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RESPONSE OF GREEN HYDRA TO FEEDING AND STARVATION AT FOUR IRRADIANCES

G. MULLER-PARKER* AND R. L. PARDY

School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588-0118

ABSTRACT

The relationship between the productivity of symbiotic algae and growth of the hydra, *Hydra viridissima* (Florida strain), was investigated in hydra maintained at four irradiances (5, 10, 15, and 30 $\mu E \cdot m^{-2} \cdot s^{-1}$) and either fed or starved. Although the productivity of fed hydra increased from 0.3 to 1.15 $\mu g\ C \cdot mg\ protein^{-1} \cdot h^{-1}$ with increase in culture irradiance, there was no significant effect of culture irradiance on population growth or on the protein biomass of individual hydra. The survival of starved hydra was similarly not affected by culture irradiance. Algal-animal biomass parameters changed in response to feeding and culture irradiance. Numbers of symbiotic algae in fed hydra declined with increased culture irradiance. The protein content of hydra starved for 28 days declined to 10% of initial levels. The relative proportion of algal to animal biomass increased in starved hydra as both algal densities and algal cell volumes were almost twice those of fed hydra. Whereas culture irradiance and feeding alter the ratio of algal to animal biomass, growth of this green hydra is only affected by feeding.

INTRODUCTION

The green hydra, *Hydra viridissima,* maintains *Chlorella*-like algae within its digestive cells. Algal photosynthesis is an important feature of the symbiosis, as photosynthetically fixed carbon is translocated from the algae to the animal host (Muscatine and Lenhoff, 1963; Mews, 1980). Numerous studies show that light sustains green hydra, especially when food is absent or in limited supply. However, the extent to which the productivity of the symbiotic algae influences the growth and survival of green hydra remains unknown. Differences in symbiont productivity can be obtained by maintaining green hydra at a series of low irradiances where photosynthesis is light-limited. Using this approach, our objective was to measure the effect of symbiont productivity on changes in the biomass of green hydra.

Growth rates of fed green and aposymbiotic (=algae-free) hydra maintained in the light showed that light enhances growth of symbiotic hydra, but only when food is limiting (Muscatine and Lenhoff, 1965b). Growth efficiencies derived for green hydra show that the contribution of symbiotic algae is significant, and that this contribution increases with a decrease in feeding frequency in the light (Stiven, 1965a). The nutritional importance of symbiont photosynthetic products is also illustrated by starvation experiments in which survival of symbiotic and aposymbiotic hydra was compared. If sufficiently illuminated, symbiotic hydra survive considerably longer periods of starvation than nonsymbiotic species or aposymbiotic animals (Muscatine and Lenhoff, 1965b; Stiven, 1965b; Kelty and Cook, 1976; Rahat and Reich, 1980).

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* Present address: University of Maryland, C.E.E.S., Chesapeake Biological Laboratory, Solomons, Maryland 20688-0038.

Similarly, symbiotic hydra starved in light retain more protein and increase glycogen stores in comparison to aposymbiotic and nonsymbiotic hydra (Cook and Kelty, 1982). Respiratory quotients of symbiotic hydra starved in light indicate that these organisms metabolize mainly carbohydrates, presumed to originate from the symbionts; hydra starved in the dark catabolize mainly fat (Pardy and White, 1977). Light is the key factor influencing the ability of symbiotic hydra to survive starvation; in the dark the algae can represent a significant energetic cost to the hydra (Douglas and Smith, 1983). In these light/dark studies, it is assumed that the productivity of symbiotic algae is responsible for the obtained results, although rates of carbon fixation were not provided.

To examine the contribution of algal productivity to hydra growth, the productivity and growth of green hydra maintained at different irradiances was measured. Since photosynthesis is dependent on irradiance at low photon flux densities, we hypothesized that light may influence the growth and survival of symbiotic hydra cultured under low irradiances. The effect of culture irradiance on the relative proportion of algal to animal biomass of fed green hydra was also measured. Furthermore, although light has been shown to enhance the survival of starved green hydra, it is not known if the total photon flux density available to the algae affects the long-term survival of starving green hydra. We addressed this question by analyzing the survival of starved green hydra acclimated to four different irradiances, and by measuring algal-animal biomass parameters of starved symbiotic hydra.

Materials and Methods

Maintenance of hydra cultures

Hydra originating from a single clone of *Hydra viridissima* (Florida strain) were used for all experiments. Approximately two years prior to these experiments a symbiosis was established between aposymbiotic hydra and cultured symbionts (NC64A) previously isolated from symbiotic paramecia (*Paramecium bursaria*). Pardy and Muscatine (1973) showed that this association formed a stable endosymbiosis, and that growth rates of these hydra were nearly identical to those of the original stock of green hydra. These hydra were designated F/NC hydra. F/NC stocks were maintained in M solution (Muscatine and Lenhoff, 1965a) in glass dishes at 21°C under continuous light ranging from 25 to 40 $\mu E \cdot m^{-2} \cdot s^{-1}$, PAR (Photosynthetically Active Radiation; 400–700 nm) provided by banks of fluorescent lamps. Hydra were cultured according to the methods of Loomis and Lenhoff (1956). Animals were fed to repletion daily (M–F) with newly hatched *Artemia* sp. nauplii, and were rinsed twice daily with fresh culture solution.

To determine the effect of irradiance on growth and biomass parameters of F/NC hydra, approximately 150 animals from stock culture were placed into each of four glass dishes (28 cm × 18 cm × 5 cm) in about 800 ml M solution. Three dishes were covered with one to three layers of fine mesh plastic screen to reduce irradiance; all dishes were covered by a Plexiglas sheet to minimize evaporation of culture medium. Photon flux density in the four dishes was measured prior to addition of hydra and culture medium with a quantum sensor connected to a Li-Cor Model 185 quantum meter. Average photon flux densities in the four experimental dishes were 30, 15, 10, and 5 $\mu E \cdot m^{-2} \cdot s^{-1}$ (PAR). Experimental dishes were maintained under continuous light at 21°C, and were rotated daily to minimize possible heterogeneity of incident irradiance.

