Review article
The Bioenergetics of Symbiotic Sea Anemones (Anthozoa: Actiniaria)

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Abstract

The bioenergetics of sea anemones and the interactions between anemones and symbiotic zooxanthellae are reviewed from the standpoint of costs and benefits to the anemone. Energy input to the anemone includes phagotrophy (consumption of particulate organic material), saprotrophy (uptake of dissolved organic material), and autotrophy (photosynthesis by zooxanthellae). Energy output includes respiration, growth and storage, reproduction, and export of carbon. Interactions between host and symbiont affect virtually all of these processes and the energetic costs of symbiosis to the host should not be ignored.

Anemones appear to benefit from translocated algal photosynthate as anemones starved in light lose weight less rapidly than anemones starved in darkness. However oxygen production during algal photosynthesis may also be important in determining the balance between aerobic and anaerobic respiration by the host. Furthermore, translocation of algal photosynthate may not equate with benefit to the host: much photosynthetic carbon is lost from the host and the remaining carbon may not contribute substantially to host bioenergetic status. The net impact of zooxanthellae on the host energy budget under field conditions is largely unknown.

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1.   Introduction

Sea anemones (Anthozoa: Actiniaria) often harbor symbiotic intracellular algal cells (=zooxanthellae: *Symbiodinium* sp.), which can attain a density exceeding 10^6 cells per mg animal protein (Muller-Parker, 1987). The intracellular locus of zooxanthellae effectively isolates them from the seawater medium common to most marine algae, so that zooxanthellae are ultimately dependent upon traffic of inorganic or organic nutrients through the host cell. This locus provides zooxanthellae with a potentially eutrophic habitat, exploitation of which is dependent upon the ability of the zooxanthellae to assimilate host organic compounds (Steen, 1987). However, zooxanthellae are photosynthetically competent and the transparency of the host tissue permits algal photosynthesis *in situ* (Muller-Parker, 1985). Therefore the net impact of zooxanthellae on the host carbon budget is dependent upon the balance between algal autotrophy, the production of fixed carbon in photosynthesis, and algal heterotrophy, the assimilation of preformed host organic carbon.

Traditional approaches to studying the anemone symbiosis have tended to yield traditional answers, largely because such a complex system can be experimentally intractable. However, several recent reports have called into question the mutualistic paradigm of anemone symbiosis (Steen, 1986a,b, 1987). In this review, the bioenergetics of symbiotic sea anemones will be examined with particular attention to the costs and benefits of symbiosis. Discussion will be limited to sea anemones (Anthozoa: Actiniaria) wherever possible, so the interested reader is referred to other reviews for a discussion of symbiotic corals and *Hydra* (Trench, 1979, 1981; Cook 1983). Throughout this review bioenergetics will be loosely defined as the biological causes and consequences of energy transformations. Because the currency of these transformations can be ATP or other energy-rich carbon compounds, the units of measurement of energy may vary widely. Results of experiments can be reported in calories or in measurements of synthesis or catabolism of organic carbon, which may or may not be standardized to some measure of animal biomass. These units can be interconverted (Strickland and Parsons, 1977), but are not strictly equivalent. Nonetheless, all results will be reported in units similar to those used by the authors of the respective studies.

This review will be limited to anemones for several reasons: anemones have recently been used extensively for laboratory experimentation because they lack the colonial growth habit or the external skeleton found in corals; anemones often reproduce asexually so that clones of animals can be used for

experiments; and anemones form a coherent taxonomic unit for discussion.

Limiting the discussion to anemones bearing zooxanthellae may also be important because of the state of confusion presently surrounding the taxonomy of zooxanthellae. Zooxanthellae isolated from various host species in two Phyla were resolved into 12 algal strains which differed in isoenzyme pattern, morphology, and infectivity to a single host (Schoenberg and Trench, 1980a,b,c). Cytogenetic analysis of 4 strains of zooxanthellae suggested that these strains were genetically isolated from each other, implying that different species of zooxanthellae may exist (Blank and Trench, 1985). Symbiotic zooxanthellae from the Caribbean jellyfish *Cassiopeia* sp. were originally described as *Symbiodinium microadriaticum*, a species long thought to be pandemic in Cnidarian symbioses (Freudenthal, 1962). However, it is probably more correct to regard zooxanthellae as a species complex (Blank and Trench, 1985), which will be designated as *Symbiodinium* sp. (Blank and Trench, 1986). By limiting this review to the anemone symbiosis, I hope to deal with coherent taxonomic groupings of both hosts and zooxanthellae, which will increase the likelihood that physiological similarities exist among the symbioses examined.

2. Energy Input to the Anemone

A. Phagotrophy

Anemone feeding

Anemones are sessile predators which capture prey by several distinct mechanisms: zooplankton may be captured from the water column in a raptorial manner, sessile prey items may be dislodged and washed into the anemone tentacles by wave action, or large motile prey may blunder onto the anemone tentacles (Sebens, 1981a). The prey is then seized and pierced by nematocyst discharge, resulting in release from the prey of substances which elicit anemone mouth opening and particle ingestion (Lenhoff, 1968; Van-Praet, 1985).

Relatively little is known about the diet or feeding rate of sea anemones in nature. Sebens (1981a) analyzed the gut contents of *Anthopleura elegantissima*, *A. xanthogrammica*, and *Metridium senile* collected in Washington state. *A. elegantissima* consumed primarily crustacean and molluscan prey, with *Mytilus edulis*, the dominant prey item, comprising 11.6% of the items found in coelenteron samples. Each *A. elegantissima* had an average of 1.01 prey items in its gut, whereas the larger *A. xanthogrammica* had an average of

0.37 items in its gut, but prey items tended to be larger. The diet of *A. xanthogrammica* was primarily composed of *Mytilus californianus* (68.9%) and various barnacles (16.3%). *M. senile* was found to consume mostly barnacle cyprids (75.2%) and nauplii (11.1%), with each anemone having an average of 5.03 items in its gut. In general, the number of captured prey was related to the feeding surface area, but prey size increased with predator size only in *A. xanthogrammica* (Sebens, 1981a).

The anemone *Anthopleura elegantissima* consumes small crustaceans, polychaetes, and gastropods, but phytoplankton, bacteria, and plant fragments may also be important in the diet (Van-Praet, 1985). Levels of digestive enzymes such as amylase and chymotrypsin undergo fluctuations suggestive of seasonal changes in the importance of plant material in the diet; amylase is proportionally more important in the summer when algal production is expected to be high (Van-Praet, 1985).

