J. Phycol. 27, 149–158 (1991)

VARIATION IN NITROGEN PHYSIOLOGY AND GROWTH AMONG GEOGRAPHICALLY ISOLATED POPULATIONS OF THE GIANT KELP, *MACROCYSTIS PYRIFERA* (PHAEOPHYTA)[1]

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ABSTRACT

Three geographically isolated populations of the giant kelp, Macrocystis pyrifera *(L.) C. Ag., were examined for responses to nitrate availability in batch culture experiments using juvenile sporophytes reared from spores in the laboratory. Although maximum rates of nitrate-saturated growth were similar among groups, there were significant quantitative differences in the response to nitrate limitation that can be related to natural patterns of nutrient availability at these sites. Plants from Santa Catalina Island (most oligotrophic) achieved maximum growth rates at ambient nitrate concentrations that were lower than those for plants from Monterey Bay, California (most eutrophic), or Refugio State Beach (near Santa Barbara, California). Tissue nitrogen and amino acid concentrations were highest in plants cultured from Santa Catalina Island populations at all external nitrate concentrations, suggesting that differences in nitrate requirements for growth may reflect the efficiency of nitrate uptake and assimilation at subsaturating nitrate concentrations. Given the different physical environments from which these plants came, the data suggest that geographically isolated populations of* M. pyrifera *have undergone genetic divergence that can be explained by ecotypic adaptation to unique habitat conditions at these sites.*

Key index words: ecotypes; geographic variation; growth; kelp; Macrocystis; *nitrate; nutrients; Phaeophyta*

The term "ecotype" was first defined by Turesson (1922) to describe genetically distinct plant populations that show some specialized adaptation to their native habitats. The presence of genetically distinct populations or races within species has been demonstrated for many taxa, including marine phytoplankton (Gallagher et al. 1984), marine macrophytes (Innes 1984), terrestrial plants (Bradshaw 1971, Hamrick and Allard 1972), insects (Powell et al. 1973), fish (Powers et al. 1986), birds (Mayr 1970), and *Homo sapiens* (Garn 1965). Although there are many examples of ecotypic differentiation within species, genetic differentiation is only one way in which populations may respond to environmental heterogeneity. Populations may retain a common genotype while phenotypic expression changes across environmental gradients. Such phenotypic changes may be reversible plastic modifications in response to changes in the environment, or they may be irreversible developmental modifications of gene expression such that individuals with identical genotypes differ phenotypically (Mayr 1970). The optimum strategy for any given population probably depends on the specific trait in question, the intensity of natural selection, and the scales of environmental variability (Levins 1968, Slatkin 1987).

The development and maintenance of geographic races within a given species depend on the strength of local selective pressures and, secondarily, on the degree of reproductive isolation (Slatkin 1987). Such selective pressures and isolating mechanisms have been linked to the occurrence of apparent ecotypes in kelp, particularly within species of the genus *Laminaria.* Differences in morphology (Chapman 1974), temperature tolerance (Gerard and DuBois 1988), photoadaptive responses (Gerard 1988), and responses to nitrogen availability (Gagné et al. 1982, Espinoza and Chapman 1983) have all been reported to be the result of genetic differentiation among geographically isolated populations. However, this is not always the case, even within species of *Laminaria* (Gerard and Mann 1979, Druehl et al. 1989).

The giant kelp, *Macrocystis pyrifera* (L.) C. Ag., exhibits many features that indicate that it, too, may be a polytypic species. It has a bipolar distribution and is most widely distributed throughout temperate waters in the southern hemisphere (Womersly 1954). In the northern hemisphere, *M. pyrifera* is distributed as discontinuous populations from Año Nuevo Island, just north of Santa Cruz in central California, to Punta San Hippolito, in Baja California, Mexico (Foster and Schiel 1985). This discon-

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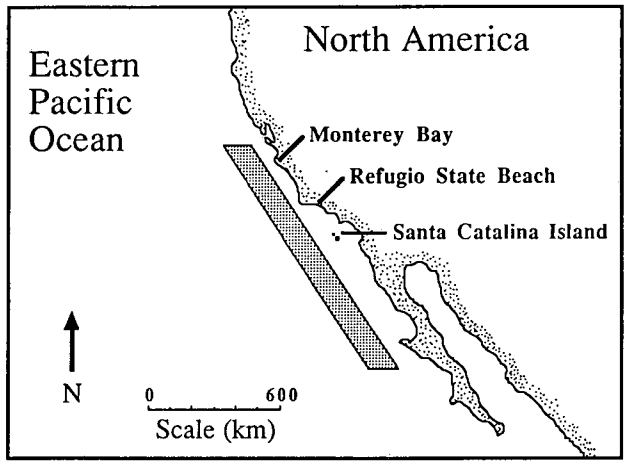

FIG. 1. Map of the California coast, showing the distribution of *Macrocystis pyrifera* (hatched bar), the location of each donor population and the Monterey Bay and Santa Catalina Island field sites.

tinuous distribution results in some degree of reproductive isolation among populations. Although spores of *M. pyrifera* are motile, their dispersal appears to be limited to a few meters from the point of release (Anderson and North 1966, Reed et al. 1988). Even though drifting bits of *M. pyrifera*, which often include fertile sporophylls, can be transported along the coast for 10–50 km (Harrold and Lisin 1989), there is probably very little genetic exchange between populations separated by a distance of 100 km or more. In addition to reproductive isolation, the local selective pressures imposed by environmental conditions can vary widely within this geographic range, which covers approximately 10° of latitude. Potentially important regulating factors include annual patterns of light availability, water temperature, and differences in water motion caused by storm activity and wave exposure (Foster and Schiel 1985). Another major difference among locations within the range of this species is the temporal pattern of dissolved nutrient availability. Geographic variation in this environmental character ranges from relatively constant and high nitrate concentrations in Monterey Bay and central California (Bolin and Abbott 1963, Strub et al. 1987) to the predictably oligotrophic conditions found during the summer months at Santa Catalina Island in the Southern California Bight (Zimmerman and Kremer 1984, 1986, Zimmerman and Robertson 1985). Populations in the former locations are probably never nutrient limited, whereas summertime nutrient limitation is a common occurrence at Santa Catalina Island.

The goal of this study was to examine three geographically isolated populations of the giant kelp, *Macrocystis pyrifera*, for evidence of a genetic basis for differences in their response to nitrate availability. Juvenile sporophytes were raised in the laboratory from spores and were examined for differences in the effect of nutrient availability on growth and nitrogen content.