Hydra maintained at the four different irradiances were fed four times weekly with *Artemia* sp. nauplii (M,W,F,Su), and were rinsed daily with M solution. After six months, the animals were subsequently starved for one month to assess the effect of starvation on biomass parameters.

Hydra population growth

Population growth of fed hydra after three months at the experimental irradiances was measured as follows: six replicate groups of three hydra, each with one bud, were selected from each treatment 24 h after feeding. Groups of animals were placed into petri dishes (3.5 cm × 1 cm) containing 5 ml culture solution and covered with screen material to provide similar irradiances as the parent cultures. Hydra were maintained under the same feeding and cleaning conditions as described for parent cultures. The positions of the four groups of dishes were rotated daily; the six replicate dishes at each irradiance were moved as a unit. Hydranths in each dish were counted daily for 8–10 days. Population growth rate constants and doubling times were calculated according to Loomis (1954).

To determine if culture irradiance affected survival of hydra under starvation, fed hydra were placed into individual petri dishes as described above and were starved for more than 80 days. Daily, the number of hydranths in each dish was counted, the culture medium was replaced, and dish positions were rotated.

Productivity of hydra

To measure photosynthesis of symbiotic algae in hydra acclimated to four irradiances, groups of twenty-five hydra (each with one fully developed bud) were incubated with $NaH^{14}CO_3$ (0.8 $\mu Ci \cdot ml^{-1}$) in 5.0 ml M solution supplemented with 5 m*M* $NaHCO_3$. Hydra maintained on a regime of 4 feedings per week were last fed 24 hours prior to an experiment. All incubations were carried out at 21°C for one hour at 1100–1200 h. The productivity of hydra at different irradiances was estimated by placing groups of hydra in beakers covered with various layers of screen. Replicate groups of hydra in beakers covered with foil were also incubated to correct for any dark fixation of $NaH^{14}CO_3$. Incubation media were sampled at the start of the experiments for total ^{14}C activity. At the end of the incubations, hydra were thoroughly rinsed with M solution and then homogenized in distilled water. Aliquots of the homogenate solutions were withdrawn for protein analysis (Lowry *et al.*, 1951) and determination of algal numbers (see methods under hydra biomass parameters). Organic ^{14}C retained by green hydra was estimated by analyzing three replicate samples (20 μl) of homogenized hydra. Samples were acidified by addition of 100 μl of 0.1 *N* HCl under gentle heat to drive off $^{14}CO_2$. Scintillation fluor (Budget-Solve, Research Products International Corp.; 5 ml) was added, and samples were analyzed in a Packard Tri-Carb liquid scintillation spectrometer (Model 3375).

After correction for dark fixation, the amounts of organic ^{14}C retained by hydra were converted to rates of carbon fixation (Vollenweider, 1969). Total CO_2 in the incubation medium was calculated from the total alkalinity, measured potentiometrically (Golterman, 1969). Productivity data were fit by linear regression to estimate photosynthetic efficiencies; all correlation coefficients were greater than 0.95.

Hydra biomass parameters

The response of symbiotic hydra to different culture irradiances was quantified using replicate samples of 100 pooled hydra which were analyzed for total protein, number of algae, and algal chlorophyll. Hydra were sampled 24 h after feeding. Sam-

pling of hydra was standardized by selecting individuals with one fully developed bud. As most 30-day starved hydra did not have buds, pooled samples consisted of single individuals. Hydra were homogenized in distilled water with a glass tissue homogenizer. The volume of the homogenate solution was recorded and aliquots removed and frozen for later protein analysis by the method of Lowry *et al.* (1951) using bovine serum albumin as a protein standard. Symbionts were isolated from the remaining homogenate solution and washed in distilled water by repeated centrifugation and resuspension of the algal pellet. The final algal pellet was suspended in a known volume of distilled water and cell concentrations determined by six replicate counts of an aliquot using an A/O Spencer Bright-Line hemacytometer and a Nikon compound microscope at 400×. The total number of symbionts per weight of hydra protein was calculated.

The number of symbionts per hydra digestive cell was estimated by placing groups of 25 hydra into maceration fluid (David, 1973) in a small glass vial. After 10 minutes cells were dissociated by gently tapping the vial on a hard surface. Using phase microscopy at 400×, numbers of algae in 100 randomly selected digestive cells were counted.

Chlorophyll content of freshly isolated symbionts was determined by overnight extraction at 4°C of known numbers of algae in absolute methanol. Absorbances of the supernatants of centrifuged methanol extracts were read in a Beckman DB spectrophotometer at 650 nm and 665 nm. The equations of Holden (1976) were used to calculate the weight of chlorophyll per cell.

Analysis of variance (ANOVA) was used to determine if culture irradiance had a significant effect on biomass parameters of fed hydra. The Least Significant Difference test was applied at the 5% significance level to data sets for which a significant F-ratio was obtained (Sokal and Rohlf, 1969). Due to the low numbers of starved hydra, it was not possible to obtain replicate samples for the biomass parameters and therefore those data could not be analyzed for statistical significance.

Cell volumes of symbionts were determined from measurements of cell diameters obtained using an ocular micrometer at 400×. The mean cell diameter was obtained from measurements of 100 randomly selected algae in hydra homogenized in M solution; algal volumes were calculated assuming spherical cells.

Results

Population growth of fed hydra and survival of starved hydra maintained at four culture irradiances

Population growth rate constants (k) of fed hydra acclimated to four culture irradiances are given in Table I. There was no significant effect of culture irradiance on k (ANOVA $F_{3,20} = 0.53$; $P = 0.67$), which averaged 0.2238 day^{-1}. Similar results were obtained with a replicate experiment in which k of hydra at the four irradiances averaged 0.2216 day^{-1}. These results show that, with a 4×/week feeding regime, there is no significant difference in population growth of hydra at culture irradiances ranging from 5 to 30 $\mu E \cdot m^{-2} \cdot s^{-1}$.