While the coelenteron of *Stoichactis giganteum* contains numerous fragments of echinoids, brittle stars, and large benthic decapods, a bacterial population in the coelenteron had a density of greater than 10^6 cells per ml (Herndl et al., 1985). However, compared with the potential nutritive value of food items in the coelenteron, bacterial carbon represented less than 1% of the calculated respiratory carbon requirement (Herndl et al., 1985).

Expansion of feeding tentacles is controlled in part by food availability, with the presence of food in the water eliciting feeding behaviour (Lewis, 1984). In *A. elegantissima* prey availability determines receptivity to feeding while food absorption efficiency is inversely related to ration size (Zamer, 1986). High intertidal anemones, which encounter less prey than low intertidal anemones, had a higher prey capture rate, absorbed food more efficiently, and had a higher growth rate than low intertidal conspecifics (Zamer, 1986).

It is difficult to assess the contribution of feeding to anemone energetics under natural conditions. While little is known about the feeding abilities and preferred food items of most anemones, even less is known about prey availability in the field. Johannes et al. (1970) sampled zooplankton over a coral reef in Bermuda and concluded that prey availability was insufficient to provide for coral respiration, even given the difficulty of sampling zooplankton distribution (Taylor, 1973). However, the existence of certain striking feeding adaptations, such as the presence of separate tentacles for feeding and for "farming" of large populations of zooxanthellae (Gladfelter, 1975; Sebens and DeRiemer, 1977; Lewis, 1984), argues that phagotrophy is important in

the energetic economy of anemones.

Digestion of zooxanthellae

Digestion of zooxanthellae has been postulated as both a means of regulating the population of endosymbiotic algae and as a possible nutritional input to the host (reviewed by Droop, 1963). Conclusive evidence of host nutritional dependence upon digestion of zooxanthellae must demonstrate the hydrolysis of algal cells and the assimilation of algal substrates by the host (Muscatine, 1974). An analysis of the frequency of phagosome-lysosome fusion in the jellyfish *Cassiopeia xamachana* showed that, although such fusion did occur, the low frequency of fusion is inconsistent with the interpretation that the animals digested intact zooxanthellae (Colley and Trench, 1985).

Protein extracts of the anemone *Phyllactis flosculifera* have a destructive effect *in vitro* on freshly isolated zooxanthellae from *P. flosculifera* and *A. tagetes*. This suggested that the anemone is able to obtain organic compounds by digestion of symbiotic zooxanthellae (Steele and Goreau, 1977). However, digestion of algal cells was only observed when zooxanthellae were exposed to host extracts *in vitro* for periods exceeding 18 hr (Steele and Goreau, 1977). It is unclear whether this phenomenon is relevant to the intact symbiosis. If expulsion or digestion of zooxanthellae is a widespread phenomenon in symbiotic anemones, this would result in underestimates of either algal productivity or the nutritional input of algae to the host (see Section IIC). However, present evidence suggests that digestion of zooxanthellae is unlikely to substantially augment energy input to the host (Cook, 1983).

B. Saprotrophy

Importance of dissolved organic material

For aquatic organisms native to habitats that may be depauperate in particulate food, it is advantageous to directly adsorb dissolved organic material (DOM) from the water column (Schlichter, 1982). Although DOM in the oceans forms an immense pool of reduced carbon, the DOM concentration of seawater is quite low, usually on the order of one to a few milligrams per liter (Wright and Stephens, 1982). Although portions of the DOM pool appear to be refractory to biological utilization, certain fractions of the total pool have a much shorter turnover time. Turnover time for dissolved free amino acids (DFAA) in surface water has been estimated to be from 1–2 days to 1 month, suggesting that DFAA is likely to be an important source of carbon for an

organism capable of DOM uptake. The major constituents of the DFAA pool are usually glycine, alanine, serine, valine, glutamic acid, and lysine. Reports of DFAA concentrations in the near shore water column, typical of what an anemone might be exposed to, generally range from 300–500 $\mu g/l$ (reviewed by Wright and Stephens, 1982).

All soft-bodied marine invertebrates thus far examined have the ability to accumulate some radioactively labeled amino acids from dilute solutions (Wright and Stephens, 1982). However, little or no information exists on the nutritional requirements of sea anemones, the availability of organic substrates in the environment, the rate of uptake of DOM at ambient concentration, or the access of accumulated products to metabolic pathways of the anemone (Wright and Stephens, 1982). Although DOM transport requires some expenditure of energy, the transport of a mole of substance against a 100,000:1 gradient requires only 7 kcal/M. This is a negligible cost when compared to the energy acquired from metabolism of the transported substrate (Wright and Stephens, 1982).

Uptake of dissolved organic material

Several studies have addressed the kinetics of DOM transport by Cnidarians. The zoanthid *Zoanthus sandwichensis* accumulates ^{14}C-glycine dissolved in seawater at a concentration of 2.16 mg/ml, and accumulates glycine or glycine metabolites into lipid (Reimer, 1971). Scyphistomae of the jellyfish *Acropora acuminata* also take up labeled glycine from solution in seawater so dissolved amino acids may be important as a source of nitrogen (Shick, 1975). The anemone *Anemonia sulcata* is able to absorb charged and neutral amino acids simultaneously, suggesting the existence of several distinct uptake systems for amino acids (Schlichter, 1978). However, the assimilation of dissolved ^{35}S-methionine into anemone tissue by *Aiptasia pulchella* is less than 1% efficient (unpublished data cited in Steen, 1986a). This demonstrates that the existence of DOM uptake does not necessarily mean that uptake is significant in the biology of the organism.

Analysis of the importance of DOM uptake by anemones is fraught with problems. At realistic environmental concentrations of DOM, epidermal uptake by invertebrates may not be able to compete with uptake by bacteria (Siebers, 1982). The effect of bacteria has often been ignored in experiments on DFAA uptake; accumulation of tritiated amino acids by *Anemonia sulcata* ectoderm (Schlichter, 1973) may actually be accumulation by epibiotic bacteria. Moreover, many of the studies of uptake by invertebrates are flawed in

design, as removal of radioactively labeled compounds from solution does not
constitute proof of net uptake of these compounds (Johannes et al., 1969).
Labelled DFAA entering animal tissue is presumably diluted by large en-
dogenous pools of unlabeled compounds so that subsequent loss of DFAA to
the medium is likely to be of low specific activity. Disappearance of label
from the medium may mask a net flux of compounds out of the organism
(Johannes et al., 1969).

