2009

Storage and abiotic regulation of investment in vegetative vs. sexual reproduction in the clonal kelp, Laminaria sinclairii

Kyle William Glenn
California State University, Monterey Bay

STORAGE AND ABIOTIC REGULATION OF INVESTMENT IN VEGETATIVE VS. SEXUAL REPRODUCTION IN THE CLONAL KELP, *LAMINARIA SINCLAIRII*

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories

And the Department of Marine Science

California State University Monterey Bay

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Kyle William Glenn

July 2009

APPROVED FOR THE DEPARTMENT OF MARINE SCIENCE

Michael H. Graham
Associate Professor of Marine Ecology
San Jose State University

Diana L. Steller
Research Faculty/ Diving Safety Officer
San Jose State University

Shannon M. Bros-Seemann
Professor of Biology
San Jose State University

APPROVED FOR THE UNIVERSITY

ABSTRACT

STORAGE AND ABIOTIC REGULATION OF VEGETATIVE VS. SEXUAL INVESTMENT IN THE CLONAL KELP, *LAMINARIA SINCLAIRII*

by Kyle William Glenn

Laboratory temperature and nutrient manipulation experiments demonstrated that investment in sexual and vegetative reproduction in the clonal kelp *Laminaria sinclairii* are regulated by different abiotic factors. Sorus production (sexual investment) and blade growth were significantly higher at 12°C compared to 17°C, regardless of nutrient concentration. Net carbon storage and depletion in rhizomes were observed in the low and high temperature treatments, respectively under low nutrients (2μM NO_3), while no trend was apparent at higher nutrient levels (12μM NO_3). Blade growth of plants in the field substantiated these results and was also negatively correlated with seawater temperature. On the other hand, rhizome elongation (investment in vegetative reproduction) in laboratory manipulations, was significantly higher at high nutrient concentrations, irrespective of temperature. This increase in rhizome growth was concurrent with an increase in rhizome percent tissue nitrogen observed at higher nutrient levels. Light level manipulation revealed no relationship between light availability and the growth response variables (sexual or vegetative reproductive output); equal investment was observed even in near darkness. However, a significant positive relationship was observed between light level and percent tissue carbon and nitrogen of rhizomes after the experiment, suggesting that the rhizome served as a storage organ of carbon and nitrogen, which allowed more regular growth when nutrients and productivity were limited. These results suggest that physiologically, *Laminaria sinclairii* is similar to aclonal kelps and that its rhizome functions similar to the stipes of other kelps with respect to carbon and nitrogen storage.

ACKNOWLEDGEMENTS

I would like to first thank my amazing committee members, Diana Steller and Shannon Bros-Seemann, for invaluable support (both academic and emotional) throughout my experience at Moss Landing Marine Laboratories and my major professor, Michael Graham, for introducing me to the kelps. In addition to my committee, I am indebted to two senior graduate students: Selena McMillan and Thew Suskiewicz for investing heavily in me and teaching me about field and experimental research and kelp culturing techniques, respectively. This project could not have been completed without the many people who helped me in the lab and field, namely: Selena McMillan, Paul Tompkins, Jonathan Pruitt, Thew Suskiewicz, Rosemary Romero, Jasmine Ruvalcaba, Sara Hutto, and Aaron Snyder. I also need to acknowledge Craig Hunter for invaluable assistance and training on the tissue chemistry analyzer and the MLML library staff for helping me track down obscure journals and seemingly ancient algal references. Lastly, and most importantly, thanks go to my mother for keeping me on course when things got rough.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure 11 Effect of daily photon dose on percent tissue nitrogen remaining in rhizomes at the end of the experiment. Area between dashed lines represents the 95% C.I. for unmanipulated field rhizomes. Values are tank means.

INTRODUCTION

In order for populations or species to persist in a given environment, some form of propagation is necessary before the death of the organism. For seaweeds, this commonly means colonizing suitable substrate, growing to reproductive maturity, and producing spores. While most seaweeds follow that general model, 1.5 billion years of evolutionary history has lead to extremely diverse and often specialized life histories. For some species, this means alternating between two functionally different life history phases, each adapted for different disturbance and resource availability regimes. Notable examples include the alternation of blade and crust morphologies in *Mastocarpus* sp. (West, 1972) and macroscopic vs. microscopic individuals in the kelps (Sauvageau, 1915, 1916). Previous studies have shown the adaptive nature of these and many other specific life history traits (Zupan and West 1988, Lubchenco and Cubit 1980, Searles 1980), however very little is known about one of the most common life history strategies in seaweeds: clonality.

Clonality has been suggested as an adaptation to stressful systems (Tatarenkov, 2005) where the cost of growth and persistence of the individual may limit reproductive investment. This is because clonality allows an organism to produce new individuals through asexual, vegetative (usually horizontal) growth. As such, some clonal organisms may rely almost solely on asexual vegetative reproduction (Smith *et al.* 2004) under stressful conditions. Because both vegetative and sexual reproduction require investment from the individual (clonal propagation and production of reproductive structures, respectively), determining the trade-offs between the two has been well studied in terrestrial botany (Haper, 1977). For instance, nutrient availability was found to be important in regulating the trade-off between sexual and asexual reproduction in a clonal herbaceous plant (Liu *et al.* 2009).

Common in marine seaweeds as well as terrestrial plants, clonality seems to have evolved multiple times in distantly related taxa (Santelices 2004). Previous studies have examined ecology and demography of clonal seaweed species (Wright and Davis 2006, Scrosati and Servière-Zaragoza 2008), but little is known about their physiology. Importantly, the abiotic factors regulating investment in vegetative vs. sexual reproduction are poorly known in seaweeds so predictions cannot be made about the relative contribution of each type of reproduction in a given environment.

Though almost entirely unstudied, clonal kelps present a very interesting opportunity for studying ecological and evolutionary aspects of clonal life histories given both their inherently high productivity and growth rates (Mann 1973) and extremely high fecundity (Chapman, 1984). Furthermore, kelps are known to respond strongly to abiotic factors to the point that one can relatively easily model their presence and success as a function of a given set of abiotic factors (Graham *et al.* 2007). Among the kelps (Laminariales, Phaeophyceae) clonality is present in three of the four families and clonal polymorphism, a unique phenomenon where one individual can develop clonally or aclonally depending on abiotic factors, is present in at least one species (Demes *et al.* 2009). The occurrence of clonality in multiple kelp clades suggests two possible evolutionary histories: that clonality evolved independently multiple times or that clonality was present in ancestral kelps.

The relatively high frequency of aclonal kelps (and absence of clonal Alariaceae, the oldest kelps [Saunders and Druehl 1992]) coupled with the observation that clonal species diverge late in phylogenetic trees (Lane *et al.* 2006) suggests that clonality has evolved independently multiple times in this order. It is interesting to note that many of the aclonal species have life history characteristics similar to traditional clonal seaweeds. Importantly, many

aclonal kelp species use their stipes as carbon and nitrogen storage organs (Germann 1989, Gerard 1982) and stipes of some perennial, aclonal species can regenerate blades and sporophylls (Setchell 1905, Dominik and Zimmerman 2006) analogous to the function of a traditional rhizome. While quantitative phylogenetic analyses are needed before directionality of clonal evolution in kelps can be determined, it is clear that meristematic regeneration and carbon and nitrogen storage have been key components of the evolutionary success of the kelps in the spatially and temporally heterogeneous temperate systems in which they occur and require investigation in clonal species.

As a clonal kelp, *Laminaria sinclairii* can invest in two reproductive pathways (Fig. 1): vegetative and sexual reproduction. Vegetative reproduction is accomplished by expansion of the rhizome which will result in the production of additional stipes and, in turn, blades. Sexual reproduction occurs through the asexual creation of haploid zoospores in clusters (sori) on blades. Once released, these zoospores will settle and develop into either male or female gametophytes, which eventually produce gametes that fuse and create a genetically novel sporophyte. Because sori are produced on blades, sorus production, and therefore investment in sexual reproduction, is constrained by blade presence. While the production of blades might occur at the expense of sori, it ultimately results in higher blade area for the production of sori, presenting an interesting physiological trade-off.

Laminaria sinclairii is one of the few clonal members of the Laminariaceae found in the low intertidal from British Columbia to Los Angeles, CA (Markham 1972). It differs from clonal congener *L. longipes* in blade size and presence of mucilage ducts (Markham 1972), which have been shown to be controlled by wave exposure (Palmisano and Sheng 1977) and temperature (Chapman 1975), respectively, within the genus. Not surprisingly, *L. longipes* is

only found in the colder waters north of the range of *L. sinclairii* and extends all the way to Alaska (Markham 1972). Unlike many intertidal seaweeds, *L. sinclairii* can withstand intense sand scouring and often appears to be growing out of sand (though rock is necessary for holdfast attachment). However, *L. sinclairii* populations can also be found attached to bare rock in areas without sand scour. This distinction is a likely cause for the conflicting results from ecological studies on this species. For instance, Markham (1973) observed that production of sori occurred seasonally and never observed a recruit in the sand-scoured populations that he studied, concluding that sporophyte production was not common. Other studies, in areas without sand scouring, have found sori present most of the year and showed that these sori produced competent zoospores resulting in high reproductive success, and therefore high sporophyte production (Dickey 1986). While these studies provide differing reproductive mechanisms for the same species, it is important to note that sand scouring might pose a physiological cost (Harrington *et al.* 2005; Umar *et al.* 1998), limiting the investment of reproductive output of *L. sinclairii*. Since recruitment of macroalgae is inhibited by sand scouring (Chapman and Fletcher 2002), it is not surprising that seaweeds capable of vegetative propagation are common in areas of high sand inundation (Eriksson and Johansson 2005). Since the success of sexual vs. vegetative reproductive mechanisms may vary under differing abiotic conditions, knowing the extent to which each reproductive mechanism is regulated by an abiotic factor, such as sand scouring, enhances our understanding population dynamics in spatially and temporal heterogeneous environments, such as the Pacific coasts.

