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The Effect of Host Feeding on the Contribution of Endosymbiotic Algae to the Growth of Green Hydra

KENNETH W. DUNN*

Department of Ecology and Evolution, State University of New York, Stony Brook, New York 11794

Abstract. Previous work has shown that the advantage conferred by endosymbiotic algae on the growth of green hydra is most evident during periods of food shortage. This advantage disappears when hydra are amply fed. However, evidence is presented here which suggests that endosymbiotic algae are not sensitive to the nutritional needs of the host. In controlled feeding studies, green hydra produced more bud tissue than did aposymbionts at all feeding levels. *Per capita* algal contribution to host growth was independent of host feeding rate. Starvation had little effect on rates of algal photosynthesis. The algae of unfed hydra translocated a larger proportion of photosynthetically fixed ^{14}C to the host than did the algae in recently fed hydra. The differences in algal translocation were small, however, and unlikely to significantly affect hydra growth rates. Evidence is presented suggesting that the decrease in the rate of algal translocation in fed hydra may result from an increased algal demand for photosynthate to support the rapid algal growth that follows host feeding.

Introduction

Studies comparing the budding rates of green and aposymbiotic (algae-free) hydra show that possession of endosymbiotic algae increases the rate of hydra bud production. The advantage conferred by endosymbiotic algae was greatest when hydra received little food. There was no difference in budding rate between amply fed green and aposymbiotic hydra (Muscatine and Lenhoff, 1965a, 1965b, Stiven, 1965). A similar phenomenon occurs in the symbiosis between *Chlorella* and *Paramecium bursaria*. Endosymbiotic algae augment the ciliate's growth at low but not high concentrations of bacterial food (Karakashian, 1963). It is generally believed that the algae augment hydra growth by providing organic materials to the host (Muscatine and Lenhoff, 1965b, Smith *et al.*, 1969, Muscatine, 1971, Thorington and Margulis, 1981). The question then arises whether the endosymbiotic algae may increase the rate of photosynthate translocation during periods when the host is without food (Smith *et al.*, 1969, Mews, 1980).

Regulation of algal translocation according to host need suggests a high degree of coevolution between the symbionts. On the other hand, feeding the host stimulates algal growth (McAuley, 1981, 1982, 1985a, 1985b, 1986a, Bossert and Dunn, 1986, Dunn, 1987). If algae need much of their photosynthate to grow, they may necessarily translocate less carbon when growing rapidly (Pardy and White, 1977; Mews, 1980). Mews (1980) showed diminished translocation by the rapidly growing algae repopulating hydra that had been artificially depleted of algae. Thus the rate of algal carbon translocation may increase when the host is without food through a mechanism which involves no algal response to host need *per se*.

To determine if the contribution of algae to host growth is influenced by host nutritional state directly, rather than by algal growth rate, one would like to vary hydra food income while holding specific algal growth rate constant. It may be possible to accomplish this by studying hydra at "steady state" with their food incomes (Otto and Campbell, 1977, Gurkewitz *et al.*, 1980). Otto and Campbell (1977) showed that after ten days on a fixed feeding regime, hydra come to a "steady state" in which the specific growth rate of hydra cells is constant across feeding rates (see also Bosch and David, 1984). In the studies described here, bud production is measured in hydra at steady state with their feeding rates. Under

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* Present address: Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.

these conditions, the contribution of algae to host bud production was either independent of, or an increasing function of host feeding rate.

One might expect that starving hydra, because of their depressed growth rates, would receive more translocated carbon from their algae than do well fed hydra. Previous examinations of the distribution of photosynthetically fixed ^{14}C in green hydra have not supported this expectation. Similar percentages of fixed carbon were found to be translocated in hydra starved one and three days (Eisenstadt, 1971), and one and nine days (Mews, 1980). Although the percentage of fixed carbon translocated was unaffected by starvation, if algae increase their rate of photosynthesis during host starvation, the actual mass of carbon translocated may have varied with the rate of algal photosynthesis. This was not measured. In studies presented here, measurements of the percentage of photosynthetically fixed ^{14}C translocated from algae to host were taken alongside measurements of the rate of algal photosynthesis, measured with a polarographic oxygen electrode. Algal photosynthesis was unaffected by starvation. However, feeding induced small, brief declines in the percentage of ^{14}C translocated from algae to host to levels below the baseline characteristic of unfed hydra.

Thus: a. the contribution of algae to hydra growth, b. the rates of algal photosynthesis and, c. the percentages of fixed carbon translocated from algae to host, provide no evidence to support the idea that endosymbiotic algae respond according to host nutritional need.

Materials and Methods

Experimental organisms

The Carolina strain of *Hydra viridissima* was obtained from the Carolina Biological Supply Co. Aposymbiotic clones were derived from individuals whose algae were removed by the method of Pardy (1976).

Stock culture conditions

Hydra were maintained in M solution (Muscatine and Lenhoff, 1965a) minus Tris buffer at 17 degrees C under continuous illumination of 15 to 25 $\mu Em^{-2}\ s^{-1}$. Stocks were fed to repletion with freshly hatched *Artemia* nauplii every Monday, Wednesday and Friday for a period of several months before the start of any study. The culture dishes were rinsed 2 hours and 10 hours after feeding and were scrubbed once a week.