Despite these difficulties an estimate of the contribution of DOM to the en-
ergetic economy of the soft coral *Heteroxenia fuscescens* was possible because
under experimental conditions this organism did not ingest particulate food
(Schlichter, 1982). Uptake of only 12 amino acids and glucose, present at
concentrations approximating "natural" environmental concentrations, sup-
plied approximately 80% of the energy demand of the organism (Schlichter,
1982). While a considerable body of work suggests that DOM uptake is
possible by many Cnidarians, further work is necessary before the ecological
significance of such uptake can be assessed.

C. Autotrophy

A great deal of research has addressed the phenomenon of translocation
of photosynthate by symbiotic zooxanthellae. The transparency of anemone
tissue permits photosynthesis of symbiotic zooxanthellae *in situ* and translo-
cation of radioactively labeled algal photosynthate to anemone tissue was
first demonstrated in *Anthopleura elegantissima* (Muscatine and Hand, 1958).
However, the existence of translocation in a symbiotic system does not im-
ply that translocation is essential to the normal maintenance of the animal
(Taylor, 1969).

Host weight loss experiments

The importance of algal photosynthate to host nutrition may be seen in
studies of host weight loss during starvation in light and darkness. Over
14 weeks of starvation, symbiotic *A. elegantissima* starved in darkness lost
35% of initial reduced weight,while anemones starved in light lost only 27%
of initial reduced weight (Muscatine, 1961). Reduced weight changes were
similarly measured in *Anemonia sulcata*: over 20 weeks anemones starved in
light lost 33% of starting weight, while dark starved anemones lost 55% of
starting weight (Taylor, 1969). Sebens (1980) found that weight change in
experimental groups of *A. elegantissima* was affected by various interactions
between temperature, feeding regime, and illumination. Weight loss during

starvation was generally less when anemones were exposed to light, but illumination had a significant effect on anemone weight change only at 5°C (Sebens, 1980).

Experimental results such as these are difficult to interpret because of a number of uncontrolled variables. Symbiotic anemones maintained in light experience elevated levels of oxygen produced by the photosynthetic activity of zooxanthellae (Muller-Parker, 1985; Clayton and Lasker, 1984; Dykens and Shick, 1982; Fitt et al., 1982). Dark maintained symbiotic anemones are not exposed to oxygen from algal photosynthesis, and because of the relatively high respiration rate of zooxanthellae (Muller-Parker, 1985) and associated coelenteric bacteria (Reimer, 1971), these anemones may even experience oxygen depletion. Oxygen availability is not the only difference between light and dark maintained anemones. Since anemone metabolic rate is directly dependent upon oxygen tension (Sebens, 1980; Sassaman and Mangum, 1972; Sassaman, 1973) anemones starved in light may have a higher metabolic rate than dark starved animals.

These uncontrolled variables between light and dark maintained anemones are further confounded by the ability of most anemones to switch between aerobic and anaerobic metabolism (Sassaman and Mangum, 1973; Shick, 1981; Ellington, 1982). Most experiments testing the ability of anemones to survive periods of starvation in darkness are done under conditions which may cause the anemones to experience some level of hypoxia. Ellington (1981) has shown that anoxia produces anaerobiosis in *Bunodosoma cavernata* in which glycolysis is the major catabolic process. During anoxia, ATP levels in *B. cavernata* fall to 50% of aerobic levels (Ellington, 1981), a decrease which may favor the general induction of catabolic processes (Atkinson, 1977). Fermentation to lactate taps only about 7% of the free energy potentially available in the glucose molecule (Zabay, 1983), so anaerobic metabolism is very inefficient compared to aerobic metabolism. Thus an anaerobic anemone must utilize much larger quantities of storage metabolites to accomplish the same cellular work as an aerobic anemone. Therefore symbiont oxygen production may spare anemone weight loss by permitting aerobic metabolism. It is possible that translocation of algal photosynthate does not contribute directly to the maintenance of anemone biomass during starvation in the light.

Photosynthesis of zooxanthellae

Extensive evidence has accumulated to show that zooxanthellae *in situ* are capable of photosynthesis, and that a certain proportion of carbon fixed in photosynthesis is translocated to the host (reviewed by Cook, 1983; Trench, 1979; Muscatine, 1974; 1980). The traditional approach to quantify algal photosynthesis and translocation of photosynthate has been to expose zooxanthellae to $^{14}CO_2$ and to compare the distribution of label between algae and host after an incubation of 0.5–2 hr. The selective appearance of labeled products in host tissue or in the incubation medium is evidence for specific release processes (Muscatine, 1965). Potential problems with this method arise because of contamination between animal and algal fractions, translocation which may occur during the process of fraction separation, unequal specific activity of product labeling in short term incubations (Cook, 1983), and bacterial metabolism of released products.

Glycerol has been identified as the major labeled extracellular product of freshly isolated zooxanthellae from a range of anemone hosts. This compound comprises between 24 and 43% of the fixed carbon released (Trench, 1979). Glycerol (43.0% of total fixed ^{14}C), succinate and fumarate (28%), malate and citrate (25.0%), glycolate (2.3%), and alanine (1.2%) are among the extracellular products released *in vitro* by zooxanthellae of *A. elegantissima* (Trench, 1971). The distribution of radioactivity among intracellular products is quite different: glycerol (3.6%), succinate, fumarate, glycolate, and other organic acids (32.8%), glutamate (6.7%), and various amino acids (6.7%) (Trench, 1971). This clearly demonstrates that translocation of photosynthate by zooxanthellae is a selective process.

Translocated glycerol appears to be rapidly catabolized by the host anemone to carbon dioxide (Battey and Patton, 1987). Treatment of the intact anemone *Condylactus gigantea* with sodium cyanide was used to partially block anemone respiration of translocated algal photosynthate. Before inhibition only 2% of the total fixed carbon was recovered from host tissue as ^{14}C-glycerol, while following NaCN treatment approximately 25% of the host radioactivity was recovered as glycerol. These results suggest that glycerol is rapidly catabolized because of the absence of accumulated pools of fatty acids or fatty alcohols in the anemone(Battey and Patton, 1987). Analysis of anemone metabolism of exogenously supplied ^{14}C-glycerol demonstrates that the host is incapable of rapidly converting glycerol to fatty acid (Battey and Patton, 1984).