MATERIALS AND METHODS

Populations from three locations in California were selected for their geographic isolation from each other and because the locations represent three different physical environments with respect to water motion and nutrient availability. The northernmost population was located in Monterey Bay (Fig. 1) at Cabrillo Point, near Hopkins Marine Station, a stenothermal environment in which nutrient concentrations are relatively constant and generally high (Bolin and Abbott 1963) and winter storms play a significant role in structuring kelp forest ecosystems (Gerard 1976, Harrold et al. 1988). The population at Refugio State Beach was located in the Santa Barbara Channel, midway between Pt. Conception and the city of Santa Barbara in a region of transition between the Oregonian and Californian biogeographic provinces (Hedgepeth 1957, Brusca and Wallerstein 1979). It is also a transition point between the outer coast of central California, which is dominated by highly energetic coastal upwelling and the California Current, and the Southern California Bight, where circulation is dominated by the Southern California counter-current and where a much greater seasonal thermal regime and frequent periods of oligotrophy exist (Jones 1971, Eppley et al. 1979, Brink and Muench 1986). The Santa Catalina Island population was located at Intake Point, near the Catalina Marine Science Center, in the center of the Southern California Bight, an environment of relatively low wave stress and nutrient availability (Zimmerman and Kremer 1984, 1986, Zimmerman and Robertson 1985).

Sporophyte cultures. To eliminate the possibility that observed differences among populations were the result of developmental gene expression caused by exposure to different environments, juvenile sporophytes from each population were cultured from spores under identical conditions in the laboratory. A sporophyll was collected by SCUBA divers from each of 10 different randomly chosen plants at each donor site. Zoospores were released by chilling the sporophylls in cold (ca. 5° C) 0.2 μm-filtered seawater (FSW) after rinsing the sporophylls in FSW and gently scrubbing them with a wet paper towel to remove epiphytes and contaminants. The density in each spore suspension was enumerated using a haemocytometer and was adjusted to approximately 10^6 spores·mL^{-1} with FSW. The suspensions were then poured onto settling racks containing rings of PVC pipe (1 inch diameter) that had been grooved, sliced into 5-mm rings, and slipped over horizontal Plexiglas bars (North 1976). Cultures from each population were maintained in separate 2-L containers in the same environmental chamber at 15° C under continuous illumination provided by 20-watt cool-white fluorescent bulbs (ca. 80 μE·m^{-2}·s^{-1}). This temperature falls within the extremes (10–23° C) for all field sites studied, although the seasonal temperature patterns differed at each site. The culture medium consisted of FSW collected from the surface at Santa Catalina Island + 100 μM NO_3^- + 8 μM PO_4^{-3} + f/2 trace metals and was changed daily. Mixing was provided by magnetic stirrers. Approximately 2 months were required to yield sporophytes of 1–2 cm in length that were suitable for growth-rate experiments.

Response to nitrate availability in laboratory cultures. Experiments were conducted to determine the growth responses of juvenile *M. pyrifera* sporophytes (1–2 cm long) to a range of quasi-constant nitrate concentrations. Individuals from each culture were grown in separate 2-L Erlenmeyer flasks containing 2000 mL of the same culture medium as described above, except that nitrate concentrations varied from 0 to 23 μM among flasks. Each plant was suspended in the flask by entwining its holdfast in the strands of a length of cotton string weighted at the bottom with a small length of glass tubing and held in place by a rubber stopper. The culture medium was changed every other day. Sporophytes depleted the nutrient supply by less than 5% between changes.

Stirring was provided by vigorous bubbling with charcoal-filtered air. The experimental incubations were carried out under an irradiance of 120 $\mu E \cdot m^{-2} \cdot s^{-1}$, provided by 20-watt cool-white fluorescent tubes. Plants were held under these conditions for 10 d to permit tissue nitrogen concentrations and growth rates to approach equilibrium with the external supply. After this 10-d period, plants were weighed and returned to the flasks for an additional 5 d, during which time the culture medium was changed daily. The 10-d preconditioning period is probably adequate. A period between 6–11 d was found to be sufficient time for similarly sized juvenile sporophytes of both *M. pyrifera* (Manley and North 1984) and *L. saccharina* (Chapman et al. 1978, Wheeler and Weidner 1983) to attain steady-state growth rates. Although the rate at which a given population makes this transition might be influenced by local conditions through ecotypic differentiation, our preliminary run of this experiment a year earlier indicated that growth rates during days 11–15 were not different from the growth rates measured during days 16–20 for plants from these sites held under the same experimental conditions.

Growth rates. Growth rates were based on an initial and final weight taken 5 d apart after the 10-d preconditioning period. Biomass-specific growth rates for each nitrate concentration were calculated as:

$$\mu = \frac{\ln(m_t) - \ln(m_0)}{t} \quad (1)$$

where m_t = the mass (g) after 5 d, m_0 = the initial mass (g), and t was the incubation time (d). Initially a nonlinear least-squares regression was used to estimate the best fit of a rectangular hyperbola to each data set. However, statistical comparisons of the estimated equation parameters between more than two populations proved intractable. Instead, the data from each population were divided at the arbitrary nitrate concentration of 5 μM. Growth rates below this concentration were taken to represent the nitrate-limited phase of growth, and those above represented the maximum growth rate. The slopes and intercepts of these two portions of the data for all groups were compared using an analysis of covariance (ANCOVA, Sokal and Rohlf 1981) and Student-Neuman-Keuls (SNK) multiple range test (Zar 1974).

Tissue nitrogen content. Each plant was split in half longitudinally after final weight determination. One half was dried at 60° C and analyzed for tissue N content by the Marine Science Institute Analytical Laboratory, University of California, Santa Barbara, using a Perkin-Elmer Model 240B elemental analyzer. Statistical significance of differences between groups for the relationship of tissue nitrogen and ambient nitrate concentration was determined by ANCOVA for a single least-squares linear regression for each group.

Free amino acids were extracted from the other half of each juvenile sporophyte in hot ethanol (80° C) and analyzed by reverse-phase HPLC after derivitization with OPA (fluoraldehyde) following the procedure of Pregnall et al. (1984). The OPA–amino acid complexes were detected using an in-line fluorometer equipped with Corning 7-54 (320 nm) excitation and 3-73 (450 nm) emission filters. Individual amino acids were identified and quantified using elution profiles and fluorometric peak heights of OPA-derivatized amino acid standards.

RESULTS

Tissue nitrogen content. Differences in the relationship between tissue nitrogen content and ambient nitrate concentration were highly significant among groups (Fig. 2, Table 1). The differences were attributable to differences in the intercept rather than in the slope of the regressions. The results of an SNK multiple range test indicated that the intercepts of all groups were significantly different from each other. Rankings were such that, at any given nitrate concentration, the tissue N content was highest in plants from the Catalina culture and lowest in the Refugio culture.