Maintenance of hydra on fixed feeding regimes

Experimental hydra were kept singly in 40 ml dishes. They were maintained on a fixed food income by pipetting a given number of freshly hatched *Artemia* nauplii directly onto the tentacles. Attached buds were not permitted to feed. Thus, bud production reflected parental investment only.

In the first study, 21 green hydra and 18 aposymbionts were fed between 1 and 5 nauplii apiece three times weekly. In the second, 23 green hydra and 20 aposymbionts were fed between 1 and 5 nauplii apiece six times weekly. In all cases the same feeding regime was enforced for ten days prior to the twenty-one day period of data collection. Ten days at a constant feeding rate has been found to be sufficient to equilibrate the size and budding rate of *Hydra attenuata* (Otto and Campbell, 1977). When experimental hydra refused to eat their alloted number of nauplii, subsequent meals were supplemented to compensate.

Measurements of hydra bud production

The number of detached buds was recorded daily for each hydra prior to feeding. On Monday, Wednesday, and Friday detached buds were removed, rinsed in distilled water, and lyophilized. Buds were later weighed individually on a Cahn G-2 microbalance.

Estimation of parental tissue

A positive relationship between parental size and feeding rate is characteristic of hydra at equilibrium with their feeding rates (Otto and Campbell, 1977, Gurkewitz *et al.*, 1980). To verify that this relationship existed in hydra whose bud production was to be measured, parental size had to be measured without harming the experimental hydra. Consequently, parental size was measured photographically. Hydra were photographed at the beginning, middle and end of each feeding experiment. Parental hydra volumes (exclusive of bud tissue) were computed from these photographs as previously described (Slobodkin and Dunn, 1983).

Calibration curves were constructed to convert photographic estimates of volume into masses. Budless adult hydra were each photographed twice, then lyophilized and weighed. Geometric mean regressions (Sokal and Rohlf, 1981) computed from these data explained 90% and 57% of the variation in the masses of green hydra and aposymbionts, respectively. These regressions were then used as calibration curves to estimate the mass of parental tissue of each experimental hydra from its photographic volume measurements.

Algal photosynthesis and translocation

To measure the effect of feeding on translocation of photosynthetically fixed carbon from algae to host, the

rates of photosynthesis and the distribution of photosynthetically fixed ^{14}C between algae and host were measured before and at various times after feeding.

Photosynthetic oxygen production was measured using a polarographic oxygen electrode chamber (volume = 6.4 ml) fitted with a YSI model 5331 oxygen probe (Dunn, 1986). Illumination from a Bausch and Lomb fluorescent lamp was passed through a Kodak 301A infrared cutoff filter to minimize heating of the incubation chamber. Photon flux at the experimental hydra was approximately 30 $\mu Em^{-2}\ s^{-1}$, an illumination level that caused no chamber heating. All incubations were done in 0.45 μ filtered M solution maintained at 17°C by circulating water from a constant temperature bath through the chamber water jacket.

For each experiment, 5 groups of 30 hydra each were assembled randomly from a pool of standard hydra (hydra with one fully formed bud) which had been unfed for 72 hours. One of these five groups was set aside for measurements on unfed hydra. The remaining 4 groups were fed and sequentially selected for measurement at the time points 12, 24, 36, and 48 hours after feeding. In one experiment hydra were fed one *Artemia* nauplius apiece and in a second, hydra were fed two nauplii apiece.

At each time point, hydra were placed in the respirometer, and all light was excluded from the chamber. Upon equilibration of the chamber temperature a constant rate of oxygen depletion was produced, reflecting respiration. Upon illumination a slower rate of oxygen depletion was immediately established and its slope, reflecting net photosynthesis (the combined rates of respiration and photosynthesis), was recorded for 15 minutes. At the end of this period, 50 μl of $Na_2{}^{14}CO_3$ (approximately 1 μCi per μl) was injected through a side port. Hydra were incubated in light in this solution for 45 minutes.

At the end of the incubation, the hydra were rinsed and then homogenized in a glass tissue homogenizer at 0°C. Algae were separated from host tissue by three rounds of centrifugation in M solution (at 600 *g*). Host and algal fractions were then frozen for later analyses. An average of 1.3% (±.15%, standard error) of the algae were found to be included in the host fraction.

At the end of each incubation, after hydra were removed from the chamber, the oxygen electrode was calibrated by means of measurements taken of air saturated distilled water at 17°C and of the deoxygenated solution after addition of a mixture of sodium dithionite and $CoCl_2$.

