of variation and there was a strong association between low CV and the *integrifolia* morphology (Fig. 9, Fig. 10). Therefore, as depth increases, there was a decreasing occurrence of the *integrifolia* morphology, supporting the hypothesis that the clonal morphology decreased in frequency with increasing depth. Additionally, this study found that there was a morphological trend with depth, characterized by jumps in frond density CV from the *integrifolia* morphology to the shallow *pyrifera* morphology to the *pyrifera* control area, so the second hypothesis that the morphology changes in a stair-step pattern with depth was accepted.

Reproductive changes of Macrocystis *with morphology*

In order to test the hypotheses that reproductive output and investment increase with depth and were significantly greater in the *pyrifera* morphology, reproductive surveys were conducted seasonally from July 2013 to April 2014. Linear regressions with depth revealed significant, increasing relationships for three of the five reproductive variables: two investment metrics (soral percent cover and total soral area), and one output variable (total reproductive output). Soral percent cover increased with depth and varied from 25% to 60% (Fig. 11b; linear regression: percent cover=0.08*depth+0.37, $F_{1,7}$=3.89, p=0.096, r^2=0.39). Total soral area increased over tenfold from shallow to deep (Fig. 11c; linear regression: soral area=12114*depth+16326, $F_{1,7}$=9.08, p=0.02, r^2=0.60). Finally, total reproductive output was most strongly positively associated with depth (Fig. 12b; linear regression: total reproduction=$4x10^6$*depth+741582, $F_{1,7}$=65.74, p<0.001, r^2=0.92).

Depth did not affect one investment metric (average soral weight) and one output metric (zoospore output). Soral weight was roughly equal across depths, with the exception of 0 m which had a much lower average soral weight, causing the appearance of an increasing trend despite the non-significant relationship (Fig. 11a; linear regression: $F_{1,7}$=2.42, p=0.17, r^2=0.29). Zoospore output was extremely variable and roughly equal across depths (Fig. 12a; linear regression: $F_{1,7}$=1.90, p=0.22, r^2=0.24). In general, variability in reproduction was highest for individuals growing in intermediate depths (1 m to 1.5 m below MLLW).

The hypotheses that reproductive investment and output varied throughout the year and were higher for the *pyrifera* morphology were tested using two-way ANOVAs.

Sexual reproductive investment and output variables were found to be controlled by either month or morphology; no interaction term was significant (Table 1, Table 2). Three reproductive parameters (average soral weight, total soral area and total reproductive output) varied throughout the year, peaking in October (Fig. 13a, Fig. 13c, Fig. 14b). Soral percent cover (Fig. 13b) and zoospore output (Fig. 14a) did not vary temporally. Soral percent cover remained nearly constant over time (two-way ANOVA: $F_{3,46}$=1.28, p=0.29), while zoospore output had high variability, particularly within the *integrifolia* morphology (two-way ANOVA: $F_{3,46}$=1.92, p=0.14).

Morphology had a significant effect on two reproductive variables: total soral area (Fig. 13c; two-way ANOVA: $F_{1,46}$=3.33, p=0.08) and total reproductive output (Fig. 14b; two-way ANOVA: $F_{1,46}$=3.77, p=0.06). In both cases, the *pyrifera* morphology reproduced significantly more than the *integrifolia* morphology. For all other reproductive variables (average soral weight, soral percent cover, and zoospore output) there was no significant difference between the morphologies (Fig. 13a, Fig. 13b, Fig. 14a).

Impacts of biomass on reproduction of Macrocystis

In order to test the hypotheses that biomass was higher in the *pyrifera* morphology and reproductive output and investment were higher in individuals with more biomass, a one-time sampling in September 2014 compared reproduction with biomass between the two morphologies. A two-way ANOVA revealed a significant interaction term between morphology (*pyrifera* vs. *integrifolia*) and morphological component (fronds vs. holdfast; Fig. 15, Table 3; two-way ANOVA: $F_{1,29}$=3.061, p=0.09). A Tukey HSD multiple comparisons test found the only significantly different

group to be the *pyrifera* fronds (Fig. 15, Table 3). There was also a significant difference in overall biomass between the morphologies (two-way ANOVA: $F_{1,29}$=10.753, p=0.003) and between the morphological components (two-way ANOVA: $F_{1,29}$=5.715, p=0.02), wherein the *pyrifera* morphology and the fronds were found to have significantly higher biomass overall than the *integrifolia* morphology and holdfast respectively. Therefore, the hypothesis that the *pyrifera* morphology had significantly higher biomass was accepted.

In order to test the hypothesis that individuals with more biomass had higher reproductive investment and output, reproductive variables were compared to frond biomass. Frond biomass was chosen as it was significantly higher than holdfast biomass for the *pyrifera* morphology, and slightly higher (if not significantly so) for the *integrifolia* individuals (Fig. 15). While the average frond biomass was significantly higher in the *pyrifera* than the *integrifolia* morphology, there was no significant difference in the average number of fronds per individual between the morphologies (Fig. 16; t-test: $t_{1,13}$= -1.721, p=0.11). Two reproductive investment variables and two reproductive output variables were compared to frond biomass. The reproductive investment variables were total sporophyll biomass (Fig. 17a) and total soral area (Fig. 17b), and the reproductive output variables were zoospore output (Fig. 18a) and total reproductive output (Fig. 18b). Individuals with no reproductive investment/output were excluded from analyses to avoid anchoring the trendline near zero and creating artificially significant results. Total soral area (Fig. 17b; linear regression: area=88.62*frond biomass-68.63, $F_{1,8}$=18.26, r^2=0.723, p=0.004) and total reproductive output (Fig. 18b; linear regression: total reproduction=109.06*frond biomass-140.83, $F_{1,8}$=4.536, r^2=0.39,

p=0.07) had positive, significant relationships with frond biomass. Soral area, in particular, was tightly linked to frond biomass. On the other hand, sporophyll biomass (Fig. 17a; linear regression: $F_{1,8}$=1.49, r^2=0.175, p=0.26) and zoospore output (Fig. 18a; linear regression: $F_{1,8}$<0.001, r^2<0.001, p=0.997) were unaffected by frond biomass, remaining relatively constant as biomass increased. Therefore, the hypothesis that reproductive investment and output increased with frond biomass was accepted for total soral area and total reproductive output, and was not accepted for total sporophyll biomass and average zoospore output.

Overall, given the significant relationship between the *pyrifera* morphology and high frond biomass, and the relationship between frond biomass and two of the reproductive variables, these data suggested that reproduction in *Macrocystis* was greater for the *pyrifera* morphology and was significantly affected by frond biomass. Individuals with higher frond biomass had increased capacity for sexual reproduction.

Impact of disturbance on recovery of clonal growth form

To test the hypotheses that the clonal *integrifolia* morphology recolonized open space primarily though regrowth of fronds from intact rhizomes rather than through the encroaching growth of bordering rhizomes or sexual recruitment, and that smaller clearings recovered more quickly than larger clearings, manual biomass removal clearings were created in the shallow *integrifolia* bed. There was no significant difference in initial frond densities between control, small (1.5 m diameter) and large (3 m diameter) experimental plots before they were cleared on 5 September 2013 (ANOVA: $F_{14,120}$=0.663, p=0.80). However, once all *Macrocystis* fronds were removed from the partially cleared plots, leaving intact rhizomes, very little frond recovery was observed

during the course of the 13 month experiment (Fig. 19). An ANCOVA was used to compare the number of fronds in control plots with the number of regrown fronds in experimental clearings (Table 4). The non-significant interaction term indicated that the slopes of the linear regressions of each treatment are equal and therefore frond numbers are changing over time at roughly the same rate between treatments (ANCOVA: $F_{1,135}$=0.392, p=0.715). The "time" term was significant indicating that regardless of treatment, frond numbers changed with time since clearing (ANCOVA: $F_{1,135}$=10.22, p=0.002). This was potentially due to the difference in frond number between control plots and experimental clearings. Finally, a significant "treatment" term suggested that the y-intercepts (starting points) differed between the treatments (ANCOVA: $F_{1,135}$=30.46, p<0.001). This was likely due to control plots still having fronds at day 5 while the experimental clearings were removed of all fronds at the start of the experiment (day 0), causing the y-intercept of the control plots to be higher than the experimental clearings. Fronds were always present in control plots, and there was no significant change in frond number in controls over time (linear regression: $F_{1,14}$=1.59, p=0.229, r^2=0.11), showing that a natural die-out of fronds was not occurring in that population over time. A potential mechanism for this lack of frond recovery was senescence of cut rhizomes (Fig. 20a); in many cases, rhizomes were seen to be overgrown, break down and eventually disappear from the clearings altogether. The lack of recovery of fronds from intact rhizomes means that the hypothesis that frond regrowth accounted for the majority of the recovery of disturbed areas was not accepted, at least for the 13 month duration of this study.

Fully-cleared plots were removed of all *Macrocystis* tissue, and rhizome encroachment from bordering individuals accounted for all of the recovery in these areas. Encroachment distances acquired through the encroachment sampling scheme (Fig. 4) were used to determine the size of each clearing at each sampling date, and that area was then converted into percentage of original clearing remaining (Fig. 21). An ANCOVA was used to compare the percent of original clearing remaining over time between the clearings sizes (Table 5). The significant interaction term indicated that the slopes of the linear regressions of each clearing size were not equal and that recovery through encroachment was significantly faster in the 1.5 m clearings than the 3 m clearings (ANCOVA: $F_{1,28}$=8.42, p=0.01). There was also a significant "time" term indicating that regardless of the size of the clearing, recovery continued through time (ANCOVA: $F_{1,28}$=57.11, p<0.001). Finally, a non-significant "size" term meant that the y-intercepts (starting points) did not differ between plot sizes (ANCOVA: $F_{1,28}$=0.24, p=0.63), which is not surprising given that all clearings started the experiment at 100% original plot size. Therefore, the second hypothesis that smaller clearings will recover quicker than larger clearings is supported, as individuals bordering the 1.5 m diameter clearings colonized significantly more space than those growing into the larger 3 m clearings.

However, encroachment rate was not always constant over time. Total encroachment peaked in late July, roughly 10 months after clearing, and decreased in subsequent samplings until the end of the experiment (Fig. 21). Polar plots comparing the start of the experiment (Day 5- 10 September 2013), date of peak encroachment (Day 319- 22 July 2014) and end of the experiment (Day 395- 6 October 2014) revealed a die-back of encroaching rhizomes along the border of the clearings (Fig. 22, Fig. 23). In

many cases, measurements taken at the end of the experiment showed the presence of encroaching rhizomes at the same locations around the border of the plots as during the peak, but the rhizomes did not encroach as far into the clearings as previously (Fig. 22, Fig. 23).

Finally, no *Macrocystis* sexual recruits were observed throughout the 15 samplings and 13 months of this experiment. Occasionally a kelp recruit was found, but subsequent samplings were unable to find the same recruit and confirm its identity as *Macrocystis*, therefore this experiment could not yield any data on *in situ Macrocystis* recruitment patterns.

DISCUSSION

Morphological plasticity is common in seaweeds and often determines the method by which physiological processes such as growth and reproduction occur (Santelices, 1990). *Macrocystis*, an ecologically important kelp genus, is characterized by several morphologies which are differentiated based on holdfast growth form (Setchell, 1932; North, 1971). I used the close proximity of two of the morphologies of *Macrocystis* (the clonal *integrifolia* form and the aclonal *pyrifera* form) in central California to explore the physiological and biological consequences of morphological plasticity. To quantify the relationship between the growth forms, I asked about the interactive effects of morphology, reproduction, biomass and growth.

This study found that the morphologies did not vary in the density of their fronds, but that the distribution of fronds was significantly more clumped for the *pyrifera* form and in deeper water. It was also observed that the morphologies switch via a stair-step that occurs around 1 m below MLLW, with possibly another morphological step from the

shallow *pyrifera* to the deep *pyrifera* that occurs around 2.5 m below MLLW. Therefore, the morphology of *Macrocystis* in Stillwater Cove changes with depth from the clonal to the aclonal form in a predictable, quantifiable way. While a narrow transition zone where the morphologies are mixed likely exists (pers. obs.), this zone was not captured in the morphological sampling, likely due to low depth replication and sampling design constraints.