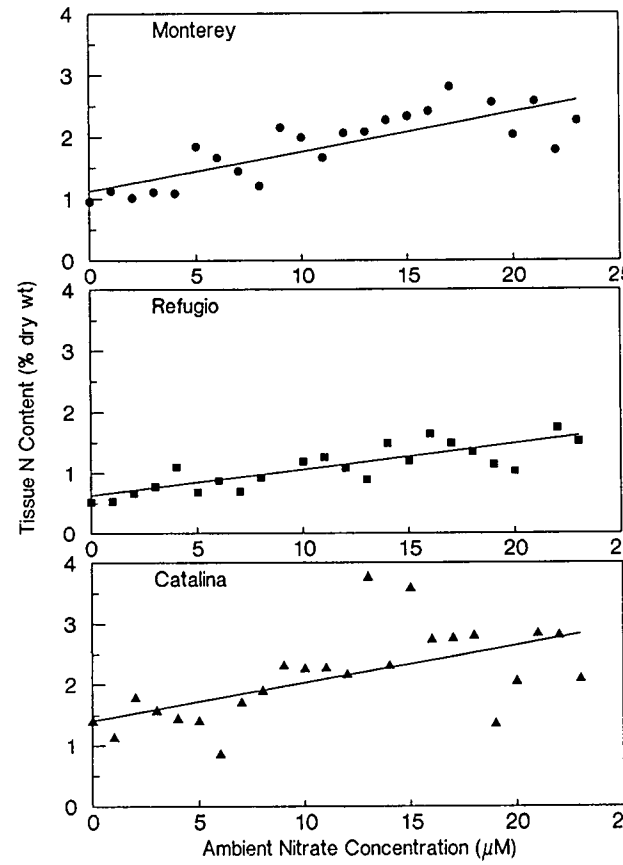

FIG. 2. Tissue N content (% of dry mass) of laboratory-reared juvenile sporophytes plotted as a function of ambient nitrate concentration. Solid lines represent a least-squares linear regression fit of each data set. Significance values, as determined by ANCOVA, are presented in Table 1.

Amino acids. The differences in amino acid content of each group reflected the observed differences in tissue nitrogen content. Amino acids were generally higher in the Catalina culture (avg = 32 $\mu mol \cdot gdw^{-1}$) than in the Monterey (avg = 11 $\mu mol \cdot gdw^{-1}$) or Refugio (avg = 11 $\mu mol \cdot gdw^{-1}$) cultures. However, there was no clear relationship between amino acid content and ambient nitrate concentration. Similarly, amino acid concentration did not appear to be related to tissue N content; however, the distribution of values within the Refugio population seemed obviously different from the other two populations (Fig. 3).

The free amino acid composition was remarkably similar among the three cultures when all 24 nitrate treatments were pooled (Fig. 4). Alanine and β-alanine were abundant in all three cultures. However, the abundance of each amino acid varied markedly among the cultures. In general, both the median concentration and the variability of the concentra-

TABLE 1. *Summary table of linear regression parameters calculated for the relationship between tissue N content and ambient nitrate concentration and the relationship between specific growth rate and ambient nitrate concentration of cultures derived from donor populations at Monterey Bay (Mon), Refugio State Beach (Ref), and Santa Catalina Island (Cat). Differences in the tissue N content and ambient nitrate concentration relationship and the specific growth rate at ambient nitrate concentrations below 5 μM were determined by analysis of covariance for the significance of differences of slopes and intercepts. Differences in the mean specific growth rate at ambient nitrate concentrations above 5 μM were determined by analysis of variance since the individual regressions were either marginally different (Refugio) or not significantly different from zero. A Student-Neuman-Keuls multiple range test was used to determine how the differences in slopes and intercepts were partitioned among Monterey, Refugio, and Catalina plants. Horizontal lines connect similar groups.* *** $P \ll 0.0005$, ** $P < 0.025$, * $P < 0.05$.

Relationship	Regression parameter	Mon	Ref	Cat	F_{calc}	df
%N vs. [NO_3]	Slope	0.0642	0.0421	0.0622	0.897	2,60
	Y-int.	1.12	0.61	1.41	35.95***	2,60
	n	23	22	24		
μ vs. [NO_3]						
<5 μM NO_3	Slope	0.0755	0.0206	0.0276	4.93*	2,9
	Y-int.	−0.1996	−0.0126	0.0140	5.77**	2,13
	n	5	5	5		
>5 μM NO_3	Mean	0.118	0.107	0.071	3.11	2,60
	n	18	17	19		

tion of each amino acid were higher in the Catalina plants than in either of the other two groups.

Growth rates. The clear differences among groups in tissue nitrogen content were not as apparent in the response of the specific growth rate to ambient nitrate concentration (Fig. 5). The growth relationship seemed to be strongly hyperbolic in the Monterey Bay culture but less so in each of the other two groups. At nitrate concentrations below 5 μM, results of an ANCOVA and a subsequent multiple range test indicated significant differences in the slopes and intercepts of the linear regressions (Table 1). The slope of this N-limited portion was higher for the Monterey Bay culture than the other two groups, which were not different from each other, and the Y-intercept of the Monterey culture was significantly lower than that for the other two groups.

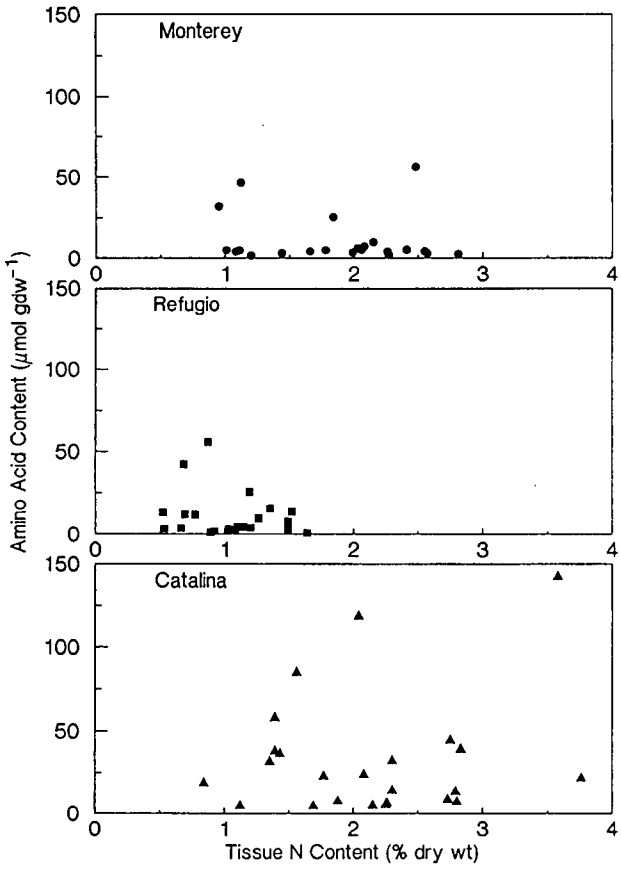

FIG. 3. Total amino acid concentration of laboratory-reared juvenile sporophytes plotted as a function of tissue nitrogen content.

The X-intercept represents the compensation quota or external nitrate concentration at which net growth is zero. The compensation quota was highest in the Monterey culture (2.62 μM) followed by the Refugio culture (0.64 μM). The Catalina culture exhibited positive growth rates down to the lowest conventionally measured nitrate concentrations. This suggests that the compensation quota for the Catalina culture is in the nanomolar range.

At nitrate concentrations above 5 μM, the slopes of the Monterey and Catalina regression lines were not significantly different from zero, indicating that growth rates were saturated with respect to nitrate concentration so that the mean values would be estimates of N-saturated specific growth rate. Although the regression line of the Refugio culture did exhibit a slope significantly greater than zero ($P < 0.005$), this is a marginal result, because there was no significant difference in the slopes among groups. Mean N-saturated specific growth rates of each group were not significantly different as determined by analysis of variance (ANOVA; Table 1). The mean N-saturated specific growth rate of the Catalina culture was depressed to some extent by the negative growth rates of four individuals (Fig. 5). However, these apparent outliers did not appear as outliers in any of the other relationships, so we decided it was inappropriate to eliminate them from the analysis.