To determine if culture irradiance had an effect on the survival of symbiotic hydra under starvation, animals maintained at the four culture irradiances for six months with a 4×/week feeding regime were isolated from parent cultures and starved. Some hydra continued to produce buds, even after 20 days without food. Data sets from five-day intervals were analyzed by the general linear models procedure (SAS, 1982). An analysis of covariance using a split-plot design with unreplicated whole plots

TABLE I

Population growth rate constants (k) and doubling times (T) of F/NC hydra fed 4×/week at 4 culture irradiances

Culture irradiance ($\mu E \cdot m^{-2} \cdot s^{-1}$)	k (day^{-1})	T (days)
5	0.2084 (±.0216)[a]	3.33
10	0.2258 (±.0094)	3.07
15	0.2308 (±.0102)	3.00
30	0.2303 (±.0135)	3.01

[a] n = 6; ±S.E.

(SAS, 1985) was used to determine if culture irradiance had a significant effect on the rate of decline of numbers of hydra. The preceeding 5-day data set was designated as a covariate. Analysis of covariance indicated that there was no significant difference between the decline in hydra populations maintained at the four culture irradiances ($F_3 = 2.16$; $P = 0.102$). Days of starvation had a highly significant effect on the decline of numbers of hydra ($F_1 = 15.70$; $P = 0.0002$). Since culture irradiance did not have a significant effect on the survival of F/NC hydra, the data for the four treatments were pooled and the decline in numbers of hydra as a function of days of starvation described by a second order polynomial equation fit to the combined data by the Proc Reg program (SAS, 1985):

$$\text{Number of hydra surviving} = 0.002406D - 0.000828D^2 + 5.76$$

$$(R = 0.802, n = 72)$$

where D = number of days. Figure 1 shows the decline in the number of hydra with days of starvation. There was no real decline in the number of hydra until Day 40 of starvation, and it took 60 days for the population of hydra to decrease to half the initial numbers.

Productivity of hydra

The productivity of algal symbionts in fed hydra was examined by measuring the amount of ^{14}C retained in hydra exposed to different irradiances. It is not clear if the retention of ^{14}C is a measure of net or gross production by the symbiotic algae since the extent of $^{14}CO_2$ recycling between animal and algae is unknown. Productivity here refers to the amount of ^{14}C retained by the combined algae and animal tissue after a one hour incubation. To determine if photosynthesis was light-limited at the four culture irradiances, the productivity of hydra from each culture irradiance was measured at several irradiances. The productivity of hydra as a function of irradiance (P-I) is shown in Figure 2. The linear rates indicate that photosynthesis of symbionts was limited by light over the range of irradiances examined. Hydra maintained at a culture irradiance of 10 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 2b) were the most productive hydra at all measured irradiances.

The photosynthetic efficiencies of hydra acclimated to the four culture irradiances (the slopes of the P-I curves in Fig. 2) are given in Figure 3. Photosynthetic efficiency declined with an increase in culture irradiance from 10 to 30 $\mu E \cdot m^{-2} \cdot s^{-1}$, but hydra maintained at 5 $\mu E \cdot m^{-2} \cdot s^{-1}$ were only half as efficient as those maintained at 10 $\mu E \cdot m^{-2} \cdot s^{-1}$ at utilizing the available light for photosynthetic carbon production.

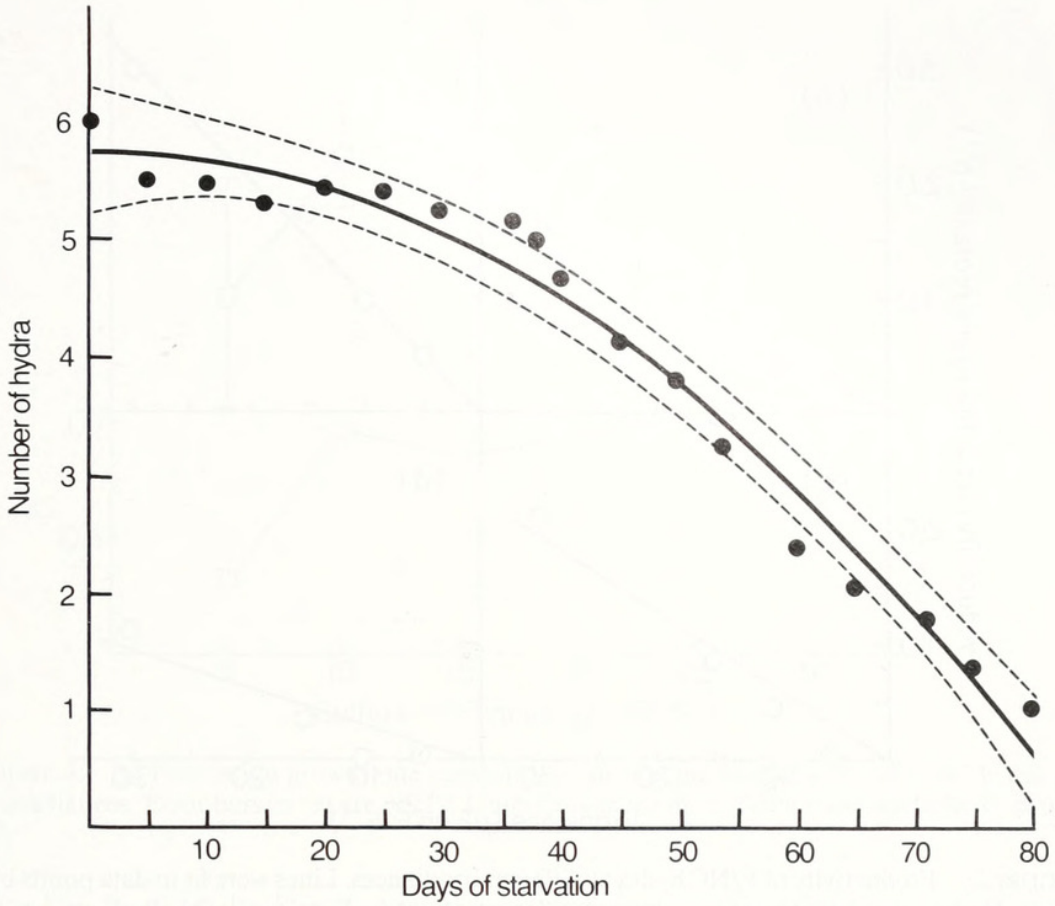

FIGURE 1. Decline in number of hydra with days of starvation. Since the survival of hydra at the four irradiances was not significantly different, the data were pooled and a second order polynomial equation fit to the combined data. Dotted lines represent the 95% confidence intervals for the equation (see text).