The environmental relationship between these morphologies is ecologically relevant because *Macrocystis* frond density affects the quality of available habitat for kelp-associated fauna, particularly mobile organisms. In the shallow *M. integrifolia* bed, fronds are close together and evenly distributed, creating habitat which could potentially be good nursery grounds for various species of kelp forest fishes (pers. obs.; Fig. 2), given the much-examined associations of fishes with *Macrocystis* (Carr, 1989; Carr, 1991). On the other hand, in the deeper *M. pyrifera* population, fronds are densely clumped into bundles that are few and far between, creating "islands" of habitat on the seafloor (Thiel and Vasquez, 2000; Fig. 2) which organisms need to cross through open water in order to reach, making them more vulnerable to predation (Anderson, 2001).

As previously noted, while many observations of morphological plasticity have been made in the terrestrial environment (Harper, 1977), most discussions of morphological variation in the marine environment has focused on rocky intertidal genera such as *Dictyota, Laurencia*, *Halimeda* (Hay, 1981) and *Ascophyllum* (Cousens, 1982). In many cases, seaweed morphological plasticity in the intertidal occurs in response to pressures from grazing and/or desiccation (Hay, 1981; Lewis, 1987), neither of which are the primary drivers of plasticity in *Macrocystis* (Mackenzie, 1993; Graham et al., 2007).

This study contributes to our knowledge of morphological gradients in the subtidal marine environment, and utilizes a novel approach to quantifying morphology in the marine environment through the calculation of the coefficient of variation from frond density measurements.

In addition to morphology, water depth significantly affected reproductive investment and output of *Macrocystis*. Average soral percent cover, total soral area, and total reproductive output all increased significantly with depth, while there was no pattern with average soral weight and zoospore output. This result indicates that shallow individuals reproduce just as much per unit area, but that deeper individuals invest in more reproductive area, which results in higher total reproductive output. While past studies have well documented the role of high irradiances on the inability of *Macrocystis* to recruit to shallow water (Deysher and Dean, 1986; Graham, 1996; Graham, 1997; Buschmann et al., 2004; Graham et al., 2007; this study), these findings show that shallow individuals produce zoospores in comparable numbers to deep individuals. Thus, the effects of high irradiance on recruitment inhibition must only affect the reproductive process after zoospores are released from the sori and not their production. Overall, this study reaffirmed the impact of water depth on reproduction in *Macrocystis*, and found that zoospore output is unaffected by depth.

In addition to finding increasing patterns of reproduction with depth, this study observed the year-round reproductive potential of *Macrocystis*, which is consistent with multiple past findings from both hemispheres (Neushul, 1963; Reed et al., 1996; Buschmann et al., 2006; Graham et al., 2007). All reproductive variables peaked in October, which is consistent with late winter/ early spring recruitment patterns of

Macrocystis along the California coast (pers. obs.; Graham et al., 2007). Despite these consistencies, the lack of an overall significant sexual reproductive advantage by the *pyrifera* morphology was surprising. Zoospore output, for example, was higher for the *integrifolia* morphology in two of the four sampled months, even though the *integrifolia* morphology was apparently unable to recruit into its own habitat (pers. obs.). Only two of the five reproductive variables were significantly affected by morphology: total soral area and total reproduction. In the case of total reproductive output, each sampled month the *pyrifera* morphology had higher output than the *integrifolia* morphology, likely due to higher total soral area, though standard error was still very high for both morphologies. Buschmann et al. (2006) found that the average total number of zoospores per individual for a perennial *pyrifera* individual was roughly $350*10^6$ zoospores, far higher than the amount found in this study which was $450*10^4$ for the *integrifolia* morphology, and $800*10^4$ for the *pyrifera* morphology. This drastic difference could be due to the use of a standardizing quadrat for sampling. Particularly in the case of the *pyrifera* morphology, the use of a quadrat limited the amount of reproductive tissue that could be collected, and very often did not include all of an individual's reproductive tissue.

Frequent production of sori by the *integrifolia* morphology suggests that sexual reproduction is not an expensive investment for this morphology, particularly as it most commonly places sori on vegetative blades rather than producing specialized sporophylls (pers. obs.). Past studies have reported *Macrocystis* frequently producing sori on vegetative blades in addition to sporophylls (Neushul, 1963; Lobban, 1978; Graham et al., 2007; Leal et al., 2014), but each of these cases addressed only the *pyrifera* morphology. The presence and viability of sori on the *integrifolia* individuals suggests

that sexual reproduction is not being selected against, and therefore must not be an expensive investment which would take resources away from growth or maintenance of the rhizome (Santelices, 1990; Pfister, 1991; Buschmann et al., 2004).

Confidence intervals were extremely high for both morphologies for all reproductive variables, likely the cause of some of the insignificant differences seasonally and morphologically. High variability could be true variation due to significant differences in individual reproductive state or possibly an artifact of undersampling, particularly for the *integrifolia* morphology, as reproductive tissue was often more difficult to find than for the *pyrifera* morphology. In particular, variability was high for individuals growing in intermediate depths (1 to 1.5 m below MLLW), which could be due to individuals in these depths living in a "transition zone" where *integrifolia* and shallow *pyrifera* individuals are mixed, and where the *pyrifera* may not display all the reproductive characteristics of deeper *pyrifera.* Overall, this study confirmed the importance of season and morphology on reproductive investment and output.

Biomass varied significantly between the morphologies; sampling detected a significant difference in total frond biomass between the morphologies, but no difference in total frond number. This suggests that a single *pyrifera* frond contains more biomass than an *integrifolia* frond. *Macrocystis* fronds grow to reach the surface and create a canopy that is several meters long (North, 1972; Lobban, 1978), thus deeper individuals would necessarily have longer fronds, and frond length has been found to correlate positively with biomass (Fox, 2013). Biomass varied more for the *pyrifera* morphology, implying a wide distribution of sizes for the *pyrifera* morphology and more similar sized individuals in the *integrifolia* morphology.

Comparisons of frond biomass with reproductive variables revealed a relationship between frond biomass and several reproductive variables. Higher biomass resources of the *pyrifera* morphology are invested into reproduction through the production of more soral area, leading to higher total reproduction of large individuals. However, consistent with the findings in the reproductive survey portion of this thesis, zoospore output was unaffected by biomass. Past studies have found that removal of vegetative biomass crashes the production of reproductive tissue in *Macrocystis* (Reed, 1987; Graham, 2002). This association between biomass and reproduction likely accounts for the within-morphology variation of reproductive variables observed during the reproductive sampling portion of this thesis. However, compared to the reproductive survey findings, it appears that reproduction was significantly underestimated in this sampling. Zoospore output values were around $50*10^3$ zoospores/mm^2 of soral tissue for the *integrifolia* morphology and $100*10^3$ zoospores/mm^2 of soral tissue for the *pyrifera* morphology. Additionally, the average total reproduction per individual (25 x 25 cm quadrat) for the *integrifolia* morphology was $10*10^3$ zoospores/individual and for the *pyrifera* morphology it was $18*10^4$ zoospores/individual. These values are more than an order of magnitude lower than those found in the reproductive survey section of this thesis. These low values could be due to an underestimation of reproductive tissue during laboratory sample processing as it is more difficult to see sori on the surface than underwater. This discrepancy may also account for the undersampling of sporophyll biomass which, surprisingly, did not show a relationship with frond biomass. Alternatively, zoospore output could have been lowered artificially due to the storage of samples submerged in seawater for 24 hours, during which time zoospores could have been releasing and were

therefore unavailable to be counted during sampling. Overall, this survey confirmed that reproductive patterns between and within the morphologies can be attributed to biomass differences.

This study used standardizing quadrats to sample the growth, reproduction and biomass of the *integrifolia* morphology. While this method is useful to approximating an "individual" when rhizomes overlap one another, the restrictive sampling of a fixed quadrat is likely not the best way to sample this morphology, or clonal species in general. Specifically this is because the use of a quadrat to represent an "individual" assumes that either 1) an entire, single individual is present within the sampling area or 2) that the rhizomes within the quadrat are capable of sharing resources through rhizome coalescence. Given the small size (25 x 25 cm) of the quadrats used in this study, and the likely large size of clonal individuals of the *integrifolia* morphology (Hargarten et al., in prep), most sampled individuals were probably fragments of the entire biological individual, rendering the first assumption unlikely. Coalescence has historically been considered a characteristic of the red algae (Santelices, 2004), however recent work by Gonzalez et al. (2015) documented the coalescence of holdfasts from two *Macrocystis* sporophytes as well as the resulting cellular modification and sharing of cytoplasm. Previous work has also noted the ability of adult *Macrocystis* individuals to fuse holdfasts *in situ* (Dayton et al., 1984). These findings imply that this study's *integrifolia* samples may indeed contain rhizomes from several individuals, but these rhizomes may be able to share resources and can therefore be considered as a single unit. Additionally, the ability to coalesce implies that fragmentation is less of an issue for the *integrifolia* morphology,

as adjacent rhizomes can share resources, allowing even the smallest rhizome fragment to have the resources to grow, reproduce or recover from disturbance.

While the morphologies of *Macrocystis* were found to be qualitatively variable in their growth form, reproductive investment and output, and biomass, an investigation into the recovery potential of the *integrifolia* morphology found that the clonal growth form is also physiologically different from the aclonal *pyrifera* form. Unexpectedly, very little frond regrowth was observed during the length of this study. When this same type of clearing was attempted in early spring (mid-March 2013), frond regeneration from pre-existing rhizomes was observed in all clearing replicates (pers. obs.). This discrepancy is potentially due to the biomass removal occurring in late summer, a time of year when *Macrocystis* individuals in central California are not accustomed to losing large proportions of their tissue (Graham et al., 2007; Reed et al., 2011). The control areas had fronds present year round, and experienced no significant upward or downward trend in frond density over time. This suggests that the inability of cut rhizomes to regenerate fronds is not due to an environmental effect on the entire population, but rather may be an issue of seasonality or potentially the lack of adequate storage in the rhizome. Rhizomes are known for their ability to store excess carbon for later allocation into growth or reproduction when needed (Harper, 1977; Santelices, 2004; Demes and Graham, 2011). While the rhizome of the *integrifolia* morphology contains enough stored resources to survive certain levels of fragmentation and biomass loss, its stores may be inadequate when all fronds are removed, too much fragmentation occurs, or if biomass loss occurs at a non-optimal time of year (Lobban, 1978; Druehl and Kemp, 1982; pers. obs.; M. Graham and R. Lagerholm, unpubl. data). The remaining rhizomes, unable to regenerate

lost fronds, slowly began to senesce over time, eventually leaving most partial clearings without any *Macrocystis* tissue at all (pers. obs., Fig. 20).

The removal of *Macrocystis* allowed other algal species to colonize the newly opened space. These algal groups included articulated corallines and fleshy red algae, primarily *Mazzaella*, *Chondracanthus* and *Rhodymenia*. *Macrocystis* and bare rock were only observed in the control plots (pers. obs.; Fig. 24). Increased recruitment of opportunistic understory algal species following a *Macrocystis* removal event has been well documented (Reed and Foster, 1984; Arkema et al., 2009). In particular, previous studies from Stillwater Cove have found a significant recruitment of the opportunistic brown alga *Desmarestia* following the removal of *Macrocystis* (Reed and Foster, 1984; Edwards, 1998; Clark et al., 2004). Observations of *Desmarestia* were conspicuously absent from within the clearings (Fig. 24), though *Desmarestia* was observed in other locations within the *integrifolia* bed (pers. obs.). This discrepancy may be due to the intentional lack of disturbance to existing turfing algae which Reed and Foster (1984) found to facilitate the recruitment of *Desmarestia*.

Due to the lack of regeneration from lost fronds, the recovery of the clearings was due to bordering individuals growing into the cleared areas, which occurred in all replicates but was only documented for the "fully-cleared" treatment. Consideration of rhizome encroachment is important as it is a type of growth unique to the *integrifolia* morphology; the *pyrifera* morphology is unable to recolonize opened space via vegetative growth, instead relying primarily on recruitment of new individuals (Dayton and Tegner, 1984; Foster and Schiel, 1985; Edwards, 2004). Encroachment was minimal until mid-winter (mid-December 2013) when rhizome growth in both clearing sizes began to

increase dramatically. This encroachment steadily increased until late July 2014 when it peaked and then sharply decreased for the rest of the experiment.