Specific growth rate can also be viewed as a func-

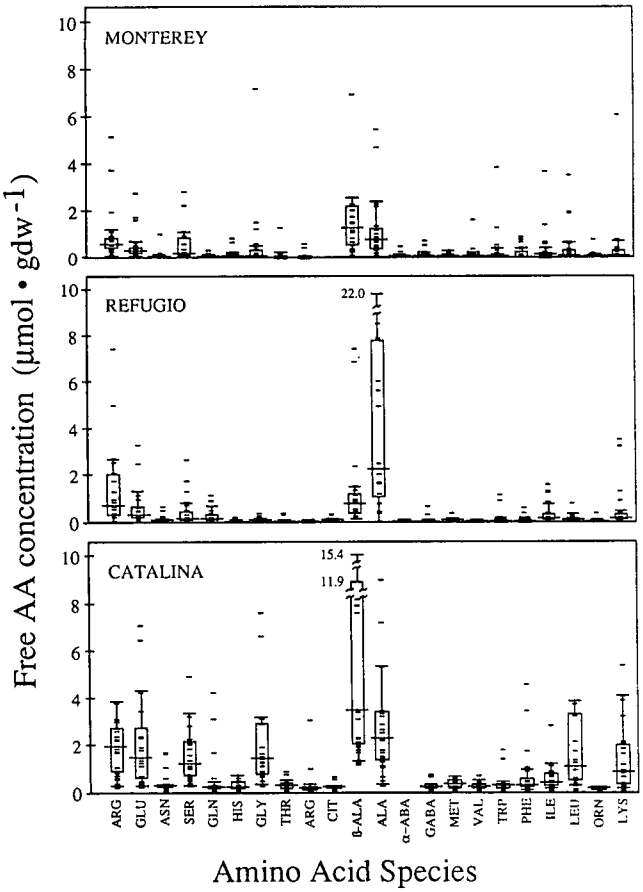

FIG. 4. Free amino acid composition of laboratory-reared juvenile sporophytes. Narrow horizontal symbols (−) represent individual free amino acid concentrations for the 24 different nitrate treatments combined. Wide horizontal line indicates median concentration for each free amino acid. The upper and lower ends (hinges) of the boxes represent the 75th and 25th percentiles of the data, respectively, which is known as the interquartile range. The vertical lines indicate the range of the data unless points exceed reasonable outer limits defined by adding or subtracting 1.5 times the interquartile range to the upper and lower hinges, respectively (Tukey 1977). Values beyond this range are considered to be outliers. There are five such values for Monterey, one for Refugio, and three for Catalina (not shown).

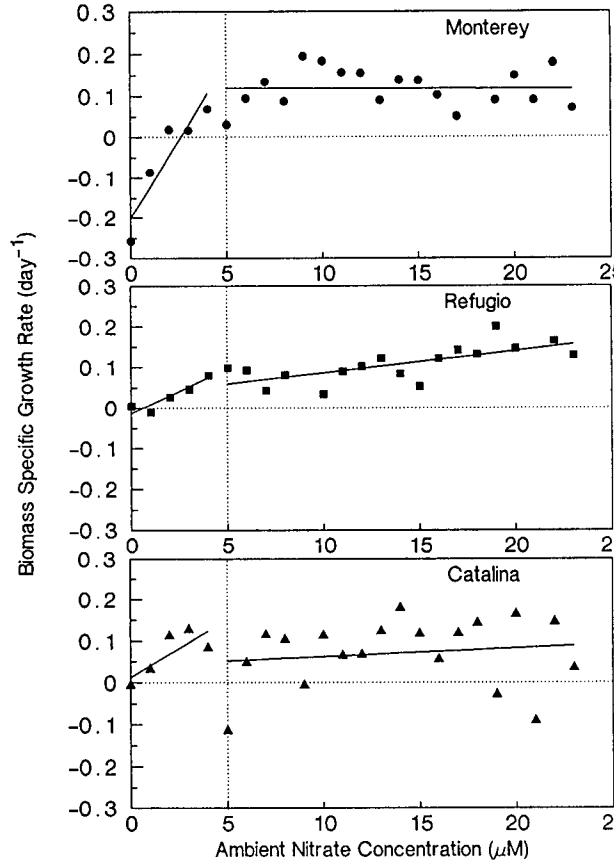

FIG. 5. Growth rates of laboratory-reared juvenile sporophytes plotted as a function of ambient nitrate availability. Solid lines represent a least-squares linear regression of all data points to the left or right of the vertical line, respectively. Significance values, as determined by ANCOVA or ANOVA, are presented in Table 1.

tion of tissue N content (Fig. 6). Although all three groups displayed a similar range of positive growth rates, the dynamic range of the tissue N content of each group varied. The dynamic range of tissue N content was highest in the Catalina plants (1–4%), intermediate in the Monterey plants (1–3%), and lowest in the Refugio plants (0.5–2%).

DISCUSSION

The data presented here suggest that isolated populations of *Macrocystis pyrifera* have evolved different responses to nitrate availability. Catalina Island plants consistently accumulated more internal nitrogen at all ambient nitrate concentrations than did the Monterey Bay or Refugio State Beach plants. There was also a tendency for the Catalina plants to store more of this nitrogen in free amino acid pools compared to the other two groups. Further, at low ambient nitrate concentrations, Catalina plants grew significantly faster than Monterey plants and also tended to grow faster than Refugio plants. Finally, the Catalina plants had the lowest compensation quota of the three groups, whereas Monterey plants had the highest. When tissue N levels are similar, as in this study, the lower the compensation quota the more effective the plants are at removing nitrate from the environment. These differences may have adaptive significance in the native environments of these plants.

The Catalina population comes from an oligotrophic environment characterized by low nitrate concentrations throughout the euphotic zone, and average surface nitrate concentrations are generally less than 1 μM (Eppley et al. 1979, Zimmerman and Kremer 1984). Although growth of individual plants is probably nutrient limited every summer (Zimmerman 1983, Zimmerman and Kremer 1986), summertime plant mortality is rare except during El Niño events (Zimmerman and Robertson 1985). Thus, the ability of this population to accumulate relatively higher internal N for a given external ni-