Gas exchange measurements have been used to estimate the net photosynthetic capacity of zooxanthellae in symbiosis with *Aiptasia pulchella* (Muller-Parker, 1985), *Anthopleura elegantissima* (Fitt et al., 1982; Shick and Dykens, 1984), and *Aiptasia pallida* (Clayton and Lasker, 1984). The net photosynthetic capacity of symbiotic *A. pulchella* was 26 μg $O_2 \cdot h^{-1}$ (mg protein)$^{-1}$, from which an assimilation number of 3.39 mg $C \cdot h^{-1}$ (mg chlorophyll a)$^{-1}$ can be calculated (Muller-Parker, 1984a). This indicates that the productivity of zooxanthellae is comparable to cultured phytoplankton, for which assimilation numbers range between 1.1 and 6.2 mg $C \cdot mg^{-1}$ chlorophyll $a \cdot h^{-1}$ (Parsons et al., 1977; Muller-Parker, 1984a). The ratio of daily net photosynthesis to respiration in whole *A. pulchella* was 1.60 or higher at all irradiances greater than 115 $\mu E\ m^{-2}s^{-1}$, indicating that this anemone is potentially phototrophic with respect to carbon (Muller-Parker, 1985). However the biomass of starved anemones showed that the mass of the anemone was not correlated with carbon fixation by the zooxanthellae (Muller-Parker, 1985).

In *A. elegantissima*, the weight-specific gross photosynthetic rate of zooxanthellae in large anemones was less than a third of that in the smallest anemones (Fitt et al., 1982). This suggests that large anemones obtain less benefit from symbiotic zooxanthellae. Feeding of the host may have a strong effect on translocation of photosynthate; the P:R ratio was 2.0 to 3.0 for starved anemones, but seldom exceeded 1.0 in fed animals. This was due to an increase in anemone respiration following feeding (Fitt et al., 1982; a similar effect was reported by Tytler and Davies, 1984). Based on oxygen flux data it was calculated that continually immersed field specimens of *A. elegantissima* obtained 34–42% of their respiratory carbon requirement from translocated algal photosynthate (Shick and Dykens, 1984).

Oxygen flux in the tissues of *A. pallida* was measured during maintenance under different host feeding regimes (Clayton and Lasker, 1984). Both anemone respiration rate and zooxanthellae density increased with increasing frequency of feeding, but algal gross photosynthesis (μg O_2 (10^6 zooxanthellae)$^{-1}$ hour^{-1}) was unaffected (Clayton and Lasker, 1984). However, zooxanthellae gross photosynthesis per anemone (μg O_2 (mg host protein)$^{-1}$ hour^{-1}) was significantly greater in fed anemones. Fed anemones had a greater density of zooxanthellae, therefore these animals received a greater quantity of translocated photosynthate from zooxanthellae than did starved animals (Clayton and Lasker, 1984). The observation that host starvation led to a decrease in zooxanthellae density (Clayton and Lasker, 1984) suggests that zooxanthellae may depend upon the host for some sub-

strate(s), the absence of which depresses algal growth (see Cook et al., in press; Section 3B).

The availability of dissolved inorganic nutrients (DIN) can have an effect on the energetic status of the host anemone in several ways: by providing limiting nutrients host productivity may be directly affected; uptake of DIN may be mediated by zooxanthellae at no direct energetic cost to the host; and uptake of DIN may enhance algal productivity thereby increasing the availability of translocated photosynthate to the host.

Zooxanthellae photosynthesis and translocation of photosynthate during illumination *in vivo* and *in vitro* is increased by ammonium at concentrations from 20 to 200 μM (Taylor, 1978). Ammonium stimulates zooxanthellae photosynthesis and translocation of photosynthate in tissue slices of tridacnid clams, but had no effect on the photosynthetic rate of zooxanthellae *in vitro* (Summons et al., 1986). These findings are consistent with the observation that coral heads with resident schools of fish grow more rapidly than coral heads without resident fish populations (Meyer et al., 1983).

Productivity of zooxanthellae

Estimates of the contribution of zooxanthellae photosynthesis to host autotrophy depend upon accurate quantitation of algal productivity. The relationship between algal photosynthetic rate and irradiance (the P/I curve) has been used to estimate productivity of zooxanthellae *in situ* (Muller-Parker, 1984a). Estimates such as this can be combined with measurement of host respiration rate and symbiont growth rate to calculate the contribution of translocated zooxanthellae photosynthate to animal respiration (CZAR; for derivation of the method see Muscatine et al., 1983). This technique, applied to corals (Muscatine et al., 1984), zoanthids (Steen and Muscatine, 1984), and anemones (Stambler and Dubinsky, 1984) suggests that 70 to 95% of algal photosynthate may be translocated to the host and that this translocated photosynthate can potentially supply 100% of the animal's daily respiratory carbon requirement (Muscatine et al., 1984). A large discrepancy exists between estimates of algal translocation by carbon budget analysis (Muscatine et al., 1984; Steen and Muscatine, 1984) and similar estimates made using the technique of $^{14}CO_2$ fixation and release of labeled organic compounds (reviewed by Cook, 1983; Trench, 1979; Muscatine, 1974). This discrepancy suggests that there may be problems with one or both techniques.

Problems associated with measuring algal photosynthetic rate by radioactive carbon fixation have been reviewed elsewhere (Peterson, 1980; Dring and

Jewson, 1982; Smith, 1982). It is not clear whether fixation of radioactive carbon more closely approximates gross or net photosynthesis, because algal respiration cannot be accurately measured (Peterson, 1980). Furthermore, under conditions of low nutrients, high irradiance, or low ratios of photosynthesis to respiration, discrepancies can arise between $^{14}CO_2$ fixation and other measures of photosynthesis (Peterson,1980). Uptake of ^{14}C by algal cells is more rapid than uptake of ^{12}C; until an equilibrium is established, ^{14}C uptake will overestimate net photosynthesis even in incubations as long as 6–12 hr (Dring and Jewson, 1982). Furthermore, algal cell growth rates are progressively overestimated as they decrease, and translocation of photosynthate can be underestimated by a factor of 2–10-fold (Smith, 1982).