Three primary abiotic factors influencing seaweed growth and reproduction are light, temperature, and nutrients (Santalices 1990), all of which vary spatially and temporally at small and large scales (Dayton *et al.* 1999, Graham *et al.* 2008). Numerous studies have demonstrated

how each of these factors, and interactions between them, regulate presence (Graham *et al.*, 2007, Glardon *et al.*, 2007), growth (Duke *et al.*, 1989, Steen and Rueness, 2004) and reproduction (Reed *et al.*1996, Guiry and Dawes 1997) of taxonomically diverse seaweed species. Specifically for aclonal kelps, temperature has been found to play a strong role in reproductive investment, with higher temperatures resulting in decreased reproductive effort (Kohtio 2008). In such situations, nutrient enrichment may help ameliorate detrimental effects of increased temperatures (North and Zimmerman 1984). However, only limited research exists on preferential investment when multiple growth forms are possible and no studies to date have examined how abiotic factors influence investment in vegetative vs. sexual reproduction in *L. sinclairii*, or any other clonal kelp.

This study aimed to explore the relative effects of temperature, nutrients, and light on the regulation of vegetative vs. sexual reproductive investment in *Laminaria sinclairii*. Specifically I asked: Is temperature or nutrients most important in controlling reproductive investment? Do different abiotic factors affect investment in vegetative vs. sexual reproduction or is all growth controlled by the same regulatory mechanisms? Does the rhizome of *L. sinclairii* provide carbon and nitrogen reserves when abiotic conditions limit growth? How does decreasing light availability affect reproductive investment and utilization of internal nutrient storage? Answers to these questions will provide novel information about the control of reproductive strategies and the use of rhizomes as storage organs in clonal kelps.

METHODS

Study Site

Pigeon Point, San Mateo CA. is an exposed outcrop in San Mateo County, CA., composed mostly of Santa Cruz mudstone, and hosts a diverse assemblage of intertidal

organisms. Notably, this site contains a large population of *Laminaria sinclairii* occurring as a narrow band along exposed rocks around 1.0m below MLLW. While Pigeon Point does experience seasonal sand scouring (Rosemary Romero, personal communication), areas populated by *L. sinclairii* were never observed to be covered in sand during this study (November 2008-May 2009). This site was used for collections of plants for laboratory experiments and for measurements of growth data in the field.

Experimental Plant Collection and Handling

Intact thalli (rhizome, stipes, and blades) were removed from the substrate using a dull knife on outgoing low tides and transported immediately back to the lab. Once back at the lab, rhizomes were cut into 3-5 stipe segments (each segment possessing one rhizome apex to ensure growth capacity of rhizomes) and placed into running ambient seawater for 24 hours for stabilization. Blades were then labeled using colored cable ties loosely attached at the stipe-rhizome interface and initial blade, sorus, and rhizome lengths were measured for each thallus.

Experimental Set-Up

All experiments were completed outdoors in an array of 16 55-gal. aquaculture tubs. To allow for precise manipulation of nutrient levels, seawater was created from InstantOcean © mix and freshwater. In nutrient manipulation experiments, two treatments were used: trace nitrate values (InstantOcean© mix alone) and enriched nitrate values (InstantOcean© + 10μM $NaNO_3$). Demes *et al.* (2009) analyzed three replicates of InstantOcean© mixed with freshwater and found an average baseline of 2.28 ± 0.05 μM NO_3^-, bringing actual treatment values to 2.28 μM and 12.28 μM NO_3^-. These values represent realistic fall and spring values, respectively (Graham *et al.*, 2008).

Temperature treatments were constructed using either aluminum coils circulating ambient seawater to serve as a temperature buffer or temperature controlled heaters set to low for the warmer tanks. To ensure that desired temperatures (11-13°C and 16-18°C for Spring and Fall, respectively) were achieved, StowAway TidBits underwater data loggers were deployed in two randomly selected tanks of each treatment. When the daily temperature cycle for the four monitored tanks was overlaid with the average monthly water temperature from 1980-2001 at the study site (Fig. 2), the cold treatments fell within the average winter temperatures whereas the warm treatments were representative of high, but naturally occurring summer temperatures (NOAA, National Data Buoy Center Station # 46012). Daily averages in each tank was averaged across treatment, bringing treatment values to 12°C and 17°C

To test the effects of decreasing light availability on reproduction and storage use, thickness of screening covering each of eight tanks was manipulated (PAR ranged from 20-2500 $\mu Em^{-2}s^{-1}$). Each tank was cooled as described above (12°C) and not enriched with NO_3^- (2.28 μM), theoretically creating both nitrogen- and light- (or productivity-) limited growth. To determine the daily photon dose for each light treatment, photosynthetically active radiation (PAR) was measured using a Biospherical Instruments Inc. Optical Sensor model # QSPL-2100 in each tank every thirty minutes from sunrise to sunset on a clear day during the experiment. A unique curve for each tank (Fig. 3) was then fit to a second order polynomial equation (all $R^2 > 0.85$, $p < 0.001$) and integrated to determine daily photon dose, which served as the independent variable in regression analyses for the effects of light on investment and storage.

In each of the two experiments, 0.5 x 0.5m^2 PVC squares were constructed to hold the thalli. Three evenly spaced, parallel polypropylene lines were tightly strung across the PVC. One rhizome was tied to each line, resulting in three thalli (subsamples) per tank. A 0.3m air

stone bubbler was placed under the PVC holders, center and perpendicular to the plant lines to allow equal aeration to the thalli.

Response Variables- Investment in vegetative vs. sexual reproduction

Three growth response variables were used in each experiment: blade growth (cm), addition of sori (cm), and rhizome elongation (cm). Rhizome growth is the only pathway by which an individual can grow clonally (vegetative reproduction), and therefore rhizome elongation was measured as the investment in vegetative reproduction. Though the rhizomes branch, a primary axis was discernable. Blade growth and sorus addition (change in sorus length) were considered together as a measure of investment in sexual reproduction because sexual reproduction (creation of sori) is constrained by blade size (see Introduction). Since apical blade erosion is known to mask growth measurements by change in blade length alone, the hole punching method described by Mann (1973) was used to measure blade growth. A hole punched above the meristem will migrate towards the tip of the blade as it grows because the growth meristem of the plant occurs at the interface between the stipe and blade. Therefore an initial hole was punched before the start of each experiment. At the end of the experiment, a second hole was punched at the same location and the distance (cm) from the bottom of the first hole to the bottom of the second hole was used as the blade growth measurement. All experiments were run for 8 days.

Response Variables- Storage and Tissue Chemistry

Before tissue analysis, rhizomes were rinsed thoroughly in seawater to remove any animals or sand residing therein. Blades were then severed from the rhizome with a razor blade and the rhizomes dried at 60°C for 48 hours. Tissue was then homogenized with a ball mill for tissue analysis. Tissue percent carbon and nitrogen were analyzed using a CHN combustion analyzer (440 CHN – O/S Elemental Analyzer). Tissue analysis was started immediately after the

termination of both experiments. Since tissue analysis was destructive, determining starting conditions of each rhizome was impossible. Therefore, 15 haphazardly selected rhizomes were collected from the field along with the experimental plants. The 95% C.I. was calculated for percent tissue carbon and nitrogen in field rhizomes allowing the determination of net carbon and nitrogen storage and depletion in experimental plants (i.e. values above the upper confidence interval suggest input to the rhizome, and values below it suggest output from the rhizome).

Field Growth

In order to compare growth in experimental plants with field values, to see how closely blade growth tracked sea water temperature, and to determine the extent to which plants in this population were reproductive, 30 haphazardly selected stipes at the field site were marked with a unique combination of colored cable ties, hole-punched 1.0cm above the blade meristem, and recorded as either sexually reproductive or not, based on the presence of sori. This was first done in December 2008 and repeated at the end of each sequential month through June 2009, reproductive status was again recorded and an additional hole was punched 1.0cm above the meristem. The distance between the two hole punches (cm) was then divided by the number of days since the last hole was punched, representing the monthly average growth rate (cm/day). Mean monthly seawater temperature was determined from data collected by the NOAA National Data Buoy Center Station 46012 (Half Moon Bay) from 1980-2001.

Culturing

To ensure that investments into sexual reproduction in *L. sinclairii* were viable, zoospores from reproductive blades were released and incubated to test for the production of the sporophytes through sexual reproduction. In December 2008, and April and June 2009, 10 haphazardly selected reproductive blades were taken from individuals at the study site and brought back to the lab. In the laboratory, the blades were then subjected to temperature,

desiccation, and light stress (Amsler and Neushul 1989) to induce zoospore release. After 24h, blades were soaked in Provasoli's nutrient enriched sterile seawater (Andersen *et al.* 2005) for 2h to create a zoospore solution, which was then pipetted into 4 petri dishes: duplicates were placed in cold (12°C) and warm (18°C) incubators. Once a week, the water in the petri dishes was replaced with fresh sterile seawater and the presence or absence of sporophytes in each dish was noted.