The productivity of green hydra at each acclimated culture irradiance (P_i) showed that hydra maintained at 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ fixed almost four times the amount of carbon fixed by hydra maintained at 5 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 4b). Hydra population growth rate constants at each culture irradiance were plotted on the same figure (Fig. 4a) as P_i to compare the two parameters. Although k was not significantly affected by culture irradiance, the curves in Figure 4 show that population growth rates of hydra (Fig. 4a) and hydra productivity (Fig. 4b) follow, in general, the same pattern.

Effect of culture irradiance on biomass parameters of fed hydra

The biomass parameters of fed hydra after one month at four culture irradiances are shown in Figure 5. The protein biomass of fed hydra (Fig. 5a) was not significantly affected by culture irradiance (ANOVA $F_{3,16} = 0.81$; $P = 0.50$). Together with the data in Table I, these results show that culture irradiance does not affect either the growth of individual hydra or that of hydra populations.

The population density of symbionts in fed hydra was affected by culture irradiance (ANOVA $F_{3,16} = 6.36$; $P = 0.005$). Hydra maintained at the highest irradiance contained significantly fewer symbionts than those maintained at the three lower irradiances (Fig. 5b). Similar results were obtained with numbers of symbionts per digestive cell (Fig. 5d). There was a significant effect of culture irradiance on the number of algae per digestive cell (ANOVA $F_{3,8} = 4.71$; $P = 0.03$), but in this case there

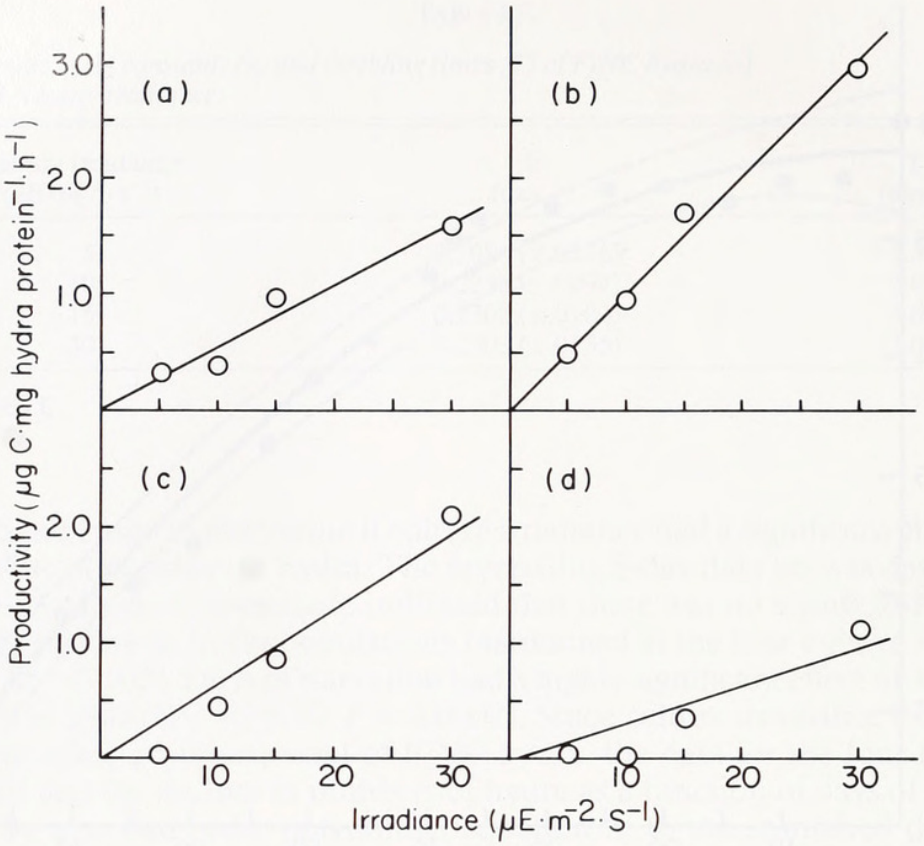

FIGURE 2. Productivity of F/NC hydra at different irradiances. Lines were fit to data points by linear regression. Hydra were maintained at culture irradiances of: (a) 5 $\mu E \cdot m^{-2} \cdot s^{-1}$; (b) 10 $\mu E \cdot m^{-2} \cdot s^{-1}$; (c) 15 $\mu E \cdot m^{-2} \cdot s^{-1}$; (d) 30 $\mu E \cdot m^{-2} \cdot s^{-1}$.

was no significant difference in algal densities of hydra maintained at 15 and 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 5d).

Total chlorophyll per algal cell (Fig. 5c) decreased significantly with increase in culture irradiance (ANOVA $F_{3,15} = 15.98$; $P < 0.001$). Symbionts isolated from hydra

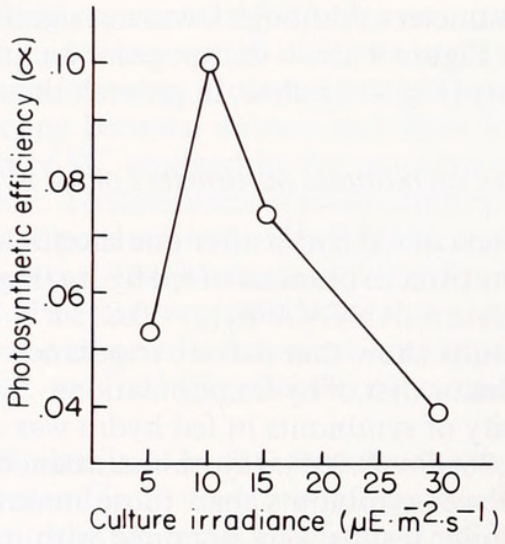

FIGURE 3. Photosynthetic efficiency (α) of F/NC hydra as a function of culture irradiance. Units of α are $\mu g\ C \cdot (\mu E \cdot m^{-2} \cdot s^{-1})^{-1} \cdot h^{-1} \cdot mg^{-1}$ protein.