Additional ^{14}C partitioning data were collected in three more experiments in which hydra were otherwise treated as described above, but no respirometry data were collected. In one experiment, hydra were starved for 72 hours, then fed a single nauplius apiece. In a second experiment, hydra were starved for 72 hours, then fed *ad lib.* In the third experiment, hydra were starved for 120 hours, then fed *ad lib.*

Triplicate samples of both animal and algal fractions for each time point were prepared for liquid scintillation counting. 0.2 ml of 6 *N* acetic acid was added to 0.2 ml aliquots from each fraction. The mixture was then placed in a warm air stream and shaken intermittently over a period of 30 minutes to allow unfixed $^{14}CO_2$ to escape. A stable transparent mixture was obtained after addition of 10 ml of scintillation fluor (8 grams Omnifluor, 2 liters toluene, 1 liter Triton X-100) and 1.2 ml deionized water. Samples were counted with a Beckman LS-100C liquid scintillation counter. Counts per minute were corrected to disintegrations per minute by the external standards channel ratio method.

Of the radioactivity found in the host fraction, 84% was assumed to have been translocated from the algae, since the host fractions of three samples of green hydra incubated in darkness contained an average of 16% (±2%, standard error) of the radioactivity present in the host fraction of identically treated but illuminated green hydra. Likewise, a sample of aposymbionts incubated with radioactive sodium carbonate accumulated 16% of the radioactivity present in the host fraction of illuminated green hydra (after correcting for differences in protein content between the two).

Measurement of algal growth

Algal growth was estimated from four to eight hemacytometer counts of both the host and the algal fractions of a particular sample under 400× epifluorescence. To avoid bias, samples were analyzed without knowing their identity.

The data in Figure 7 on algal growth are also presented as part of another report (Dunn, 1987).

Protein determination

Triplicate protein determinations were made of both host and algal fractions by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Sample absorption values were read at 750 nm to avoid interference from chlorophyll.

The difference between the protein content of hydra before and 12 hours after feeding (thus following regurgitation) was used as a measure of food intake for hydra fed *ad lib.* In the two experiments described above in which each hydra was fed one nauplius, the protein content of an individual *Artemia* nauplius was calculated according to this procedure to be 1.6 and 1.8 μg of protein

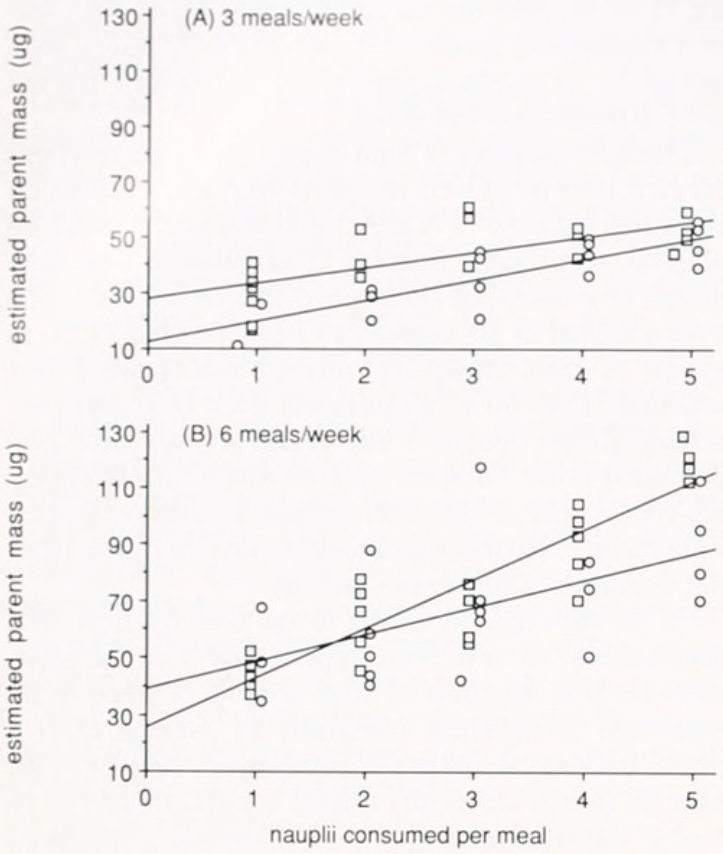

Figure 1. Estimated masses of green (□) and aposymbiotic (○) hydra at various steady state feeding rates for hydra fed three times weekly (A) and six times weekly (B). Masses were derived from photographic size estimates of parental tissue only (exclusive of bud tissue) by the method described in Slobodkin and Dunn (1983). Plotted values represent the means of determinations made at the beginning, middle, and end of the 21-day experiment. Least squares regressions (shown) yielded R^2 values of 0.51 and 0.82 for green hydra fed three times and six times weekly, respectively, and 0.69 and 0.33 for aposymbionts fed three times and six times weekly, respectively. In this and following figures, plotted values for green hydra and aposymbionts have been slightly offset horizontally for clarity.

per nauplius. These values agree well with a direct determination of 1.8 μg of protein per nauplius.

Results

The effect of feeding rate on hydra bud production

Parental body size of both green and aposymbiotic *Hydra viridissima* was an increasing function of steady state feeding rate (Fig. 1).