A possible explanation for this rhizome die-back in mid-summer is high water temperatures. *In situ* water temperature measurements from a location in outer Stillwater Cove at a depth of 5m found that average daily water temperatures nearly topped 18°C on several occasions, and averaged over 15°C during the mid-summer to early fall (Fig. 25). Given the shallow location of the *integrifolia* morphology, it is possible that water temperatures in the *integrifolia* bed exceeded those at the location in the outer cove. Though *Macrocystis* is better adapted to warm water conditions than other kelps (Graham et al., 2007), the inverse relationship between water temperature and nutrient concentrations causes *Macrocystis* in California to become nitrate limited at around 16°C (Zimmerman and Robertson, 1985; Graham et al., 2007). This lack of nutrient availability not only inhibits vegetative growth, it decreases individual fertility (Gerard, 1982; Deysher and Dean, 1986). While summer rhizome die-back may be a naturally occurring phenomenon in the *integrifolia* population, the sampling of these clearings did not exceed a year, and so observations from each season were not replicated.

Additionally, rhizome encroachment occurred at a surprisingly slow rate; after a full year of regrowth, the small clearings were only 7% recovered, while the large clearings were only 2% recovered. Given this rate of recovery, it will take approximately 30 years for the small and 100 years for the large clearings to recover fully. The small clearings had a recovery rate of approximately 2.6 cm/year and the large clearings recovered at a rate of 1.5 cm/year. In accordance with findings from the partial clearings, these results imply that the *integrifolia* morphology recovers optimally when some tissue

is left intact. When large amounts of biomass are removed, this morphology has trouble recovering on both an individual and population-scale. The slow recovery of this highly disturbed population also likely accounts for the patchy distribution of individuals in the *integrifolia* bed (pers. obs.) and corresponds with global distribution patterns of this morphology. The *integrifolia* morphology generally occurs in protected sites globally (North, 1971; Abbott and Hollenberg, 1976; Graham et al., 2007). It is likely due to the slow recovery of disturbed individuals that this morphology is most successful in protected areas; the decreased fragmentation potential in areas of decreased wave exposure allows enough individual longevity to create stable, perennial populations (Graham et al., 2007).

Finally, clearings made in the *integrifolia* bed resulted in no sexual recruitment of *Macrocystis* even with the new availability of space, light, and comparable zoospore output with the *pyrifera* morphology. This phenomenon has been well documented in the literature from California (Setchell, 1932; Graham, 1996; Graham, 1997; M. Graham and R. Lagerholm, unpubl. data). However, recruits are frequently seen in *integrifolia* morphology populations in southern Canada (Lobban, 1978; Druehl and Wheeler, 1986). Previous studies have also noted recruitment pulses following the removal of adults in deeper *Macrocystis* beds (Dayton and Tegner, 1984; Foster and Schiel, 1985; Edwards, 2004). The discrepancy between the lack of recruitment in shallow habitats in California and the presence of shallow recruits at higher latitudes in Canada are most likely due to the extremely high irradiances in lower latitude *integrifolia* beds. Graham (1996) found that high PAR prohibited microscopic stage (gametophyte and microscopic sporophyte) growth until a depth of 3-4m which is well below the lowest extent of the *integrifolia* bed

in Stillwater Cove. As discussed previously, water temperature could also be a contributing factor (Gerard, 1982; Deysher and Dean, 1986).

Due to the *integrifolia* morphology's general inability to propagate into its intertidal bed through regular sexual recruitment events, it seems likely that another mechanism regulates the perpetuation of this population. Individuals likely persist for long periods of time, fragmenting occasionally resulting in a population dominated by several genetic individuals, which account for most of the biomass within this population (Hargarten et al., in prep**).** These individuals are potentially the result of infrequent recruitment pulses, permitted by events such as El Niño which decrease irradiance levels in shallow water for prolonged periods of time. Overall, these results suggest that the *integrifolia* morphology heavily relies on vegetative growth to maintain its populations, a strategy that is unavailable to the aclonal *pyrifera* morphology, which relies on annual sexual recruitment events. Due to their differences in growth, reproduction and recovery, it is clear that the morphologies vary greatly in their individual and population dynamics.

CONCLUSIONS

Investigations into morphological gradients and their consequences are common in the terrestrial environment (Harper, 1977) but are lacking in marine systems. This study helps to fill this void by exploring the individual and population dynamics of two of the common morphologies of *Macrocystis*, an ecologically important genus in nearshore temperate ecosystems (Dayton, 1985; Graham et al., 2007).

This study found that the morphologies differ from one another in their frond distribution, and there is a switch from clonal to aclonal with increasing depth. The *pyrifera* morphology invested in more reproductive area, resulting in higher total

individual reproduction, which was also true at greater depths. For both morphologies, reproduction was seasonally variable and peaked in October. Reproductive area and total reproduction were also positively correlated with frond biomass, and the *pyrifera* morphology had significantly more frond biomass than the *integrifolia* morphology. Finally, this study found that the *integrifolia* morphology recovered poorly on an individual and population scale when large amounts of biomass was removed and appears to be incapable of sexually recruiting to shallow locations in California. Overall, this study found that the two morphologies of *Macrocystis,* while being genetically interchangeable, are morphologically, reproductively, and physiologically variable and that there are physiological and biological consequences to this variation in growth form.

Given the recent synonymization of these two species (Demes et al., 2009), there have been very few studies which directly compare the dynamics of these two morphologies. Integrative studies like these are needed to understand the relationship between these morphologies and their impacts on the nearshore environment.

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FIGURES

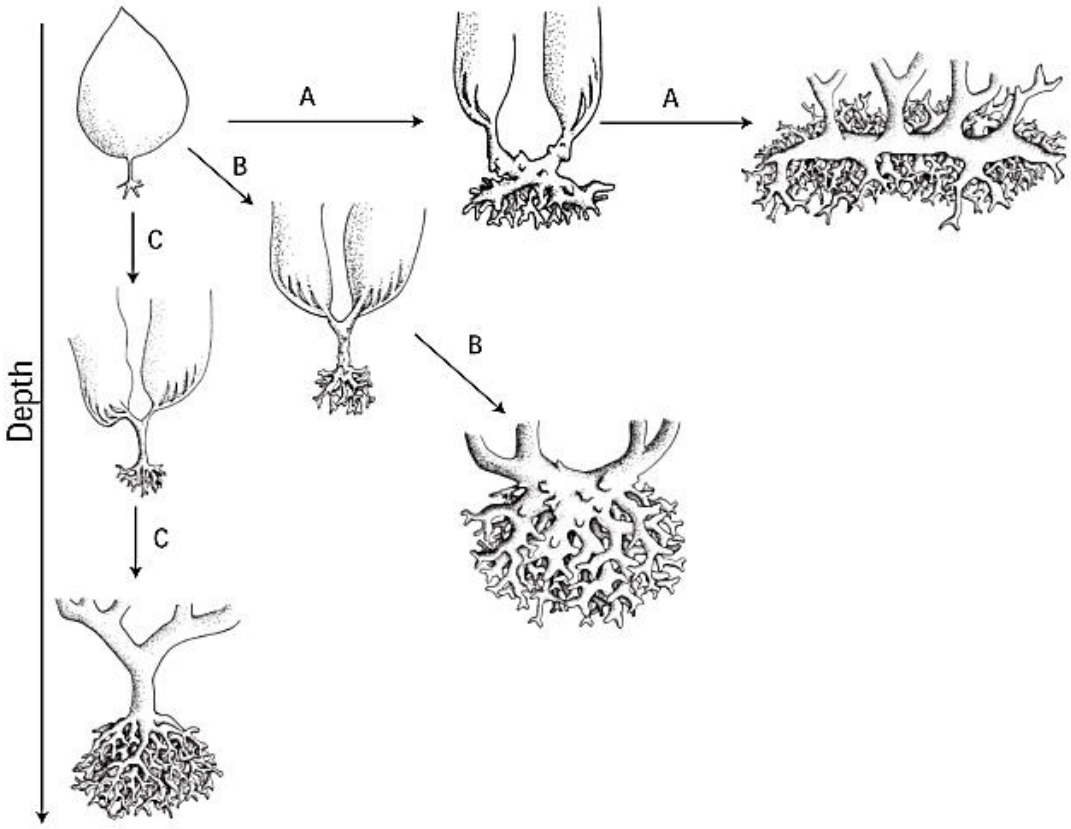


Figure 1. Depth of recruitment determines holdfast morphology and location of primary dichotomies of *Macrocystis* morphologies (adapted from Demes et al., 2009). A) *M. integrifolia* morphology, B) *M. angustifolia* morphology and C) *M. pyrifera* morphology.

Figure 2. Graphic representation of the proposed depth relationship between the two morphologies of *Macrocystis*; the clonal *integrifolia* morphology grows at the shallow extent, there is a narrow mixed morphology zone in the middle, followed by the aclonal *pyrifera* morphology growing at the deep end of the population. (created by Catherine Drake).

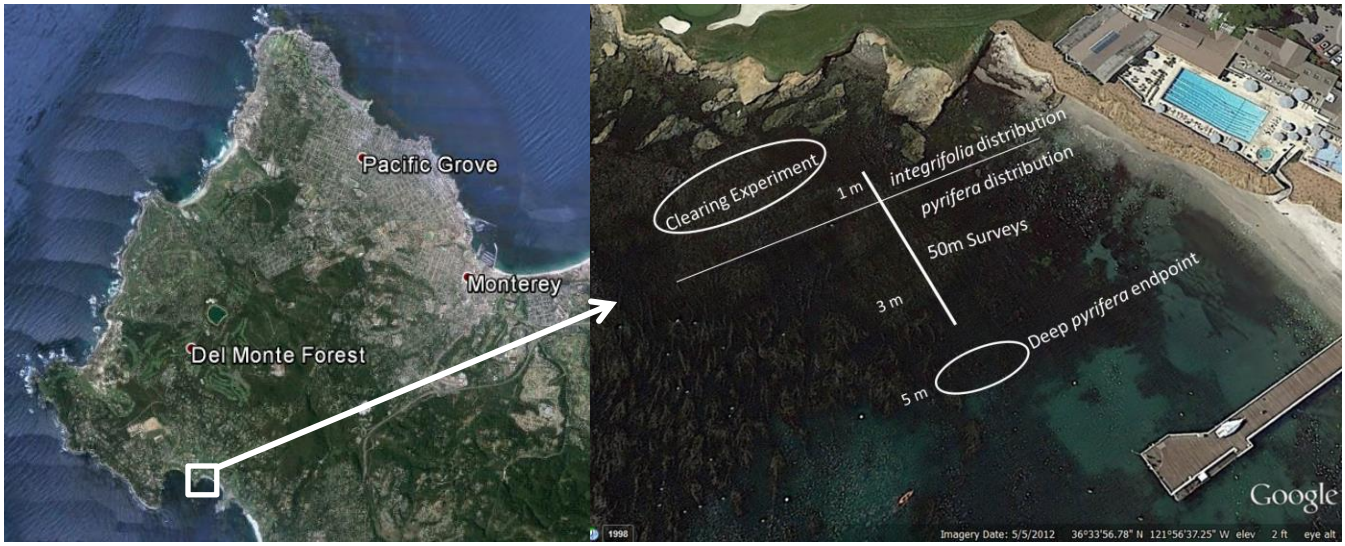


Figure 3. Site map of inner Stillwater Cove, Pebble Beach, California. The distribution of the morphologies is shown as well as the locations of the transects for morphology, seasonal reproduction and biomass surveys, the *pyrifera* control and the location of the clearing experiment.

