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Abstract
A study was undertaken to determine if natural growth irradiance regimes influence the pigment content and/or photosynthesis-irradiance relationships of the tropical brown seaweed, Lobophora variegata. Pigment analyses were performed on samples collected from a series of depths between 9 and 49m. Chlorophyll a, chlorophyll c, and fucoxanthin were constant on a thallus weight basis, but showed small decreases when expressed in terms of thallus area. The beta-carotene content of L. variegata was much greater in shallow than in deep-water samples regardless of the pigment units employed. Experimental reduction of the light regime of the shallow population, produced a significant decrease in beta-carotene content within 2 days. In 4 days, beta-carotene had decreased by 55% on a thallus weight basis, while no significant changes were found in any of the other three pigments.

Photosynthesis-irradiance (P-I) relationships were determined in situ for Lobophora variegata populations from a series of depths between 4.6 and 36.6m. Determinations were made for each population at its native depth, as well as at the spectral regimes of several other depths. The shallowest population examined was found to have a substantially higher rate of light saturated photosynthesis (P(\text{max})) compared with populations from greater depths. Further reductions of P(\text{max}) with depth were evident on a thallus area basis, but not on a thallus weight basis. The efficiency of low-light photosynthesis, indicated by the initial slope (alpha) of the P-I curve, was found to increase with depth regardless of the depth from which the L. variegata samples were collected.

Keywords
Biology, Botany
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PHOTOADAPTIVE RESPONSES IN THE TROPICAL BROWN SEAWEED,
LOBOPHORA VARIEGATA (LAMOUR.) WOMERS.

BY

CHRISTOPHER DAVID NEEFUS
B.A. (Biology), Boston University, 1971

A DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfilment of
the Requirements for the Degree of

Doctor of Philosophy
in
Botany

May, 1982
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ABSTRACT

PHOTOADAPTIVE RESPONSES IN THE TROPICAL BROWN SEAWEED, 
LOBOPHORA VARIEGATA (LAMOUR.) WOMERS.

By

Christopher David Neefus

University of New Hampshire, May, 1982

A study was undertaken to determine if natural growth 
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In 4 days, beta-carotene had decreased by 55% on a thallus 
weight basis, while no significant changes were found in any 
of the other three pigments.
Photosynthesis-irradiance (P-I) relationships were determined in situ for Lobophora variegata populations from a series of depths between 4.6 and 36.6m. Determinations were made for each population at its native depth, as well as under the spectral regimes at several other depths. The shallowest population examined was found to have a substantially higher rate of light saturated photosynthesis ($P_{\text{max}}$) compared with populations from greater depths. Further reductions of $P_{\text{max}}$ with depth were evident on a thallus area basis, but not on a thallus weight basis. The efficiency of low-light photosynthesis, indicated by the initial slope (alpha) of the P-I curve, was found to increase with depth regardless of the depth from which the L. variegata samples were collected.

The results indicate a gradual decrease with depth in the number of photosynthetic units (PSU's) on a thallus area basis, which is correlated with a decrease in thallus weight to area ratio. Growth in very high irradiance regimes appears to stimulate synthesis of beta-carotene and of photosynthetic electron transport or dark reaction constituents. The primary function of beta-carotene in shallow Lobophora variegata populations may be to provide protection from photodynamic injury, although there is some evidence to suggest that it has a significant role in
photosynthetic light collection. The increase in alpha with incubation depth suggests that the pigment antennae and thallus form of *L. variegata* are phylogenetically adapted for maximum low-light efficiency in deep-water spectral regimes.
PHOTOADAPTIVE RESPONSES IN THE TROPICAL BROWN SEAWEED, 
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PART 1.

PIGMENT CONTENT AND IN VIVO THALLUS ABSORPTION.
1.1 INTRODUCTION

Differences in photosynthetic pigment content with water depth or with reduced light levels have been found to occur in various green, red, and brown seaweeds. Rhee and Briggs (1977) found higher levels of phycoerythrin and chlorophyll in *Chondrus crispus* from shaded environments. *Dictyota dicotoma, Udotea petiolata, Ulva fenestrata,* and species of *Enteromorpha* have been shown to produce greater amounts of pigments in deep or shaded locations than in high light regimes (Titlyanov and Lee, 1978; Perez-Bermudez et al., 1981). Ramus and coworkers (1976a, 1977) have reported similar findings for levels of all major pigments in *Ulva lactuca, Codium fragile, Porphyra umbilicalis, Chondrus crispus, Fucus vesiculosus,* and *Ascophyllum nodosum.* Furthermore, they were able to demonstrate that the pigment content of the same six species could be changed by moving them to different light regimes. Wheeler (1980) produced a similar response in natural populations of *Macrocystis pyrifera* by vertically transplanting young sporophytes.

In addition to influencing total photosynthetic pigment content, the depth to which a seaweed is acclimated may affect the relative amounts of the various pigments present.
For algal species in which pigment content increases with depth, it has often been found that ratios of accessory pigments to chlorophyll a also increase (Duncan, 1973; Ramus et al., 1976a; Rhee and Briggs, 1977; Wheeler, 1980). In some cases, this results in spectral absorbance characteristics for the deeper plants which are more complementary to the spectral distribution of ambient light (Ramus et al., 1976b). Dring (1981) has recently cautioned that this apparent "chromatic adaptation" may be "merely an accidental byproduct" of physiological adaptation to low light levels since, in some species, similar changes in pigment ratios have been produced by reducing total irradiance without altering spectral distribution. There are also examples of species which have a maximum ratio of carotenoid:chlorophyll a in high light regimes; this has generally been attributed to a greater requirement in saturating light for the protective ability that carotenoids provide against photodynamic injury (Ramus et al., 1977; Krinsky, 1978; Titlyanov and Lee, 1978; Ley and Butler, 1980). Overall, however, differences with depth in total or relative pigment content, whether brought about by changes in total irradiance or spectral distribution, can generally be interpreted as photoadaptive responses to compensate for limiting light conditions, and therefore, to extend vertical distribution.
Lobophora variegata (Lamour.) Womers. is a brown seaweed in the order Dictyotales and is widespread in the tropics (Womersley, 1967). The species occurs over an extremely large range of depths; it is common intertidally in exposed areas (Taylor, 1972), and attached specimens have been collected at a depth of 220m (Earle, personal communication). The present study was initiated to determine if photoadaptive changes in pigment content and composition occur with depth in L. variegata that might contribute to its unusual vertical distribution.
1.2 MATERIALS AND METHODS

SITE DESCRIPTION

All of the samples of *Lobophora variegata* used in the present study were collected from the fringing reef at Bloody Bay (19°41'25''N, 80°04'12''W) off the north shore of Little Cayman Island, British West Indies. The same site was used for *in situ* shading experiments to examine photoadaptive changes in thallus pigmentation. Abundant populations of the decumbent form of *L. variegata* grow at 7-10m along the top of the deep fore-reef and extend downward along the vertical face to depths greater than 50m. The water temperature over this depth range was constant (29.5°±1°C). The water was extremely clear, with visibility consistently in excess of 30m. Sky conditions were generally clear prior to and during the field study. Most of the field work was completed during July 1980, although some additional pigment analyses were conducted in August 1981.
SAMPLE COLLECTION

The samples of Lobophora variegata used for pigment analysis and in vivo absorption scans were collected by SCUBA divers. Whole thalli were carefully removed from their substrata and brought to the surface in black plastic bags filled with seawater. The samples were transported to the laboratory where they were cleaned of visible epiphytes. Pigment analyses were begun within 2 hours of sample collection, except in August 1981 when extractions were delayed 24 hours while the samples were shipped via air for analyses at the University of New Hampshire.

PIGMENT ANALYSIS

The pigment contents were determined using a modification of the method described by Seeley et al. (1972). Discs, 17mm in diameter, were cut from Lobophora variegata samples, with 3 or 4 generally being removed from each thallus. The discs were rinsed quickly in freshwater and blotted dry. For each replicate, 10 to 14 discs (approximately 0.4g fresh weight) were weighed and placed in a 20ml polypropylene beaker with 5ml of dimethyl sulfoxide (DMSO). After 5 minutes, the DMSO extract was poured off, adjusted to volume, and diluted 1:5 with 4:1 DMSO:water. Concentrations of chlorophyll a, chlorophyll c, and fucoxanthin in the DMSO extract were calculated from
absorbances at 665, 631, 582 and 480nm read with a Beckman DB-G or a Beckman Model 35 dual beam spectrophotometer. The discs were further treated with two 5 minute extractions in 100% acetone. This was followed by re-hydration of the tissue in water and a final 1 hour extraction in acetone. At this point, the discs appeared colorless and were discarded. The acetone extracts were combined, adjusted to volume, shaken with 5ml hexane and 3.75ml water. The two phases were separated. The hexane phase was extracted twice with 1.7ml of 75% methanol and twice with 1.7ml of 80% methanol. The methanol and aqueous acetone phases were combined, adjusted to 25ml with acetone, and absorbance readings at 664, 631, 581 and 470nm were used to determine the concentrations of chlorophyll a, chlorophyll c, and fucoxanthin. Beta-carotene and chlorophyll a contained in the hexane phase were analyzed by diluting 1:5 with acetone and reading absorbance at 661 and 480nm. The concentrations in all phases were used in calculating thallus content of each pigment on an area basis (mg pigment per m² thallus) and a weight basis (mg pigment per g fresh weight).

SHADING EXPERIMENTS

Shading experiments were conducted in situ on a flat area of the reef at a depth of 9m. Pieces of substrata, with attached samples of Lobophora variegata, were placed under 30cm diameter plexiglass domes. The domes were
secured to the reef using pieces of stainless steel wire-rope and masonry nails. A space was left at the bottom edge of each dome to allow for water exchange. At the beginning of the experiment, one of the three domes was covered with a vinyl neutral density filter (Roscovin N9, Rosco Laboratories Inc., Port Chester, N.Y., U.S.A.) held in place by a 30cm diameter stainless steel band clamp. The filter reduced the quantum irradiance levels in the dome to approximately 13% of ambient. After 2 days, a second dome was covered with a similar filter. At the end of 4 days, the samples were removed from the 2 filtered domes and from the clear (control) dome, and pigment analyses were performed as described above.

THALLUS ABSORPTION MEASUREMENTS

Thallus absorption spectra of Lobophora variegata were measured in vivo with a method similar to that used by Ramus et al. (1976a) and to the opal glass method of Shibata et al. (1954). A strip of thallus was placed against the window of a 1cm spectrophotometer cuvet filled with filtered seawater. A very slightly oversized strip of membrane filter (Metricel GA-6, Gelman Sciences, Inc.) was carefully pressed into place against the sample. The sample cuvet and a reference cuvet, containing only seawater and a filter strip, were positioned at the detector end of the beam paths in a Beckman Model 35 spectrophotometer. The cuvets were
oriented such that the filter strips faced the detector and the thallus strip faced the source. Absorption scans were made from 400 to 700 nm. As pointed out by Shibata et al. (1954), the filter strips uniformly diffuse the light leaving each cuvet irrespective of whether or not the light entering the cuvet is scattered by a thallus strip. Close contact between the thallus strip and the filter ensures that the light transmitted by the sample reaches the diffuser. The method should provide a good estimate of transmittance, while absorbance is likely to be overestimated since the sample reflects and scatters some light back toward the source.