While problems with carbon budget analysis cannot be fully discussed here, several potential problems deserve mention:

(a) *Sensitivity of the carbon budget analysis to variations in t_d.* The duration of mitosis (t_d) is an essential parameter for calculation of the zooxanthellae cell-specific growth rate (Muscatine et al., 1983), however it has not been directly measured in symbiotic zooxanthellae. The maximum value of t_d was estimated to be 11 hr by calculation from measured values of mitotic index (Wilkerson et al., 1983). However, on the basis of measured growth rate of log-phase zooxanthellae *in vitro*, t_d has been estimated to be 4.9 hr (Steen, 1987). A decrease in the estimate of t_d will increase the estimate of the cell-specific growth rate, and the extent of translocation to the host will thus be overestimated in carbon budget calculations (Steen, 1987).

(b) *Errors in estimating algal respiration rate* (Shick and Dykens, 1984). The carbon budget analysis assumes that the biomass specific respiration rate of host and symbiont is equal (Muscatine et al., 1983), an assumption which does not appear to be valid. The respiration rate of zooxanthellae freshly isolated from *A. pulchella* is 5.7 times higher than the estimated respiration rate of zooxanthellae *in situ* (Muller-Parker, 1984a). Similarly, the respiration rate of zooxanthellae isolated from the coral *Stylophora pistillata* was nearly eight times higher than the estimated *in situ* respiration rate (McCloskey and Muscatine, 1984). If the respiration rate of zooxanthellae freshly isolated from *S. pistillata* is equivalent to the respiration rate of zooxanthellae *in situ*, then *in situ* zooxanthellae respiration would account for approximately 50% of the total coral respiration (McCloskey and Muscatine, 1984). Respiration rates of freshly isolated zooxanthellae from the coral *Montastrea annularis* is an order of magnitude higher than predicted from the algal biomass ratio (Smith and Muscatine, 1986). In general, weight-

specific respiration rates of unicellular organisms are much higher than those of multicellular invertebrates (Prosser, 1973).

(c) *Host respiration rate changes with nutritional state.* The carbon budget analysis assumes that host respiration, measured over a diel period in a respirometer, is relatively constant (Muscatine et al., 1983). However, immediately after feeding, the respiration rate of *Anemonia sulcata* is elevated for between 24 and 72 hr (Tytler and Davies, 1984). The respiration rate of fed *A. elegantissima* is elevated 30–80% relative to starved anemones, irrespective of the presence of zooxanthellae (Fitt and Pardy, 1981).

(d) *Reverse flux of organic material from host to zooxanthellae.* The carbon budget method assumes that zooxanthellae are able to completely satisfy all of their nutritional needs by photosynthesis without heterotrophic uptake of carbon from the host. However, Cook (1971) has shown that zooxanthellae symbiotic with *Aiptasia* sp. will accumulate radioactive label when the host is fed ^{35}S methionine-labeled food. Steen (1986a) calculated that *A. pulchella* zooxanthellae obtain 2.8–6.4% of their growth requirement for protein by heterotrophic uptake of amino acids from the host, even though zooxanthellae were exposed to 70 μE m^{-2}s^{-1} of light (12 hr light: 12 hr dark). Zooxanthellae from the jellyfish *Cassiopeia xamachana* have an active transport mechanism for alanine uptake (Carroll and Blanquet, 1984), while zooxanthellae from the clam *Tridacna maxima* have active transport mechanisms for cysteine, methionine, and taurine (Deane and O'Brien, 1981).

Problems clearly exist with both the carbon budget analysis and the use of ^{14}CO$_2$ to estimate productivity of and translocation by zooxanthellae. Since it is unknown which of these techniques is more accurate, the discrepant estimates of the extent of translocation by zooxanthellae cannot be resolved at present.

Impact of zooxanthellae on host bioenergetics

Questions clearly remain about the extent to which anemones benefit from the translocation of algal photosynthate. For example, the protein biomass of *A. pulchella* starved under four different irradiances (ranging from 45 to 320 μE m^{-2}sec^{-1}) was not significantly different even though the photosynthetic rate clearly differed at the different irradiances (Muller-Parker, 1985). It was concluded that sea anemone growth was not directly related to the productivity of zooxanthellae in this association (Muller-Parker, 1985).

A new approach to evaluate the impact of symbiotic algae on the metabolism of a sea anemone has recently emerged. *In vivo* ^{31}P nuclear mag-

netic resonance (NMR) spectroscopy is capable of measuring tissue levels of phosphorylated metabolites. Although phosphorus is present in measurable concentration in only a few metabolites, many of these metabolites are involved in the energy metabolism of the cell. Therefore it is possible to analyze energy production and utilization in a living organism as a function of the relative levels of adenylates such as ATP and ADP (Gadian, 1982). NMR spectroscopy has enormous potential as a means to attack the question of how the rates, reactions, and regulatory processes described *in vitro* actually affect the physiology of the organism *in vivo*.

Symbiotic *A. pulchella* were fed to repletion, then starved under high irradiance (300–320 μE m^{-2}s^{-1}) or low irradiance (70–80 μE m^{-2}s^{-1}) conditions before collection of *in vivo* NMR spectra (Steen, 1986b). The host adenylate ratio (ATP: ATP + ADP) declined significantly under both treatments, but no significant difference could be detected between treatments (Steen, 1986b). Photosynthesis-irradiance curves derived for intact *A. pulchella* show that photosynthesis increases with increasing irradiance (Muller-Parker, 1984a, 1985), suggesting that translocation of algal photosynthate also increases with irradiance. The inability to detect differences in the bioenergetic status of anemones exposed to different irradiances suggests that translocation of photosynthate from symbiotic zooxanthellae did not affect the energetic status of the host. These results are corroborated by data showing that the mean blotted wet weight of starved *A. pulchella* declined at the same rate under high and low irradiances. Therefore, translocated algal photosynthate did not have a sparing effect on loss of host biomass in this anemone. During experimental incubations anemones received clean water regularly and bacterial populations were kept to a minimum by removing egested food 6 hr after feeding (Steen, 1986b). Under these conditions anemones probably did not experience hypoxia (see Section 3A).

Translocation of photosynthate from zooxanthellae to host has traditionally been viewed as the major energetic advantage of symbiosis to the anemone host. However, data supporting the hypothesis that translocated photosynthate is "junk food" for the host have come from many studies (Davies, 1984; Muscatine et al., 1984; Falkowski et al., 1984; Muller-Parker, 1985; Steen, 1986b; Edmunds and Davies, 1986; Zamer and Shick, 1987). More work is required before the impact of algal photosynthesis on host bioenergetics can be quantitatively assessed.