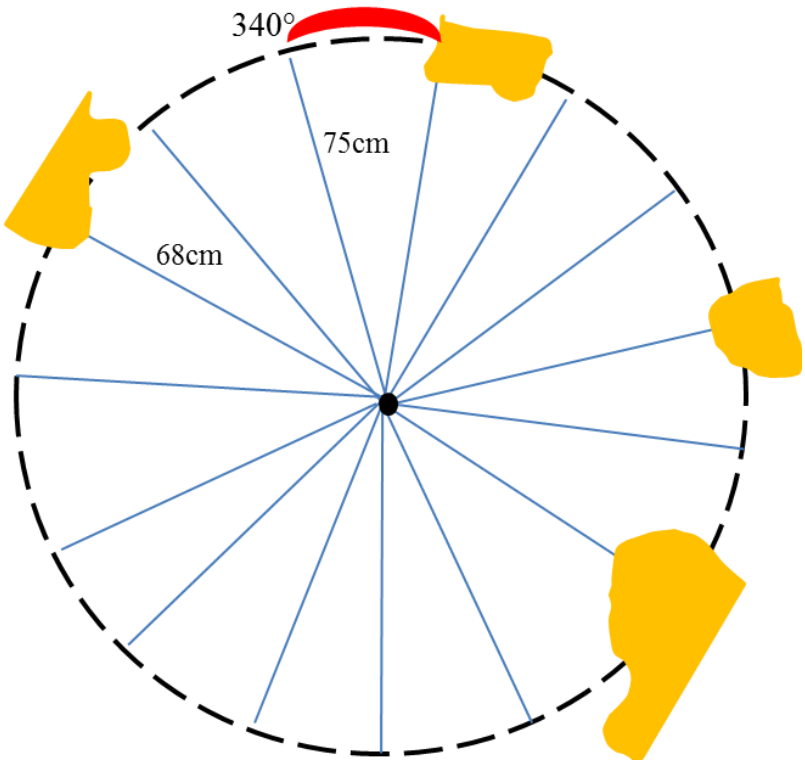


Figure 4. Diagram of the method for determining encroachment distance into fully-cleared plots. A random heading (e.g. 340°) was selected for each sampling, and the first encroachment measurement was taken on this heading. A tape measure (blue lines) was used to measure the distance from center to the edge of the clearing (dashed black line, e.g. 75cm) or to the leading edge of an encroaching rhizome (yellow polygons, e.g. 68 cm). Each subsequent point was a set distance from the previous using a curved piece of pipe to equally separate the measurements (red polygon) and fifteen measurements were taken per clearing to cover the entire circumference.

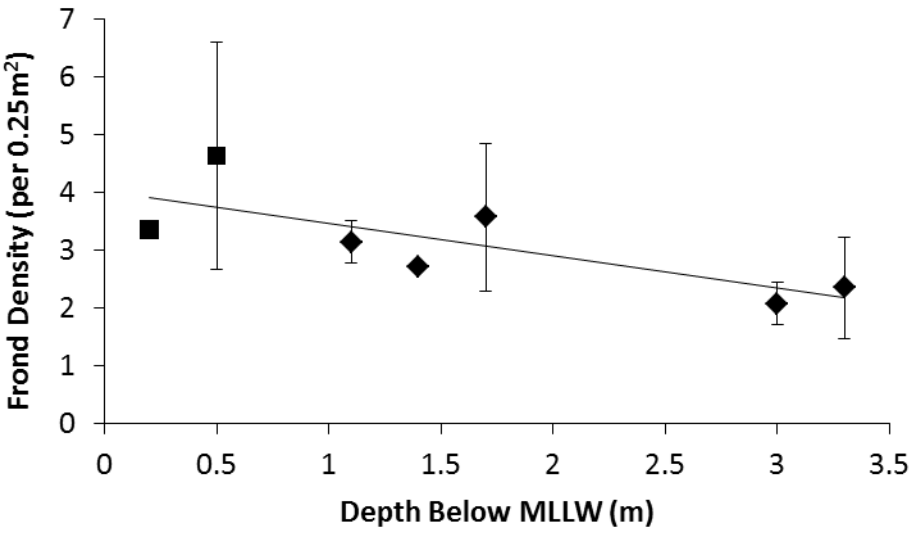


Figure 5. Effect of depth on average frond density per $0.25m^2$ (linear regression: density = -0.56 * depth + 4.02, $F_{1,6}=7.20$, $p=0.04$, $r^2=0.59$). Squares indicate *integrifolia* morphology transects and diamonds indicate *pyrifera* morphology transects. Error bars are ±SE for depths that were replicated during sampling.

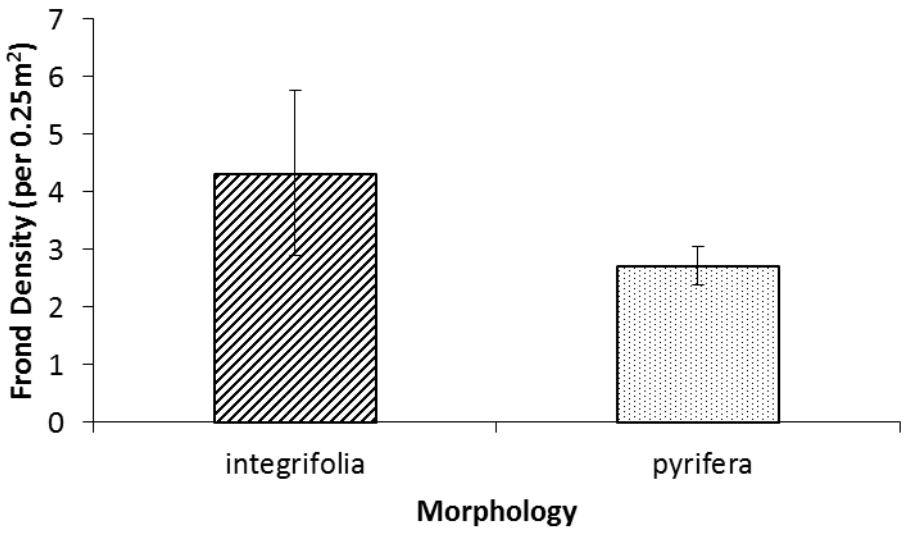


Figure 6. Effect of morphology on average frond density per 0.25 m^2 (t-test: $t_{1,17}$=1.70, p=0.11). Error bars are ±SE.

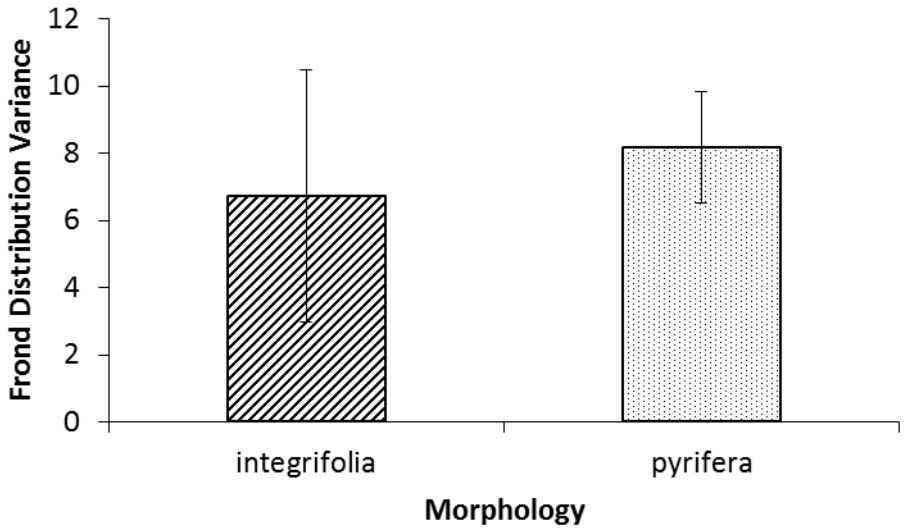


Figure 7. Effect of morphology on variance in frond distribution (t-test: $t_{1,17}$= -0.39, p=0.70). Error bars are ±SE.

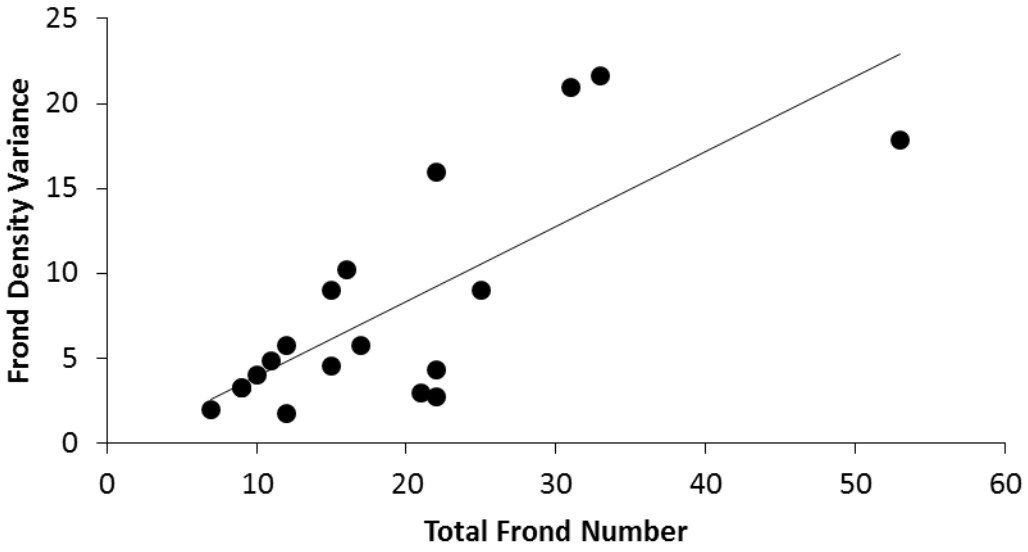


Figure 8. Variance in frond density increases with increasing total frond number (linear regression: frond variance=0.44*frond number-0.55, $F_{1,18}$=22.24, p<0.001, r^2=0.57, df=18).

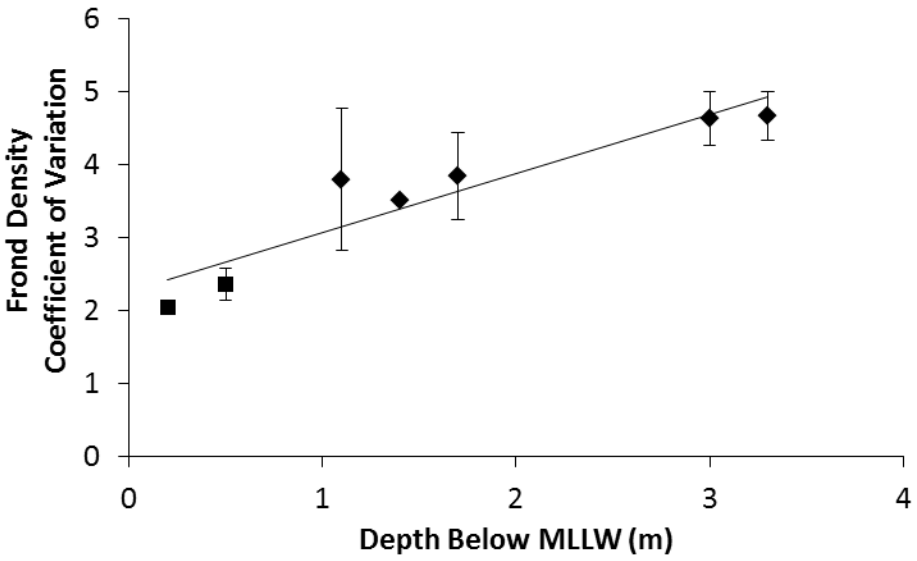


Figure 9. Effect of depth on frond distribution coefficient of variation (CV) values (linear regression: CV=0.81*depth+2.26, $F_{1,6}$=35.48, p=0.002, r^2=0.88). Squares indicate *integrifolia* transects, and diamonds indicate *pyrifera* transects. Error bars are ±SE for depths that were replicated during sampling.