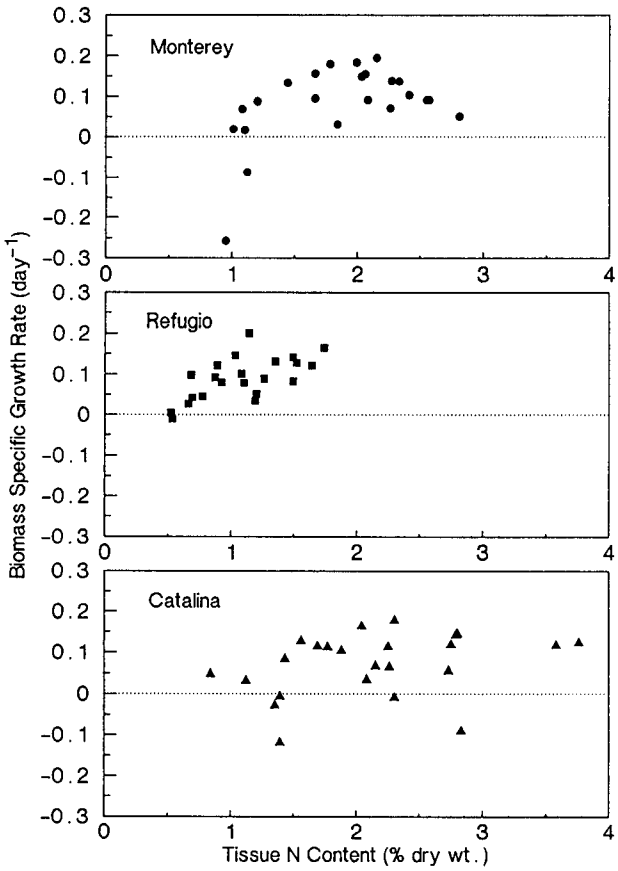

FIG. 6. Growth rates of laboratory-reared juvenile sporophytes plotted as a function of tissue N content (% of dry mass).

trate concentration may permit maximum growth rates at ambient nitrate concentrations as low as 2 μM. Growth rate of adult *M. pyrifera* at Santa Catalina Island was found to be N saturated at nitrate concentrations as low as 1 μM (Zimmerman and Kremer 1984, 1986).

In contrast, nitrate concentrations in the surface layer of Montery Bay usually range from 3 to 5 μM and can exceed 20 μM for extended periods during the spring and summer upwelling season (Bolin and Abbott 1963). Thus, growth of the Monterey Bay population *in situ* may never be nutrient limited even though growth rates of this population in culture were nitrate limited up to an ambient nitrate concentration of at least 5 μM. The significantly higher slope of this relationship compared to that of the other groups also suggests that growth of the Monterey population is extremely sensitive to changes in ambient nitrate at these limiting concentrations. The negative growth rates at low nitrate concentrations and the high sensitivity to subsaturating nitrate concentrations might be expected, because plants in Monterey Bay may rarely experience these conditions. Although the tissue N and amino acid content of the Monterey plants were intermediate between those of the Catalina and Refugio plants, growth rates at limiting nitrate concentrations were significantly lower than in either of the other groups. This suggests a less efficient utilization of internal N for growth compared to the other groups.

The average nutrient environment at Refugio State Beach appears to be intermediate between Monterey Bay and Santa Catalina Island. However, sporophytes cultured from the Refugio population did not respond in an intermediate fashion. Although periods of low nitrate availability are common in the Santa Barbara Channel, they are not as persistent as the oligotrophic conditions that dominate the hydrography near Santa Catalina Island during the summer. Water upwelled near Pt. Conception is frequently driven into the Santa Barbara Channel, exposing the Refugio population to periods of nutrient availability that are as high as those found in Monterey Bay (Dugdale 1985, Atkinson et al. 1986, Brink and Muench 1986). Growth of the Refugio culture was not as responsive to external nitrate concentrations as were the other two cultures, although, like the Catalina population, these plants appear to be capable of maintaining positive growth rates at very low external nitrate concentrations. Unlike the Catalina or Monterey cultures, however, these plants appeared to be relatively inefficient at accumulating nitrogen from the environment. The low response to nitrate concentration and the low tissue N content in the Refugio culture have been found in other kelp species adapted to a very high nutrient environment (Druehl et al. 1989). Even though the tissue N content of these plants was about half that of the other two groups, growth rates were similar; this suggests a highly efficient utilization of internal N for growth. This appears to be consistent with the boom-and-bust cycle of nutrient availability that may be typical of the Santa Barbara Channel.

The best approach to compare these three groups statistically would have been to derive the values of the Monod equation parameters from nonlinear least-squares regression analysis and thus compare the total response directly. Unfortunately, it is impossible to compute the pooled variance term required by ANOVA from the estimated standard deviation computed as described by Zimmerman et al. (1987). Rather than applying multiple comparison tests without initial verification or significant differences by ANOVA, we fit our data to a two-phase model that could be analyzed by established linear methods (regression and ANCOVA). Although 5 μM was chosen arbitrarily after inspection of the growth curves, it leads to more conservative conclusions than would be drawn if each group were divided independently. Breaking the data sets at 5 μM seems most appropriate for the Refugio culture and less so for the other two groups. Comparison of the mean growth rate at the higher nitrate concentrations (>10 μM) with the apparent nitrate-limited rates suggests that growth may have been nitrate limited up to a concentration of 7–8 μM in the Mon-

TABLE 2. *The amount of unexplained variation for simple linear regressions fit to relationships between tissue N content and ambient nitrate concentration, amino acid content and ambient nitrate concentration, amino acid content and tissue N content, and specific growth rate at ambient nitrate concentration greater than 5 μM for plants derived from donor populations at Monterey Bay, Refugio State Beach, and Santa Catalina Island.*

Relationship	Figure	Residual mean square		
		Monterey	Refugio	Catalina
%N vs. [NO_3]	2	0.1126	0.0432	0.3687
Amino acid vs. [NO_3]	Not shown	225.9	196.5	1345.4
Amino acid vs. %N	3	235.1	199.2	1314.8
μ vs. [NO_3] > 5 μM	5	0.0024	0.0011	0.0070

terey culture, whereas nitrate limitation in the Catalina culture does not seem apparent above 2–3 μM nitrate. By using the more appropriate breakpoint, growth of the Monterey culture would be N limited over a wider range of nitrate concentrations, and growth of the Catalina culture would be N limited over a narrower range of nitrate concentrations than were currently considered. These differences still indicate that the Monterey plants are N limited at nitrate concentrations that are lower than those commonly encountered in their native environment, whereas the Catalina plants are able to maintain positive growth rates at nitrate concentrations that are below the conventional limits of detection, a condition not uncommon at Santa Catalina Island in the summer.

The lack of strong Monod-type responses obscured by the variability in the relationship between growth and ambient nitrate concentration may be further evidence of differentiation among the groups. Typically, juvenile sporophytes have been found to require 6–11 d for growth rate and internal N reserves to come into equilibrium with ambient N supply (Chapman et al. 1978, Wheeler and Weidner 1983, Manley and North 1984). Although the growth rate of the Monterey culture seemed to exhibit a reasonable hyperbolic-type response to nitrate concentration, the relationship was weaker for the Refugio culture and almost nonexistent for the Catalina culture. Despite published data and direct evidence from our preliminary experiments, these results suggest that the growth of the latter two cultures was supported by internal reserves not depleted during the 10-d preconditioning period. A trend toward slower utilization of N reserves to provide a buffer for periods of low N availability would be consistent with lower N availability in the native environment. Such would be the case for Catalina plants, where long periods of low N availability are only periodically punctuated by very brief pulses of nitrate from below the thermocline (Zimmerman and Kremer 1984). The selective pressures of such an environment might tend to favor plants with the ability to maximize rates of nitrate accumulation at very low concentrations. This seems to be the case for the Catalina culture and, to a lesser extent, for the Refugio culture. Catalina plants accumulated higher (or depleted less of their) N reserves as free amino acids, yet maintained growth rates comparable to (or higher than) the other groups at the same nitrate concentrations.