3. Energy Expenditure by the Anemone

A. *Respiration*

Gas flux measurements

Oxygen consumption and carbon dioxide production during cellular respiration have been measured for several symbiotic anemones. However, distinguishing animal respiration from algal respiration and photosynthesis requires elaborate calculations (Muscatine, 1980) that have made such measurements problematic. Anemone respiration rate is dependent upon anemone size, metabolic activity, state of expansion or contraction, ambient illumination, temperature, and oxygen tension (Sebens, 1981a). Measurements of anemone respiration rate are complicated by a number of factors.

(a) Mesoglea, which represents a variable fraction of the total mass of various anemone species, is metabolically less active than gastroderm or ectoderm tissue (Shick et al., 1979). Unless anemone weight-specific respiration is corrected for mesogleal weight, it will be in error by an amount which varies depending upon the proportion of anemone weight contributed by mesoglea.

(b) Gastroderm appears to be the most metabolically active tissue (Sassaman and Mangum, 1972; Shick et al., 1979), yet because of its location, oxygen consumption is difficult to measure directly. If short-term oxygen consumption measurements are made, metabolic rate may be underestimated because diffusion of oxygen to the gastroderm is slow.

(c) Oxygen production by photosynthetic zooxanthellae can produce hyperbaric levels of dissolved oxygen in the coelenteron of anemones. An oxygen microelectrode inserted into the gastrodermal tissue of *A. elegantissima* recorded an oxygen partial pressure of 328 mm Hg, more than twice the normal atmospheric pressure of oxygen (Dykens and Shick, 1982). This may or may not offset the potential error noted in (b) above.

(d) Anemone postural changes have a strong effect on the oxygen consumption rate measured (Shick et al., 1979). Therefore, any measurement of anemone oxygen consumption in which the anemone posture is not accounted for may be seriously in error.

(e) Anemone metabolic rate is affected by algal photosynthesis. *Anemonia sulcata* starved in darkness for 25 days had a significantly lower rate of respiration than anemones starved in light (Tytler and Davies, 1984), suggesting that anemone respiration rate is directly affected by algal photosynthesis. Respiration rate of *A. pulchella* was 1.5 times higher after exposure to saturating irradiance than after maintenance in darkness (Muller-Parker, 1984).

Protocols for measurement of respiration must be explicit as to the irradiance regime experienced by the anemone during measurement.

(f) Anemones are able to tolerate anoxia or hypoxia by switching over from aerobic to anaerobic metabolism (Ellington, 1982). If respiration is measured in a closed vessel which becomes depleted of oxygen, oxygen consumption rate will decline while anaerobic processes increase and an oxygen debt can be incurred (Sassaman and Mangum, 1972, 1973; Shick et al., 1979; Shick, 1981). Anaerobic respiration will be discussed more fully in the following pages.

(g) Starvation can lead to changes in the energy metabolism of anemones. Rates of oxygen and carbon dioxide exchange in *A. elegantissima* were measured and used to calculate values for anemone respiratory quotient (RQ; the molar ratio of CO_2 produced to O_2 consumed) (Fitt and Pardy, 1981). However, calculation of RQ values assumes that anemones remained fully aerobic, as during anaerobic glycolysis carbon dioxide can be evolved in the absence of oxygen consumption (Zubay, 1983). Even partial anaerobic respiration will lead to an underestimation of anemone respiration.

The oxygen consumption rate of symbiotic *Anthopleura elegantissima* starved in darkness was measured to be between 750 and 1233 μl O_2 (gm submerged weight)$^{-1}$ hour^{-1} (Fitt and Pardy, 1981). Oxygen consumption measurements of expanded *A. elegantissima* in darkness were measured to be 502 μl O_2 (gm dry weight)$^{-1}$ hour^{-1} (Shick et al., 1979). The oxygen consumption rate of non-symbiotic *Metridium senile* was 598 μl O_2 (gm dry weight)-1 hour^{-1} in the same study and feeding is known to strongly affect anemone metabolism (Tytler and Davies, 1984). Weight-specific oxygen consumption was 119.9 and 247.4 μl O_2 (gm wet weight)$^{-1}$ hour^{-1} for non-symbiotic *Diadumene leucolena* and symbiotic *A. elegantissima*, respectively (Shick and Brown, 1977; Brown, unpub., cited in Shick et al., 1979). The infaunal non-symbiotic anemone *Haloclava producta*, which encounters interstitial water of very low oxygen content (0–10% of the water column), has a weight-specific oxygen consumption rate of only 30 μl O_2 (gm wet weight)$^{-1}$ hour^{-1} (Sassaman and Mangum, 1972). Acclimation to oxygen apparently can affect the weight-specific oxygen consumption rate, as *Actinia equina* from the sub-tidal environment consumed more oxygen than intertidally acclimatized anemones of the same species (344 vs. 261 μl O_2 gm^{-1} hour^{-1}; Shick, 1981). In general, the rate of aerobic metabolism of cnidarians appears to be comparable to that of other aquatic invertebrates (Brafield and

Chapman, 1965; Prosser, 1973).

Oxygen consumption and hypoxia

Oxygen availability may affect anemone metabolism by controlling the balance between aerobic and anaerobic metabolism. Evidence to support this hypothesis is incomplete but can be drawn from several studies. For example, the respiration of *A. elegantissima* has been characterized by measurement of the mass-specific rate of oxygen flux and the mass-specific rate of heat dissipation from metabolic processes (Shick and Dykens, 1984). Coordinated measurement of both parameters indicates that anemones remain fully aerobic during 15 hr of air exposure. Therefore measurement of oxygen uptake in organisms under these conditions could be used to quantify energy metabolism. Anaerobic metabolism was avoided by behavioural responses to exposure; intertidally acclimatized anemones pump water onto the oral disc, and back into the coelenteron, by alternating contraction and expansion of the body column musculature (Shick and Dykens, 1984). These observations highlight the complex interplay between host respiration and behaviour, and environmental conditions.