Statistical Analyses

Univariate 2-way fixed factor analysis of variance (ANOVA) were used to test the effect of temperature, nutrients, and the temperature*nutrient interaction on rhizome elongation (vegetative reproduction) and percent tissue carbon and nitrogen in the rhizome (tissue storage). Given a potential positive relationship between blade growth and sorus addition, blade growth was planned to be used as a covariate for sorus addition, however the weak relationship between the two made this impossible. Instead, 2-way fixed factor multivariate analysis of variance (MANOVA) was used to test the effects of temperature, nutrients, and an interaction term on both variables. When interactions were significant, variance components (ω^2) were calculated to determine relative magnitude of effects for each factor (Graham and Edwards, 2001). Negative variances components were set to 0.

In the light manipulation experiment, univariate regression analyses were performed on each of the growth (blade, sorus, and rhizome) and storage (percent tissue nitrogen and carbon) response variables to investigate the effects of light on growth and storage with a constant set of abiotic factors. Since light decreased exponentially with the addition of screens, the log transformed daily photon dose per tank was used as the independent variable in the light experiment. Linear regression analysis was also used to test the effects of mean monthly sea

water temperature on mean monthly blade growth of plants at the field site. In all analyses, an effect was considered to be significant when $p < 0.05$. All statistical analyses were run in SYSTAT 12.

RESULTS

Temperature vs. Nutrients

Investment in vegetative and sexual reproduction were both found to be controlled by either temperature or nutrients alone; no interaction term was found to be significant for growth responses (Table I, II). Temperature had a significant negative effect on the multivariate vector of blade growth and sorus addition (proxies for investment in sexual reproduction), with mean values twice as high in cooler temperatures (Table II, Fig. 6). Nutrient conditions had no significant effect on blade growth or sorus addition. In order to explore why these two variables showed the same response but were not correlated, as expected, Pearson's correlation analysis was used to test the relationship between variance of sorus addition and mean blade growth. A significant ($r = 0.546$, $df = 15$, $p = 0.029$) positive trend was discovered. Visual inspection of results indicated that most of the variance was due to less common high values among more regular, lower values. Because nearly all blades already had sori and could all theoretically produce sori, this result suggests that as blade growth increases, the likelihood of putting on sori increases. On the other hand, only nutrient levels had a significant effect on clonal investment (rhizome elongation) (Table Ia, Fig. 7). Specifically, a more than three-fold increase in mean rhizome elongation was found in conditions with enriched nutrients ($p = 0.044$).

Percent tissue nitrogen in rhizomes at the end of the experiment was significantly higher (Table Ib, $p = 0.015$) in treatments with higher nutrients (Fig. 8); the interaction term was not significant. Furthermore, treatments with higher nutrients showed mean tissue enrichment above

the upper 95% C.I. for rhizomes at the time of collection. A significant interaction between temperature and nutrients (Table Ic, $p = 0.011$) was detected for percent tissue carbon in rhizomes (Fig. 9). However, this interaction effect ($\omega^2 = 17\%$ of variance explained) was overshadowed by the strong effect of temperature ($\omega^2 = 48\%$ of variance explained, Table Ic).

Light manipulation

Linear regression analyses on log transformed daily photon dose on each of the three growth response variables showed no significant trends (Table III). Linear regression analyses on the percent tissue carbon and nitrogen of rhizomes at the end of the experiment both showed significantly positive trends (Table III). With increasing light availability, percent tissue carbon in the rhizome increased (Fig 10, $p = 0.043$). However, only the two highest light levels resulted in percent tissue carbon values outside of what was expected due to variability of plant starting conditions alone. These two values were comparable to values from the same treatment combination in the temperature and nutrient combination (Fig. 9). Percent tissue nitrogen showed the same increasing trend with increasing light (Fig. 11), and was also significant ($p = 0.049$). While only two of the intermediate light level treatments resulted in tissue nitrogen values outside of the range of what is to be expected based solely on individual starting conditions, the values from the three highest light levels (which were comparable to same temperature and nutrient combination in the previous experiment[Fig. 8]) group together higher than do those from the five lower light level treatment, which are clustered at the lower end of the "naturally occurring" nitrogen values zone.

In situ growth and reproduction

The overall average growth rate (± SD) for plants in the field from January to April was 0.390 ± 0.059 cm·day^{-1}. Linear regression analysis showed a significant negative effect (y = -

0.1064x +1.7015, F=25.725, p= 0.037, r^2= 0.928) of mean monthly seawater temperature on mean monthly blade growth (Fig 4), despite the limited sample size (N = 4). As illustrated by Fig. 5, blades with sori were not only present in all months, but were also more abundant than blades without sori, except in February (Fig. 5).

Spores successfully settled into gametophytes and grew to completely cover all four petri dishes after only 2-3 weeks. At this time, numerous new sporophytes had been produced in the cold (12°C) incubator. In the warm (17°C) incubator, female gametophytes started to lose their pigmentation after 16 days and no sporophytes were observed.

DISCUSSION

Temperature vs. nutrient effects on vegetative vs sexual reproduction

Laminaria sinclairii exhibits a classic kelp physiology. Temperature, rather than nutrients, was the major factor driving investment in sexual reproduction in *L. sinclairii*. At temperatures where growth is highest, carbon accumulates in the rhizome as storage material to be used when temperature is not optimal. The strong effect of temperature, and lack of effect of nutrients, on blade growth and sorus production observed in this study is comparable to previous findings on closely related aclonal species (Gagné *et al.*, 1982).

Clonal growth in *Laminaria sinclairii* was found to be more dependent on nutrient availability and appeared to occur to a much greater extent when there was more nitrogen in the rhizome than was required for regular growth. The finding that clonal growth (rhizome elongation) was highest in higher nutrient levels and was concurrent with nitrogen enrichment of rhizome tissue (above what was to be expected based on starting conditions) implies that clonal growth occurs most when the rhizomes have more than enough nutrients stored to supply the rest

of the plant. This finding presents an argument that blade growth and sorus production are more important in terms of resource allocation than is clonal growth.

This scenario is interesting for a clonal organism and may be a strong argument against ancestral clonal kelps. For instance, one would imagine that if a clonal life history strategy were significant in the evolution of a lineage, then clonal growth would likely be the most important growth for resource allocation. Likewise, sexual reproduction is thought to be more important in aclonal organisms, as failure to reproduce before dying is increased. Since clonal growth was only observed with tissue rhizome enrichment, it seems likely that kelps evolved aclonally, with a need to sexually reproduce, and clonal evolutionary events are derived traits to allow persistence in stressful environments.

Light effects on growth and storage

When light and nutrients are limiting, seaweeds may use storage carbon and nitrogen to sustain growth (Gerard, 1987). These findings show that the rhizome system serves the same storage functions as the stipes of many aclonal kelps (Chapman and Craigie, 1977). Full orthogonal replication of light, temperature, and nutrients was unfeasible, yet the cold, low nutrient setting for the light experiment was ideal to test the effects of light on growth and stored nutrients since the temperature and nutrient experiment demonstrated net accumulation of carbon into the rhizomes (over-production) only under cold, nutrient-limited conditions (Fig. 9). Accordingly, the tank without screening (unmanipulated control) resulted in tissue carbon and nitrogen values comparable to those of the same treatment (low temperature and low nutrients) in the temperature and nutrient manipulation experiment. Any deviation from the unmanipulated control tank was therefore interpreted as due to light and productivity limitation.

Growth was observed in all light treatments, even when the highest PAR values were around $10\mu Em^{-2}s^{-1}$. This observation and the significant positive relationship between ending percent tissue carbon and light suggested that previously stored carbon was responsible for much of the growth. However, since percent tissue carbon in the rhizomes after the experiment was not outside of the range of values expected solely based on starting conditions, it appeared that carbon storage in other parts of the plant, probably stipes (Gerard 1982), was a major contributor to this growth. Net accumulation of carbon into the rhizomes was observed at the two highest light levels, suggesting that photosynthetic rates were more than high enough to account for the growth seen in these tanks.

The positive relationship between light level and ending rhizome percent tissue nitrogen, coupled with the clustering of values resulting from the five lowest light levels towards the lower confidence interval of starting conditions, suggested that nitrogen reserves in the rhizome were used by the plant to a greater extent with decreasing light. It has been previously reported among algae that nitrogen limitation decreases efficiency of photosystem production (Kolber *et* al 1988). One potential explanation of this finding is that the plant used stored nitrogen to increase photosystem production in low light, allowing higher productivity with the same light availability. The ambient light tank (no screens) resulted in ending tissue carbon and nitrogen concentrations comparable to those of the same treatment (cold, low nutrients) in the temperature and nutrient manipulation experiment.

In situ growth and reproduction

In light of the strong effect of temperature on blade growth in laboratory experiments ($p = 0.001$), it is not surprising that a significant relationship between mean monthly temperature and mean monthly blade growth was found with only four data points. Mean blade growth in the

field over the four months was twice as high as blade growth observed in any treatment, after correcting for number of days. This discrepancy was not unexpected since the wave energy of the intertidal surf zone could not be replicated and maintained in the experimental tanks. However, since all treatments experienced the same hydrodynamic conditions, this problem does not affect the questions asked.