Free amino acid concentrations can be correlated with internal nitrogen content in laminae of mature *M. pyrifera* (Gerard 1982, Zimmerman and Kremer 1986). However, this does not appear to be true for the three populations of juvenile sporophytes cultured in this experiment. Free amino acids accounted for only 1–2% of the total nitrogen in the juvenile sporophytes, whereas they compose around 10% of the nitrogen content of adult laminae (Gerard 1982, Zimmerman and Kremer 1986). Although amino acid concentrations of juveniles were an order of magnitude lower than in adult lamina, growth rates of the juveniles were an order of magnitude higher than rates typically measured in adult plants (Gerard 1976, Coon 1981, Jackson 1987). Thus, rather than accumulating in storage pools, the free amino acids were probably being combined rapidly into protein to support growth. The amino acid composition, and particularly the abundance of alanine and β-alanine, in tissue extracts from all three cultures is similar to the amino acid composition of hydrolyzed *M. pyrifera* protein (Mateus et al. 1976). This contrasts strongly with the amino acid composition of tissues containing large amounts of amino acids for storage or translocation, such as germinating seeds (Elmore and King 1978), vascular plant phloem (Pate et al. 1974), and sieve tube exudates from *M. pyrifera* (Schmitz and Srivastava 1979) in which aspartate and the di-amino acids asparagine and glutamine are generally the most abundant forms.

A consistent difference among the groups was the greater variability of the response of Catalina plants. In every relationship examined, the Catalina plants were the most variable and the Refugio plants were the least variable (Table 2). We believe this to be a real result, because this difference was also seen in our preliminary experiment, but the reasons for this are not clear.

The response of these populations to limiting nitrate concentrations in laboratory cultures appears to be reflected in the results of experiments in which we attempted to transplant juveniles to field sites at Santa Catalina Island and Monterey Bay. Hundreds of juvenile sporophytes cultured from each donor population were transplanted to Santa Catalina Island on 10 occasions between 1986 and 1989. As with all juvenile plants in natural environments,

mortality was high, and, regardless of donor population, most individuals in each transplant disappeared within 7 d. Notably, the only individuals that survived longer than 2 weeks were individuals from the Santa Catalina Island donor population, and six of those have survived to reproductive maturity.

In addition to nutrient limitation, water motion is an extremely important factor in determining kelp mortality (Rosenthal et al. 1974, Dayton et al. 1984, Dayton and Tegner 1984). Observations of a single Catalina sporophyte grown to maturity among plants from Monterey Bay in the Kelp Forest Exhibit tank at the Monterey Bay Aquarium suggest that, although rates of frond initiation and growth of the Catalina plant were indistinguishable from native Monterey Bay plants, the holdfast of the Catalina plant was smaller and its rate of growth was lower than the Monterey Bay plants. Although a large, rapidly growing holdfast may be advantageous for plants subjected to the high water motion environment of Monterey Bay, it may be a significant liability in the relatively calm environment of Santa Catalina Island. The nitrogen content of holdfast tissue is high (2–3% of dry mass); it is not labile and cannot be mobilized to support growth upon nitrogen starvation (Gerard 1982).

Ecotypic variations in plant species can permit survival in marginal habitats (Gerard et al. 1987, Gerard and DuBois 1988) or expansion into new habitats (Slatkin 1987). However, the differences are often quite subtle unless viewed under extreme conditions. Growth performance of the three populations examined here appears to be equivalent under ideal conditions. However, the Catalina population appears to have a significant advantage under conditions of low nutrient availability typical of its native habitat, whereas prolific holdfast growth may confer a significant advantage to the Monterey Bay population in its native environment. How these three populations fare under other conditions remains unexamined. Quantification of the performance of such population-level traits may be useful for matching different genotypes to specific local conditions. Such information would be of value to habitat restoration efforts, and the previous lack of such information may explain the mixed success rates among such projects (North 1969, Soule et al. 1978, Wilson 1982, LOSL 1983).

Using the criteria first defined by Turesson (1922), the three populations of *Macrocystis pyrifera* examined in this study appeared to show ecotypic differentiation with regard to the dynamics of nitrogen utilization, because apparent specializations of each group may be adaptive. Efficient accumulation of tissue N has obvious adaptive significance under the oligotrophic conditions that prevail at Santa Catalina Island, whereas an inability to grow at nitrate concentrations below 5 μM and an apparently lower growth efficiency would not be liabilities to survival in the more eutrophic environments of Monterey Bay and the Santa Barbara Channel.

The ability of *Macrocystis pyrifera* to thrive under a broad range of environmental conditions argues for the existence of a diversity of genotypes, some of which may be highly adapted to local conditions. The evidence presented here suggests that ecotypic differentiation provides an important mechanism by which *Macrocystis pyrifera* can occupy a wide range of environmental conditions.

We thank V. Anderson, R. Horvath, M. Reeder, D. Robertson, C. Staller, and G. Tellegen for assistance with field collections; J. Watanabe, Director of Research, Monterey Bay Aquarium (MBA), for providing access to facilities of the Monterey Bay Aquarium; C. Dea for assistance with amino acid analyses; and W. North for helpful comments and suggestions. We thank R. C. Dugdale and F. P. Wilkerson for helpful comments on the manuscript. This research was supported by the National Oceanic and Atmospheric Administration's National Sea Grant College Program, Department of Commerce, under grant number NA 86-AA-D-SG119; the State of California Resources Agency, Project No. R/RD-30 from the Sea Grant Institutional Program, University of Southern California; and Contract No. N00014-88-K-0445 from the Office of Naval Research. Funding for C.D.K. was provided by a Sea Grant Traineeship, scholarships from the ARCS Foundation and the Joseph Drown Foundation, and a Sigma Xi Grant-in-Aid-of-Research. This is contribution No. 140 from the Catalina Marine Science Center, University of Southern California.

Anderson, E. K. & North, W. J. 1966. *In situ* studies of spore production and dispersal in the giant kelp, *Macrocystis. Proc. Int. Seaweed Symp.* 5:73–86.

Atkinson, L. P., Brink, K. H., Davis, R. E., Jones, B. H., Paluszkiewicz, T. & Stuart D. 1986. Mesoscale hydrographic variability in the vicinity of Points Conception and Arguello during April–May 1983: the OPUS 1983 experiment. *J. Geophys. Res.* 91:12899–918.

Bolin, R. L. & Abbott, D. P. 1963. Studies on the marine climate and phytoplankton of the central coastal area of California, 1954–1960. California Cooperative Oceanic Fisheries Investigations Progress Report, 1 July 1960 to 30 June 1962. Mar. Res. Comm., Calif. Fish & Game, Sacramento, pp. 23–45.

Bradshaw, A. D. 1971. Plant evolution in extreme environments. *In* Creed, R. [Ed.] *Ecological Genetics and Evolution.* Blackwell, Oxford, 391 pp.

Brink, K. H. & Muench, R. D. 1986. Circulation in the Point Conception–Santa Barbara region. *J. Geophys. Res.* 91:877–95.