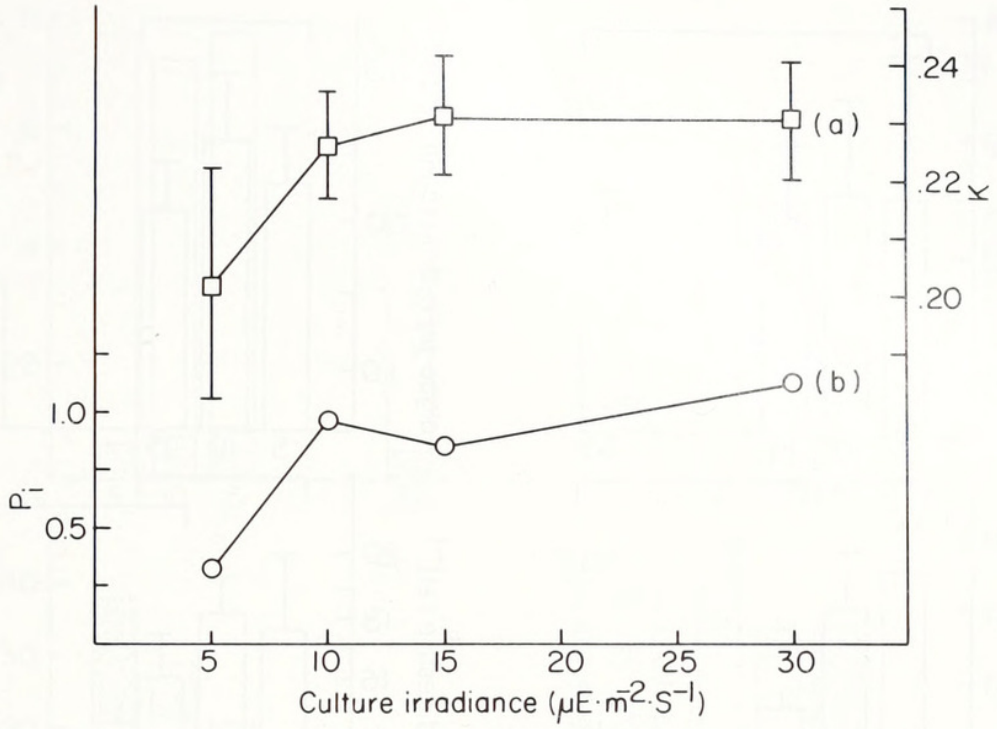

FIGURE 4. (a) Population growth rate constants, k, and (b) productivity, P_i, of F/NC hydra at four culture irradiances. Error bars in (a) are ±S.E.. Units for k same as in Table I and units for P_i same as in Figure 2.

maintained at 15 and 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ contained significantly less chlorophyll than those isolated from hydra maintained at the two lower irradiances (Fig. 5c). Data for chlorophyll *a* and chlorophyll *b* gave similar results to those for total chlorophyll, and are not included. There was no significant effect of irradiance on the ratio of chlorophyll *a* to chlorophyll *b* (ANOVA $F_{3,15} = 0.88$; $P = 0.47$), which averaged 1.19 (±0.17; n = 19, ±S.D.). Cell volumes of algae isolated from fed hydra increased with increase in culture irradiance (Table II).

Effect of culture irradiance on biomass parameters of 28-day starved hydra

Biomass parameters of hydra starved for 28 days were compared to initial parameters obtained from the same populations after two days of starvation (Fig. 6). The protein biomass of starved hydra declined to about 10% of initial levels after 28 days of starvation (Fig. 6a).

Algal densities estimated from the number of symbionts per weight of hydra protein in 28-day starved hydra were twice as high as those of 2-day starved hydra (Fig. 6b). Increased numbers of symbionts per digestive cell were also found in 28-day starved hydra (Fig. 6d).

Chlorophyll per algal cell also increased after 28 days of starvation (Fig. 6c). There were proportional changes in chlorophyll *a* and chlorophyll *b*, with the ratio of chlorophyll *a*:*b* remaining constant at 1.12 for algae isolated from all starved hydra. As was found for symbionts from fed hydra, cell volumes increased with increased irradiance (Table II). However, cell volumes of symbionts from hydra starved for 30 days were greater than corresponding cell volumes of symbionts from fed hydra (Table II).

DISCUSSION

The nutritional contribution of symbiont productivity to growth of green hydra was evaluated by measuring changes in biomass of hydra maintained under differ-