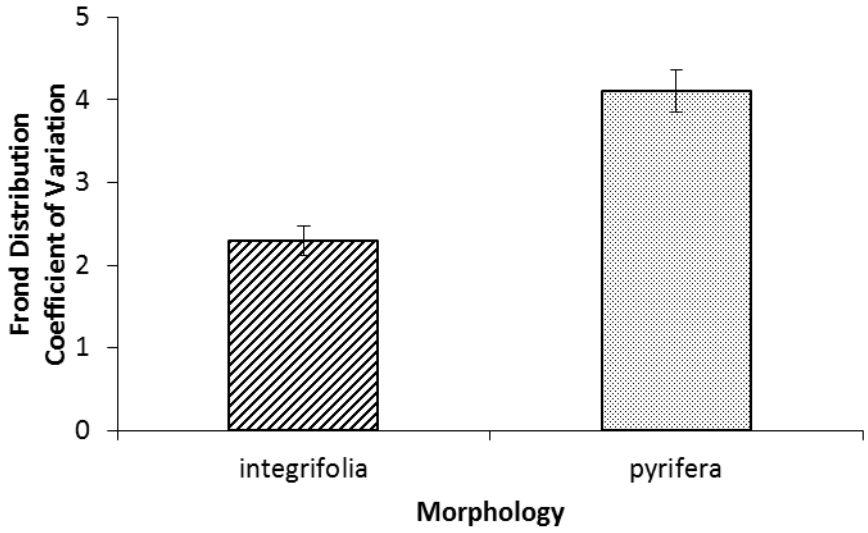
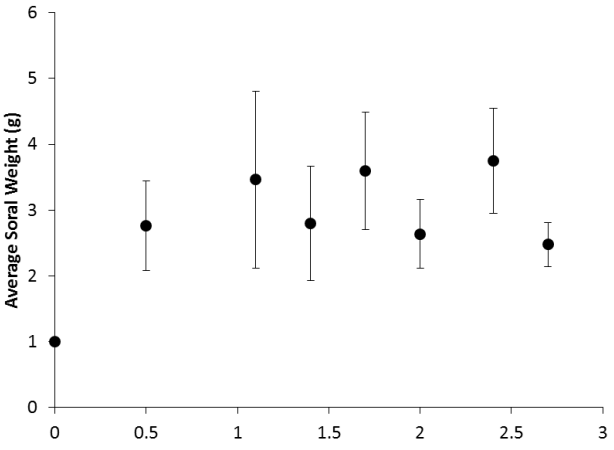
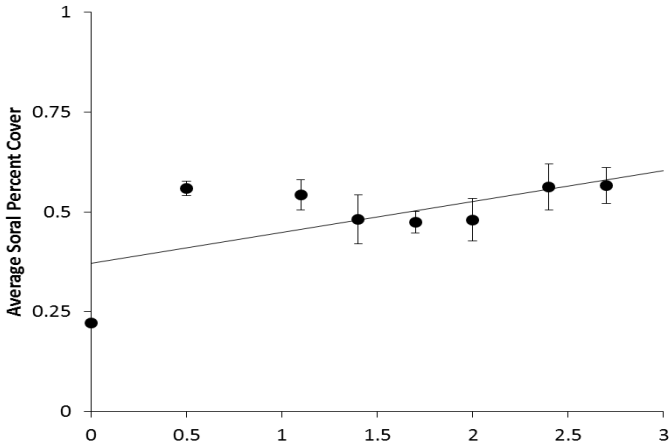


Figure 10. Effect of morphology on frond density coefficient of variation (CV) values (t-test: $t_{1,17}$= -4.68, p=0.01). Error bars are ±SE.

a)



b)



c)

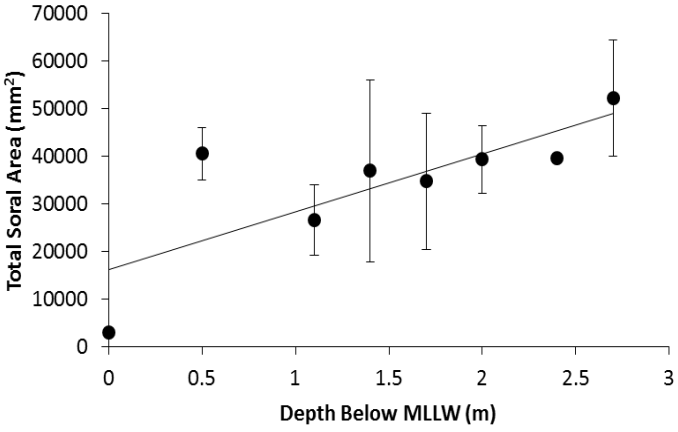
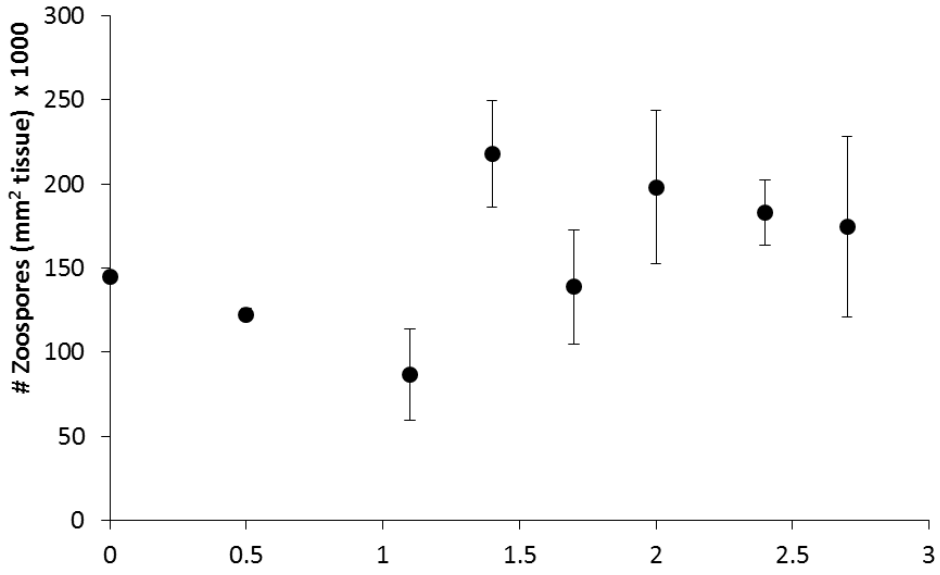


Figure 11. Effect of depth on three metrics of reproductive investment a) average soral weight ($F_{1,7}$=2.42, p=0.17, r^2=0.287, df=7), b) average soral percent cover (linear regression: percent cover=0.08*depth+0.37, $F_{1,7}$=3.89, p=0.09, r^2=0.393, df=7), and c) total soral area (linear regression: area=12114*depth+16326, $F_{1,7}$=9.08, p=0.02, r^2=0.602, df=7).

a)



b)

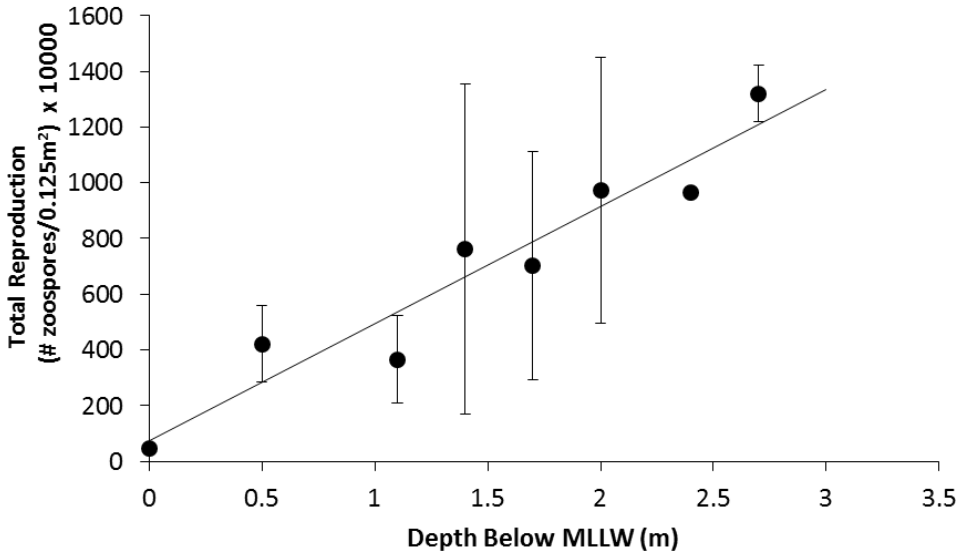
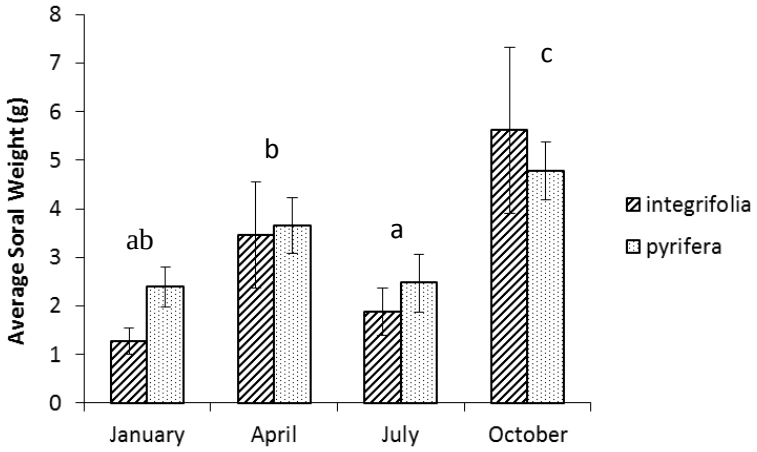


Figure 12. Effect of depth on two metrics of reproductive output a) average zoospore output (per mm^2 tissue; $F_{1,7}$=1.90, p=0.22, r^2=0.240, df=7) and b) total reproductive output (per 0.125m^2 quadrat; linear regression: total reproduction=4x10^6*depth+741582, $F_{1,7}$=65.74, p<0.001, r^2=0.92, df=7).

a)



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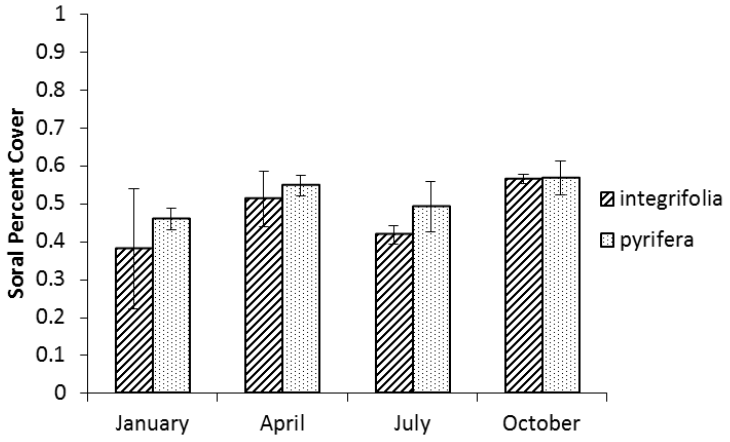