The experimental hydra produced more buds when provided with more food. For any food income, green hydra appeared to produce more buds than did aposymbiotic hydra (Fig. 2). (Because some of the cells of these meristic data have zero variance, the statistical significance of these regressions or the differences between them cannot be tested parametrically.) Bud size is likewise an increasing function of feeding rate for both forms of hydra (Fig. 3). The green hydra made larger buds than did the aposymbiont (ANCOVA, $P < .025$ in the low frequency feeding study, $P < .001$ in the high frequency feeding study).

To assess the contribution of algae to host growth, estimates of host tissue production were derived from measurements of the mass of buds produced. An average of 63% of the Carolina strain's protein was found in the host fraction when algae were removed by centrifugation. McAuley (1986b) showed that another 17% of hydra protein is lost by the host fraction to the algal fraction by this procedure. Accordingly, I assumed that 80% of the total protein produced by the Carolina strain is of host origin. Assuming that this protein ratio is proportional to the biomass ratio of host tissue to total hydra tissue, rough estimates of host tissue production can be made. Note that these estimates could be confounded by systematic variation in the biomass ratio with feeding rate (see Discussion).

When hydra were fed daily, the beneficial effect of algae on host tissue production was evident at all food lev-

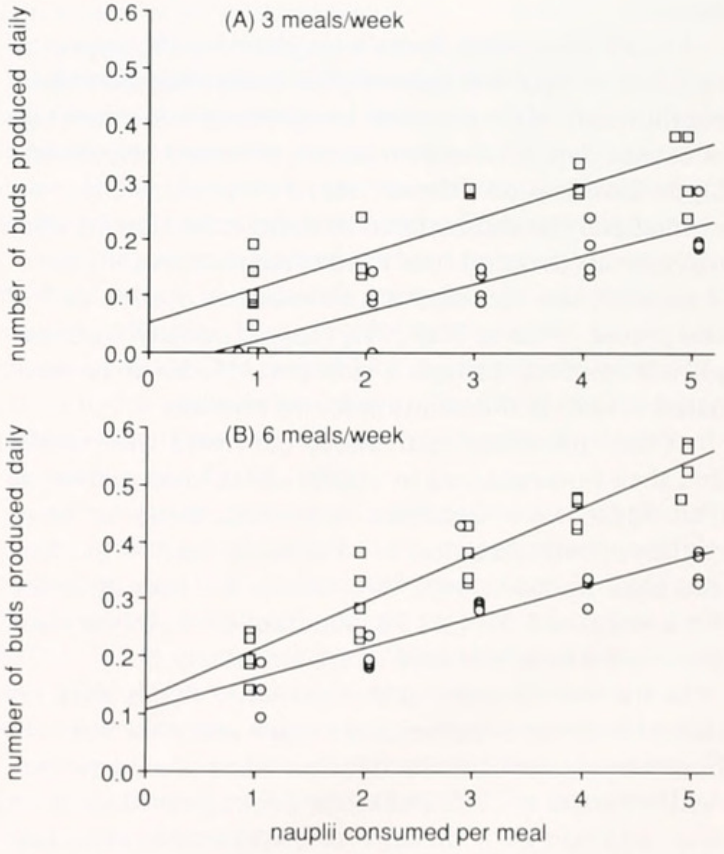

Figure 2. The numbers of buds produced by green (□) and aposymbiotic (○) hydra during 21 days at various steady state feeding levels for hydra fed three times (A) and six times weekly (B). Least squares regressions (shown) yielded R^2 values of 0.75 and 0.90 for green hydra fed three times and six times weekly, respectively, and 0.74 for aposymbionts fed three times or six times weekly, respectively.