QUANTUM IRRADIANCE MEASUREMENTS

Measurements of total downwelling quantum irradiance at the sample collection depths and in the shaded domes were made with a Li-cor LI 185 Quantum Meter (Lambda Instruments, Lincoln, Nebraska, U.S.A.) with a submersible sensor (LI 192S). The spectral distribution of light at various depths was determined with a submersible spectroradiometer designed and built at the Harold E. Edgerton Research Laboratories of the New England Aquarium, Boston (Neefus and McLeod, 1974). Both instruments were calibrated in units of quantum irradiance over a 400-700 nm spectral bandwidth. Each instrument was equipped with a cosine irradiance collector, which should simulate quite closely, the light collecting geometry of the flat, decumbent Lobophora variegata thallus.
Diffuse spectral attenuation coefficients (k), determined at 10nm intervals were used to calculate estimates of spectral irradiance at depths other than measurement depths. Attenuation coefficients were calculated as:

\[ k = \frac{\ln(n_1/n_2)}{(z_2-z_1)} \]

where \( n_1 \) and \( n_2 \) are spectral quantum irradiances at depths \( z_1 \) and \( z_2 \) meters, respectively (Tyler and Smith, 1970; Tyler, 1976).
1.3 RESULTS

Thalli of *Lobophora variegata* from shallow (about 9m), unshaded locations had a darker, more orange coloration than deep-water specimens (>30m). A continuous gradation of color was not found, rather with increasing depth, a progressively lower frequency of dark, orange individuals was present.

Shallow and deep specimens, examined microscopically in cross section, were five cell layers thick, with a central medullary layer bordered on either side by single layers of subcortex and cortex. In shallow samples, the lower subcortical and cortical layers were orange-brown and optically dense relative to the upper two layers which appeared lighter and fairly green.

In addition to differences in coloration, the shallow samples were generally smaller in diameter. At 9m the suborbicular to kidney-shaped thalli were commonly 3 to 5cm across, while those at 49m were more typically 6 to 8cm. The thalli of deep-water samples tended to have more perfect margins, while the shallow samples were frequently jagged or cleft. Deep samples also appeared to be thinner and more translucent than shallow samples.
PIGMENT CONTENT

Photosynthetic pigment content was determined for *Lobophora variegata* samples collected at depths ranging from 9 to 49m. An initial set of analyses was conducted in July 1980, using plants collected from 9, 32, and 49m. In August 1981, a second pigment content profile was made using smaller depth intervals near the surface (i.e. 9, 14, 18, 23, and 44m).

No significant differences (p<0.05) in chlorophyll a, chlorophyll c, or fucoxanthin content were found in either set of determinations when calculations were based on thallus fresh weight (Figure 1-1, Table 1-1). In contrast, beta-carotene levels were substantially higher in the shallowest samples of each series. In the July 1980 data, the mean beta-carotene at 9m was 0.869mg·gfw⁻¹. The level dropped to 0.102mg·gfw⁻¹ at 32m, and no significant difference was found between 32 and 49m. The August 1981 profile indicated that the decrease in beta-carotene occurred between 9 and 14m, where the mean values dropped from 1.002 to 0.098mg·gfw⁻¹, and that no further significant change occurred below this depth.

Small, but significant (p<0.05), differences with depth were found in chlorophyll a, chlorophyll c, and fucoxanthin when pigment content was expressed on a basis of thallus
area, rather than weight (Figure 1-2, Table 1-II).

Chlorophyll a values in the July 1980 data decreased from 102.1mg·m⁻² at 9m to 79.8mg·m⁻² at 49m. Chlorophyll c content decreased from 48.8 to 33.2mg·m⁻² between 9 and 32m, but increased to 44.3mg·m⁻² at 49m; only the value at 32m was significantly different (p<0.05) from the value at each other depth. Fucoxanthin content at 9m was 77.7mg·m⁻² and declined to 60.4mg·m⁻² at 49m. Trends in beta-carotene content expressed on a thallus area basis were not different than those found using thallus weight. Levels of beta-carotene dropped from 117.6mg·m⁻² at 9m to 12.7 and 14.6mg·m⁻² at 32 and 49m, respectively.

Ratios of thallus weight to area were calculated for all _L. variegata_ samples from the July 1980 and August 1981 profiles. The mean values and 95% confidence intervals at each depth are included in Table 1-II. In the 1980 samples, an 11% decrease in weight per unit area occurred between 9 and 49m; the change appeared to be fairly linear.

**SHADING EXPERIMENTS**

The results of shading experiments carried out in situ at a depth of 9m, are shown in Figure 1-3. After two days in a light regime 87% below normal ambient quantum irradiance, _Lobophora variegata_ showed significantly lower (p<0.05) levels of beta-carotene relative to controls.
Samples kept under shaded domes for four days had a mean beta-carotene content of 0.388 mg gfw⁻¹, which is 55% lower than the 0.862 mg gfw⁻¹ found in samples from clear control domes. No significant differences (p<0.05) in chlorophyll a, chlorophyll c, or fucoxanthin were found between shaded samples and controls.

THALLUS ABSORPTION

In vivo absorption spectra of Lobophora variegata specimens from 9 and 44m are compared in Figure 1-4. Absorbance values for the 9m thallus exceeded those for the 44m thallus throughout the visible spectrum (400 to 700 nm). In addition, the absorption spectrum for the 9m samples had a wide shoulder between 480 and 550 nm, which was not apparent for the 44m sample. Over the blue and green regions, the absorption spectrum of the 44m sample was generally narrower than in the 9m thallus, and had a more clearly defined peak at about 438 nm and shoulder at 460 nm.

PREDICTED QUANTUM ABSORPTION

From measurements of downwelling spectral quantum irradiance made in the water column, and from in vivo thallus absorption spectra, it is possible to estimate the amount and spectral distribution of light that would be absorbed by and/or transmitted by a thallus at various
depths. Midday spectral quantum irradiance data from 9m were used to predict curves for curves for quanta absorbed and transmitted by a *Lobophora variegata* thallus grown at that depth (Figure 1-5). The total quanta available, calculated by integrating the 9m spectral irradiance curve, is $535.1 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Of this amount, it was predicted that $522.9 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ would be absorbed by the thallus and $12.2 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ would be transmitted.

The same method was used to predict the light absorbed and transmitted by a thallus from 44m moved to 9m (Figure 1-6). Under these conditions, the deep-water thallus would absorb only $365.4 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, while $169.7 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ would pass through it. Most of the light transmitted by the thallus would be in the 500 to 600nm region of the spectrum, with a maximum quantum flux density at 560nm. The same thallus at its native depth would transmit only $15.0 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a spectral peak at 490nm (Figure 1-7). Finally, if a thallus growing at 9m were moved to 44m, it would absorb $54.6 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of the available $55.5 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and transmit $0.9 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Figure 1-8).

A summary of the total quanta available at 9 and 44m and the predicted values of quanta absorbed and transmitted at each depth for *Lobophora variegata* thalli from 9 and 44m is given in Table 1-III. The same table also indicates the amount of light absorbed as a percentage of the total quanta
available. A thallus from 44m would absorb 68.3% of the quanta incident upon it at 9m, while at 44m it absorbs 73.0%, a difference of 4.7%. The difference for 9m thallus predictions is only 0.7%, since the absorbed light is 97.7% and 98.4% of that incident at 9 and 44m, respectively.
1.4 DISCUSSION

In addition to serving as light harvesting pigments, carotenoids in the photosynthetic antenna complex perform an important protective role, described in detail by Krinsky (1978). Through very rapid quenching of both triplet chlorophyll and singlet oxygen, carotenoids are capable of preventing photodynamic chlorophyll destruction which might otherwise occur at high irradiance levels. Although this protective function of carotenoids is attractive as an explanation of the elevated beta-carotene levels found in shallow populations of Lobophora variegata, it does not explain the observations noted for thallus cross-sections. If it is assumed that the relatively dense orange-brown coloration of the lower cortex and subcortex indicate higher levels of carotenoids, why would the lower layers seemingly require greater protection? Furthermore, it is the beta-carotene molecules associated with the reaction center chlorophylls that act as "safety valves" to protect the photosynthetic apparatus, and singlet oxygen is very effectively quenched by low concentrations of beta-carotene (Foote and Denny, 1968; Cogdell, 1978; Oquist et al., 1980). Therefore, the levels of beta-carotene found in the shallow L. variegata samples may be greatly in excess of the amount
required for photoprotection. Although beta-carotene undoubtedly does protect *L. variegata* from photodynamic injury, additional explanations should be considered for the changes in pigmentation observed with depth.

For a seaweed growing at any depth, there exists a theoretical physiological limit, beyond which the energy required to synthesize and maintain additional photosynthetic apparatus per unit area becomes greater than the additional energy that would be trapped. Ramus et al. (1976b) concluded that the strategy of seaweeds is not to maximize the amount of light harvesting pigments produced, but rather to optimize this amount with respect to the ambient light regime.

*Lobophora variegata* growing at 9m transmits only a small amount of the light it receives; 12.2uE·m⁻²·s⁻¹ according to the midday estimate (Table 1-III). Although it is somewhat coincidental, since beta-carotene content does not decrease linearly with depth, nearly the same amount of light, 15.0uE·m⁻²·s⁻¹, would be transmitted at midday by a thallus growing at 44m (Table 1-III). These light levels are approximately equal to ambient quantum irradiance levels at a depth of 65m (Table 1-III), which is just below the lower distributional limit of *L. variegata* at the study site. Perhaps at each depth, the light transmitted by the thallus is an amount that cannot be efficiently captured.
If 9m populations of *Lobophora variegata* had the same pigment content and spectral absorption characteristics as deep growing thalli, the predicted quantity of light transmitted would be 169.7uE\cdot m^{-2}\cdot s^{-1} (Table 1-III). This is a substantial amount of light. In fact it is more than three times the total quantum irradiance available to the populations growing successfully at 44m. Thus, by containing additional amounts of all pigments (on a thallus area basis), but primarily beta-carotene, the shallow plants are able to collect considerably more light. In contrast, if the light harvesting ability of a 44m plant were increased, the additional light collected could not exceed 15.0uE\cdot m^{-2}\cdot s^{-1} regardless of the amount of additional pigments produced.