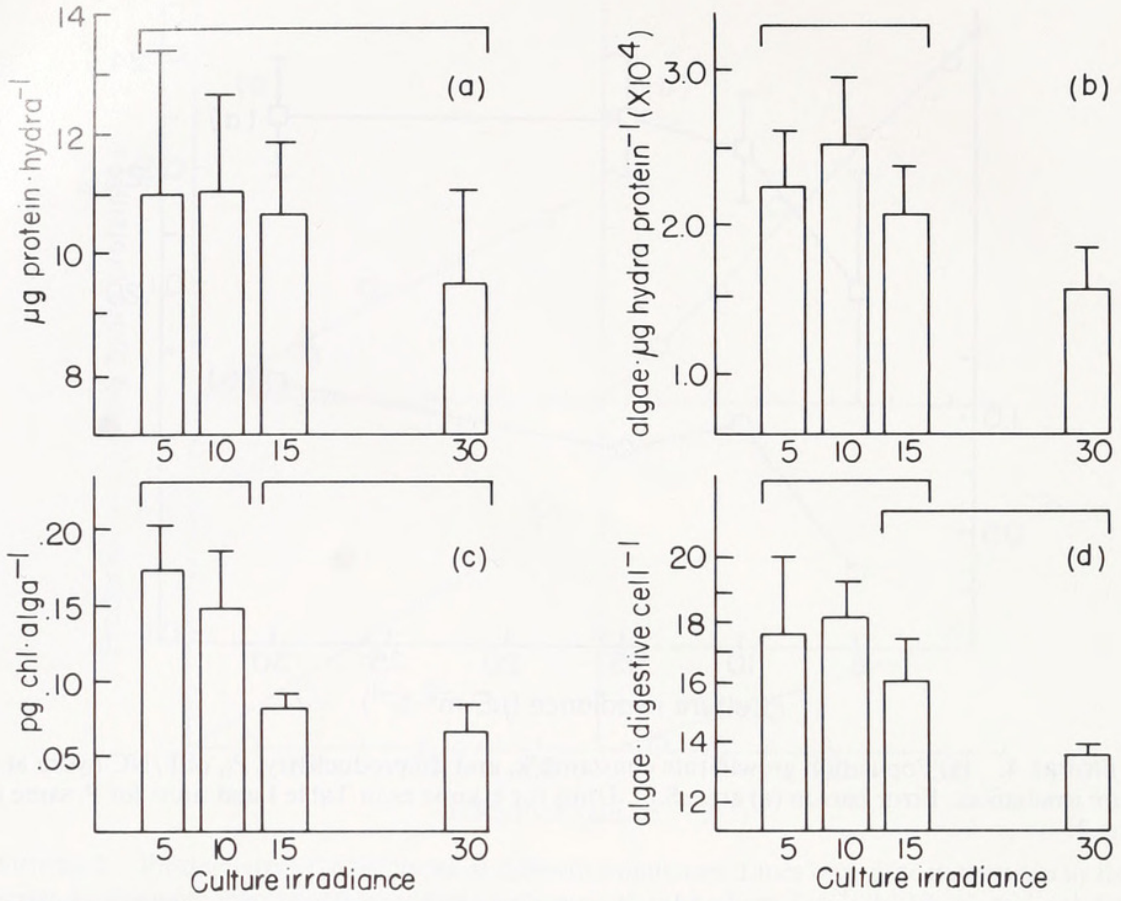

FIGURE 5. Final biomass parameters of F/NC hydra maintained at four irradiances for 4 weeks and fed 4×/week with *Artemia* sp. nauplii. Each bar in 5(a)–5(c) represents the mean of 5 replicate samples of 100 hydra each. Bars in 5(d) are mean values for 3 replicate samples of 100 digestive cells each. Error bars represent standard deviations of the mean. The horizontal brackets connect data sets which were found to be not significantly different at the $P > 0.05$ level.

ent irradiances. The results show that culture irradiances ranging from 5 to 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ had no significant effect on both growth rate of fed green hydra and survival of starved green hydra. While the productivity of fed green hydra increased with an increase in culture irradiance, hydra growth was independent of culture irradiance. These results suggest that the amount of carbon fixed by the symbiotic algae and potentially available to the hydra via translocation or digestion is not transferred

TABLE II

Cell volumes of symbiotic algae from F/NC hydra either fed or starved for 30 days at four culture irradiances

Culture irradiance ($\mu E \cdot m^{-2} \cdot s^{-1}$)	Algal cell volume (μm^3)	
	From fed hydra	From starved hydra
5	10.4	18.5
10	12.1	20.6
15	15.6	21.5
30	19.0	27.2

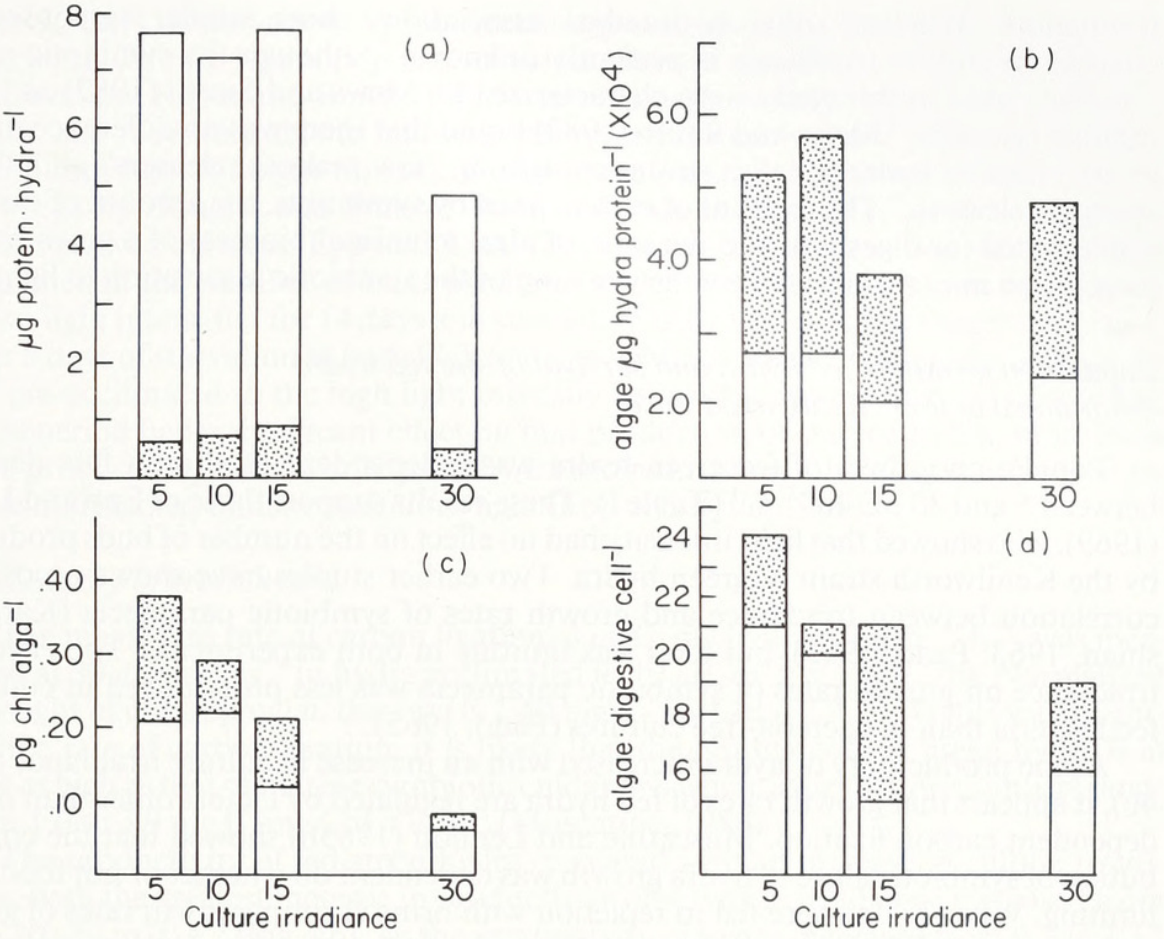

FIGURE 6. The effect of starvation on biomass parameters of F/NC hydra maintained at four irradiances. Initial biomass parameters were measured in 2-day starved hydra (□); final biomass parameters in 28-day starved hydra (□).