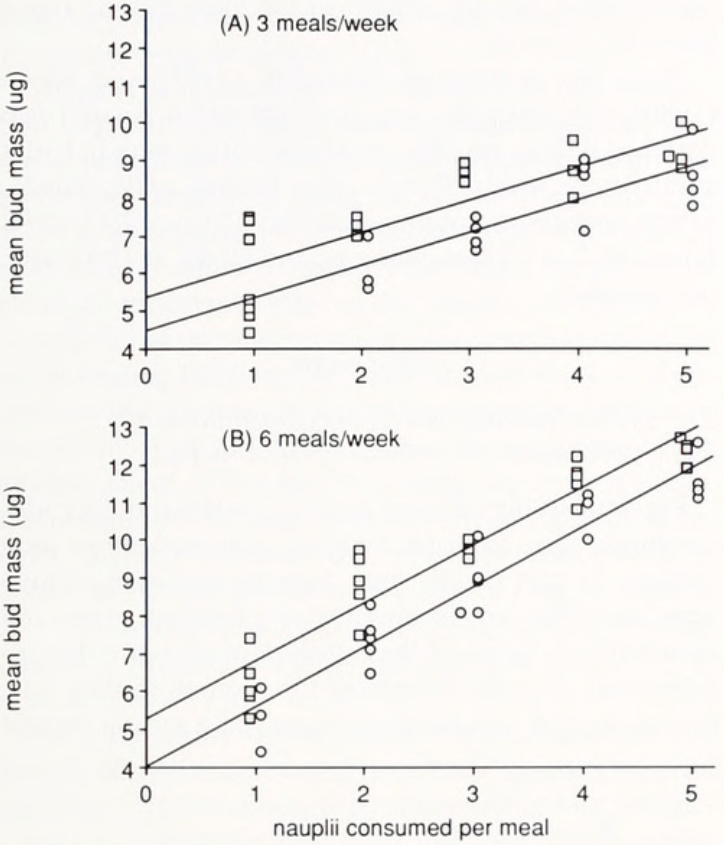

Figure 3. Mean masses of buds produced by green (□) and aposymbiotic (○) hydra during 21 days at various steady state feeding levels for hydra fed three times (A) and six times weekly (B). Least squares regressions (shown) yielded R^2 values of 0.70 and 0.90 for green hydra fed three times and six times weekly, respectively, and 0.65 and 0.91 for aposymbionts fed three times and six times weekly, respectively.

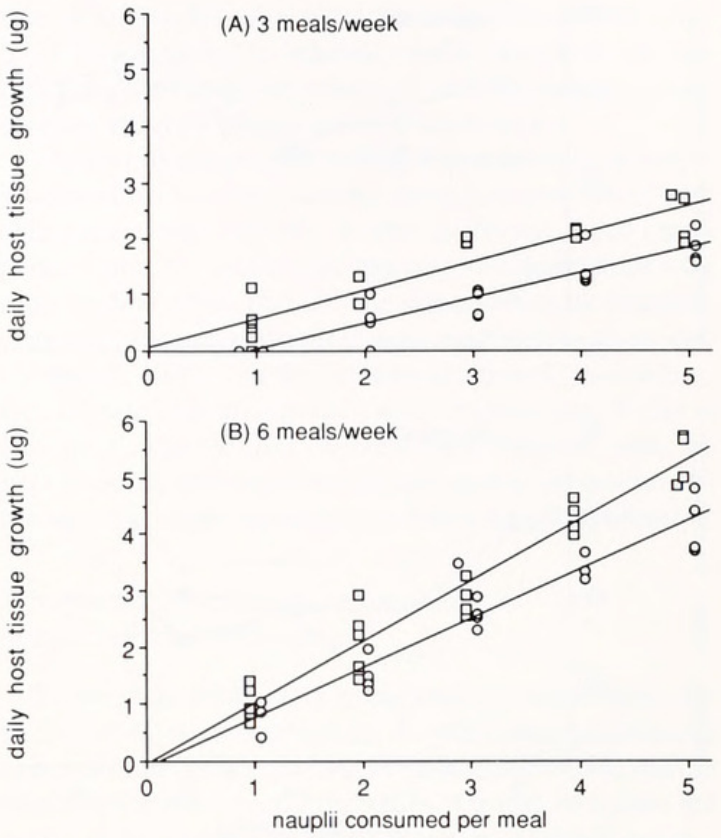

Figure 4. Estimated total amount of host tissue produced by green (□) and aposymbiotic (○) hydra during 21 days at various steady state feeding levels for hydra fed three times (A) and six times weekly (B). Least squares regressions (shown) yielded R^2 values of 0.85 and 0.94 for green hydra fed three times and six times weekly, respectively, and R^2 of 0.82 and 0.90 for aposymbionts fed three times and six times weekly, respectively.

els (Fig. 4b), and the benefit increased as food level increased (slopes differ significantly, $P < .05$, ANCOVA). For hydra fed every other day (Fig. 4a), green hydra produced more animal tissue than did aposymbionts ($P < .001$, ANCOVA), but the increment of difference was independent of food level (slopes do not differ significantly. $P > .25$, ANCOVA). There is no evidence at either feeding frequency that the contribution of algae to host tissue production increases as host food level decreases.

The effect of host feeding on algal photosynthesis

The rates of photosynthesis and of respiration were measured as a function of time after feeding for hydra consuming either one or two *Artemia* nauplii apiece. For each sample, the rate of gross photosynthesis was derived by subtracting the rate of oxygen depletion in the dark (due to respiration) from the rate of oxygen depletion in the light (due to the combination of respiration and photosynthesis). As shown in Figure 5, feeding appeared to have little effect on either the photosynthetic rates or the respiratory rates of starved hydra, at least at the level of resolution of twelve hours.

In all cases, hydra consumed oxygen, indicating that

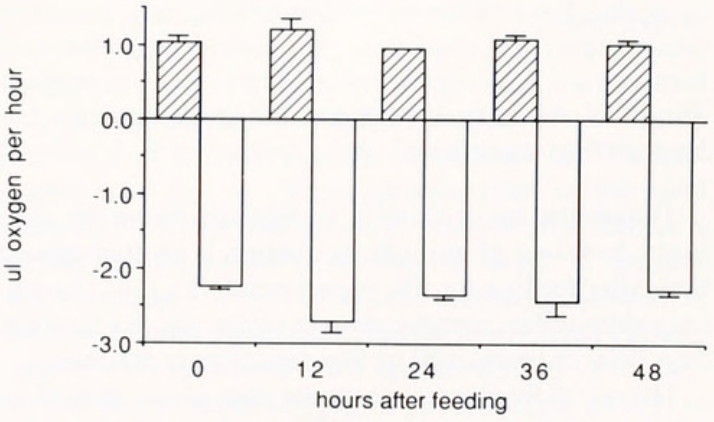

Figure 5. Gross photosynthetic oxygen production (hatched bars) and respiratory oxygen consumption (open bars) of samples of 30 hydra apiece at various times after feeding. Data shown are from two experiments. In one, hydra were fed a single *Artemia* nauplius apiece, in the other, hydra consumed an average of two nauplii apiece. Since no differences between the two feeding conditions were apparent, the data were pooled to yield the means and standard errors shown.