The spectral distribution of downwelling quantum irradiance at 9m (Figure 1-6) is broad compared with that at 44m (cf. Figure 1-7). At 9m, a thallus with the spectral absorption characteristics of those growing at 44m, would transmit the greatest amounts of light in the blue-green (480 to 590nm) region of the spectrum (Figure 1-7). Because light in this spectral region is more effectively absorbed by carotenoids than by chlorophylls (Goedheer, 1970), elevated levels of beta-carotene would result in a thallus absorption spectrum that is better suited to the 9m spectral regime. Evidence of this can be seen in the spectral distribution curve for light transmitted by a 9m thallus at its native depth (cf. Figures 1-6 and 1-7).
With increasing depth, the spectral distribution of ambient light becomes narrower with proportionally less light available above 500nm (Figure 1-7). Consequently, the ability of a thallus to absorb light above 500nm becomes progressively less important with depth. A thallus from 44m, with its lower beta-carotene content, would absorb 68% of the light available at 9m while at its native depth, it would absorb 73% of the light it receives (Table 1-III). These results support the prediction made by Dring (1981), that in clear oceanic water, thin brown seaweeds are chromatically better adapted for photosynthesis at greater depth. This is not meant to imply that additional beta-carotene would not improve the absorption characteristics of Lobophora variegata thallus growing at 44m relative to its ambient light regime; the light it transmits does overlap with the spectral region of carotenoid absorption (Figure 1-7). What is suggested, is that the spectral absorption characteristics of L. variegata thalli that lack elevated beta-carotene levels, are best suited to the spectral regime in deep water.

Although the evidence presented might lead one to a rather unusual interpretation of the concept of complementary chromatic adaptation, the results of the shading experiments indicate that beta-carotene content in Lobophora variegata responds to changes in total quantum irradiance levels that occur without alteration of the
spectral distribution. This is not surprising since similar
dependence of carotenoid levels on total irradiance has been
reported for other species of brown seaweeds, as well as for
species of red and green algae (Ramus et al., 1977;
Titlyanov and Lee, 1978; Ley and Butler, 1980). Enhanced
chromatic suitability is provided by elevated beta-carotene
levels in shallow _L. variegata_ populations, however, as
Dring (1981) has suggested, this may be a "by-product" of a
response to the quantity of light received.

Ramus et al. (1976b) pointed out that cells within a
seaweed thallus are subject to shading by overlying layers;
consequently, the irradiance throughout the thallus is
heterogeneous with respect to both quantity and spectral
quality. In the case of _Lobophora variegata_, the upper
cortex and subcortex receive more incident light than the
lower layers. If the pigmentation of all cell layers were
equal, the upper half of the thallus would absorb more light
than the lower half; the difference would be amplified in
highly pigmented thalli. This inequality would be reduced
if the upper cell layers produced less pigment than the
lower layers. Furthermore, if the composition of the
pigment antennae in the lower layers were relatively high in
carotenoids, the spectral absorbance characteristics would
complement the spectral distribution of light transmitted by
chlorophylls of the upper layers. The effectiveness would
be greatest in shallow water, where the ambient spectral
regime is broad and contains light in the absorption bands of the chlorophylls and carotenoids. It is not known what triggers the differences observed between the upper and lower layers of *L. variegata* thalli from 9m. It would be interesting to determine quantitatively whether differences in pigmentation occur in response to differences in the quantity or the spectral quality of light in various cell layers.

In summary, beta-carotene in *Lobophora variegata* may perform a more significant role in shallow-water light regimes than it could at depth. The protective function of carotenoids is more important at high light levels. Greater thallus light absorption over a broader spectral range produces a substantial gain in the amount of energy collected in shallow water, while in deep water, the gain would be quite small. In a relatively broad spectral regime, a predominance of beta-carotene in lower cell layers of the thallus, might allow absorption of light transmitted by chlorophyll in the upper layers; with depth, the ambient spectral distribution becomes narrow and both pigments must compete for light in the same spectral range.

Embodied in the classical concept of chromatic adaptation, is the idea that seaweeds become more dependent on accessory pigments with depth. In the case of *Lobophora variegata*, the opposite appears to be true. The results of the present study also have interesting implications for the
concept of "intensity" adaptation; *L. variegata* exists over an extensive depth range while, when expressed on a thallus area basis, its contents of all photosynthetic pigments decrease with depth.
TABLE 1-1: Mean pigment content per gram fresh weight of Lobophora variegata at depths ranging from 9 to 49m. 95% confidence intervals are indicated in parentheses.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Chlorophyll a (mg·g⁻¹)</th>
<th>Chlorophyll c (mg·g⁻¹)</th>
<th>Fucoxanthin (mg·g⁻¹)</th>
<th>Beta-Carotene (mg·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 1980</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.76 (0.72-0.81)</td>
<td>0.36 (0.33-0.39)</td>
<td>0.58 (0.54-0.61)</td>
<td>0.87 (0.72-1.02)</td>
</tr>
<tr>
<td>32</td>
<td>0.76 (0.63-0.86)</td>
<td>0.27 (0.21-0.33)</td>
<td>0.50 (0.39-0.61)</td>
<td>0.10 (0.050-0.16)</td>
</tr>
<tr>
<td>49</td>
<td>0.66 (0.61-0.72)</td>
<td>0.37 (0.32-0.42)</td>
<td>0.50 (0.45-0.55)</td>
<td>0.12 (0.10-0.14)</td>
</tr>
<tr>
<td>August 1981</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.63</td>
<td>0.32</td>
<td>0.49</td>
<td>1.00</td>
</tr>
<tr>
<td>14</td>
<td>0.91 (0.73-1.09)</td>
<td>0.24 (0.17-0.32)</td>
<td>0.54 (0.47-0.61)</td>
<td>0.098 (0.058-0.139)</td>
</tr>
<tr>
<td>18</td>
<td>0.65 (0.49-0.82)</td>
<td>0.15 (0.06-0.24)</td>
<td>0.39 (0.27-0.52)</td>
<td>0.067 (0.023-0.111)</td>
</tr>
<tr>
<td>23</td>
<td>0.70 (0.52-0.87)</td>
<td>0.21 (0.06-0.36)</td>
<td>0.40 (0.31-0.49)</td>
<td>0.058 (0.043-0.073)</td>
</tr>
<tr>
<td>44</td>
<td>0.73 (0.61-0.84)</td>
<td>0.24 (0.19-0.29)</td>
<td>0.44 (0.37-0.51)</td>
<td>0.042 (0.013-0.071)</td>
</tr>
</tbody>
</table>
TABLE I-II: Mean pigment content per unit thallus area, and thallus weight to area ratios of *Lobophora variegata* at depths ranging from 9 to 49m. 95% confidence intervals are indicated in parentheses.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Chlorophyll a (mg·m⁻²)</th>
<th>Chlorophyll c (mg·m⁻²)</th>
<th>Fucoxanthin (mg·m⁻²)</th>
<th>Beta-Carotene (mg·m⁻²)</th>
<th>Weight:Area (g·m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 1980</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>102.1 (98.6-105.6)</td>
<td>48.8 (44.6-53.0)</td>
<td>77.7 (72.9-82.5)</td>
<td>117.6 (95.9-139.3)</td>
<td>134.8 (129.4-140.2)</td>
</tr>
<tr>
<td>32</td>
<td>93.7 (81.8-105.6)</td>
<td>33.2 (29.1-37.3)</td>
<td>61.4 (48.9-73.9)</td>
<td>12.7 (6.8-18.6)</td>
<td>126.8 (112.8-140.8)</td>
</tr>
<tr>
<td>49</td>
<td>79.8 (75.4-84.2)</td>
<td>44.3 (39.6-49.0)</td>
<td>60.4 (56.3-64.5)</td>
<td>14.3 (12.0-17.3)</td>
<td>120.5 (116.5-124.5)</td>
</tr>
<tr>
<td>August 1981</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>129.2 (106.3-152.1)</td>
<td>34.5 (25.4-43.6)</td>
<td>77.3 (68.5-86.1)</td>
<td>14.0 (8.0-20.0)</td>
<td>142.7 (133.7-151.7)</td>
</tr>
<tr>
<td>14</td>
<td>93.2 (69.7-116.7)</td>
<td>22.2 (3.1-41.3)</td>
<td>56.0 (42.6-69.5)</td>
<td>9.4 (4.5-14.3)</td>
<td>146.7 (80.9-212.5)</td>
</tr>
<tr>
<td>18</td>
<td>99.5 (81.9-117.1)</td>
<td>29.1 (12.1-46.1)</td>
<td>56.9 (47.9-66.0)</td>
<td>8.2 (7.2-9.3)</td>
<td>145.3 (122.6-168.0)</td>
</tr>
<tr>
<td>23</td>
<td>84.7 (80.1-89.3)</td>
<td>27.5 (25.5-29.5)</td>
<td>50.9 (46.1-55.7)</td>
<td>4.9 (1.9-7.8)</td>
<td>116.7 (99.3-134.1)</td>
</tr>
</tbody>
</table>


TABLE 1-III: Mean total quantum irradiance at midday, and predicted quanta absorbed and transmitted at 9 and 44 m by *Lobophora variegata* thalli from each depth.

<table>
<thead>
<tr>
<th>Depth</th>
<th>Total Quantum Irradiance (m)</th>
<th>Total Quantum Absorbed (μE m⁻² s⁻¹)</th>
<th>Percent of Ambient Quantum Absorbed (%)</th>
<th>Total Quantum Transmitted (μE m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>535.1</td>
<td>522.9</td>
<td>97.7%</td>
<td>12.2</td>
</tr>
<tr>
<td>44</td>
<td>55.5</td>
<td>54.6</td>
<td>98.4%</td>
<td>0.9</td>
</tr>
<tr>
<td>65</td>
<td>13.8*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Extrapolated value.
FIGURE 1-1: Mean pigment content per gram fresh weight of *Lobophora variegata* as a function of growth depth. Error bars indicate 95% confidence intervals. July 1980 values are connected by solid lines, those from August 1981 are connected by dashed (---) lines.
FIGURE 1-1

Pigment Content (mg·g⁻¹)

- Chlorophyll a
- Chlorophyll c
- Fucoxanthin
- Beta-Carotene

Depth (m)
FIGURE 1-2: Mean pigment content per unit thallus area of Lobophora variegata as a function of growth depth. Error bars indicate 95% confidence intervals. July 1980 values are connected by solid lines, those from August 1981 are connected by dashed (---) lines.
Pigment Content (mg·m⁻²)

FIGURE 1-2

Depth (cm)

Beta-Carotene
Fucaxanthin
Chlorophyll a
Chlorophyll b
FIGURE 1-3: Mean pigment content per gram fresh weight of Lobophora variegata as a function of time in experimentally reduced light regime (13% of ambient). Error bars indicate 95% confidence intervals.
FIGURE 1-3

Chlorophyll a

Chlorophyll c

Fucoxanthin

Beta-Carotene

Pigment Content (mg·g⁻¹)