directly into animal biomass of the symbiotic association. Similar results were obtained in a previous study on the symbiotic sea anemone *Aiptasia pulchella,* where changes in sea anemone biomass in fed and starved animals were not related to the productivity of the symbiotic algae at different irradiances (Muller-Parker, 1985).

Hydra were maintained at irradiances below those required for light-saturated photosynthesis by the algae. Symbiotic algae were smaller at lower irradiances, and responded to decreases in irradiance with proportional increases in chl *a* and chl *b*. The greatest increase in productivity occurred between hydra maintained at 5 and 10 $\mu E \cdot m^{-2} \cdot s^{-1}$, and photosynthetic efficiency declined with further increase in culture irradiance.

Algal-animal biomass parameters of green hydra changed in response to feeding and culture irradiance. Algal density in fed hydra changed in response to culture irradiance, as both the number of algae per weight of hydra protein and the number of algae per digestive cell were lower for hydra maintained at the highest irradiance (Fig. 5b, d). In spite of lower algal densities, changes in the ratio of plant to animal biomass were less pronounced, since algal cell volumes increased with increase in culture irradiance (Table II). Algal densities of 28-day starved hydra were twice those of fed hydra (Fig. 6b). Since algal cell volumes from these hydra were much greater than those of algae from fed hydra (Table II), the ratio of plant to animal biomass increased greatly with starvation of the host.

We have measured the response of one hydra-algal symbiosis to different culture

irradiances. Whether other hydra-algal associations show similar responses to changes in culture irradiance is presently unknown. Although the symbiotic algae (NC64A) used in this study were characterized by Mews and Smith (1982) as "low maltose releasers," Mews and Smith (1982) found that there was no difference in the growth rates of hydra (English strain) containing "low maltose releasers" and "high maltose releasers." The amount of carbon fixed by symbionts, the amount of carbon translocated (or digested), and the ratio of algal to animal biomass of a given hydra association may variously affect the response of the symbiotic association to light.

Population growth of fed hydra and survival of starved hydra maintained at four culture irradiances

Population growth of fed green hydra was independent of photon flux density between 5 and 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Table I). These results support those of Epp and Lytle (1969), who showed that light intensity had no effect on the number of buds produced by the Kenilworth strain of green hydra. Two earlier studies have shown a positive correlation between irradiance and growth rates of symbiotic paramecia (Karakashian, 1963; Pado, 1965), but food was limiting in both experiments. The effect of irradiance on growth rates of symbiotic paramecia was less pronounced in cultures fed bacteria than in bacteria-free cultures (Pado, 1965).

As the productivity of hydra increased with an increase in culture irradiance (Fig. 4b), it appears that growth rates of fed hydra are regulated by factors other than light-dependent carbon fixation. Muscatine and Lenhoff (1965b) showed that the contribution of symbiotic algae to hydra growth was dependent on whether or not food was limiting. When hydra were fed to repletion with brine shrimp, growth rates of green and aposymbiotic hydra were identical. When food was limited, growth rates of green hydra exceeded those of aposymbiotic individuals (Muscatine and Lenhoff, 1965b). Similar results were obtained with symbiotic and aposymbiotic paramecia (Karakashian, 1963). Thus, the similarity in growth rates for the four experimental hydra populations may not be surprising, as equal rations of brine shrimp were provided to all cultures. Although the number of brine shrimp ingested by hydra maintained at the four irradiances was not measured, it is likely that irradiance had no effect on the ability of hydra to capture shrimp since Clayton (1984) found the number of shrimp captured and ingested by green hydra to be the same under both dark and light maintenance conditions.

All other studies on the effect of light on growth of green hydra have dealt with variations in daily photoperiod. No significant difference in budding rate was found for Kenilworth green hydra maintained on either a 12:12 h L:D photoperiod or continuous light (Epp and Lytle, 1969). Douglas and Smith (1984) found that green hydra maintained under continuous light grew more slowly than those maintained on a 12:12 h L:D photoperiod. These studies support the results obtained here, *i.e.*, that there is no positive correlation between total irradiance and growth rates of fed green hydra.

Survival of starved green hydra was not affected by culture irradiance. Although symbiotic hydra starved in the light survive longer than those starved in the dark (Stiven, 1965b; Rahat and Reich, 1980), these results indicate that the total photon flux density does not have a significant impact on the length of time symbiotic hydra survive. It would be interesting to determine the effect of very low irradiances (0–5 $\mu E \cdot m^{-2} \cdot s^{-1}$) on the survival and productivity of starved symbiotic hydra.

The decline in numbers of hydra with days of starvation (Fig. 1) was described by a second order polynomial equation. As some hydra continued to produce buds for

up to 20 days of starvation, hydra mortality was somewhat offset by the production of new individuals. Hydra populations did not decline appreciably until the 40th day of starvation. Rahat and Reich (1980) observed buds on green hydra starved for six weeks, and they were able to maintain starved symbiotic hydra in M solution under continuous light for eight months. Unfed hydra cultures were maintained for up to 10 weeks by Douglas and Smith (1984), although survival data were not reported for the starved cultures. Epp and Lytle (1969) showed that there was no significant difference in the number of buds produced by Kenilworth green hydra maintained at two light intensities for 14 days and starved. In their study, bud production stopped after 8 days of starvation in both high and low light cultures. However, the hydra were not pre-acclimated to the high light intensity before the experiment was conducted. Photoperiod had a significant effect on bud production of starved hydra; more buds were produced by Kenilworth green hydra maintained on a 12:12 L:D cycle than by those maintained under continuous light (Epp and Lytle, 1969).