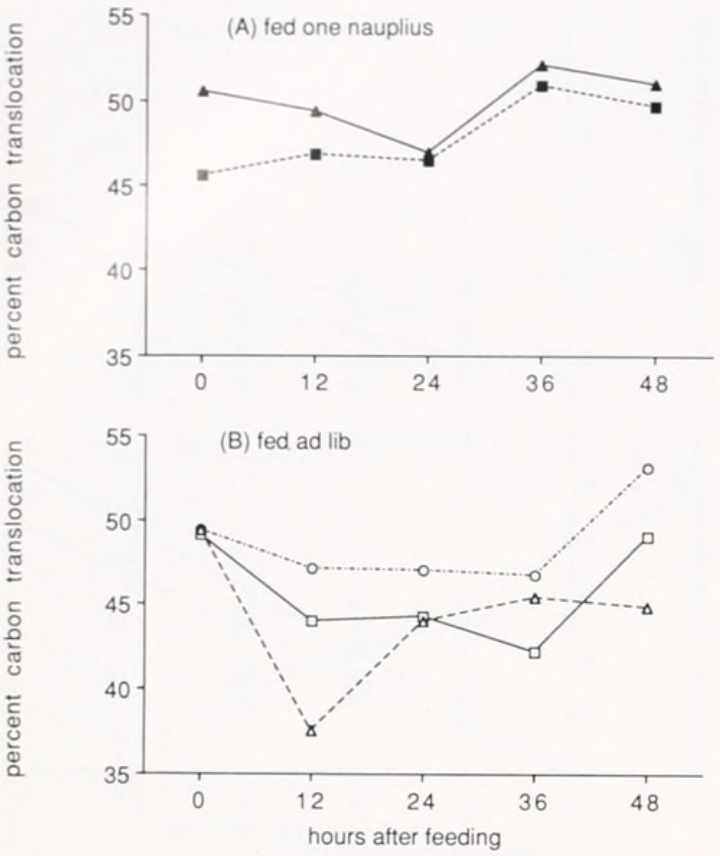

Figure 6. Percentage of photosynthetically fixed ^{14}C found in the host fraction of 30 hydra at various times after feeding. A. Each hydra fed one *Artemia* nauplius apiece. B. Hydra fed *ad lib*, each consuming two nauplii (○), or an average of three nauplii (△), or four nauplii (□). Hydra were starved for three days prior to experimental feeding in all cases except for the hydra which ate an average of three nauplii, which had been starved for five days.

30 $\mu Em^{-2}\ s^{-1}$ is below this association's light compensation point. Phipps and Pardy (1982) report a compensation point of 175 $\mu Em^{-2}\ s^{-1}$ for the Florida strain of *H. viridissima*.

The effect of feeding on photosynthate partitioning between host and algae

The percentage of fixed ^{14}C translocated from the algae to the host in a 45 minute incubation is plotted against time after feeding for five experiments (Figs. 6a, b). Initial values reflect translocation in hydra starved for three days (four experiments) or five days (one experiment).

The *ad lib* feeding experiments were characterized by a decline in the percentage of ^{14}C translocated during the interval from 12 to 36 hours following a meal (Fig. 6b). The mean percentage translocated during that interval varied inversely with both the average meal size of the hydra (Fig. 7a) and with the net algal growth rate during the interval (Fig. 7b). However, the mean percentage translocated was not related to the algal mitotic index (data not shown).

As in the studies of Eisenstadt (1971) and Mews (1980), extending the period of starvation beyond two days had little or no effect on translocation rates in hydra studied here; within 48 hours after feeding *ad lib*, translocation appears to stabilize near 50% (51.0 ± 2.1% at 48 hours, 48.7 ± 1.1% at 72 hours, and 49.4% at 120 hours, one sample).