The observation that sori were present on blades less than one month after blade production in February indicates that sexual reproduction was not limited to older blades in early winter in the Pigeon Point population. It is important to note that no individuals in this population were observed to be covered with sand. If sand scouring and burial poses a physiological cost to *L. sinclairii* plants, it is not surprising that buried populations only sexually reproduce at the beginning of winter (Markham, 1973) whereas populations attached to rock have a much longer reproductive season (Dickey, 1986). Settlement and growth of gametophytes observed in both temperatures and the production of new sporophytes in the cold incubator suggests that the sori produced by plants in the field in late December 2008 and April 2009 were competent and that sexual reproduction is a viable reproductive mechanism for *L. sinclairii* at Pigeon Point. While it is interesting to note that fertilization success occurred in colder temperatures when investment in zoospore production was highest, the replication needed to say this with certainty was not obtained.

Conclusions

Physiologically, *Laminaria sinclairii* behaved comparably to aclonal kelps species. Temperature was most important in regulating soral output, with higher reproductive investment occurring at lower temperatures. When blade growth was highest, a net accumulation of carbon was observed in the rhizome while net depletion of rhizome carbon reserves was concurrent with

conditions that were not optimal for blade growth. Materials stored in the rhizome were depleted at an increasing amount with decreasing light, further supporting that the rhizome of *L. sinclairii* serves as a storage organ. The observation that vegetative reproduction (rhizomatous expansion) occurred highest when rhizomes experienced nitrogen enrichment above field values suggests that the rhizome's primary function is to provide the stipes and blades with carbon and nitrogen for sexual reproduction and to reproduce vegetatively secondarily, when more than enough nutrients are stored. Secondary clonal reproduction is consistent with the hypothesis that clonality has evolved independently multiple times in the kelps from an aclonal ancestor. Multiple evolutionary events of clonality in the kelps seems likely given that most aclonal kelp species can store carbon and nitrogen in their stipes, translocate carbon and nutrients, and regenerate blades and sporphylls, all vital precursors in the evolution of clonality.

LITERATURE CITED

Amsler, CD, M Neushul. 1989. Chemotactic effects of nutrients on spores of the kelps *Macrocystis pyrifera* and *Ptreygophora californica*. *Mar. Biol.* 102: 557-564.

Andersen, RA, JA Berges, PJ Harrison, MM Watanabe. 2005. Recipes for freshwater and seawater media. Pp. 429-538 in RA Andersen, ed. Algal culturing techniques. Elsevier, Amsterdam.

Chapman, ARO. 1975. Inheritance of mucilage canals in *Laminaria* (Section Simplices) in Eastern Canada. *Br. Phycol. J.* 10: 219-223.

Chapman, ARO and JS Craigie. 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.* 40: 197-205.

Chapman, ARO. 1984. Reproduction, recruitment, and mortality in two species of *Lamrinaria* in southwest Nova Scota. *J. Exp. Mar. Biol. Ecol.* 78: 99-109.

Chapman, AS, RL Fletcher. 2002. Differential effects of sediments on survival and growth of *Fucus serratus* embryos (Fucales, Phaeophyceae). *J. Phycol.* 38: 894-903.

Dayton, PK, MJ Tegner, PB Edwards, KL Riser. Temporal and spatial scales of kelp demography: the role of oceanographic climate. *Ecol. Monogr.* 69: 219-250.

Demes, KW, SS Bell, and CJ Dawes. 2009. The effects of phosphate on the biomineralization of the green alga, *Halimeda incrassata* (Ellis) Lam. *J. Exp. Mar. Biol. Ecol.* 374(2): 123-127.

Demes, KW, MH Graham, and TS Suskiewicz. *In Press*. Phenotypic plasticity reconciles incongruous molecular and morphological taxonomies: giant kelp, Macrocystis (Laminariales, Phaeophyceae), is a monospecific genus. *J. Phycol.*

Dickey, K. 1986. The role of light in the gametogenesis of *Laminaria sinclairii*. M.S. Thesis, University of California Santa Cruz.

Dominik, CM, RC Zimmerman. 2006. Dynamics of carbon allocation in a deep-water population of the deciduous kelp *Pleurophycus gardneri* (Laminariales). *Mar. Ecol. Prog. Ser.* 309:143-157.

Duke, CS, W Litaker, J Ramus. 1989. Effect of temperature on nitrogen-limited growth rate and chemical composition of *Ulva curvata* (Ulvales: Chlorophyta). *Mar. Biol.* 100: 143-150.

Eriksson, BK, G Johansson. 2005. Effects of sedimentation on macroalgae: species-specific responses are related to reproductive traits. *Oecologia* 143: 438-448.

Gagné, JA, KH Mann, ARO Chapman. 1982. Seasonal patterns of growth and storage in *Laminaria longicruris* in relation to differing patterns of availability of nitrogen in the water. *Mar. Biol.* 69: 91-101.

Gerard, VA. 1982. Growth and utilization of internal nitrogen reserves by the giant kelp *Macrocystis pyrifera* in a low-nitrogen environment. *Mar. Biol.* 66: 27-35.

Germann, I. 1989. Aspects of carbon metabolism in relation to autumnal blade abscission in the kelp *Pleurophycus gardneri* (Phaeophyceae, Laminarlaes). *Mar. Ecol. Prog. Ser.* 54:179-183.

Glardon, CG, LJ Walters, PF Quintana-Ascencio, LA McCauley, WT Stam, and JL Olsen. 2007. Predicting risks of invasion of macroalgae in the genus *Caulerpa* in Florida. *Biol. Invasions* 10(7): 1147-1157.

Graham, MH and MS Edwards. 2001. Statistical significance versus fit: estimating the importance of individual factors in ecological analysis of variance. *Oikos* 93(3): 505-513.

Graham MH, BS Halpern, MH Carr. 2008. Diversity and dynamics of Californian subtidal kelp forests. Pp. 103-134 in McClanahan, TR and GR Branch (eds), Food Webs and the Dynamics of Marine Benthic Ecosystems, Oxford University Press.

Graham, MH, BP Kinlan, LD Druehl, LE Garske and S Banks. 2007. Deep-water kelp refugia as potential hotspots of tropical marine diversity and productivity. *P Natl Acad Sci USA.* 104:16576-16580

Guiry, MD, CJ Dawes. 1997. Daylength, temperature, and nutrient control of tetrasporogenesis in *Asparagopsis armata* (Rhodophyta). *J. Exp. Mar. Biol. Ecol.* 158: 197-217.

Haper, JL. 1977. Population biology of plants. Academic Press. London, UK.

Harrington, L, K Fabricius, G Eaglesham, A. Negri. 2005. Synergistic effects of diuron and sedimentation on photosynthesis and survival of crustose coralline algae. *Mar. Pollut. Bull.* 51: 415-427.

Kohtio, D. 2008. Population biology of *Undaria pinnatifida* in central California. M.S. Thesis, San Jose State University.

Kolber, Z, J Zehr, P Falkowski. 1988. Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in Photosystem II. *Plant Physiol.* 88: 923-929.

Lane, CE, C Mayes, LD Druehl, and GW Saunders. 2006. A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) suuports substantial taxonomic re-organization. *J. Phycol.* 42: 493-512.

Lubchenco, J, J Cubit. 1980. Htereomorphic life histories of certain marine algae as adaptations to variations in herbivory. *Ecology* 61: 676-687.

Mann, KH. 1973. Seaweeds: Their productivity and strategy for growth. *Science* 182: 975-981.

Markham, JW. 1972. Distribution and taxonomy of *Laminaria sinclairii* and *L. longipes* (Phaeophyceae, Laminariales). *Phycologia* 11(2): 147-157.

Markham, JW. 1973. Observations on the ecology of *Laminaria sinclairii* on three northern Oregon beaches. *J. Phycol.* 9: 336-341.

North, WJ, RC Zimmerman. 2004. Influences of macronutrients and water temperatures on summertime survival of *Macrocystis* canopies. *Hydriobiologia* 116-117: 419-424.

Palmisano, JF and YS Sheng. 1977. Blade width of *Laminaria longipes* (Phaeophyceae, Laminariales) as an indicator of wave exposure. *Syesis* 10:53-56.

Reed, DC, AW Ebeling, TW Anderson, M Anghera. 1996. Differential reproductive esponses to fluctuating resources in two seaweeds with different reproductive strategies. *Ecology* 77: 300-316.

Santalices, B. 1990. Patterns of reproduction, dispersal, and recruitment in seaweeds. *Oceanogr. Mar. Biol.* 28: 177-276.

Santalices, B. 2004. A comparison of ecological responses among aclonal (unitary), clonal and coalescing macroalgae. *J. Exp. Mar. Biol. Ecol.* 300: 31-64.

Saunders, GW, LD Druehl. 1992. Nucleotide sequences of the small-subunit ribosomal RNA genes from selected Laminariales (Phaeophyta): Implications for kelp evolution. *J. Phycol.* 28: 544-549.

Sauvageau, C. 1915. Sur les gametophytes de deux Laminaires (*L. flexicaulis* et *L. saccharina*). *Compt. Rend. Acad. Sci.* 161:796-799.

Sauvageau, C. 1916. Sur les plantules de quelques Laminaires. *Comp. Rend. Acad. Sci.* 163: 522-524.