Time (days)
FIGURE 1-4: *In vivo* thallus absorption spectra of *Lobophora variegata* specimens collected from depths of 9 and 44m.
Absorbance (A) vs. Wavelength (nm)

FIGURE 1-4
FIGURE 1-5: Midday quantum spectral irradiance at a depth of 9m (octagons), and predicted spectral distributions of quanta absorbed (triangles) and transmitted (squares) at 9m by a *Lobophora variegata* thallus growing at that depth. The numbers next to each curve indicate the total quanta (uE m⁻² s⁻¹) in the 400-700nm spectral band.
FIGURE 1-5
FIGURE 1-6: Midday quantum spectral irradiance at a depth of 9m (octagons), and predicted spectral distributions of quanta absorbed (triangles) and transmitted (squares) at 9m by a Lobophora variegata thallus from a depth of 44m. The numbers next to each curve indicate the total quanta (uE·m⁻²·s⁻¹) in the 400-700nm spectral band.
FIGURE 1-6
FIGURE 1-7: Midday quantum spectral irradiance at a depth of 44m (octagons), and predicted spectral distributions of quanta absorbed (triangles) and transmitted (squares) at 44m by a Lobophora variegata thallus growing at that depth. The numbers next to each curve indicate the total quanta (µE·m⁻²·s⁻¹) in the 400-700nm spectral band.
FIGURE 1-8: Midday quantum spectral irradiance at a depth of 44m (octagons), and predicted spectral distributions of quanta absorbed (triangles) and transmitted (squares) at 44m by a Lobophora variegata thallus from a depth of 9m. The numbers next to each curve indicate the total quanta (uE·m⁻²·s⁻¹) in the 400-700nm spectral band.
FIGURE 1-8
1.5 LITERATURE CITED


PHOTOADAPTIVE RESPONSES IN THE TROPICAL BROWN SEAWEED,
LOBOPHORA VARIEGATA (LAMOUR.) WOMERS.

PART 2.

EFFECT ON IN SITU PHOTOSYNTHESIS-IRRADIANCE RELATIONSHIPS
2.1 INTRODUCTION

In the preceding paper (Part I), it was shown that the photosynthetic pigment content of the tropical brown seaweed, Lobophora variegata (Lamour.) Womers., exhibits differences with depth that are inconsistent with classical concepts of both quantitative ("intensity") photoadaptation and complementary chromatic photoadaptation. Rather than increasing with depth, chlorophyll a, chlorophyll c, fucoxanthin and beta-carotene decreased on a thallus area basis. In addition, the relative content of the accessory pigment beta-carotene was much greater in the shallow populations than at depth. The gradual changes in pigment content with depth can be attributed to morphological, rather than physiological, differences because the changes were paralleled by a decrease in thallus weight to area ratio, and because pigment contents did not change on a thallus weight basis. An exception was found in beta-carotene content which was high in the shallow (9m) population, but dropped 90% by 14m regardless of the thallus units chosen. Physiological control of beta-carotene content was indicated by the loss of the pigment from thalli under experimentally reduced light regimes.
Lobophora variegata has a remarkable depth range; it occurs intertidally (Taylor, 1972) and has been collected at a depth of 220m (Earle, personal communication). In light of this, it was suggested that the differences in pigment content and in weight to area ratio might have implications for photosynthetic performance and possibly represent photoadaptive responses to growth irradiance conditions.

Differences in pigment content in response to depth or light regime are generally interpreted as differences in either the density or the size of photosynthetic units (PSU's) in the thallus, or both (Prezelin, 1976; Ramus and coworkers, 1976a, 1976b, 1977; Wheeler, 1980). The density of PSU's (number per unit of thallus) can potentially affect the light saturated rate of photosynthesis ($P_{\text{max}}$) as well as photosynthetic performance at lower light level (Prezelin, 1976). This would be true whether PSU density is determined physiologically or morphologically, as long as photosynthetic rate and PSU density are expressed in terms of the same denominator (eg. per thallus area). The size of the PSU, or more specifically the amount of pigment associated with its light-harvesting antennae, has an effect on light limited photosynthetic rate, but not on $P_{\text{max}}$ (Prezelin, 1976).
Although quantitative changes in pigmentation are probably the most frequently reported photoadaptive responses in seaweeds (e.g. Duncan, 1973; Ramus and coworkers, 1976a, 1977; Rhee and Briggs, 1977; Titlyanov and Lee, 1978; Wheeler, 1980; Perez-Bermudez et al., 1981), growth irradiance has been shown to influence, in algae as well as in higher plants, a number of other responses that may affect photosynthetic performance and/or photosynthetic capacity ($P_{\text{max}}$). Among these responses are: alterations in chloroplast orientation (Nultsch and Pfau, 1979, 1981; Ruffer et al., 1981); changes in the amount of photosynthetic electron transport or dark reaction constituents (Beardall and Morris, 1976; Boardman, 1977); variations in morphology (see Luning, 1981); changes in the relative distribution of pigments between photosystems I and II (Ley and Butler, 1980); conformational changes in thylakoid membranes (Govindjee et al., 1979; Jeffreys and Vest, 1978); and, possibly, differences in the distribution of pigments among various parts of the thallus (see Part I).

The present study was designed to examine the totality of effects produced by those photoadaptive responses that occur in Lobophora variegata, with respect to photosynthetic performance and capacity. Although some photoadaptive responses are controlled by total irradiance levels, which means that they should technically be regarded as quantitative photoadaptation (Dring, 1981), their effects on
photosynthesis may depend upon the spectral distribution of light available. It was therefore important that the photosynthetic response of the seaweed be examined in situ, rather than under a more arbitrary spectral regime imposed by laboratory conditions. By using neutral density filters to vary total photon flux density, photosynthesis-irradiance relationships have been determined in the present study for \textit{L. variegata} populations from a series of depths. Determinations were made for each population first at its native depth, and secondly, under the spectral regimes at various other depths. The data obtained allow the comparison of populations acclimated to different light regimes on the basis of: light saturated photosynthetic rates ($P_{\text{max}}$); photosynthetic performance in response to different quantum flux densities at several spectral regimes; and photosynthetic performance in response to different spectral distributions at constant total quantum flux density. This information, combined with the results of the preceding pigment studies (Part I), provides insight about the photodaptive "strategy" of \textit{L. variegata} and contributes to an explanation of its extensive vertical distribution.
2.2 MATERIALS AND METHODS

SITE DESCRIPTION

Photosynthesis studies were carried out in situ on the coral reefs of the small island of Utila, one of the Islas de la Bahia off the Caribbean coast of Honduras. The field work was conducted during July and August of 1977 through 1979. Two sites were used; the first was located off the southeastern point of the island (16°04'55''N, 86°53'30''W) where a nearly vertical reef extends from the surface to a depth of about 20m. Abundant populations of the decumbent form of *Lobophora variegata* were present from 4m to the bottom of the reef where the substrata changed to sand. The second site was located at Turtle Harbor along the north shore (16°06'45''N, 86°57'15''W); here, the vertical "wall" starts at approximately 6m and descends beyond 60m. The lower distributional limit of *L. variegata* at this site was found near 50m. Temperature and salinity at both sites were consistently found to be 29°C(+1°C) and 36ppt (+1ppt) respectively, and no differences occurred with depth from 4 to 40m. Underwater visibility generally exceeded 25m, although some fluctuations were noted following infrequent storms or high winds. Sky conditions were generally clear,
with some cloud build-up towards late afternoon. Overcast skies and rainy weather were rare.

PHOTOSYNTHESIS MEASUREMENTS

Photosynthetic rates were calculated from oxygen exchange in 310ml "BOD" bottles. Dissolved oxygen rates were determined with a modified Winkler method adapted for microburette (Gilmont 2.0ml Micrometer Burette) titration (See APPENDIX). Incubations were set up underwater by SCUBA divers. At each incubation depth, a rack of six BOD bottles was clipped to loops in a weighted line suspended from the surface and tethered to a coral outcropping below. A second set of racks was attached to a similar, adjacent line. Vinyl neutral density filters (ROSCOVIN, Rosco Laboratories Inc., Port Chester, N.Y., U.S.A.) were attached to bottles in each rack to produce a series of stepwise reductions in total irradiance. A "dark bottle", covered with a triple layer of black vinyl tape, was included in each rack to determine dark (mitochondrial) respiration. Water in each bottle was exchanged at depth with the surrounding medium by means of a 1 liter syringe type, suction gun; additional bottles ("initials"), filled in the same way, were immediately taken to the surface and "fixed" for Winkler titration.
Samples of *Lobophora variegata* were selected by divers for uniform size and condition, carefully removed from the reef, and gently freed of visible epiphytes and debris. When samples were collected below the incubation depth, they were transported to the BOD bottles in black plastic bags. An entire thallus (approximately 30 to 50mg dry wt) was enclosed in each bottle on one of the two lines. The bottles on the second line were incubated without samples and served as "blanks" to correct for D.O. changes not resulting from photosynthesis or respiration of the seaweed; generally, no significant differences in D.O. were found among the blanks or between the blanks and initials. Incubations lasted 3 to 4 hours and were centered around solar noon. At the end of this period, the racks were retrieved to the surface where aliquots of water were transferred from each enclosure to 60ml D.O. bottles and "fixed" for subsequent titrations.

Apparent photosynthetic rates were calculated on the basis of both thallus area and thallus ash-free dry weight. After incubation, each *Lobophora variegata* sample was placed on a sheet of photographic paper (Kodak Studio Proof) and exposed briefly to direct sunlight; the area of the image was determined with a compensating polar planimeter (Keuffel and Esser Model 62 0005). The samples were dried in a vacuum oven at 90°C for 48h, weighed, combusted in a muffle furnace at 550-600°C, and weighed again.
BOTTLE EFFECT DETERMINATIONS

Littler (1979) has demonstrated that a number of "bottle effects" can influence seaweed photosynthetic rate measurements in closed containers, and has made suggestions to minimize such errors. A method similar to that described by Littler (1979) was used to determine if elevated oxygen tension in the bottles caused suppressed rates of measured photosynthesis in *Lobophora variegata*. Samples were enclosed in BOD bottles filled with seawater containing low (0.9 mg·l⁻¹), saturated (7.5 mg·l⁻¹), and high (20.0 mg·l⁻¹) levels of dissolved oxygen. Incubations (4h) were carried out at the surface under 0.3 neutral density filtration (approximately 50% transmission).

To determine whether depletion of nutrients in the enclosures during the course of the incubation had an effect on photosynthetic rate, samples were incubated in bottles containing seawater enriched with nitrate and phosphate by 0, 12.5, 25, 50 and 100 mg·l⁻¹. Enrichment solutions were made using the sodium salt of each nutrient. Incubations were carried out at 9m for 4h.