Discussion

The effect of feeding rate on the contribution of endosymbionts to the growth of green hydra

Previous studies showed that the contribution of endosymbiotic algae to hydra budding increased at low food intakes. In the steady state feeding studies presented here, the difference in budding rate between green and aposymbiotic forms of the Carolina strain was either independent of, or an increasing function of, feeding rate. It may be that well-fed hydra enjoyed a greater benefit

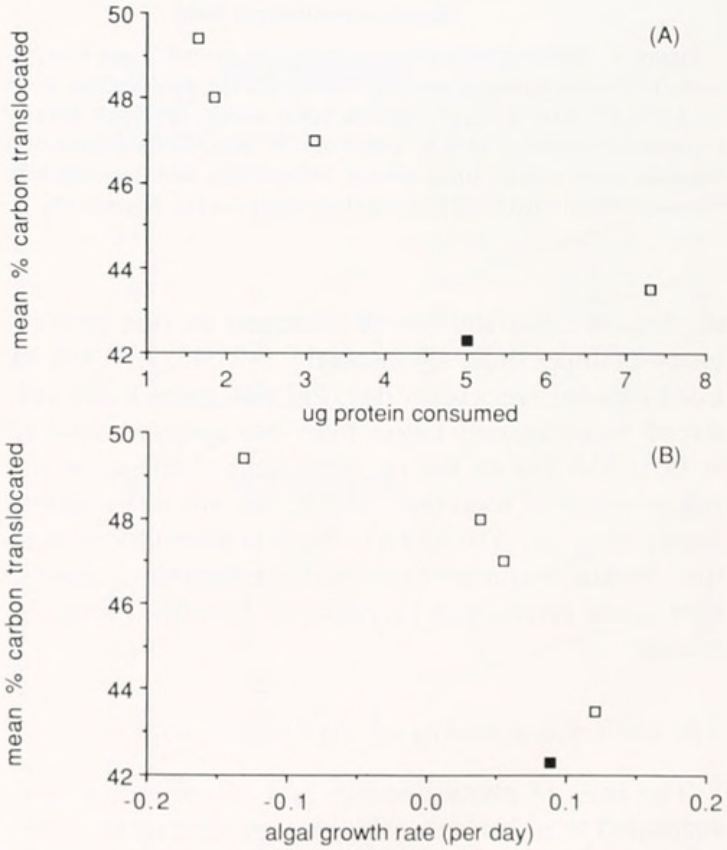

Figure 7. Mean percentage of photosynthetically fixed ^{14}C translocated to the host during the period from 12 to 36 hours after feeding as a function of the average amount of protein ingested by hydra in the immediately preceding meal (A), and as a function of the interval average algal growth rate (B). Hydra were starved for three days prior to experimental feeding in all cases except for one (■), in which hydra had been starved for five days.

from their algae by virtue of larger algal populations concommitant with their larger size.

Stiven (1965) found that the efficiency of bud production in green hydra (computed as calories of buds produced per calorie of *Artemia* nauplii consumed during a given interval of time, using the conversion coefficients given in Slobodkin, 1964) increased from 50% when fed daily to 61% when fed every other day. The beneficial effect of endosymbionts on the budding of the steady state hydra of the present study is likewise more obvious when feeding frequency is reduced from daily to semi-daily. However, it is less a consequence of an increase in the efficiency of green hydra than a consequence of the adverse effect of infrequent feeding on aposymbionts. Decreasing the feeding frequency from daily to semi-daily slightly increases the efficiency (calculated as above) of green hydra from 66% to 68% (not significant, $P > .75$, ANCOVA), but lowers aposymbiont budding efficiency from 43% to 35% (nearly significant, $.05 < P < .1$, ANCOVA).

For quantifying the beneficial effect of algae on the growth of hydra, the rate of host tissue production is an arguably better criterion than is the rate of bud production, which includes both host and algal components. However, the conclusion remains the same regardless of whether hydra growth is quantified in terms of number of buds, mass of buds, or mass of host tissue produced per day; the augmentation of hydra growth caused by algae is either independent of, or an increasing function of, hydra feeding rate.

However, it should be noted that an implicit assumption in the correction applied to convert the mass of buds produced into mass of host tissue produced is that the ratio of algal to host biomass is independent of hydra feeding rate. Large systematic variation in this ratio could seriously confound this correction. While no evidence exists that directly pertains to this, it is generally noted that starvation increases the ratio of algal to host biomass (Muscatine and Pool, 1979; Douglas and Smith; 1984, McAuley, 1985a; Muller-Parker and Pardy, 1987). If the same sort of variation occurs in steady state hydra such that the amount of algal tissue increases relative to host tissue at low feeding rates, it would only serve to accentuate the results already found, that is, the beneficial effect of algae increases with hydra feeding rate.

Many of the experimental hydra developed testes during the daily feeding experiments. This sexuality may have resulted from the constant feeding regime imposed on the hydra (Rutherford, *et al.*, 1983) since non-sexual animals were chosen for the experiment. Sexuality did not measurably affect asexual reproduction by these hydra. The proportion of the experimental interval in which hydra were observed to exhibit testes was not correlated to the efficiency of bud production of either form of *H. viridissima* (correlation coefficients were not significantly different from zero, $r^2 = .04$ for each form, arcsine-square-root transformed proportions).

The results suggest an increased contribution of endosymbionts to bud production seen at low host food levels (Muscatine and Lenhoff, 1965a, b; Stiven, 1965) may pertain only to hydra that are not at steady state with their feeding rates. The cell-specific growth rate of steady state hydra is independent of host feeding rate (Otto and Campbell, 1977), but the cell-specific growth rate of non-steady state hydra may vary with feeding rate. If this is true, it is possible that the beneficial effect of algae on host budding depends less on feeding rate *per se* than on the specific cellular growth rate of one or both symbionts.