Scrosati,R and E Servière-Zaragoza. 2008. Ramet dynamics for the clonal seaweed *Pterocladiella capillacea* (Rhodophyta): A comparison with *Chonrdus crispus* with *Mazzaella cornucopiae* (Gigartinales). *J. Phyol.* 36: 1061-1068.

Searles, RB. 1980. The strategy of the red algal life history. *Am. Nat.* 115: 113-120.

Setchell, WA. 1905. Regeneration among kelps. Univ. Calif. Publ. Bot. 2(5): 139-168.

Smith, JE, CL Hunter, EJ Conklin, R Most, T Sauvage, C Squair, CM Smith. 2004. Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on O'ahu, Hawai'i. *Pacific Science* 58: 325-343.

Steen, H and J Rueness. 2004. Comparison of survival and growth in germlings of six fucoid species (Fucales, Phaeophyceae) at two different temperature and nutrient levels. *Sarsia* 89(3): 175-183.

Tatarenkov, A, L Bergström, RB Jönsson, EA Serrão, L Kautsky, K Johannesson. 2005. Intriguing asexual life in marginal populations of the brown seaweed *Fucus vesiculosus*. *Mol. Ecol.* 14: 647-651.

Umar, MJ, LJ McCook, IR Price. 1998. Effects of sediment deposition on the seaweed *Sargassum* on a fringing coral reef. *Coral Reefs* 17: 169-177.

West, JA. 1972. The life history of *Petrocelis franciscana*. *Br. Phycol. J.* 7: 299-308.

Wright, JT, AR Davis. 2006. Demographic feedback between clonal growth and fragmentation in an invasive seaweed. *Ecology* 1744-1754.

Zupan, JR, JA West. 1988. Geographic variation in the life history of *Mastocarpus papillatus* (Rhodophyta). *J. Phycol.* 24: 223-229.

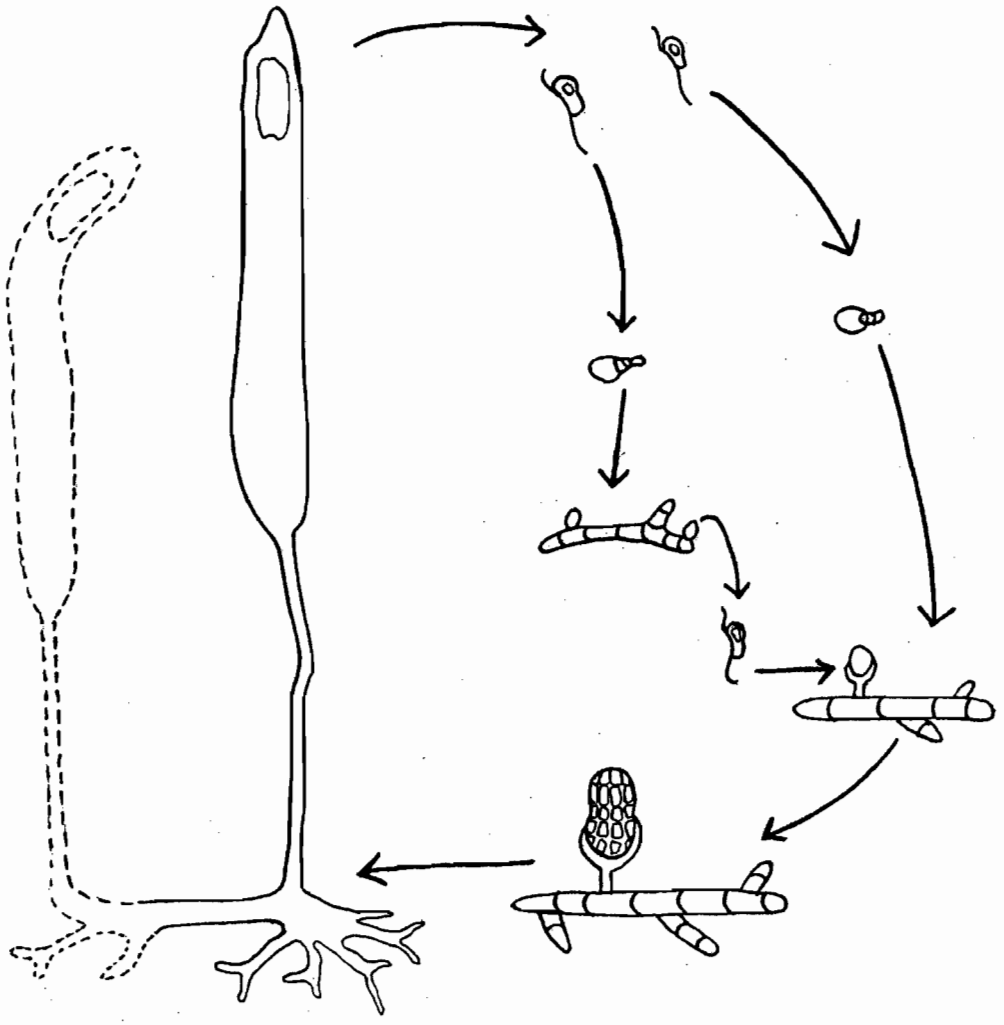

Figure 1 Life history of *Laminaria sinclairii*. Adult sporophyte on left produces spores which settle into male and female gametophytes which produce sperm and eggs respectively. Fertilization of the egg by spermatozoids results in production of a new sporophyte. Dotted lines represent the ability of *L. sinclairii* to produce new individuals through vegetative (clonal) reproduction.

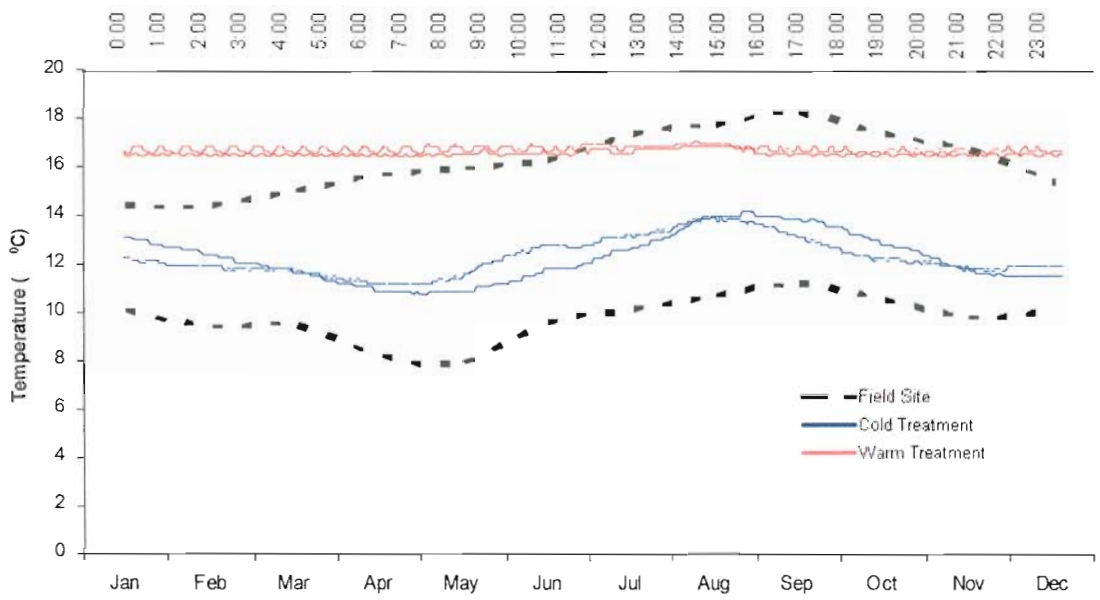

Figure 2 Temperature time series for the field collection site and experiment temperature treatments. Dashed black lines represent +/- 2 S.D. for monthly averages (bottom x-axis) of seawater temperature at the field site for 1980-2001. Blue and red lines represent the temperature in four randomly selected cooled and warm tanks, respectively, over a 24h period (top x-axis).

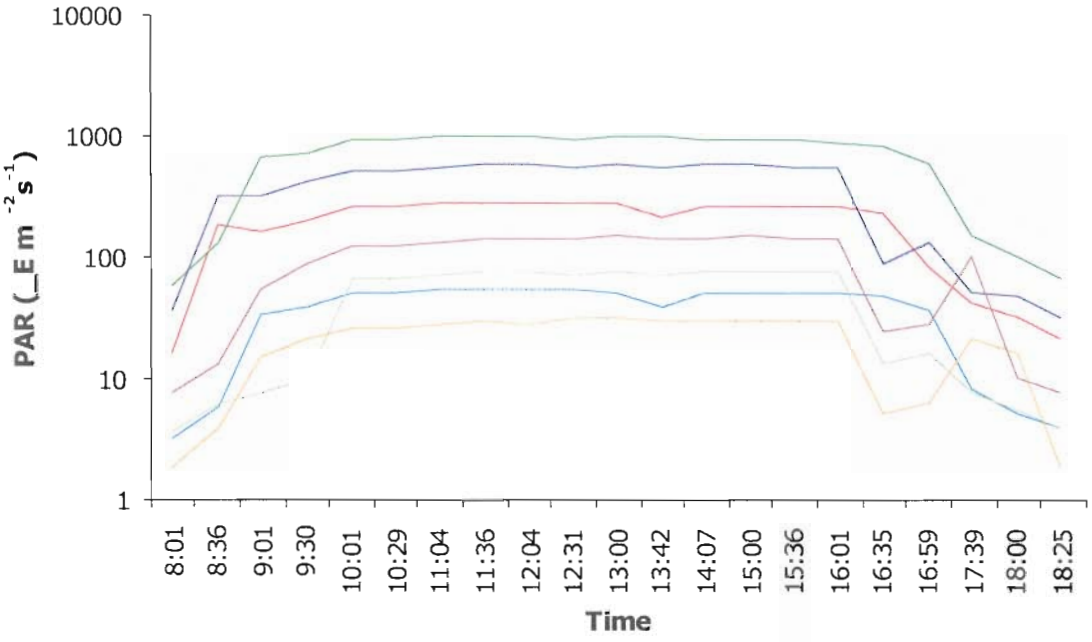

Figure 3 Photosynthetically active radiation (PAR) within each light treatment (tank) on March 17, 2009. PAR is on a logarithmic scale to facilitate visual comparison.