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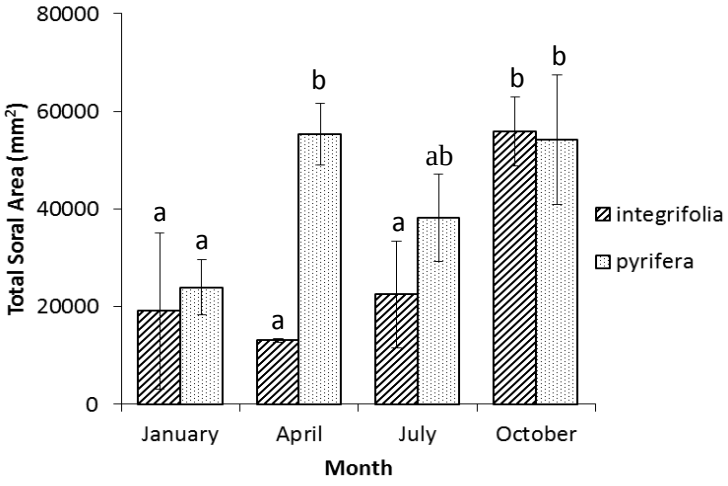
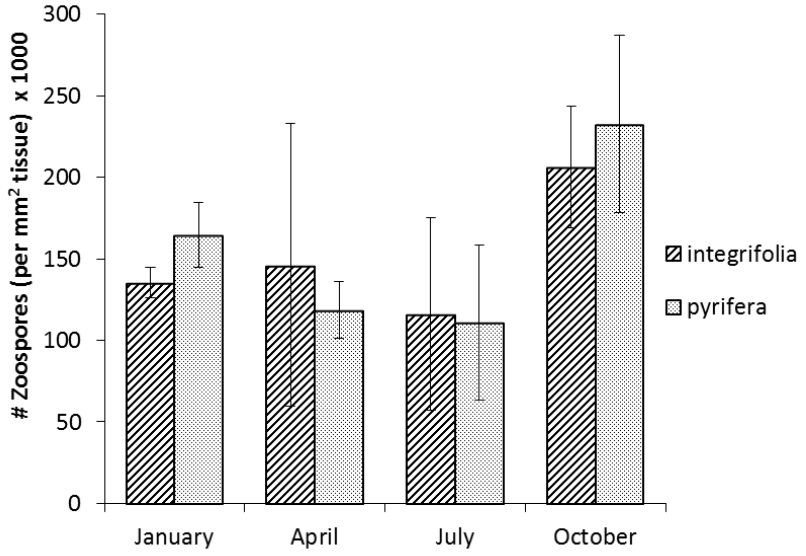


Figure 13. Effects of morphology and month on reproductive investment: a) average soral weight, b) soral percent cover and c) total soral area. Letters above bars represent significant differences ($p<0.1$, Tukey HSD). Error bars are ±SE. See Table 1 for full ANOVA results.

a)



b)

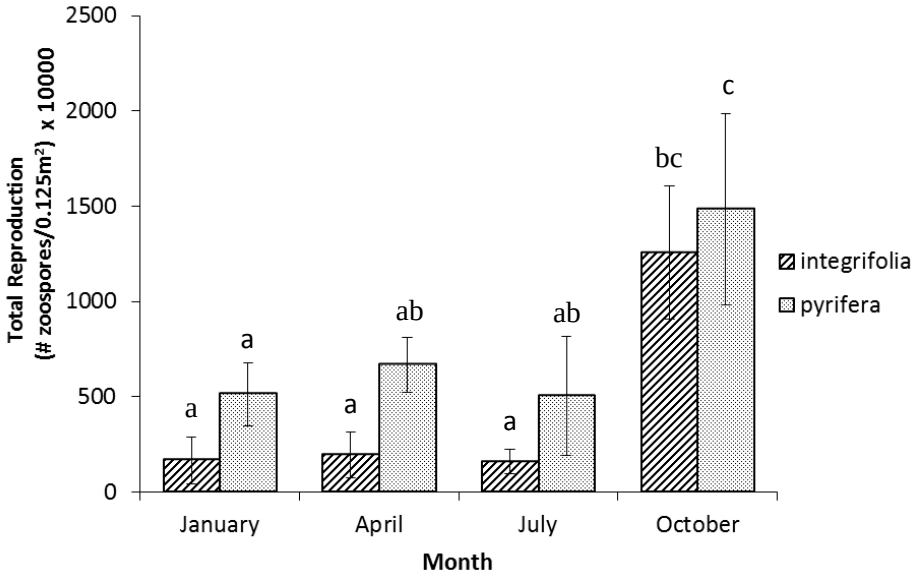


Figure 14. Effects of morphology and month on two metrics of reproductive output: a) zoospore output and b) total reproduction. Letters above bars represent significant differences ($p<0.1$, Tukey HSD). Error bars are ±SE. See Table 2 for full ANOVA results.

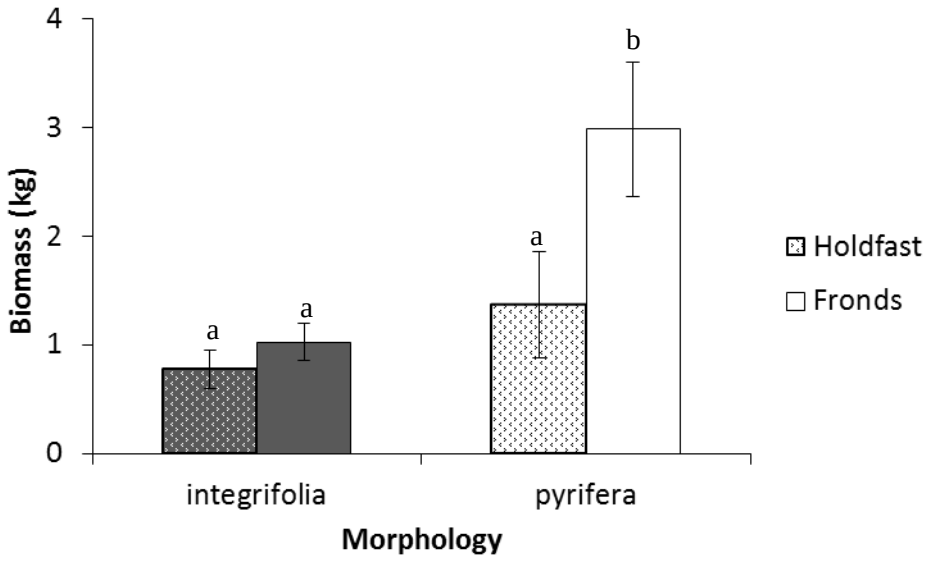


Figure 15. Average biomass values for holdfast and fronds between the morphologies. Letters above bars represent significant differences ($p<0.1$, Tukey HSD). Error bars are ±SE. See Table 3 for ANOVA and Tukey HSD post hoc comparisons results.

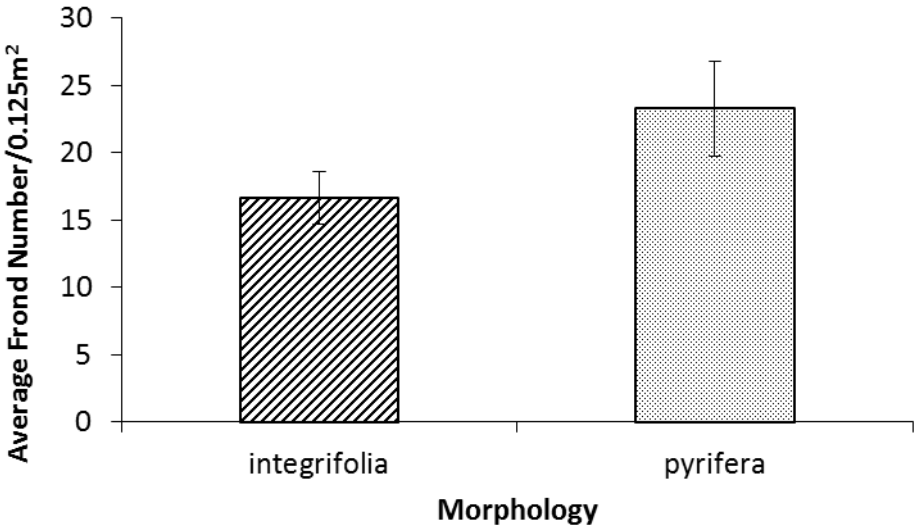
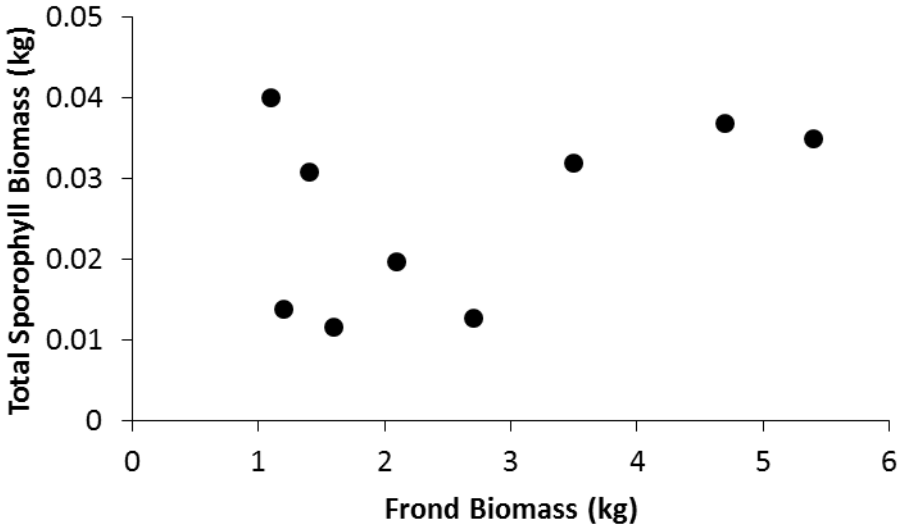


Figure 16. Effect of morphology on average number of fronds per 0.125m^2 quadrat (proxy for individual; t-test: $t_{1,13}$= -1.72, p=0.11). Error bars are ±SE.

a)



b)

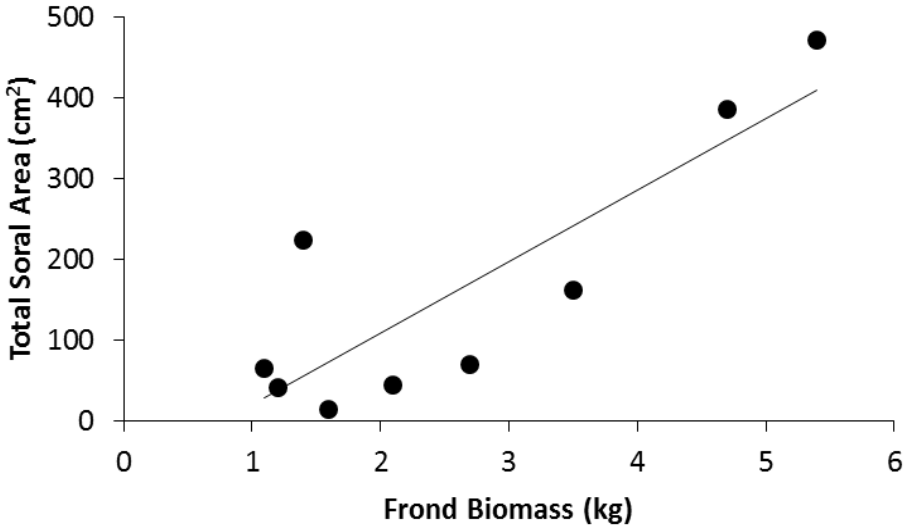
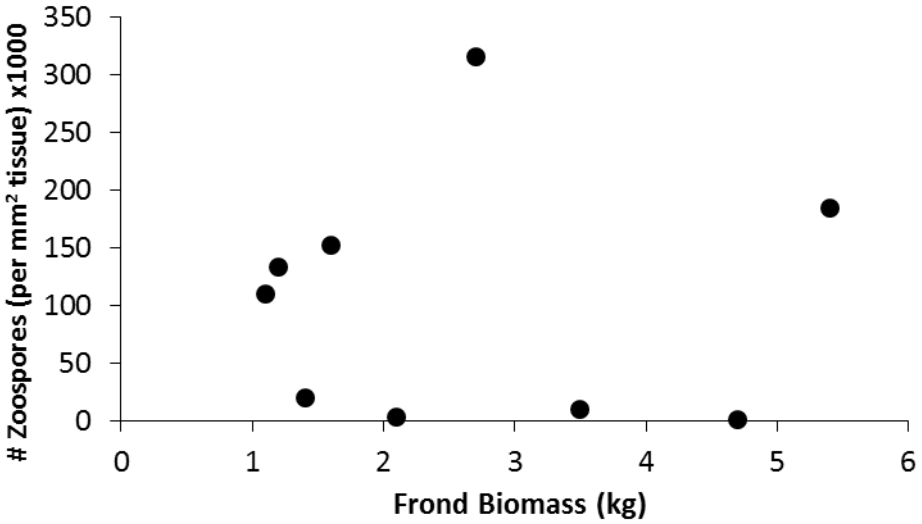


Figure 17. Effect of frond biomass on two variables of reproductive investment: a) total sporophyll biomass ($F_{1,7}$=1.49, p=0.26, r^2=0.18) and b) total soral area (linear regression: soral area=89.01*frond biomass–70.3, $F_{1,7}$=18.26, p=0.004, r^2=0.72).