The effect of incubation period duration was determined by comparing measured photosynthetic rates of samples enclosed in BOD bottles for 1, 2, 3, 4 and 5h. These incubations were carried out at 9m.
The thallus weight to bottle volume ratio selected for the photosynthesis measurements was somewhat higher than that suggested by Littler (1979). Littler's recommended value (0.030 g dry wt·l⁻¹) was intended for incubations in clear, unfiltered bottles at surface irradiance levels. In the present study, larger *Lobophora variegata* samples were required to produce measurable responses in experimentally reduced light regimes. Samples used were generally between 20 and 50 mg dry wt which is equivalent to 0.060 to 0.150 g dry wt·l⁻¹.

**QUANTUM IRRADIANCE MEASUREMENTS**

During incubations, total downwelling and upwelling quantum flux density measurements were made using a Li-cor LI 185 Quantum Meter (Lambda Instruments, Lincoln, Nebraska, U.S.A.) with a submersible sensor (LI 192S) positioned by a diver at the level of each bottle rack. One set of measurements was made immediately after the incubations were set up, and a second shortly before the bottles were returned to the surface. Surface quantum irradiance measurements were made at half hour intervals throughout the incubation period.

Midway through the incubation, a series of upwelling and downwelling spectral quantum irradiance measurements were made using a submersible spectroradiometer which was
designed and built at the Harold E. Edgerton Research Laboratory of the New England Aquarium, Boston (Neefus and McLeod, 1974). This instrument, as well as the Li-cor Quantum Meter, had a cosine irradiance collector. The collector geometry was considered to be particularly appropriate for determining the light received by the flat, decumbent thalli of *Lobophora variegata*.

Diffuse attenuation coefficients for the intervals between quantum irradiance measurement depths were calculated from the equation:

$$k = \ln\left(\frac{n_1}{n_2}\right) / (z_2 - z_1)$$

where $n_1$ and $n_2$ are quantum flux densities at depths $z_1$ and $z_2$ meters, respectively (Smith and Tyler, 1970; Tyler, 1976).

Spectral absorption scans (400 - 700nm) of the vinyl neutral density filters were made using a Beckman Model 35 Spectrophotometer. This information was used in conjunction with spectral quantum irradiance measurements to determine the reduction in quantum flux density for filtered incubation enclosures at each depth.
DATA ANALYSIS

Data reduction, analysis, and plotting were performed on the DEC-10 Computer System and Calcomp-936 plotter at the University of New Hampshire. With the exception of the curve-fitting procedures described below, all operations were done with FORTRAN software developed specifically for the present study.

Direct, non-linear parameter estimation was used to fit the hyperbolic tangent model of Jassby and Platt (1976) to the photosynthesis-irradiance (P-I) data. The model was used in the form:

\[ P_{\text{app}} = P_{\text{gmax}} \cdot \tanh(\alpha I / P_{\text{gmax}}) - R \]  \hspace{1cm} (eq. 2)

where \(P_{\text{app}}\) is the rate of "apparent" photosynthesis; \(I\) is total quantum flux density; \(P_{\text{gmax}}\) is "gross" photosynthetic capacity; \(\alpha\) is the initial slope of the P-I curve and is proportional to photosynthetic efficiency at low light levels; and \(R\) is an estimate of oxygen uptake at zero irradiance and therefore approximates dark (autotrophic) respiration. The model was fitted to "apparent" photosynthesis data. Because \(P_{\text{gmax}}\) is implicitly equivalent to the sum of "apparent" photosynthetic capacity (\(P_{\text{max}}\)) and \(R\), and because \(R\) is not the respiration rate at saturating light levels, it was considered most accurate to convert the fitted values of \(P_{\text{gmax}}\) ("gross") to \(P_{\text{max}}\) ("apparent").
The parameters $P_{\text{max}}$, alpha, and $R$ were estimated by the computer program MLAB (Knott, 1979), which employs the Marquardt-Levenberg curve-fitting algorithm to minimize the sum of squared errors (SSE). Using the "grand SSE" criterion suggested by Lederman and Tett (1981), eight different photosynthesis-irradiance models, given in Jassby and Platt (1976), were compared for their abilities to fit the 16 data sets (254 data points) collected for *Lobophora variegata*. Although differences were not statistically significant ($p<0.05$), the hyperbolic tangent model (eq. 2) fit slightly better than the seven other models when photosynthesis was expressed on a thallus area basis, and it ranked third when thallus dry weight was used.

Significant differences among fitted P-I curves for *Lobophora variegata* collected and incubated at various depths were determined using a pairwise comparison described by Neter and Wasserman (1974). An $F$ value was calculated using:

$$F = \frac{(SSE_1 + SSE_2) - SSE_{1&2}}{(n_1 + n_2 - 3) - (n_1 + n_2 - 6)} \times \frac{SSE_{1&2}}{(n_1 + n_2 - 6)} \quad (\text{eq. 3})$$

where $SSE_1$ is the sum of squared errors term for one P-I curve; $SSE_2$ is the same term for the second P-I curve; $SSE_{1&2}$ is the sum of squared errors for a curve fitted to the pooled data from curves 1 and 2; $n_1$ and $n_2$ are the number of
data points for curves 1 and 2, respectively. The two curves (1 and 2) were considered different if 
\( F > F(0.95,3,n_1+n_2-6). \)

Confidence intervals were calculated for \( P_{\text{max}}, \alpha, \) and \( R \) using 95% t-values and the "normal error" standard error calculated by MLAB for each parameter estimate. Differences between parameter estimates were considered significant (p<0.05) if their confidence intervals did not overlap.

There is an inherent limitation in the in situ method used to determine photosynthesis-irradiance relationships. Light is attenuated as it passes through seawater, and therefore the range of quantum irradiance levels that can be produced with neutral density filters becomes increasingly restricted with depth. Consequently, for deeper incubations, it was not possible to measure photosynthetic rates at saturating light levels. An advantage of using a direct method to fit a P-I model to the data, is that it allows estimation of \( P_{\text{max}} \) as long as data are available above the initial linear region of the curve. This does, however, represent an extrapolation of the data, and precautions must be observed in interpreting \( P_{\text{max}} \) in these cases. The confidence intervals calculated by MLAB for \( P_{\text{max}} \) provide an indication of the precision of the estimates.
2.3 RESULTS

BOTTLE EFFECTS

Oxygen tension in the incubation bottles was found to have no significant effect (p<0.05) on the measured photosynthetic rate of *Lobophora variegata*. Mean values (n=9) for apparent photosynthesis (P\textsubscript{app}) were 87.9, 78.2, and 111.4mg O\textsubscript{2}m\textsuperscript{-2}h\textsuperscript{-1} at initial dissolved oxygen concentrations of 0.9, 7.5, and 20.0mg l\textsuperscript{-1}, respectively (Table 2-I). Although the mean P\textsubscript{app} at 20.0mg l\textsuperscript{-1} appeared somewhat elevated, the results at this oxygen level were variable and the confidence interval for the mean was quite wide (CI\textsubscript{0.95} = 85.8 to 136.9) in relation to those at the other two concentrations (CI\textsubscript{0.95} = 78.5 to 97.2 at 0.9mg l\textsuperscript{-1}, and 69.9 to 85.8 at 7.5mg l\textsuperscript{-1}).

Nutrient enrichment of seawater in the incubation enclosures did not enhance the measured photosynthetic rate for *Lobophora variegata*. Even at the highest level of nitrate addition (100mg l\textsuperscript{-1}) the mean P\textsubscript{app} was 217.0mg O\textsubscript{2}m\textsuperscript{-2}h\textsuperscript{-1} compared with 216.3mg O\textsubscript{2}m\textsuperscript{-2}h\textsuperscript{-1} for no enrichment (Table 2-I). Phosphate addition, surprisingly, resulted in a reduction in P\textsubscript{app} from 245.7 without
enrichment to 87.5mg O₂·m⁻²·h⁻¹ at 100mg·l⁻¹; smaller additions had less effect (Table 2-I).

Incubations of 1 and 2h yielded slightly lower measured \( P_{\text{app}} \) compared with longer periods, however, this was based on only 2 replicates and the differences were not statistically significant \((p<0.05)\). The mean values were 211.1, 209.6, 264.4, 262.6, and 242.2mg O₂·m⁻²·h⁻¹ at 1, 2, 3, 4, and 5h, respectively (Table 2-I). The changes in dissolved oxygen during 1 and 2h incubations were relatively small \(<0.76mg O_2·l^{-1})\), while 5h incubations ended with final dissolved oxygen levels as high as 10.4mg O₂·l⁻¹. Since these tests were done at moderate quantum irradiance levels, it was considered likely that the response at low light would be difficult to measure using short (1 or 2h) incubations, and that long periods at high quantum irradiance might cause bubble formation. Consequently, 3 to 4h incubation periods were used throughout the remainder of the study.

QUANTUM IRRADIANCE

For each in situ photosynthesis experiment, total and spectral quantum irradiance measurements were used to calculate the mean downwelling plus upwelling quantum
irradiance at the surface and at each incubation depth. The quantum irradiance values calculated for all of the photosynthesis experiments have been averaged and the means are shown as a function of depth in Figure 2-1. Between 9 and 36m, total quantum irradiance decreased exponentially with a mean diffuse attenuation coefficient (k) of 0.0686. A steeper slope found between 0 and 9m (k=0.129) was most likely due to heterochromatic attenuation (Smith and Tyler, 1970). The mean quantum irradiance at the surface was $2071.5 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, while at 36.6m, the deepest incubation depth, the level was reduced to $97.3 \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. With the attenuation coefficient calculated for the depth interval from 27.0 to 36.6m (k=0.0663), it was predicted that 1% of the surface quantum irradiance would reach a depth of 59.9m.

Mean spectral quantum irradiance curves for depths ranging from 0 to 36.6m are shown in Figure 2-2. With depth, the spectral distribution of light became increasingly narrow with a peak at 480nm. Attenuation of light at wavelengths greater than 580nm was very rapid.

PHOTOSYNTHESIS-IRRADIANCE CURVES

The photosynthesis-irradiance data for Lobophora variegata collected and incubated at 4.6m are shown in Figure 2-3 with the "least squares" fitted hyperbolic tangent model of Jassby and Platt (1976). The RMS error
\[ (=\text{SSE}/(n-3)) \text{ of the fit was 61.97mg O}_2\cdot\text{m}^{-2}\cdot\text{h}^{-1}. \] The estimated value for \( P_{\text{max}} \) ("apparent") was 361.4mg O\(_2\)\cdot m\(^{-2}\)\cdot h\(^{-1}\) with a 95% confidence interval (CI\(_{0.95}\)) of 302.7 to 420.1mg O\(_2\)\cdot m\(^{-2}\)\cdot h\(^{-1}\). The initial slope (alpha) of the fitted curve was \[ 1.12(\text{mg O}_2\cdot\text{m}^{-2}\cdot\text{h}^{-1})/(\text{uE}\cdot\text{m}^{-2}\cdot\text{s}^{-1}), \] with a CI\(_{0.95}\) ranging from 0.70 to 1.54. Respiration was estimated to be 44.5 (CI\(_{0.95}=0.2\) to 88.1) mg O\(_2\)\cdot m\(^{-2}\)\cdot h\(^{-1}\), slightly higher than the mean value of 37.0 (CI\(_{0.95}=23.5\) to 50.6) mg O\(_2\)\cdot m\(^{-2}\)\cdot h\(^{-1}\) calculated from autotrophic respiration measurements in "dark bottles".