Brusca, R. C. & Wallerstein, B. R. 1979. Zoogeographic patterns of idoteid isopods in the northeast Pacific, with a review of shallow water zoogeography of the area. *Bull. Biol. Soc. Wash.* 3:67–105.

Chapman, A. R. O. 1974. The genetic basis of morphological differentiation in some *Laminaria* population. *Mar. Biol. (Berl.)* 24:85–91.

Chapman, A. R. O., Markham, J. W. & Luning, K. 1978. Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. *J. Phycol.* 14: 195–8.

Coon, D. 1981. Studies of whole plant growth in *Macrocystis angustifolia. Bot. Mar.* 24:19–27.

Dayton, P. K., Currie, V., Gerrodette, T., Keller, B. D., Rosenthal, R. & Ven Tresca, D. 1984. Patch dynamics and stability of some California kelp communities. *Ecol. Monogr.* 54:253–89.

Dayton, P. K. & Tegner, M. J. 1984. Catastrophic storms, El Niño and patch stability in a southern California kelp community. *Science (Wash. D.C.)* 224:283–5.

Druehl, L. D., Harrison, P. J., Lloyd, K. E. & Thompson, P. A. 1989. Phenotypic variation in N uptake by *Laminaria groenlandica* Rosenvinge (Laminariales, Phaeophyta). *J. Exp. Mar. Biol. Ecol.* 127:155–64.

Dugdale, R. C. 1985. The effects of varying nutrient concentration on biological production in upwelling regions. California Cooperative Oceanic Fisheries Investigations Report, La Jolla, Calif. 26:93–6.

Elmore, C. D. & King, E. L. 1978. Amino acid composition of germinating cotton seeds. *Plant Physiol.* 62:531–5.

Eppley, R. W., Renger, E. H. & Harrison, W. G. 1979. Nitrate and phytoplankton production in southern California coastal waters. *Limnol. Oceanogr.* 24:483–94.

Espinoza, J. & Chapman, A. R. O. 1983. Ecotypic differentiation of *Laminaria longicruris* in relation to seawater nitrate concentration. *Mar. Biol. (Berl.)* 74:213–18.

Foster, M. S. & Schiel, D. R. 1985. The ecology of giant kelp forests in California: a community profile. U.S. Fish Wildl. Serv. Biol. Rep. 85(7.2); 152 pp.

Gagné, J. A., Mann, K. H. & Chapman, A. R. O. 1982. Seasonal patterns of growth and storage in *Laminaria longicruris* in relation to differing patterns of availability of nitrogen in the water. *Mar. Biol. (Berl.)* 69:91–101.

Gallagher, J. C., Wood, A. M. & Alberte, R. S. 1984. Ecotype differentiation in the marine diatom *Skeletonema costatum*: influence of light intensity on the photosynthetic apparatus. *Mar. Biol. (Berl.)* 82:121–34.

Garn, S. M. 1965. *Human Races.* Thomas, Springfield, Illinois, 155 pp.

Gerard, V. A. 1976. Some aspects of material dynamics and energy flow in a kelp forest in Monterey Bay, Calif. Ph.D. thesis, University of California, Santa Cruz, 173 pp.

——— 1982. Growth and utilization of internal nitrogen reserves by the giant kelp, *Macrocystis pyrifera* in a low nitrogen environment. *Mar. Biol. (Berl.)* 66:27–35.

——— 1988. Ecotypic differentiation in light-related traits of the kelp *Laminaria saccharina*. *Mar. Biol. (Berl.)* 97:25–36.

Gerard, V. A. & DuBois, K. R. 1988. Temperature ecotypes near the southern boundary of the kelp *Laminaria saccharina*. *Mar. Biol. (Berl.)* 97:575–80.

Gerard, V. A., DuBois, K. & Greene, R. 1987. Growth responses of two *Laminaria saccharina* populations to environmental variation. *Hydrobiologia* 151/152:229–32.

Gerard, V. A. & Mann, K. H. 1979. Growth and production of *Laminaria longicruris* (Phaeophyta) populations exposed to different intensities of water movement. *J. Phycol.* 15:33–41.

Hamrick, J. L. & Allard, R. W. 1972. Microgeographical variation in allozyme frequencies in *Avena barbata*. *Proc. Nat. Acad. Sci. U.S.A.* 69:2100–4.

Harrold, C. & Lisin, S. 1989. Radio-tracking rafts of giant kelp: local production and regional transport. *J. Exp. Mar. Biol. Ecol.* 130:237–51.

Harrold, C., Watanabe, J. & Lisin, S. 1988. Spatial variation in the structure of kelp forest communities along a wave exposure gradient. *Mar. Ecol. (Naples)* 9:131–56.

Hedgepeth, J. W. 1957. Marine biogeography. *In* Hedgepeth, J. W. [Ed.] *Treatise on Marine Ecology and Paleoecology,* Vol. 1, Ecology. Geol. Soc. Am. Mem., New York, pp. 359–82.

Innes, D. J. 1984. Genetic differentiation among populations of marine algae. *Helgol. Meersunters.* 38:401–17.

Jackson, G. A. 1987. Modeling the growth and harvest yield of the giant kelp *Macrocystis pyrifera*. *Mar. Biol. (Berl.)* 95:611–24.

Jones, J. H. 1971. General circulation and water characteristics in the Southern California Bight. Southern California Coastal Water Research Project, Long Beach, 37 pp.

Levins, R. 1968. *Evolution in Changing Environments. Some Theoretical Explorations.* Princeton University Press, Princeton, New Jersey, 120 pp.

LOSL (Lockheed Ocean Science Laboratories). 1983. Final Report on Pendleton Artificial Reef. Mimeo. Rep. Encinitas, California, n.p.

Manley, S. L. & North, W. J. 1984. Phosphorous and the growth of juvenile *Macrocystis pyrifera* (Phaeophyta) sporophytes. *J. Phycol.* 20:389–93.

Mateus, H., Regenstein, J. M. & Baker, R. C. 1976. The amino acid composition or the marine brown alga *Macrocystis pyrifera* from Baja California, Mexico. *Bot. Mar.* 19:155–6.

Mayr, E. 1970. *Populations, Species and Evolution.* Harvard University Press, Cambridge, Massachusetts, 453 pp.

North, W. J. 1969. Kelp habitat improvement project: annual report 1968–1969. W. M. Keck Laboratory of Environmental Health Engineering. California Institute of Technology, Pasadena, 142 pp.

——— 1976. Aquaculture techniques for creating and restoring beds of giant kelp, *Macrocystis* spp. *J. Fish. Res. Board Can.* 33: 1015–23.

Pate, J. S., Sharkey, P. J. & Lewis, O. A. M. 1974. Phloem bleeding from legume fruits: a technique for study of fruit nutrition. *Planta (Berl.)* 120:229–43.

Powell, J. R., Levine, H. & Dobzhansky, T. 1973. Chromosomal polymorphism in *Drosophila pseudoobscura* used for diagnosis of geographic origin. *Evolution* 26:553–9.