a)



b)

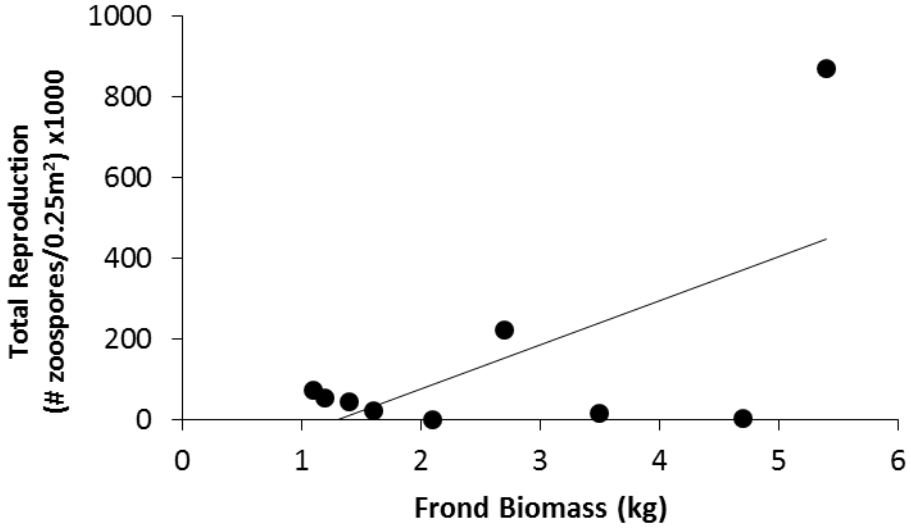


Figure 18. Effect of frond biomass on two variables of reproductive output: a) zoospore output ($F_{1,7}$<0.001 p=0.99, r^2<0.001) and b) total reproductive output (linear regression: total output=109.1.1*frond biomass-140.83, $F_{1,7}$=4.54, p=0.07, r^2=0.39).

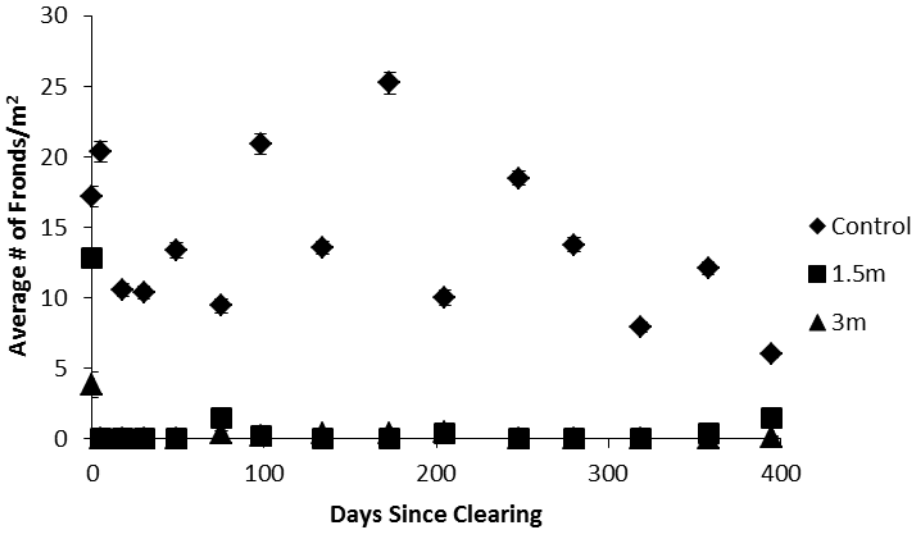
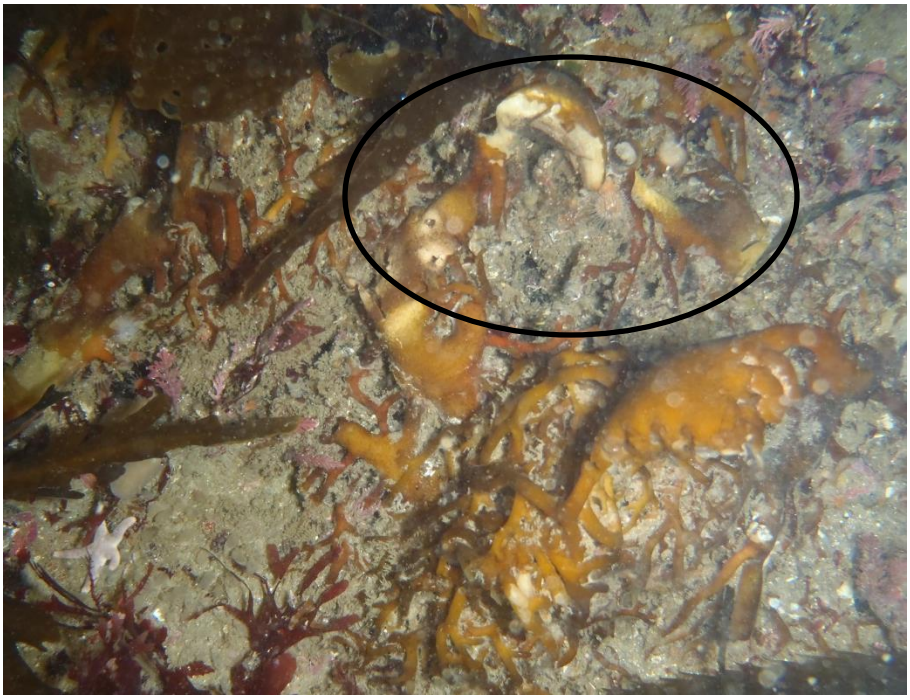


Figure 19. Effect of time on average number of fronds per m^2 of clearing by treatment. Diamonds represent control plots ($F_{1,14}$=1.59, p=0.23, r^2=0.11), squares represent 1.5 m diameter clearings ($F_{1,14}$=1.19, p=0.30, r^2=0.08) and triangles represent 3 m diameter clearings ($F_{1,14}$=1.57, p=0.23, r^2=0.11). Error bars are ±SE. See Table 4 for ANCOVA results.

a)



b)

Figure 20. Senescence is a potential explanation for die-off of cut rhizomes in partial clearings. Underwater photographs of a) senescing rhizomes (indicated by black circle) taken on December 12, 2013, 98 days after clearing and b) healthy rhizomes taken on September 23, 2013, 18 days after clearing.

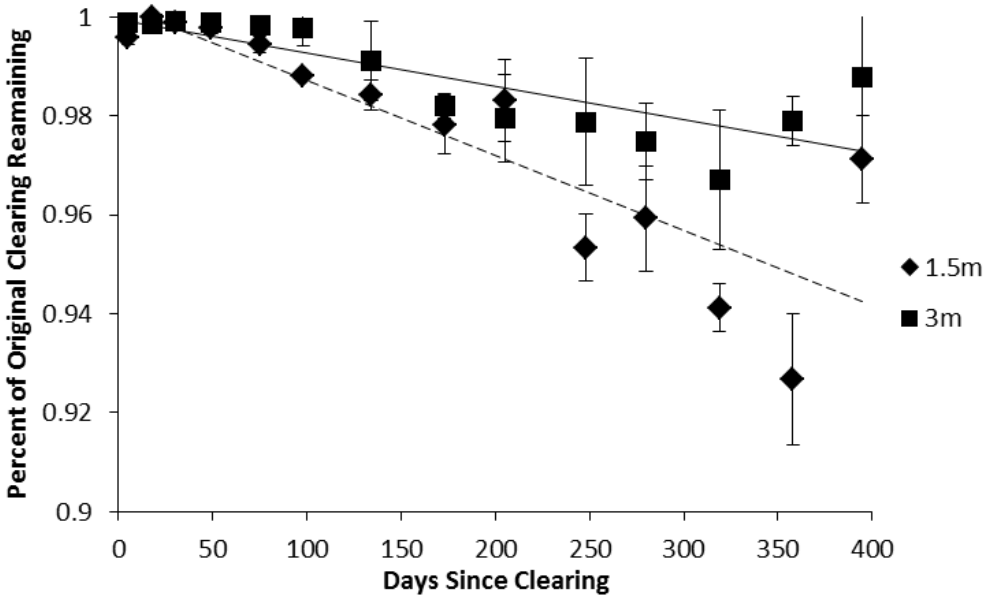


Figure 21. Effect of time on percent of original clearing remaining, taking into account encroachment by bordering rhizomes. Diamonds represent the smaller 1.5m diameter clearings, with the dashed line as the linear regression (linear regression: $F_{1,13}$=35.42, p<0.001, r^2=0.75). Peak encroachment occurs at day 319, and encroachment decreases through the end of the experiment. Squares represent the larger 3m diameter clearings, with the solid line as the linear regression (linear regression: $F_{1,13}$=23.77, p<0.001, r^2=0.67). Error bars are ±SE. See table 5 for ANCOVA results.

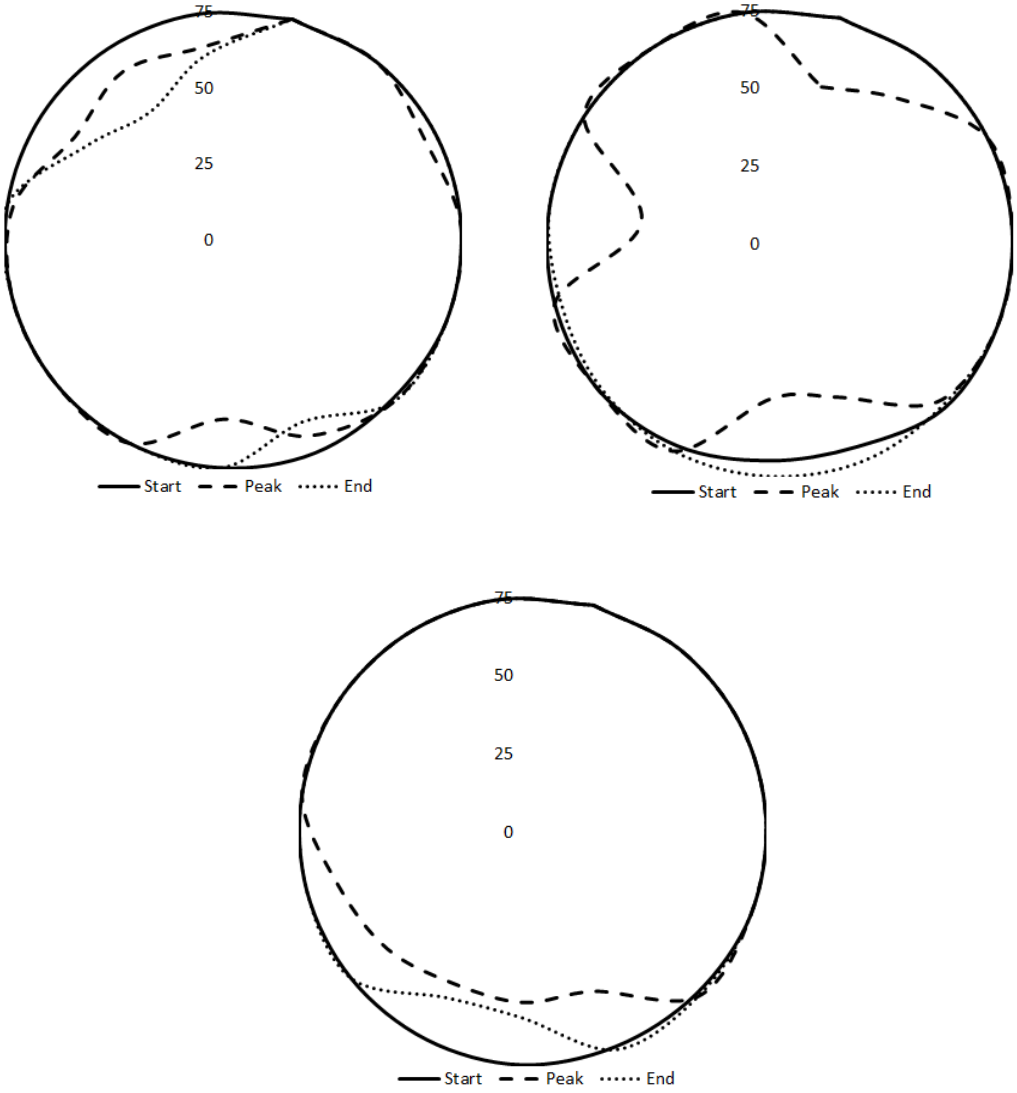


Figure 22. Clearings recovered primarily through vegetative encroachment of bordering individuals. Polar plots depict encroachment distances for the three 1.5 m diameter full clearings. Lines indicate the beginning of the experiment (Day 5, 10 September 2013; solid line), date of peak encroachment (Day 319, 22 July 2014; dashed line), and end date of the experiment (Day 395, 6 October 2014; dotted line). Axis indicates encroachment distance in centimeters from the center of each clearing.