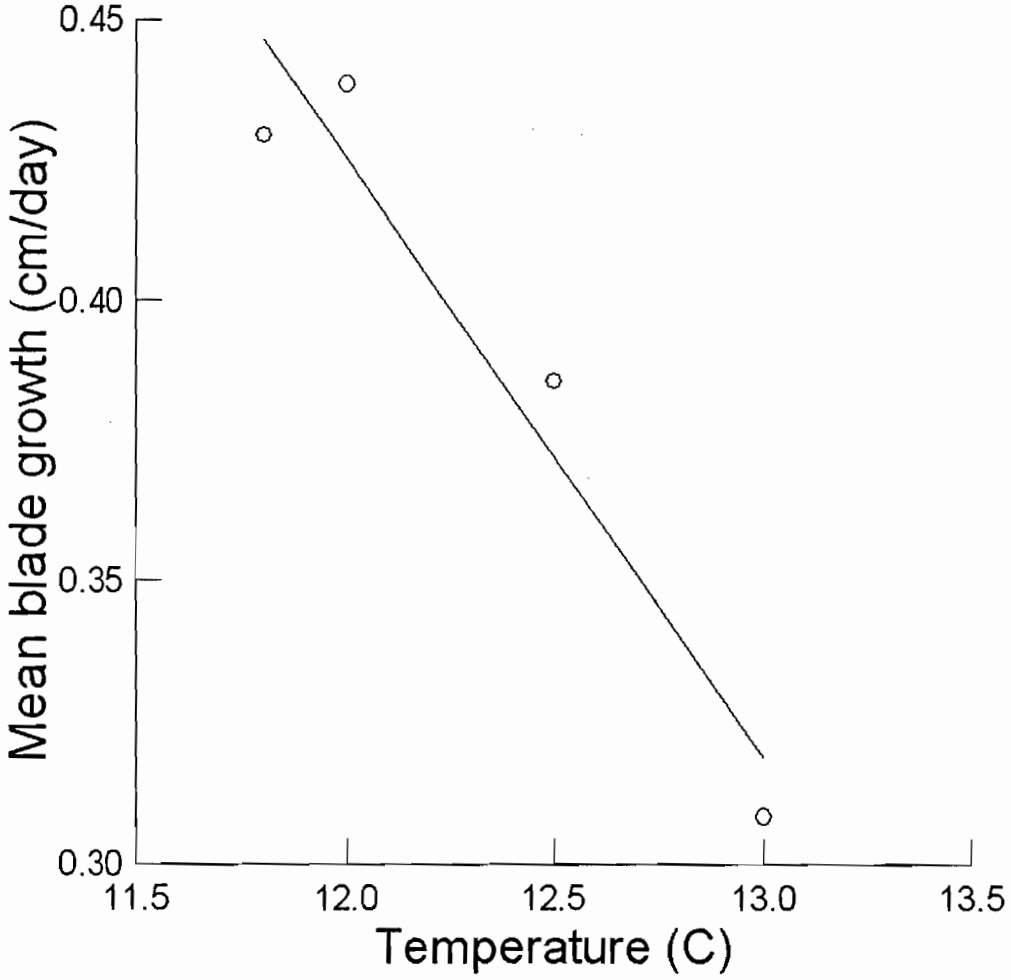

Figure 4 Average monthly blade growth of individuals tagged in the field (cm/day) vs. mean monthly seawater temperature (°C) at the field site.

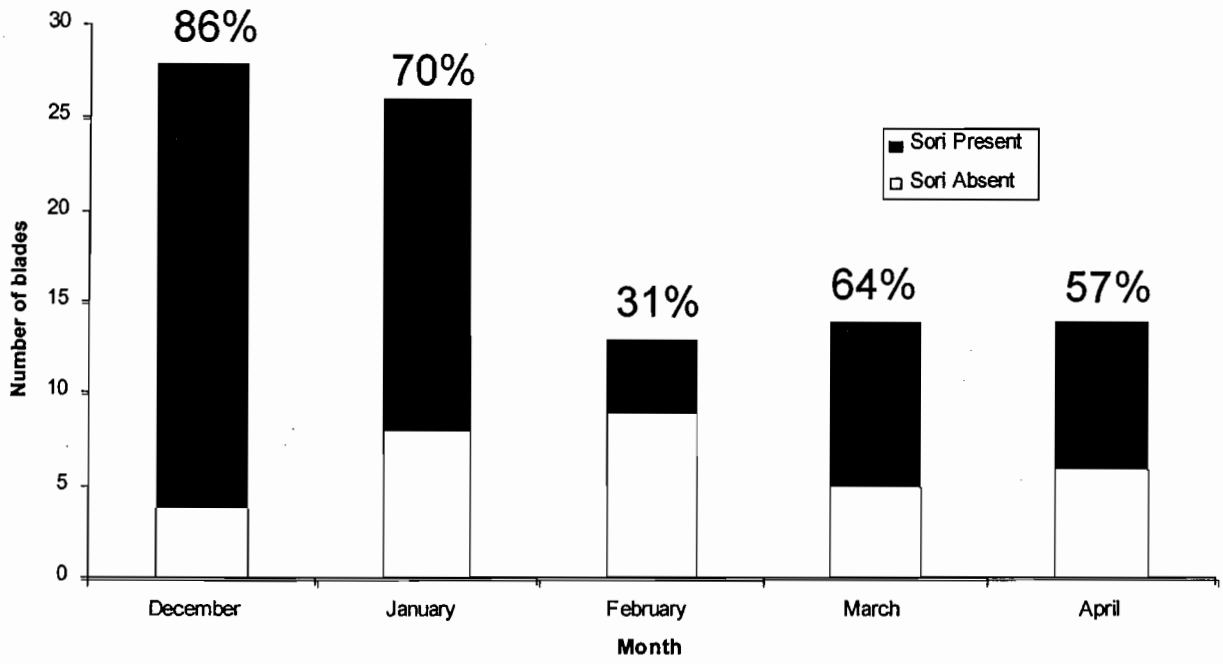

Figure 5 Number of blades on 30 haphazardly selected *Laminaria. sinclairii* stipes at the Pigeon Point during the experiment (December 2008- April 2009). Black portions represent frequency of reproductive blades (sori present) vs. the white portions, which represented non-reproductive blades (sori absent). The decline in total number of blades from January to February 2009 was due to wave-induced mortality from large swell events, not differential sampling effort. Values are percent of total blades present with sori present.

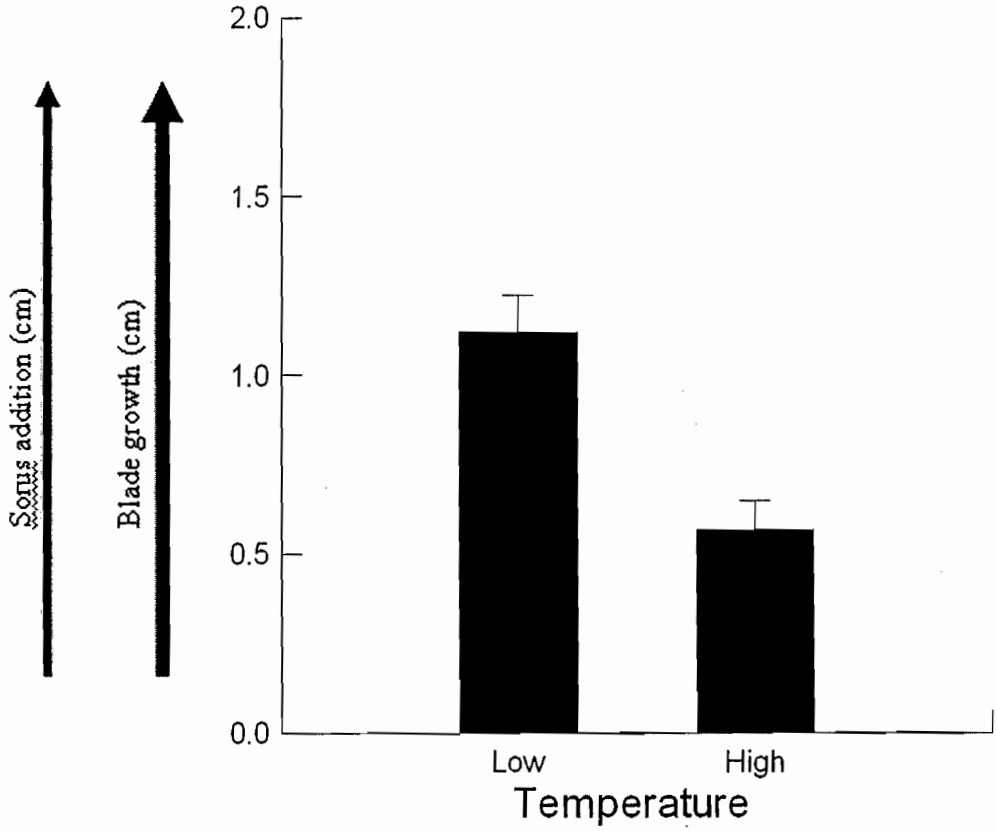

Figure 6 Effect of temperature on blade growth (cm) and sorus addition (cm) in the laboratory. Values on y-axis are mean MANOVA canonical scores ± SE (n = 8). Arrow directions, values, and thickness represent loadings (correlation coefficients) of original variables to multivariate axis.