Anemones appear to be faced with the problem of delivering oxygen to a gastrodermal site of consumption from an epidermal site of availability (Sassaman and Mangum, 1972). Hypoxia usually induces a dramatic increase in anemone body volume which increases the surface area for gas exchange while reducing diffusion distances to metabolically active tissue (Shick et al., 1979). Internal mixing of the coelenteron water and access of enteric water to spaces between mesenteries may be facilitated by lack of complex internal organization in anemones (Sassaman and Mangum, 1972; 1974). There may be a greater dependence upon direct irrigation of the coelenteron and gas exchange across the tentacles in large anemones (Shick et al., 1979).

The body surface of anemones is covered with a copious layer of mucus which may diminish oxygen uptake (Shick, 1982). A 5 μm thick layer of mucus on the secondary lamellae of carp gill leads to an 81% increase in the diffusion resistance to oxygen (Ultsch and Cros, 1979). Most of this increased resistance to diffusion is due to mucus forming a non-convective (unstirred) layer on the surface of the gill, rather than to differences in the oxygen diffusion constant of mucus and water (Ultsch and Gros, 1979). The unstirred layer over fish gill was measured with microelectrodes sensitive to gradients of Na^+, K^+, and Ca^+; this layer varied in thickness between 50 μm and 1,200 μm, and was capable of supporting a stable, linear, diffusion gradient

of ions (Shephard, 1982). Examination of scanning and transmission electron micrographs of *M. senile* which underwent special treatment to leave intact the superficial mucus layer (Figs. 1 and 3; Bunde et al., 1978) suggests that this mucus layer is continuous and on the order of 10–100 μm in thickness. While diffusion measurements similar to those on fish gill (Shephard, 1982) have not yet been made on anemones, it is likely that a substantial unstirred layer exists which may hinder diffusion of oxygen.

Oxygen availability may therefore be limiting to anemone tissues under certain circumstances. Microelectrode measurements of oxygen partial pressure in the coelenteron of *A. elegantissima* show that during illumination hyperbaric levels of oxygen may be produced within 1 min, but that upon cessation of illumination the coelenteric level of oxygen may be depleted to levels below ambient in about 4 min (Dykens and Shick, 1982). This rapid decrease in oxygen partial pressure suggests that oxygen consumption by the gastrodermis is very rapid and/or that the water in the coelenteron is rapidly turned over by irrigation. Calculations based on coelenteron volume and the oxygen consumption rate of *Bunodosoma cavernata* suggest that this anemone may be able to deplete the coelenteron of oxygen in as little as 7.5–15 min (Ellington, 1981).

Several other lines of evidence suggest that oxygen limitation may be more important than previously thought. Zooxanthellae photosynthesis produces oxygen, therefore phototaxis of *A. elegantissima* can modulate oxygen production by symbiotic anemones (Fredericks, 1976). Symbiotic *A. elegantissima* do not show phototactic behavior at high ambient oxygen concentrations, but are phototactic under conditions of diminished oxygen availability, while non-symbiotic anemones are not. Oxygen is thus clearly implicated as a factor controlling phototaxis. Field observations show that aposymbiotic anemones space themselves significantly farther apart than do symbiotic anemones, suggesting that symbiotic anemones have better oxygen availability than aposymbionts (Fredericks, 1976).

Photosynthesis of reef-building corals may be diffusion limited, as the metabolic activity of the coral *Acropora formosa* is higher in stirred water than in unstirred water, whether in saturating or sub-saturating light, or in darkness (Dennison and Barnes, 1988). Water motion may affect coral metabolism by altering the thickness of the boundary layer adjacent to the animal tissue. The thickness of this layer will affect diffusion of CO_2 for photosynthesis or of O_2 for respiration (Dennison and Barnes, 1988). Light may alleviate problems of oxygen delivery because oxygen will be produced

in situ by photosynthesis of zooxanthellae.

Oxygen appears to have a stimulatory effect on the calcification rate of fragments of the coral *Stylophora pistillata* (Rinkevich and Loya, 1984). After an incubation period of 24 hr in darkness, all samples in an aerated tank were found to have calcified significantly more than the control,non-aerated tank. These results suggest that light does not directly enhance calcification in hermatypic corals, but that light enhances oxygen production which then indirectly stimulates coral metabolism (Rinkevich and Loya, 1984).

Anaerobic metabolism

Studies of the metabolic response of anemones to declining oxygen tension show that they are able to survive long-term (11 day) exposure to hypoxic conditions by switching over to anaerobic metabolism (Sassaman and Mangum, 1973). The hypothesis that anemone survival under low oxygen conditions is due to aerobic metabolism requires that the aerobic metabolic rate decrease by 89.4–99.7% (Sassaman and Mangum, 1973),which is clearly unlikely. An oxygen debt response is therefore common following exposure of anemones to hypoxic conditions. In an oxygen debt response the oxygen consumption rate following hypoxia exceeds that of the same animals prior to hypoxia (Brafield and Chapman, 1965; Sassaman and Mangum, 1972, 1973; Shick, 1981). These results indicate that anaerobic metabolic pathways are evoked by hypoxic conditions. Certain molluscs perform aerobic and anaerobic metabolism simultaneously even under well oxygenated conditions (Bayne et al., 1976; Booth and Mangum, 1978). Whether this occurs in anemones is unknown.

Exposure of *Anthopleura elegantissima* to hypoxic conditions in darkness results in an oxygen debt (Shick and Brown, 1977; Shick, 1981). While this debt is usually eliminated by exposure of symbiotic anemones to light, inhibition of photosynthesis with DCMU results in an oxygen debt response even in light. Exposure of *Bunodosoma cavernata* to 18 hr of anoxia produced an oxygen debt response which increased as the duration of anoxic exposure increased (Ellington, 1982). In this study, alanine, glutamate, and propionate accumulated as end products of anaerobic metabolism, with alanine levels elevated 20-fold following 18 hr of anoxia. Alanine levels increased, perhaps as the result of anaerobic metabolism of glycogen during anoxia, but then declined rapidly after anemones were returned to normoxic conditions (Ellington, 1982). Changes in the adenylate energy charge (Atkinson and Chapman, 1979) and the ratio of tissue ATP:ADP occurred in less than

1 hr of anoxic incubation; adenylate energy charge fell from 0.78 in aerobic *B. cavernata* to 0.54 in anoxic anemones after less than 6 hr (Ellington, 1981).