Powers, D. A., Ropson, I., Brown, D. C., Van Benden, R., Cashon, R., Gonzalez-Villasenor, L. I. & DiMichelle, J. A. 1986. Genetic variation in *Fundulus heteroclitus*: geographic distribution. *Am. Zool.* 26:131–44.

Pregnall, A. M., Smith, R. D., Kursar, T. A. & Alberte, R. S. 1984. Metabolic adaptation of *Zostera marina* (eelgrass) to diurnal periods of root anoxia. *Mar. Biol. (Berl.)* 83:141–7.

Reed, D. C., Laur, D. R. & Ebeling, A. W. 1988. Variation in algal dispersal and recruitment: the importance of episodic events. *Ecol. Monogr.* 58:321–35.

Rosenthal, R. J., Clark, W. D. & Dayton, P. K. 1974. Ecology and natural history of a stand of giant kelp, *Macrocystis pyrifera,* off Del Mar, California. *Fish. Bull.* 72:670–84.

Schmitz, K. & Srivastava, L. M. 1979. Long distance transport in *Macrocystis integrifolia*. I. Translocation of ^{14}C-labeled assimilates. *Plant Physiol.* 63:995–1002.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science (Wash. D.C.)* 236:787–92.

Sokal, R. R. & Rohlf, F. J. 1981. *Biometry,* 2nd ed. W. H. Freeman and Co., New York, 859 pp.

Soule, D. F., Oguri, M. & Soule, J. D. 1978. On-site mitigation: kelp transplant project for Los Angeles harbor. Final Report. Institute for Marine and Coastal Studies, University of Southern California, Los Angeles, 131 pp.

Strub, P. T., Allen, J. S., Huyer, A. & Smith, R. L. 1987. Seasonal cycles of currents, temperatures, winds and sea level over the northeast Pacific continental shelf: 35°N to 48°N. *J. Geophys. Res.* 92:1507–26.

Tukey, J. W. 1977. *Exploratory Data Analysis.* Addison-Weseley, Reading, Massachusetts, 506 pp.

Turesson, G. 1922. The genotypical response of the plant species to its habitat. *Hereditas* 3:211–350.

Wheeler, W. N. & Weidner, M. 1983. Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. *J. Phycol.* 19:92–6.

Wilson, K. 1982. The kelp forests of Palos Verdes Peninsula 1982. *In* Bascom, W. [Ed.] *Coastal Water Research Project Biennial Rep. 1981–1982.* Southern California Coastal Water Research Project, Long Beach, pp. 67–70.

Womersly, H. B. S. 1954. The species of *Macrocystis* with special reference to those on southern Australian coasts. *Univ. Calif. Publ. Bot.* 27:109–32.

Zar, J. H. 1974. *Biostatistical Analysis.* Prentice-Hall, Inc., New Jersey, 620 pp.

Zimmerman, R. C. 1983. Seasonal patterns in the productivity of a giant kelp (Macrocystis pyrifera) forest: the effect of

nutrient availability. Ph.D. thesis, University of Southern California, Los Angeles, 182 pp.
Zimmerman, R. C. & Kremer, N. J. 1984. Episodic nutrient supply to a kelp forest ecosystem in southern California. *J. Mar. Res.* 42:591–604.
——— 1986. *In situ* growth and chemical composition of the giant kelp, *Macrocystis pyrifera*: response to temporal changes in ambient nutrient availability. *Mar. Ecol. Prog. Ser.* 27:277–85.
Zimmerman, R. C. & Robertson, D. L. 1985. Effects of El Niño on local hydrography and growth of the giant kelp, *Macrocystis pyrifera*, at Santa Catalina Island, California. *Limnol. Oceanogr.* 30:1298–1302.
Zimmerman, R. C., SooHoo, J. B., Kremer, J. N. & D'Argenio, D. Z. 1987. Evaluation of variance approximation techniques for non-linear photosynthesis-irradiance models. *Mar. Biol. (Berl.)* 95:209–15.

J. Phycol. **27**, 158–165 (1991)

NITROGENASE CONFINED TO RANDOMLY DISTRIBUTED TRICHOMES IN THE MARINE CYANOBACTERIUM *TRICHODESMIUM THIEBAUTII*[1]

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ABSTRACT

Nitrogenase reductase (Fe-protein) was detected in the marine planktonic cyanobacterium Trichodesmium. *The molecular weight was about 38 kD, as shown by western blotting using anti-*Rhodospirillum rubrum *nitrogenase reductase antiserum. The enzyme was confined to a limited number (ca. 10–40%) of randomly distributed trichomes in the* Trichodesmium *colonies, as shown by immunogold localization and transmission electron microscopy. Associated microorganisms had little or no nitrogenase. Nitrogenase showed a diel cycle in localization: present throughout the cytoplasm of cells in N_2-fixing (daytime) colonies but at the periphery of non-N_2-fixing (nighttime) colonies. This structural arrangement of N_2-fixing trichomes and nitrogenase is novel and different from the previously held paradigm for this and other diazotrophic cyanobacteria.*

Key index words: cyanobacterium; immunolocalization; nitrogenase; nitrogen fixation; Oscillatoria; *TEM;* Trichodesmium

Trichodesmium spp. are filamentous, non-heterocystous colony-forming cyanobacteria that comprise a major fraction of the planktonic flora of tropical seas (Fogg 1987, Carpenter 1983). *Trichodesmium* colonies are important in the global nitrogen budget, frequently form massive blooms, and are responsible for fixing 4.8 Tg of N_2 annually, about one quarter the total of the world's oceans (Capone and Carpenter 1982). It has been debated whether nitrogen fixation in *Trichodesmium* colonies is located in *Trichodesmium* or in other prokaryotic organism(s) associated with the colonies. Nitrogenase is an oxygen-labile enzyme, and *Trichodesmium* is unusual in that, if fixing nitrogen, it appears to lack any of the known strategies employed by other cyanobacteria for protecting nitrogenase. *Trichodesmium* colonies fix nitrogen under aerobic conditions in the light, the period when oxygenic photosynthesis also occurs (Carpenter 1983), whereas all other cyanobacteria examined separate the two processes spatially by forming thick-walled heterocysts (Wolk 1982) and temporally by fixing N_2 in the dark (Mullineaux et al. 1981, Mitsui et al. 1986, Stal and Krumbein 1987) or by confining the process to microaerobic conditions only (Stewart and Lex 1970, Weisshaar and Böger 1983).

We used western blotting and immunocytochemistry to determine the occurrence and localization of nitrogenase within the *Trichodesmium* colonies. Evidence is presented for the confinement of nitrogenase to the *Trichodesmium* trichomes but only to a restricted number. These trichomes show a random distribution within each colony; we suggest that these trichomes may be equivalent to the nitrogen-fixing heterocysts of other cyanobacteria. Furthermore, we noted significant diel changes in the subcellular distribution of nitrogenase within cells.

MATERIALS AND METHODS

Trichodesmium thiebautii Gomont colonies were collected from the upper 10 m with a 1-m-diameter, 250-μm-mesh net. Sampling took place 20 km SE of Bermuda in July 1989 (R/V "Weatherbird") and in the southwestern Sargasso Sea in September 1989

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