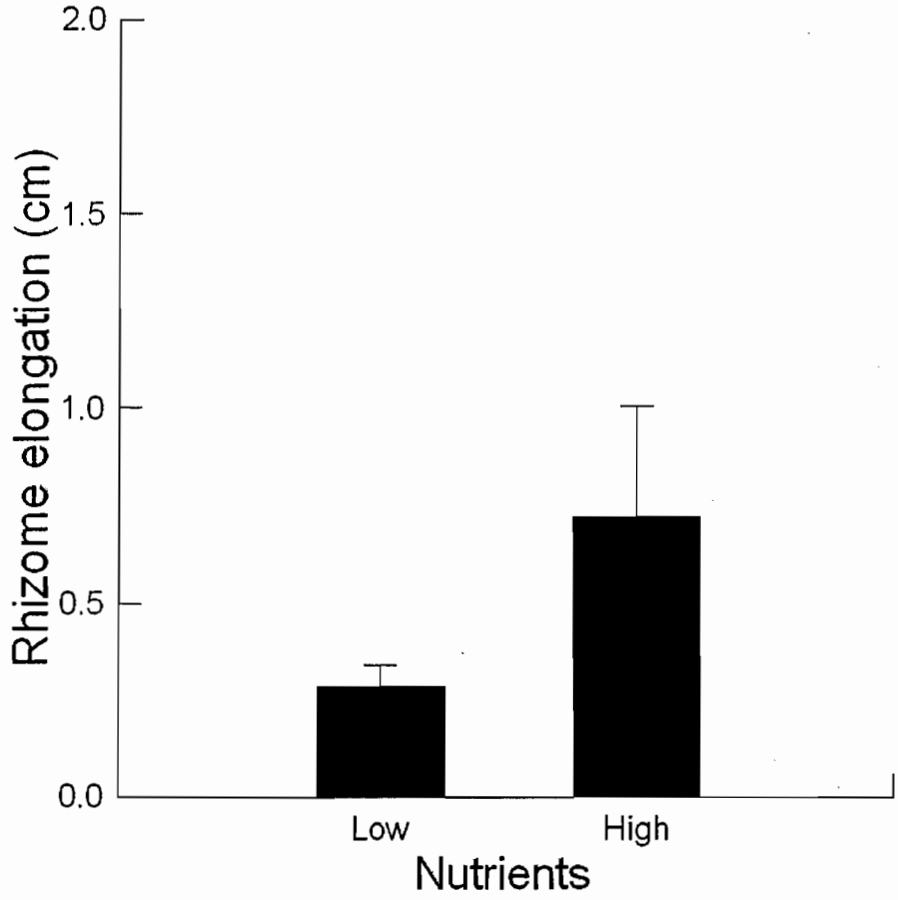

Figure 7 Effect of nutrients on rhizome elongation, as change in rhizome length (cm). Values are means ± SE (n = 8).

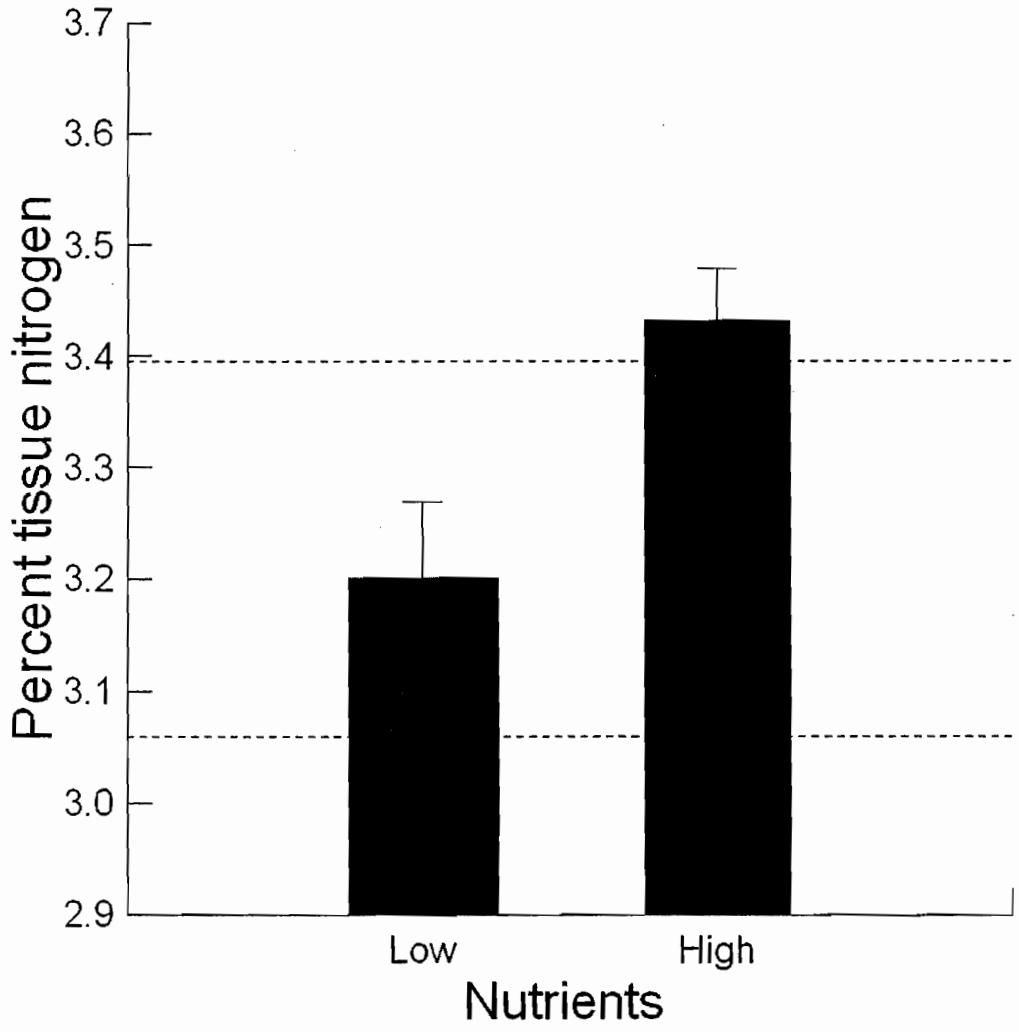

Figure 8 Effect of nutrients on percent tissue nitrogen in rhizomes. Area between dashed lines represents the 95% C.I. for unmanipulated field rhizomes. Values are means ± SE (n = 8).

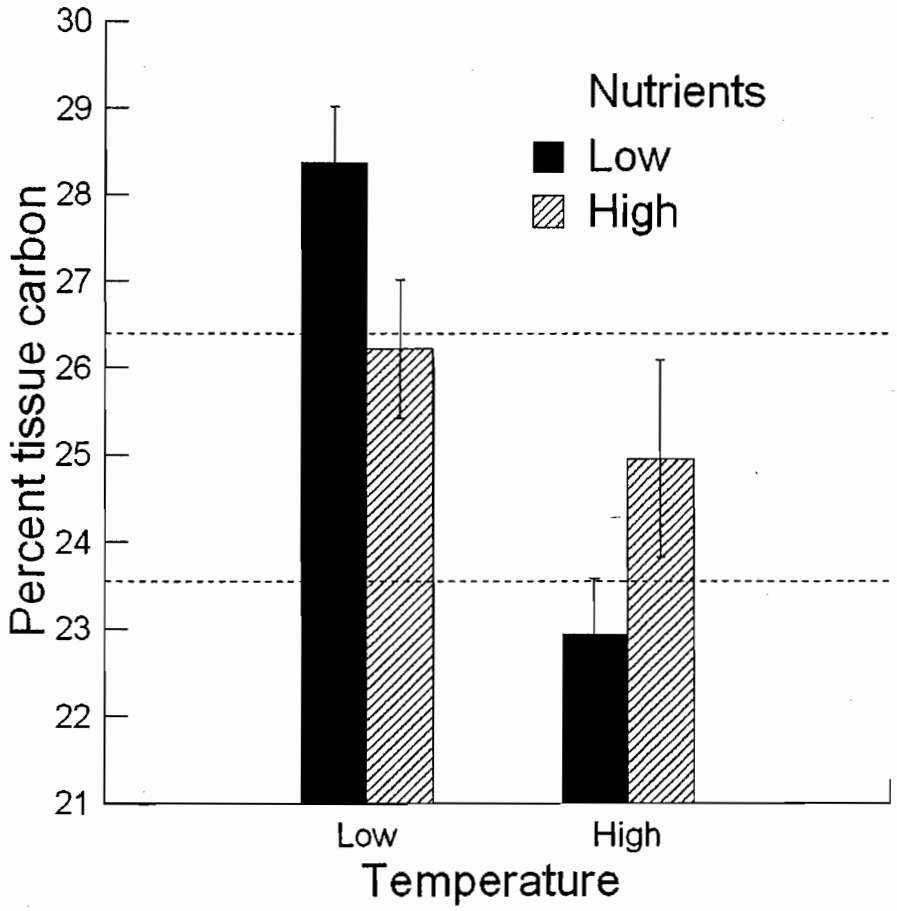

Figure 9 Effect of temperature and nutrients on percent tissue carbon in rhizomes. Area between dashed lines represents the 95% C.I. for unmanipulated field rhizomes. Values are means ± SE (n = 4).