The curves fitted to photosynthesis data for \textit{Lobophora variegata} populations at 4.6, 9.1, 18.3, and 36.6m incubated at their native depths, are shown in Figures 2-4 and 2-5. Goodness of fit information (RMS error), as well as parameter estimates and confidence intervals, are included in Tables 2-II and 2-III. For the curves fitted to photosynthesis data expressed on a thallus area basis (Figure 2-4), the light saturated photosynthetic rates \( (P_{\text{max}}) \) decreased as a function of depth, with the greatest difference occurring between 4.6 and 9.1m. The initial slope of the P-I relationship was lowest for the shallow (4.6m) population and became progressively steeper at greater depths. Statistical comparison, based on sum of squared error terms (SSE's), indicated that each curve was significantly different \((p<0.05)\) from each of the other three with the exception that the 4.6 and 9.1m curves were
not different from one another (Table 2-II). The estimated values for $P_{\text{max}}$ ("apparent") decreased with depth from $361.4 \text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ at 4.6m to $212.1 \text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ at 36.6m (Table 2-II). The limited range of quantum irradiance levels available at 36.6m was responsible for a relatively wide confidence interval around $P_{\text{max}}$. Consequently, the estimate at that depth was not statistically different ($p < 0.05$) from $P_{\text{max}}$ at 4.6m, although the difference between the estimates at 4.6 and 18.3m ($240.7 \text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) was significant. Estimates of the parameter alpha increased for each successive incubation depth, resulting in a nearly 3-fold change in initial slope over the full depth range. The estimated values of alpha at 4.6m (1.12) and 36.6m (3.23) were significantly different at the 95% confidence level. No consistent trend was found with depth for respiration ($R$). In general, $R$ appeared to be a fairly unstable parameter of the model with a high dependency on the fitted value of alpha; confidence intervals for $R$ were frequently very broad.

When photosynthesis was expressed on the basis of thallus weight, the P-I curves of *Lobophora variegata* populations at their native depths had nearly equal rates of light saturated photosynthesis at 9.1, 18.3, and 36.6m, while a substantially higher rate was apparent at 4.6m (Figure 2-4). $P_{\text{max}}$ of the 4.6m fit was $19.9 \text{mg} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ compared with values ranging from 9.9 to $10.6 \text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$.
at the other three depths. The initial slopes (alpha) of the fitted curves did not increase substantially between 4.6 and 18.3m, but the estimate at 36.6m was more than twice the value at 4.6m. Again, no trend was apparent in R. Based on pairwise comparisons of SSE's, each of the fitted curves was significantly different ($p \leq 0.05$) from each of the other three. Differences in $P_{\text{max}}$ were significant ($p \leq 0.05$) when the 4.6m estimate was compared with those from 9.1 and 18.3m, but not from 36.6m. The increase in alpha between 4.6 and 36.6m was significant at the 90%, but not the 95%, confidence level.

To determine the effects of spectral distribution on the photosynthesis-irradiance relationship of the 4.6m *Lobophora variegata* population, samples from this depth were incubated at 4.6, 9.1, 13.7 and 18.3m. The curves fitted to the data expressed on a thallus area basis and on a thallus weight basis are shown in Figures 2-6 and 2-7, respectively. On either basis, the curves for each depth were not significantly different ($p \leq 0.05$) from the curves for any other depth. Parameter estimates of $P_{\text{max}}$ and R were independent of incubation depth as they did not change significantly ($p \leq 0.05$) with depth on either an area (Table 2-II) or weight (Table 2-III) basis. Initial slopes (alpha) were lowest for the two 4.6m curves and generally increased with depth, although the differences were not significant at the 95% confidence level.
The dependence of the photosynthesis-irradiance relationship on spectral distribution was also examined in the 18.3m Lobophora variegata population by incubating samples at the same series of depths: 4.6, 9.1, 13.7 and 18.3m. In this case, significant differences (p<0.05) were found between most of the fitted curves. With photosynthesis on an area basis, each of the curves (Figure 2-8) was found to be significantly different (p<0.05) from each other with the exception that the 4.6 and 9.1m curves were not different from one another (Table 2-II). On a thallus weight basis (Figure 2-9), differences were insignificant (p<0.05) between 4.6 and 9.1m, 9.1 and 13.7m, and 13.7 and 18.3m, but the other three possible combinations were different at the 99% confidence level (Table 2-III). Although the individual parameter estimates (Tables 2-II and 2-III) were insufficiently precise to isolate the cause of the differences, a trend was again apparent for alpha, which increased more than 2-fold over the entire depth range regardless of whether the data was expressed on an area or a weight basis.

Samples from 36.6m were incubated at a series of four depths ranging from 9.1 to 36.6m, and the fitted curves are shown in Figures 2-10 and 2-11. Although the RMS errors indicate that the model fit the data very well, the small numbers of data points used in the fits resulted in relatively imprecise parameter estimates (Tables 2-II and
On a thallus area basis, an increase in alpha was again noted with depth, but confidence intervals were very broad and overlapped considerably.

The P-I curves obtained for Lobophora variegata samples from 4.6, 18.3 and 36.6m incubated at 18.3m are compared in Figure 2-12. It appeared from the initial slopes of these curves that the low light efficiency of L. variegata was independent of population depth when all samples were incubated in the same spectral regime. This was not so apparent, however, when similar comparisons were made with other incubation depths. Tables 2-II and 2-III include curve fit information on incubations conducted at 4.6m using samples from 4.6 and 18.3m; at 9.1m using samples from 4.6, 9.1, 18.3 and 36.6m; and at 18.3m using samples from 4.6, 18.3 and 36.6m. Although alpha appeared to decrease with population depth between 4.6 and 18.3m, it was generally as high at 36.6m as it was at 4.6m.
Inherent in the general concept of seaweed photoadaptation is the postulate that it leads to more effective utilization of available light and therefore acts to extend species distribution into limiting light regimes, either high or low, that would otherwise prohibit growth. Two clear trends were observed for the dictyotalean seaweed *Lobophora variegata* that indicate enhancement of photosynthetic performance in the light regimes at each extreme of its vertical range. First, the shallow population growing in a high light regime has a greater rate of light saturated photosynthesis compared with deep populations; and secondly, at their respective depths, deep populations can utilize low irradiance levels more efficiently than can shallow populations.

Dependence of $P_{\text{max}}$ on growth irradiance has been demonstrated in several other algae as well as in some higher plants. Björkman et al. (1972) found that growth in reduced light regimes was accompanied by a decreased rate of light saturated photosynthesis in the vascular herb *Atriplex patula* (Chenopodiaceae). Similar results were reported by Beardall and Morris (1976) for cultures of the marine diatom
Phaeodactylum tricornutum. Shallow-water populations of the red seaweeds Chondrus crispus and Ptilota serrata have been shown to have greater photosynthetic capacities, expressed on a thallus weight basis, relative to deep populations (Mathieson and Norall, 1975a, 1975b). Ramus et al. (1976b) and Ramus and Rosenberg (1980) reported reduced rates of light saturated photosynthesis, on a chlorophyll a basis, for deep-water samples of Dictyota dicotoma and Ulva lactuca compared with samples from shallow locations.

In the present study, the greatest reduction of $P_{\text{max}}$ for Lobophora variegata populations occurred over the shallowest depth interval examined (4.6 to 9.1m). Below this interval, further reductions were found on a thallus area basis, but $P_{\text{max}}$ remained constant when expressed in terms of thallus weight. The differences found in $P_{\text{max}}$ were independent of the spectral distribution and range of quantum flux densities used for incubation. In other studies, changes in $P_{\text{max}}$ for different species have been attributed to changes in the number of photosynthetic units (PSU's) produced (Prezelin, 1976) or to changes in the amount of photosynthetic electron transport or dark reaction constituents present (Beardall and Morris, 1976; Boardman, 1977). Prezelin (1976) has pointed out that a change in the number of PSU's is likely to produce a change in photosynthetic pigment content. As described in the preceding study (Part 1), chlorophyll a, chlorophyll c and
fucoxanthin content of _L. variegata_ decreased with depth on a thallus area basis, but not when expressed in terms of thallus weight. It is evident that the small changes in $P_{\text{max}}$ below the shallowest depth interval can be attributed to a reduction with depth in the number of PSU's per unit area of thallus. Also, the number of PSU's remains constant on a thallus weight basis, and the weight to area ratio of the thallus decreases with depth (see also Part I). No evidence is available to suggest that the number of PSU's can be physiologically altered in response to changes in light regime. It may be, instead, that the reduction in PSU's/area results from a decrease in cell size with depth. This would be consistent with the change in thallus weight to area ratio and it would account for a constant number of PSU'S on a thallus weight basis. However, differences in weight to area ratios could have resulted from differences in the density of cellular contents or from changes in cell wall thickness. The nature of the weight:area differences does have some metabolic implications because the ratio of PSU's to other actively metabolizing cellular components could be affected.

Over the shallowest depth interval, the relatively large change found in $P_{\text{max}}$ was significant on both a thallus area and weight basis. Consequently, it cannot be attributed entirely to a change in thallus weight to area ratio. In the preceding study (Part I), the only pigment
that was found to be elevated in the shallow populations of Lobophora variegata on both an area and weight basis was beta-carotene. Although this may imply an increase in the size of the PSU pigment antennae, it does not indicate an increase in the number of PSU's per unit of thallus. Because the size of the pigment antennae would not be expected to effect \( P_{\text{max}} \), it is more likely that the elevated photosynthetic capacity in shallow populations is the result of photo-induced synthesis of additional electron transport components or soluble proteins, such as RUBP carboxylase, involved in dark reactions.