The effect of host feeding on the rate of algal carbon translocation

The translocation data presented here qualititatively fit the model presented earlier in which algae translocate more carbon during periods of slow growth than during periods of rapid growth; the algae of unfed or poorly fed hydra translocated a larger proportion of their fixed carbon than algae in recently fed and well-fed hydra, respectively. The simplest interpretation of these data is that the algae in well-fed hydra retain more of their photosynthate to support their own rapid growth. Since the carbon fixation rates of endosymbionts are relatively constant regardless of host nutritional condition, slowly growing algae in poorly fed hydra may translocate more of their photosynthate.

Using ^{14}C as a tracer to estimate the rates of carbon translocation from algae to host incurs certain errors (see Mews, 1980; Muscatine *et al.*, 1984 for discussion). In particular, the specific activity of newly fixed carbon or of translocated carbon may both vary (*e.g.*, when hydra respiratory $^{12}CO_2$ production changes or if translocated carbon contains some variable fraction of previously fixed algal ^{12}C). Consequently, the amount of ^{14}C translocated may not be strictly proportional to the total amount of carbon translocated. Translocated carbon is quantified here as the percentage of fixed ^{14}C translocated from algae to host, a variable that is independent of variations in the specific activity of newly fixed carbon. Mews (1980) showed that the specific activity of translocated maltose varied with time of incubation as well as with intensity of illumination. These variables were carefully controlled in my studies, but comparisons of percent translocation rates in different incubations may still be compromised if the specific activity of translocated carbon varied despite these precautions. Muscatine *et al.* (1984) suggest that while the ^{14}C tracer technique may

be adequate for "short term relative comparisons," their "growth rate method" is to be preferred for estimation of absolute amounts of translocation integrated over protracted periods of time.

Considered quantitatively, the changes in percent translocation rates with host nutritional state were small. It seems unlikely that they explain the dramatic changes in the algal contribution to budding seen in the non-steady state feeding studies of Muscatine and Lenhoff (1965a, b) and Stiven (1965). During the 36 hour decline in percent translocation following feeding, the algae of hydra ingesting an average of four nauplii apiece translocated approximately 44% of their photosynthate, while those in hydra eating only one nauplius translocated approximately 49%. Assuming equivalent carbon fixation rates at these two feeding levels, this represents an 11% increase in the amount of photosynthate translocated. From Stiven's (1965) regressions, green hydra and aposymbionts produced buds at essentially the same rate when fed four nauplii a day. Green hydra receiving one nauplius a day produced buds 2.5 times as fast as did comparably fed aposymbionts. It seems unlikely that an 11% difference in carbon translocation could alone account for such a difference in host growth.

Muscatine and Lenhoff (1965b) found that populations of aposymbionts and green hydra grow identically when fed daily, but green hydra grow twice as fast as aposymbionts when fed every two days. We assume that hydra fed every other day receive translocated carbon as in the *ad lib* feeding experiments shown in Figure 6. Assuming that daily feeding maintains translocation rates at the levels observed from 12 to 24 hours after feeding, daily fed hydra receive only 5% less translocated carbon than hydra fed every 2 days. Again, it seems unlikely that a 5% difference in translocation rate could have such a dramatic effect on hydra growth rate.

Even considering possible differences in culture conditions between this and previous studies, these calculations suggest that the enhanced beneficial effect of algae on host budding (Muscatine and Lenhoff, 1965a, b; Stiven, 1965) at low feeding frequencies may depend on an algal factor other than carbon translocation. The importance of algal carbon translocation to the growth of regularly fed green hydra has been questioned by Mews and Smith (1982) and Muller-Parker and Pardy (1987). Mews and Smith (1982) found no relation between the rates of translocation and host budding in several artificial green hydra associations. In studies of an artificial association between aposymbiotic *H. viridissima* and algae isolated from symbiotic *Paramecium bursaria*, Muller-Parker and Pardy (1987) found that hydra raised at 30 $\mu Em^{-2} s^{-1}$ fixed almost four times as much carbon as those raised at 5 $\mu Em^{-2} s^{-1}$, but grew only 10% faster. They concluded that "the growth rates of fed hydra are regulated by factors other than light-dependent carbon fixation."

The light intensity in the studies reported here was below the association's compensation light intensity where photosynthetic oxygen production matches respiratory oxygen consumption. Therefore, hydra satisfied a certain fraction of their respiratory carbon requirement through feeding. If the experiments had been conducted at a higher illumination, algal photosynthesis and translocation may have satisfied the association's respiratory needs so that heterotrophy would not be required for maintenance. However, as discussed above, increased carbon translocation might not result in increased hydra growth.

The amount of algal disintegration in green hydra can be substantial and may increase when hydra are underfed (Dunn, 1987). Disintegration of algae may provide the host with a variety of nutrients, some of which may be more limiting to bud production than reduced carbon. Insofar as disintegrating algae are incapable of fixing carbon, this form of nutrient translocation will not be detectable in short-term radioactive carbon partitioning assays.

Acknowledgments

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