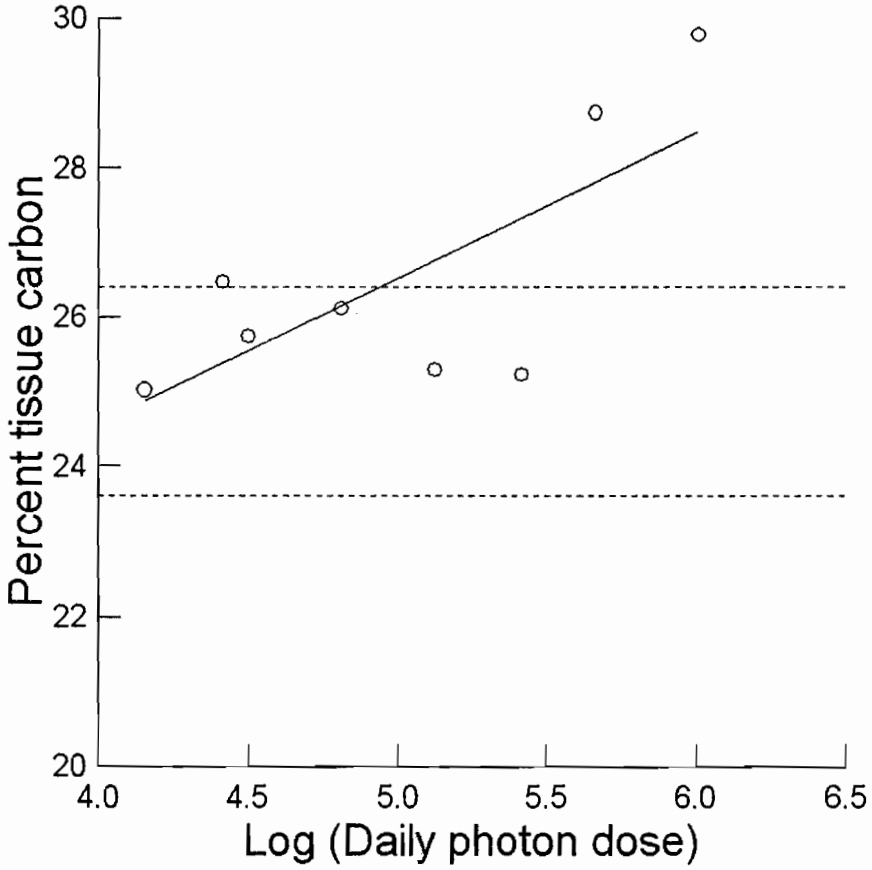

Figure 10 Effect of daily photon dose on percent tissue carbon remaining in the rhizome after the experiment. Area between dashed lines represents the 95% C.I. for unmanipulated field rhizomes. Values are tank means.

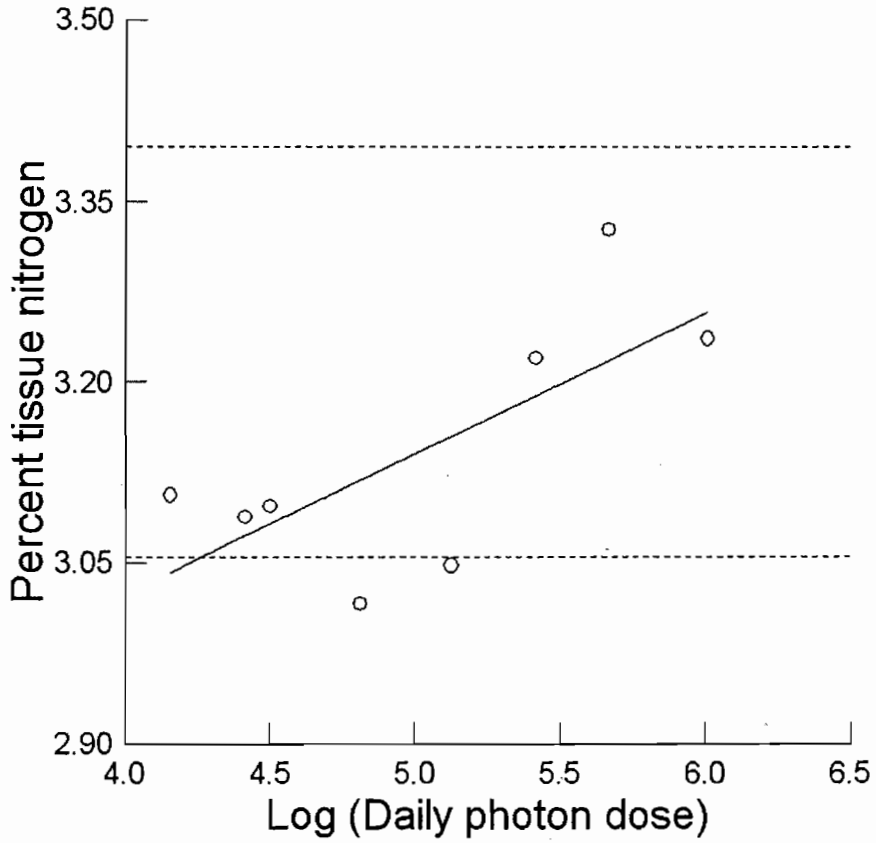

Figure 11 Effect of daily photon dose on percent tissue nitrogen remaining in rhizomes at the end of the experiment. Area between dashed lines represents the 95% C.I. for unmanipulated field rhizomes. Values are tank means.

Table I Analysis of variance from temperature and nutrient manipulation experiment: (A) rhizome elongation, (B) ending rhizome percent tissue nitrogen, and (C) ending rhizome percent tissue carbon.

A.

Source of variation	df	MS	F	p
Temperature	1	0.514	1.553	0.236
Nutrients	1	1.669	5.045	0.044
T * N	1	0.825	2.494	0.14
Error	12	0.331		

B.

Source of variation	df	MS	F	p
Temperature	1	0.009	0.323	0.58
Nutrients	1	0.215	8.11	0.015
T * N	1	0.004	0.158	0.698
Error	12	0.026		

C.

Source of variation	df	MS	F	p	ω^2
Temperature	1	44.739	23.232	< 0.001	48.08
Nutrients	1	0.02	0.01	0.921	0
T * N	1	17.347	9.008	0.011	17.32
Error	12	1.926			34.61

Table II Multivariate analysis of variance for blade growth and sorus addition from temperature and nutrient manipulation experiment: (A) temperature, (B) nutrients, and (C) temperature*nutrients.

A	Temperature				
		Univariate Statistics			
	Source	df	MS	F-ratio	p-value
	Blade	1	0.821	21.417	0.001
	Error	12	0.038		
	Sorus	1	1.497	6.254	0.028
	Error	12	0.239		
		Multivariate Statistic			
	Statistic	df	Value	F-ratio	p-value
	Pillai Trace	2, 11	0.654	10.388	0.001
B	Nutrients				
		Univariate Statistics			
	Source	df	MS	F-ratio	p-value
	Blade	1	0.06	1.557	0.236
	Error	12	0.038		
	Sorus	1	0.251	1.048	0.326
	Error	12	0.239		
		Multivariate Statistic			
	Statistic	df	Value	F-ratio	p-value
	Pillai Trace	2, 11	0.143	0.919	0.428
C	Temperature*Nutrients				
		Univariate Statistics			
	Source	df	MS	F-ratio	p-value
	Blade	1	0	0.001	0.972
	Error	12	0.038		
	Sorus	1	0.305	1.272	0.281
	Error	12	0.239		
		Multivariate Statistic			
	Statistic	df	Value	F-ratio	p-value
	Pillai Trace	2, 11	0.107	0.659	0.537

A.	Regressions: Field Site	slope	y-intercept	F	p	r^2
	Blade growth vs temperature	-0.1064	1.7015	25.725	0.037	0.928
B.	Regression: Light manipulation	slope	y-intercept	F	p	r^2
	Blade growth vs Light	-0.003	0.7073	0.006	0.942	0.001
	Sorus addition vs Light	0.2375	-0.209	0.878	0.385	0.128
	Rhizome elongation vs Light	-0.201	1.4891	0.869	0.387	0.127
	% tissue carbon vs Light	1.9452	16.787	6.521	0.043	0.521
	% tissue nitrogen vs Light	0.1163	2.5588	6.071	0.049	0.503

Table III. Linear regression of (A) mean monthly blade growth rate (dependent variable) vs. mean monthly temperature (independent variable) at field site and (B) growth and storage response variables (dependent variables) vs. log transformed daily photon dose (Light) in the light manipulation experiment.