A correlation between growth irradiance and the ability to utilize low light levels has been shown for other algal species. P-I curves for the diatom, Phaeodactylum tricornutum and the marine dinoflagellate, Glenodinium sp. grown in dim light were found to have relatively steep slopes, on a cell volume basis, when compared with high-light cultures (Beardall and Morris, 1976; Prezelin, 1976). Ramus and coworkers (1976b, 1977) found that during deep-water incubations, the photosynthetic performances of several red, green, and brown seaweeds from deep or shaded locations exceeded, on a thallus weight basis, those of the same species from shallow, high-light regimes. In each of the above studies, enhanced low-light utilization was attributable, at least in part, to an increase in the size or number of PSU's per unit weight or volume, indicated by elevated pigment levels.
In the present study, when samples of *Lobophora variegata* were incubated at their native depths, the P-I curves obtained for thalli growing in deep water had steeper initial slopes than the curves for those in shallow locations. The enhanced low-light efficiency in deep water is apparently not caused by changes in the number or size of PSU's, as the results of the preceding study (Part 1) indicated that the pigments of *L. variegata* do not increase with depth. Furthermore, it was found that the low-light photosynthetic efficiency of even the shallow population increased when incubations were conducted at greater depths. Thus, the low-light efficiency of *L. variegata* is, at least to some degree, independent of growth irradiance, and is a function of the spectral distribution of light available for photosynthesis. Predictions in the preceding study indicated that *L. variegata* would absorb a greater percentage of ambient light in deep water than in shallow. Although the size and composition of the pigment antennae in *L. variegata* may be constant below the shallowest depth, it appears to be chromatically adapted for maximum light harvesting efficiency in deep water spectral regimes. A supporting conclusion was reached by Dring (1981) who predicted that the photosynthetic efficiency of thin brown seaweeds would be greatest in deep, clear oceanic waters.
The results presented for *Lobophora variegata* are not sufficiently conclusive to suggest that low-light photosynthetic efficiency is totally independent of growth irradiance conditions. In view of the elevated beta-carotene content and increased thallus absorption found in the preceding study (Part 1) for the shallow *L. variegata* population, it was somewhat surprising in the present study that alpha for the shallow population did not always exceed alpha for other populations when incubated at the same depth. It is possible of course that elevated beta-carotene levels were not present in the shallow population used for photosynthesis studies. If elevated beta-carotene levels were, indeed, present, then the failure to find greatly enhanced low-light efficiency for shallow populations may indicate inefficient transfer of excitation energy from beta-carotene to chlorophyll. Perhaps the major role of beta-carotene in the shallow populations of *L. variegata* is protection from photodynamic injury by excessive light (Krinsky, 1978). Alternatively, because light energy harvested by beta-carotene is transferred mainly to photosystem I (Goedheer, 1970), it is possible that in shallow populations this energy is being used primarily to drive cyclic electron flow which would not be detected by oxygen exchange measurements.
In summary, the photosynthesis-irradiance relationship of *Lobophora variegata* is dependent upon the spectral distribution of light available for photosynthesis and upon the depth at which the thallus has grown. The dependence of photosynthetic performance on spectral distribution has apparently been brought about through phylogenetic adaptation of the pigment antenna and of thallus form. The result of this, an increase with depth in low-light photosynthetic efficiency, has undoubtedly influenced the lower vertical limit of the species distribution. The depth at which an *L. variegata* thallus grows has been shown to affect its capacity for light saturated photosynthesis. Greatly increased rates of $P_{\text{max}}$ were found only in the population from shallow water where the maximum quantum irradiance was well above that required for saturation. This apparently results from increased synthesis of photosynthetic electron transport or dark reaction components. High photosynthetic capacity would be of no benefit to deep-water populations because quantum irradiance levels are never saturating and the energy required to produce and maintain this capacity would be wasted. It is unclear why higher photosynthetic capacities were not found in populations at intermediate depths where quantum irradiance levels were at or above saturation for at least part of the day. A high growth rate would be an advantage at depths where grazing and competitive pressures are greatest, or perhaps more energy is required by shallow
populations to synthesize replacement components for those destroyed by excessive light. It therefore remains to be shown whether increased photosynthetic capacity in the shallow *L. variegata* population represents a physiological optimization or a maximization required for survival.
TABLE 2-1: Effects of oxygen tension, nutrient addition, and incubation period on the measured photosynthetic rate ($P_{\text{app}}$) of Lobophora variegata.

<table>
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<tr>
<th>Dissolved Oxygen (mg·l$^{-1}$)</th>
<th>Mean $P_{\text{app}}$ (mg O$_2$·m$^{-2}$·h$^{-1}$)</th>
<th>95% Confidence Interval</th>
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<tr>
<td>0.9</td>
<td>87.9</td>
<td>(78.5-97.2)</td>
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<td>7.5</td>
<td>78.2</td>
<td>(69.9-85.8)</td>
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<td>20.0</td>
<td>111.4</td>
<td>(85.8-136.9)</td>
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<table>
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<th>Nitrate Addition (mg·l$^{-1}$)</th>
<th>Mean $P_{\text{app}}$ (mg O$_2$·m$^{-2}$·h$^{-1}$)</th>
<th>Range</th>
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</thead>
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<tr>
<td>0.0</td>
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<td>(207.0-225.6)</td>
</tr>
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<td>12.5</td>
<td>193.4</td>
<td>(86.4-300.4)</td>
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<td>25.0</td>
<td>163.0</td>
<td>(144.1-181.9)</td>
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<tr>
<td>50.0</td>
<td>241.2</td>
<td>(230.9-252.4)</td>
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<tr>
<td>100.0</td>
<td>217.0</td>
<td>(120.2-313.8)</td>
</tr>
</tbody>
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<table>
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<tr>
<th>Phosphate Addition (mg·l$^{-1}$)</th>
<th>Mean $P_{\text{app}}$ (mg O$_2$·m$^{-2}$·h$^{-1}$)</th>
<th>Range</th>
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</thead>
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<tr>
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<td>(180.3-311.2)</td>
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<tr>
<td>12.5</td>
<td>170.8</td>
<td>(160.8-180.9)</td>
</tr>
<tr>
<td>25.0</td>
<td>147.2</td>
<td>(135.5-159.0)</td>
</tr>
<tr>
<td>50.0</td>
<td>139.8</td>
<td>(120.8-158.8)</td>
</tr>
<tr>
<td>100.0</td>
<td>87.5</td>
<td>(70.3-105.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incubation Period (h)</th>
<th>Mean $P_{\text{app}}$ (mg O$_2$·m$^{-2}$·h$^{-1}$)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>211.1</td>
<td>(203.8-218.3)</td>
</tr>
<tr>
<td>2</td>
<td>209.6</td>
<td>(192.3-227.0)</td>
</tr>
<tr>
<td>3</td>
<td>264.4</td>
<td>(246.2-282.5)</td>
</tr>
<tr>
<td>4</td>
<td>262.6</td>
<td>(226.9-298.4)</td>
</tr>
<tr>
<td>5</td>
<td>242.2</td>
<td>(221.4-262.2)</td>
</tr>
</tbody>
</table>
TABLE 2-II: Values of photosynthesis-irradiance relationship parameters $P_{\text{max}}$, $\alpha$, and $R$ determined on a thallus area basis for *Lobophora variegata* from a series of depths. 95% confidence intervals of parameter estimates are shown in parentheses. The final column indicates the results of curve comparisons based on SSE's.

<table>
<thead>
<tr>
<th>Sample Depth (m)</th>
<th>Incub. Depth (m)</th>
<th>$P_{\text{max}}$ (mg $O_2 \cdot m^{-2} \cdot h^{-1}$)</th>
<th>$\alpha$ (0.07-1.54)</th>
<th>$R$ (mg $O_2 \cdot m^{-2} \cdot h^{-1}$)</th>
<th>RMS Error</th>
<th>n</th>
<th>Different From (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a 4.6 4.6</td>
<td>361.4 (302.7-420.1)</td>
<td>1.12 (0.07-1.54)</td>
<td>44.5 (0.2-88.8)</td>
<td>61.97</td>
<td>40</td>
<td>c*,d</td>
<td></td>
</tr>
<tr>
<td>b 9.1 9.1</td>
<td>267.9 (225.8-310.2)</td>
<td>1.44 (0.95-1.93)</td>
<td>73.2 (39.6-106.8)</td>
<td>48.27</td>
<td>45</td>
<td>c*,d*</td>
<td></td>
</tr>
<tr>
<td>c 18.3 18.3</td>
<td>240.7 (208.1-272.0)</td>
<td>1.52 (1.12-1.92)</td>
<td>38.4 (12.8-64.0)</td>
<td>29.09</td>
<td>37</td>
<td>a,b,d*</td>
<td></td>
</tr>
<tr>
<td>d 36.6 36.6</td>
<td>212.1 (110.4-313.8)</td>
<td>3.23 (1.62-4.85)</td>
<td>50.6 (15.6-85.5)</td>
<td>20.33</td>
<td>10</td>
<td>a,b*,c*</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at 0.01 level
TABLE 2-III: Values of photosynthesis-irradiance relationship parameters $P_{max}$, alpha, and $R$ determined on a thallus ash-free dry weight basis for Lobophora variegata from a series of depths. 95% confidence intervals of parameter estimates are shown in parentheses. The final column indicates the results of curve comparisons based on SSE's.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Incub. Depth (m)</th>
<th>P_{max} (mg O_2 g^{-1} h^{-1})</th>
<th>alpha (mg O_2 g^{-1} h^{-1})</th>
<th>R (mg O_2 g^{-1} h^{-1})</th>
<th>RMS Error</th>
<th>n</th>
<th>Different From (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>4.6</td>
<td>19.9 (16.6-23.2)</td>
<td>0.052 (0.033-0.071)</td>
<td>1.98 (-0.27-4.24)</td>
<td>3.38</td>
<td>40</td>
<td>b*,c*,d*</td>
</tr>
<tr>
<td>b</td>
<td>9.1</td>
<td>9.9 (8.4-11.5)</td>
<td>0.051 (0.034-0.069)</td>
<td>2.49 (1.26-3.71)</td>
<td>1.79</td>
<td>45</td>
<td>a*,c*,d*</td>
</tr>
<tr>
<td>c</td>
<td>18.3</td>
<td>10.6 (8.7-12.4)</td>
<td>0.058 (0.038-0.078)</td>
<td>1.24 (-0.14-2.61)</td>
<td>1.63</td>
<td>37</td>
<td>a*,b*,d*</td>
</tr>
<tr>
<td>d</td>
<td>36.6</td>
<td>10.4 (3.4-17.5)</td>
<td>0.106 (0.064-0.147)</td>
<td>1.70 (0.71-2.70)</td>
<td>0.61</td>
<td>10</td>
<td>a*,b*,c*</td>
</tr>
<tr>
<td>a</td>
<td>18.3</td>
<td>8.1 (5.9-10.4)</td>
<td>0.029 (0.012-0.047)</td>
<td>1.59 (-0.34-3.53)</td>
<td>1.23</td>
<td>14</td>
<td>c*,d*</td>
</tr>
<tr>
<td>b</td>
<td>18.3</td>
<td>9.5 (7.1-11.9)</td>
<td>0.043 (0.016-0.070)</td>
<td>1.68 (-0.38-3.74)</td>
<td>1.46</td>
<td>15</td>
<td>a*</td>
</tr>
<tr>
<td>c</td>
<td>18.3</td>
<td>11.3 (8.0-14.6)</td>
<td>0.045 (0.020-0.070)</td>
<td>0.45 (-1.64-2.52)</td>
<td>1.43</td>
<td>13</td>
<td>a*</td>
</tr>
<tr>
<td>d</td>
<td>18.3</td>
<td>10.9 (7.9-13.8)</td>
<td>0.067 (0.027-0.105)</td>
<td>0.80 (-1.67-3.27)</td>
<td>1.55</td>
<td>15</td>
<td>a*,b*</td>
</tr>
<tr>
<td>a</td>
<td>36.6</td>
<td>8.4 (-2.4-19.3)</td>
<td>0.081 (-0.127-0.290)</td>
<td>3.32 (-7.53-14.17)</td>
<td>1.56</td>
<td>5</td>
<td>none</td>
</tr>
<tr>
<td>b</td>
<td>36.6</td>
<td>7.6 (4.3-10.9)</td>
<td>0.072 (-0.004-0.148)</td>
<td>1.77 (-1.07-4.61)</td>
<td>0.59</td>
<td>5</td>
<td>none</td>
</tr>
<tr>
<td>c</td>
<td>36.6</td>
<td>10.7 (5.1-16.3)</td>
<td>0.066 (0.035-0.096)</td>
<td>1.45 (0.16-2.73)</td>
<td>0.29</td>
<td>5</td>
<td>d</td>
</tr>
<tr>
<td>d</td>
<td>36.6</td>
<td>10.0 (-0.7-20.7)</td>
<td>0.101 (0.013-0.189)</td>
<td>1.50 (-0.72-3.73)</td>
<td>0.48</td>
<td>5</td>
<td>c</td>
</tr>
</tbody>
</table>