Productivity of green hydra

The maximum rate of carbon fixation, 3 μg C · mg hydra protein^{-1} · h^{-1}, was measured at 30 μE · m^{-2} · s^{-1} in hydra acclimated to 10 μE · m^{-2} · s^{-1} (Fig. 2b). Normalized to weight of chlorophyll *a*, this rate is 1.40 mg C · mg Chl *a*$^{-1}$ · h^{-1}. As this was a light-limited rate of carbon fixation, it is likely that the productivity of green hydra is at least as high as that of marine symbiotic cnidarians where assimilation numbers range from 1.0 to 3.9 mg C · mg Chl *a*$^{-1}$ · h^{-1} (Muscatine, 1980).

The productivity of fed green hydra increased with an increase in culture irradiance, with the greatest increase in productivity observed as irradiance increased from 5 to 10 μE · m^{-2} · s^{-1} (Fig. 4b). As the productivity of hydra increased from 0.3 to 1.15 μg C · mg protein^{-1} · h^{-1}, and neither population growth nor hydra protein biomass were significantly different at the four irradiances, increased carbon fixation by the symbiotic algae did not result in an increase of hydra biomass. However, the curves in Figure 4 suggest that at low irradiances productivity and growth of green hydra may be related. It is not known if photosynthetically fixed carbon was stored in algal lipid or carbohydrate pools, as these were not measured. Other sinks for photosynthetically fixed carbon in symbiotic associations are listed by Muscatine *et al.* (1984). Algal-derived carbon acquired by the animal may be respired or released as particulate or dissolved organic carbon. These parameters were not measured in this study. An energy budget constructed for the symbiotic coral *Pocillopora eydouxi* suggested that about 51% of the photosynthetically fixed energy is used in respiration, 0.9% in growth, and 48% is unaccounted for and probably excreted (Davies, 1984).

Figure 2 shows that light-saturated rates of photosynthesis were not obtained over the range of irradiances measured. Phipps and Pardy (1982) found that light-saturated photosynthesis in green hydra maintained at 60 μE · m^{-2} · s^{-1} was obtained at irradiances exceeding 500 μE · m^{-2} · s^{-1}. In general, photosynthetic efficiency increased with decreasing culture irradiance, suggesting that algae in hydra compensate to culture irradiance. Hydra maintained at 10 μE · m^{-2} · s^{-1} were the most efficient at utilizing the available light for carbon fixation (Fig. 3). The low photosynthetic efficiency of hydra maintained at 5 μE · m^{-2} · s^{-1} is puzzling, and cannot be explained by any differences in biomass parameters of these hydra (Fig. 5).

Effect of culture irradiance on biomass parameters of fed hydra

Environmental factors can alter the algal-animal biomass ratio of symbiotic hydra. The role of light has previously been addressed by comparing the biomass param-

eters of hydra maintained in light and in dark. When hydra are transferred from light to continuous darkness the number of algae per digestive cell goes down (Pardy, 1974; McAuley, 1981, 1985b; Steele and Smith, 1981; Douglas and Smith, 1984). When hydra are returned to the light, algal densities return to previous levels (Pardy, 1974; Douglas and Smith, 1984). The effect of irradiance on algal densities in hydra has not been previously examined, although at high light intensity symbiotic hydra lose their algae (Pardy, 1976; Steele and Smith, 1981). In this study the density of symbiotic algae in hydra maintained at four irradiances was estimated with two methods; by calculating the number of algae per μg hydra protein and by counting the number of algae in digestive cells. Similar results were obtained with both methods. Hydra maintained at the highest irradiance contained fewer algae than those maintained at the three lower irradiances (Figs. 5b and 5d).

Differences in the relative proportion of algal and animal biomass may be less pronounced than suggested by the algal density data, as algal cell volumes in fed hydra maintained at 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ were almost twice those of algae from hydra maintained at 5 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Table II). As cultured symbiotic algae (NC64A) acclimated to different irradiances exhibited a similar reduction in cell volume with decrease in culture irradiance (unpubl. data), these data strongly suggest that culture irradiance, and not some influence of the host, determined the cell size of symbionts *in situ*. Others have found that algae from hydra maintained in the dark were smaller than those from light-maintained hydra (Pardy, 1981; Douglas and Smith, 1984; McAuley, 1985b).

Symbiotic algae adapted to low light with an increase in chlorophyll (Fig. 5c) and a decrease in algal cell volume. The cell volume data in Table II suggests that changes in chlorophyll between algae isolated from hydra maintained at the four irradiances would be even greater if chlorophyll were normalized to cell weight or carbon.

Effect of irradiance on biomass parameters of starved hydra

The protein biomass of F/NC hydra starved for 28 days declined to about 10% of initial levels (Fig. 6a). The final protein biomass of starved hydra was similar in hydra maintained at the four irradiances, but hydra maintained at 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ lost proportionately less protein than the other groups.

Algal densities in hydra starved for 28 days were twice as high as those of fed hydra (Fig. 6b). The difference between initial and final algal densities was less pronounced in the comparison of numbers of algae per digestive cell (Fig. 6d). A similar increase in the number of symbiotic algae per digestive cell in starved hydra was found by Muscatine and Pool (1979), Douglas and Smith (1984), and McAuley (1985a). Douglas and Smith (1984) found that this increase in algal density resulted from a decline in protein content and not from an increase in numbers of algae. Algal cells isolated from starved hydra were larger than those isolated from fed hydra (Table II); similar results were obtained by Douglas and Smith (1984) and by McAuley (1985a). Increase in algal cell size and numbers of algae result in a greater proportion of algal to animal biomass in starved hydra.

This study has attempted to define the role of irradiance in the green hydra symbiosis by comparing growth, productivity, survival to starvation, and algal-animal biomass parameters of F/NC hydra maintained at culture irradiances ranging from 5 to 30 $\mu E \cdot m^{-2} \cdot s^{-1}$. The results indicate that irradiance does not have an effect on the biomass of fed or starved hydra, but that algal-animal biomass parameters are influenced by irradiance and feeding regime.

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