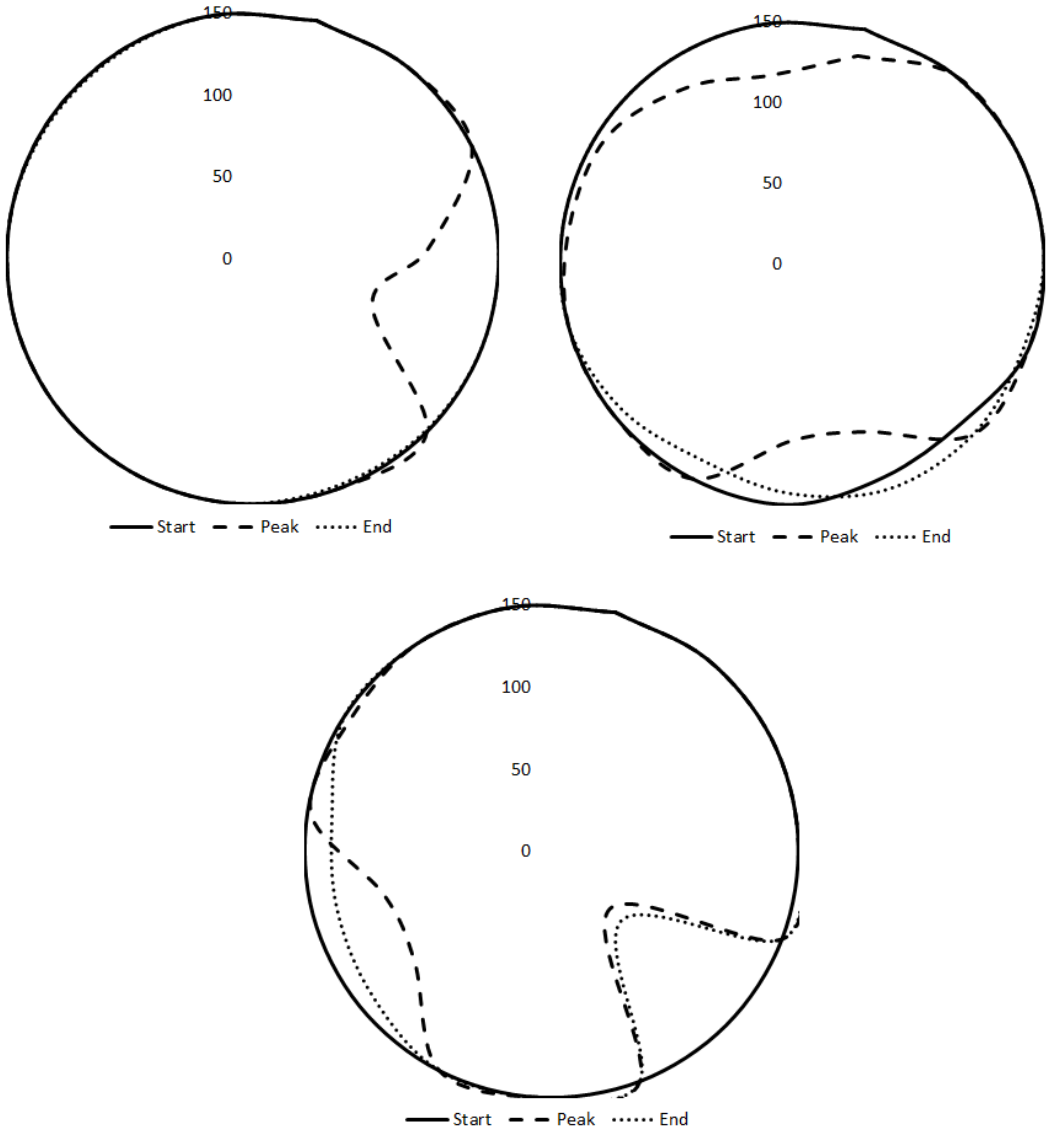


Figure 23. Clearings recovered primarily through vegetative encroachment of bordering individuals. Polar plots depict encroachment distances for the three 3 m diameter full clearings. Lines indicate the start date of the experiment (Day 5, 10 September 2013; solid line), date of peak encroachment (Day 319, 22 July 2014; dashed line), and end date of the experiment (Day 395, 6 October 2014; dotted line). Axis indicates encroachment distance in centimeters from the center of each clearing.

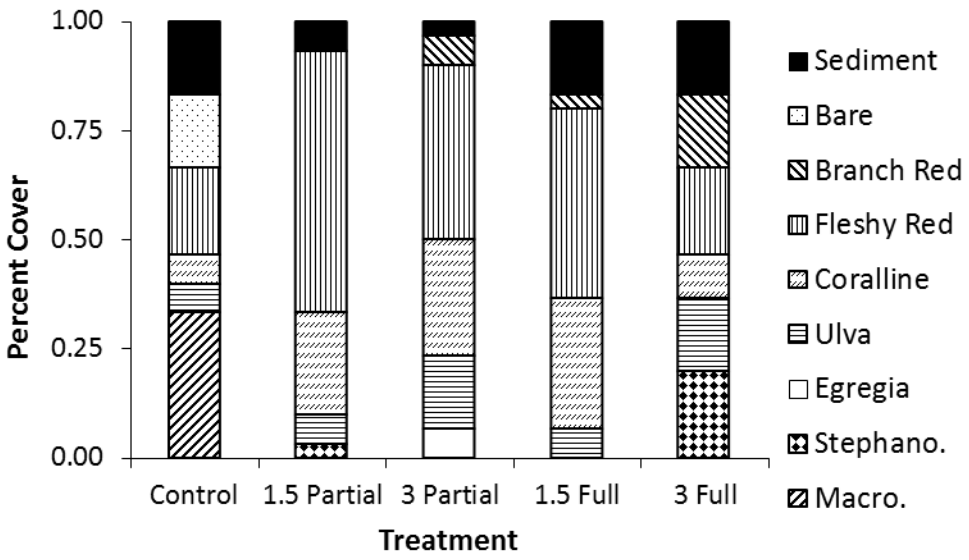


Figure 24. Manual *Macrocystis* removal in clearings resulted in a general lack of recolonization by *Macrocystis*. This vacancy allowed other algal species to colonize the open space. Random point contact data by treatment from June 2014 revealed that bare rock and *Macrocystis* were only observed in control plots.

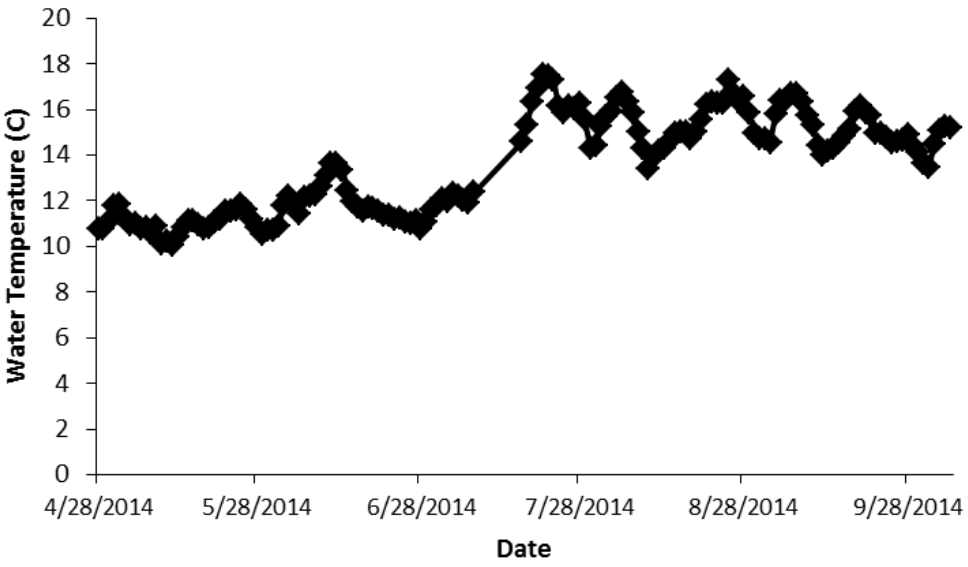


Figure 25. A possible explanation for rhizome die-back in mid-summer is high water temperatures. Daily water temperatures at 5m below the surface from a location in outer Stillwater Cove averaged over 15°C from July to October 2014.

TABLES

Table 1. Results of two-way Analysis of Variance (ANOVA) tests comparing morphology and month effects on reproductive investment variables from reproductive surveys: a) average soral weight, b) average soral percent cover, c) total soral area.

a) Average soral weight

	df	MS	f	p
month	3	27.329	10.526	<0.001
morphology	1	1.936	0.746	0.393
month*morph	3	2.172	0.836	0.482
error	39	2.596		

b) Average soral percent cover

	df	MS	f	p
month	3	0.024	1.281	0.294
morphology	1	0.006	0.326	0.571
month*morph	3	0.013	0.675	0.573
error	39	0.019		

c) Total soral area

	df	MS	f	p
month	3	1.95 E +9	3.465	0.025
morphology	1	1.87 E +9	3.335	0.075
month*morph	3	8.21 E +8	1.458	0.241
error	39	5.63 E +8		

Table 2. Results of two-way Analysis of Variance (ANOVA) tests comparing morphology and month effects on reproductive output variables from reproductive surveys: a) zoospore output and b) total reproductive output.

a) Zoospore output

	df	MS	F	p
month	3	20599.702	1.924	0.142
morphology	1	1529.720	0.143	0.707
month*morph	3	1304.655	0.122	0.947
error	39	10705.383		

b) Total reproductive output

	df	MS	F	p
month	3	9.229	5.689	0.003
morphology	1	6.107	3.765	0.061
month*morph	3	0.866	0.534	0.622
error	39	1.622		

Table 3. Results of two-way Analysis of Variance (ANOVA) tests comparing component and morphology biomass. Tukey HSD results show *pyrifera* fronds are the source of significance from the ANOVA.

	df	MS	F	p
component	1	6.488	5.715	0.024
morphology	1	12.206	10.753	0.003
component*morph	1	3.474	3.601	0.092
error	26	1.135		

Tukey HSD Multiple Comparisons Test:

			p
integrifolia fronds	vs	*pyrifera* fronds	p=0.008
integrifolia fronds	vs	*integrifolia* holdfast	p=0.965
pyrifera fronds	vs	*pyrifera* holdfast	p=0.041
pyrifera fronds	vs	*integrifolia* holdfast	p=0.002
integrifolia fronds	vs	*pyrifera* holdfast	p=0.922

Table 4. Results of Analysis of Covariance (ANCOVA) test comparing the effects of time and treatment (control, 1.5m diameter and 3 m diameter) on average number of fronds per m^2.

	df	MS	f	p
Treatment	2	35.432	30.462	<0.001
Time	1	11.885	10.218	0.002
Treatment*Time	2	0.392	0.337	0.72
Error	129	1.163		

Table 5. Results of Analysis of Covariance (ANCOVA) test comparing the effects of time and original clearing size (1.5 m and 3 m diameter) on percent of clearing remaining.

	df	MS	f	p
Size	1	2.28 E -5	0.238	0.63
Time	1	0.005	57.111	<0.001
Size*Time	1	0.001	8.417	0.01
Error	24	9.58 E -5		