* Significant at 0.01 level
FIGURE 2-1: Mean quantum irradiance (400-700nm) at midday as a function of water depth at Utila, Honduras. Error bars are 95% confidence intervals.
FIGURE 2-1

Quantum Flux Density (µE·m⁻²·s⁻¹)

Depth (m)
FIGURE 2-2: Mean spectral quantum irradiance at midday at depths of 0, 4.6, 9.1, 18.3, and 36.6m at Utila, Honduras.
FIGURE 2-2

Quantum Flux Density (uE·m⁻²·s⁻¹·nm⁻¹)

Wavelength (nm)
FIGURE 2-3: Photosynthesis-irradiance data, on a thallus area basis, of Lobophora variegata collected and incubated at a depth of 4.6 m, and the fitted hyperbolic-tangent model. Parameter values of the model are:

\[ P_{\text{max}} = 361.4 \text{mg O}_2 \cdot \text{m}^{-2} \cdot \text{h}^{-1} \]

\[ \alpha = 1.12 (\text{mg O}_2 \cdot \text{m}^{-2} \cdot \text{h}^{-1})/({\mu E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}) \]

\[ R = 44.5 \text{mg O}_2 \cdot \text{m}^{-2} \cdot \text{h}^{-1}. \]

The RMS error of the fit was 61.97.
Quantum Flux Density (uE·m⁻²·s⁻¹)

**FIGURE 2-3**
FIGURE 2-4: Photosynthesis–irradiance relationships, on a thallus area basis, of Lobophora variegata populations at 4.6, 9.1, 18.3, and 36.6m, incubated at their native depths. Solid lines indicate the range of irradiance values used in each fit. Curve parameter and RMS error values of the fits are given in Table 2-II.
Quantum Flux Density (uE·m⁻²·s⁻¹)

$P_{\text{app}}$ (mg O₂·m⁻²·h⁻¹)

FIGURE 2-4
FIGURE 2-5: Photosynthesis-irradiance relationships, on an ash-free dry weight basis, of Lobophora variegata populations at 4.6, 9.1, 18.3, and 36.6m, incubated at their native depths. Solid lines indicate the range of irradiance values used in each fit. Curve parameter and RMS error values of the fits are given in Table 2-III.
FIGURE 2-5

Quantum Flux Density (\(\text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\))

P_{\text{app}} (\text{mg O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1})

- 36.6m
- 18.3m
- 9.1m
FIGURE 2-6: Photosynthesis-irradiance relationships, on a thallus area basis, of Lobophora variegata from a depth of 4.6 m, incubated at 4.6, 9.1, 13.7, and 18.3 m. Solid lines indicate the range of irradiance values used in each fit. Curve parameter and RMS error values of the fits are given in Table 2-II.
FIGURE 2-6
FIGURE 2-7: Photosynthesis-irradiance relationships, on an ash-free dry weight basis, of Lobophora variegata from a depth of 4.6m, incubated at 4.6, 9.1, 13.7, and 18.3m. Solid lines indicate the range of irradiance values used in each fit. Curve parameter and RMS error values of the fits are given in Table 2-III.
FIGURE 2-7

Quantum Flux Density (uE·m⁻²·s⁻¹)

Papp (mg O₂·g⁻¹·h⁻¹)

4.6m 18.3m 9.1m 13.7m
FIGURE 2-8: Photosynthesis-irradiance relationships, on a thallus area basis, of Lobophora variegata from a depth of 18.3m, incubated at 4.6, 9.1, 13.7, and 18.3m. Solid lines indicate the range of irradiance values used in each fit. Curve parameter and RMS error values of the fits are given in Table 2-II.
Quantum Flux Density $C_uE^*m^{-2}s^{-1}$

**FIGURE 2-8**
FIGURE 2-9: Photosynthesis-irradiance relationships, on an ash-free dry weight basis, of Lobophora variegata from a depth of 18.3m, incubated at 4.6, 9.1, 13.7, and 18.3m. Solid lines indicate the range of irradiance values used in each fit. Curve parameter and RMS error values of the fits are given in Table 2-III.
FIGURE 2-9

Quantum Flux Density (\text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1})

\( P_{\text{app}} \) (\text{mg} \text{O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1})

- 18.3m
- 13.7m
- 9.1m
- 4.6m
FIGURE 2-10: Photosynthesis-irradiance relationships, on a thallus area basis, of Lobophora variegata from a depth of 36.6m, incubated at 9.1, 18.3, 27.4, and 36.6m. Solid lines indicate the range of irradiance values used in each fit. Curve parameter and RMS error values of the fits are given in Table 2-II.
FIGURE 2-10

Quantum Flux Density (uE·m⁻²·s⁻¹)

P_app (mg O₂·m⁻²·h⁻¹)
FIGURE 2-11: Photosynthesis-irradiance relationships, on an ash-free dry weight basis, of Lobophora variegata from a depth of 36.6m, incubated at 9.1, 18.3, 27.4, and 36.3m. Solid lines indicate the range of irradiance values used in each fit. Curve parameter and RMS error values of the fits are given in Table 2-III.
FIGURE 2-11
FIGURE 2-12: Photosynthesis-irradiance relationships, on a thallus area basis, of Lobophora variegata from depths of 4.6, 18.3, and 36.6m, incubated at a depth of 18.3m. Solid lines indicate the range of irradiance values used in each fit. Curve parameter and RMS error values of the fits are given in Table 2-II.


APPENDIX
DISSOLVED OXYGEN

Modification of Winkler Method

New England Aquarium
Harold E. Edgerton Research Laboratory
Central Wharf
Boston, Massachusetts, U.S.A.

Reagents:

1. Manganese Chloride, 3M; dissolve 600g of MnCl₂·4H₂O in 600ml distilled water. When dissolved, filter through a glass fiber filter and make up to 1 liter.

2. Sodium Iodide, 4M with Sodium Hydroxide, 8M; dissolve 600g NaI in 600ml of distilled water and note color. If yellowish-brown in appearance, throw it out and use a new reagent. Next add 320g NaOH. Cool the mixture and dilute to 1 liter. Store in a dark bottle.

3. Sulfuric Acid, 23%.

4. Starch Indicator; add to a 100ml beaker 1g of soluble potato starch and 5mg of mercuric chloride (for a preservative). Add a little distilled water to make a thick paste. Add 100ml of boiling distilled water and stir for 1 minute. Cool and place in a small dropper bottle.

5. Sodium Thiosulfate, 0.025N; dissolve 6.25g of sodium thiosulfate crystals and 0.625g of sodium borate (for a preservative) in 1 liter of distilled water. Store in a dark bottle.

6. Potassium bi-iodate, 0.025N; certified standard solution.

Procedure:

Standardization

1. Fill a 60ml glass stoppered sample bottle with S.O.W. (substitute ocean water) and insert the stopper.
2. Remove the stopper and add 0.6ml of 23% sulfuric acid.

3. Add 0.5ml of the sodium iodide-hydroxide reagent; mix.

4. Add 0.5ml of the manganese chloride reagent.

5. Insert stopper being careful not to enclose any air bubbles; shake.

6. Using a volumetric pipette, transfer 20ml of the solution to a 50ml beaker.

7. Add 1.0ml (volumetric pipette) of standard potassium bi-iodate to the beaker.

8. Using a microburette and with stirring, titrate the liberated iodine with sodium thiosulfate solution until yellow color has almost disappeared.

9. Add 4 drops of the starch indicator and titrate until solution turns from blue to colorless.

NOTE: This titration should be reproducible to within +0.005ml.

Unknown Samples

1. In obtaining water samples, great care must be taken to avoid entrained air bubbles in the sample bottles. Carefully fill a 60ml glass stoppered bottle so that no air bubbles are trapped.

2. Immediately remove the stopper and introduce 0.5ml manganese chloride followed by 0.5ml sodium iodide-hydroxide reagent.

3. Replace stopper carefully, shake thoroughly and store in the dark. One may proceed this far in the field and complete the procedure in the laboratory up to 8 hours later.

4. After precipitate has settled, shake thoroughly again and allow the precipitate to settle a second time.

5. After a minimum of 1 hour after the precipitate has thoroughly settled, add 0.6ml of 23% sulfuric acid, replace the stopper, and shake thoroughly. If the precipitate does not completely disappear, add more sulfuric acid, dropwise, until the solution clears (2.4<pH<2.6).

6. Transfer 20ml (volumetric pipette) into a 50ml beaker and titrate, with stirring, with the 0.025N sodium thiosulfate solution until the yellow color has almost disappeared.

7. Add 4 drops of starch indicator and titrate until colorless.
Calculations:

Standardization

To determine the factor for parts per million of dissolved oxygen per ml of sodium thiosulfate, use the following:

\[ F = \frac{139.95}{20 (0.0698) X} = \frac{10.0251}{X} \]

where \( X \) = volume of sodium thiosulfate to titrate standard bi-iodate
20 = volume of the sample (ml)
0.698 = ml O\(_2\)/mg (at STP)
139.95 = (meq O\(_2\)/l) (meq bi-iodate used)

Unknown Samples

Multiply the amount of sodium thiosulfate (ml) used to titrate the sample by the factor \( F \) determined by standardization.

D.O. (mg·l\(^{-1}\)) = \( F \times \) ml thio. to titrate sample