The adenylate energy charge of the anemone *Metridium senile* appears to govern the poise of intermediary metabolism between catabolic and anabolic activities, while the overall metabolic rate is more a function of the total adenylate concentration (Walsh and Somero, 1981). Starvation of *M. senile* is accompanied by a large decrease in adenylate energy charge that may suppress biosynthetic activity while facilitating a high level of catabolism (Walsh and Somero, 1981). Extension of these conclusions to *B. cavernata* (data of Ellington, 1981, 1982) suggests that anaerobic anemones undergo catabolic processes to a greater extent than aerobic anemones. If anemones become even partially hypoxic in the absence of algal photosynthetic oxygen, then increased weight loss by anemones maintained in darkness may be due to oxygen depletion favoring anemone catabolic processes (see Section 3A).

Although Cnidarians are seldom found where environmental oxygen is chronically low (Muscatine, 1971), the existence of sophisticated metabolic adaptations to deal with hypoxia (Ellington, 1977, 1980, 1982) suggests that anemones may nonetheless experience some measure of hypoxia. It is postulated that endogenous oxygen production in a symbiotic anemone may be significant because it permits aerobic metabolism and consequently a higher net energy yield than would be possible anaerobically (Shick and Brown, 1977).

Algal heterotrophy

The impact of zooxanthellae photosynthesis on anemone respiration has already been discussed (Section 2C). However, the energetic impact of algal uptake of host carbon has often been ignored (Steen, 1986). *Aiptasia pulchella* fed ^{35}S methionine-labeled food translocated labeled material to the zooxanthellae (Cook, 1971; Steen, 1986a). Even when anemones were illuminated on a 12 hr light: 12 hr dark photoperiod, up to 6.4% of the zooxanthellae growth requirement for protein was satisfied by heterotrophy (Steen, 1986a). Aposymbiotic *A. pulchella* starved in darkness suffered a lower mortality rate than symbiotic anemones under the same conditions, suggesting that heterotrophy by zooxanthellae can impose a fatal metabolic burden on these anemones under certain experimental conditions (Steen, 1986a).

This evidence is consistent with findings that cultured zooxanthellae derived from the same host could grow by photoautotrophy or facultative het-

erotrophy (Steen, 1987). Zooxanthellae maintained at $5–7$ μEin m^{-2} sec^{-1} grew heterotrophically when supplied with 100 μM glycerol, glycolate, acetate, malate, or propionate, and grew in darkness on 100 μM propionate (Steen, 1987). Cultured zooxanthellae derived from the giant clam *Tridacna maxima* have active transport systems for methionine, cysteine, and taurine (Deane and O'Brien, 1981). Zooxanthellae freshly isolated from the jellyfish *Cassiopeia xamachana* accumulated ^{14}C alanine by active uptake from the incubation medium (Carroll and Blanquet, 1984). This uptake was inhibited by a low molecular weight protein fraction derived from homogenate of the anemone, suggesting that some host fraction regulates reverse translocation of nutrients from host to alga (Carroll and Blanquet, 1984). These results suggest that under certain circumstances the host may incur a metabolic cost from having symbiotic algae (Steen, 1986a).

In vivo ^{31}P nuclear magnetic resonance (NMR) spectroscopy was used to quantify the adenylate levels of symbiotic and aposymbiotic *A. pulchella* starved in darkness (Steen, 1986b). Host adenylate ratio (ATP : ATP + ADP) declined significantly with starvation in both symbiotic and aposymbiotic hosts. However, this decline was significantly more rapid in animals bearing symbiotic zooxanthellae. Symbiotic algae in darkness cause more rapid depletion of host energy reserves, possibly by drawing on host pools of organic substrates. Although these results imply that zooxanthellae can function as parasites, this does not exclude the possibility that zooxanthellae nonetheless confer an overall benefit on the host (Steen, 1986b).

B. Growth and storage

Energetics of host growth

Assimilated nutrients in excess of the amount needed for respiration and maintenance of anemone tissue can be directed towards tissue growth in juveniles or, in mature anemones, can be utilized to develop nutrient stores or reproductive tissue (Smith, 1986). Large body size is directly related to fitness in an anemone in which the volume of gametes produced is determined by body mass (Sebens, 1981b). Maximum body size is determined by characteristics of a particular habitat such as physiological stress and prey availability. Optimum body size of *A. elegantissima* is therefore presumed to be the size at which the difference between energy intake through prey capture and energy expenditure in respiratory losses is maximized (Sabens, 1981a). Prey capture by *A. elegantissima* was proportional to weight to the 0.33 power, while respiratory loss increased as the 0.77 power of weight.

Therefore there will be some size beyond which respiratory costs will far outstrip the prey capture ability of the animal (Sebens, 1981a). Maximum size is dynamic as transplanted individuals eventually reach a size near the mean for adults in the new habitat and fluctuate around that size. Optimum body size may decrease as prey availability declines, energetic costs increase, or both occur simultaneously (Sebens, 1981a).

Annual fluctuations in lipid content of field-collected *A. elegantissima* were measured through 3 years by ether extraction of whole anemones (Jennison, 1979). Lipid levels were found to fluctuate in a manner paralleling the reproductive cycle. Lipid content was not dependent upon sex of the anemone, but was proportional to the size of the anemone (Jennison, 1979). These results tend to corroborate the hypothesis that large body size is directly related to reproductive fitness (Sebens, 1981a).

Adult polyps of the anemone *Aulactinia stelloides* devote a greater proportion of translocated ^{14}C-photosynthate to lipid than do juvenile polyps; higher levels of lipid synthesis in adult polyps may be a response to the depletion of storage lipid that occurs during reproduction (Smith, 1986). Estimates of the percent translocation of algal photosynthate to the host were lower for zooxanthellae in juvenile polyps than for the zooxanthellae of adult polyps. Zooxanthellae in juvenile polyps therefore were able to exhibit a higher growth rate than zooxanthellae in adults (Smith, 1986). Decreased growth rate of zooxanthellae in adult anemones may result in increased availability of carbon to the host if zooxanthellae photosynthetic performance is unchanged (Smith and Muscatine, 1986).

Energetics of zooxanthellae growth

The impact of host feeding on the physiology of zooxanthellae in *A. pallida* has been examined by maintaining animals in nutrient depleted surface waters of the Sargasso Sea (Cook et al., in press). In such water the concentrations of dissolved inorganic nitrogen and phosphorus are typically close to the limits of detection and far below levels of nutrients which are routinely found in recirculating seawater systems (Muller-Parker, pers. comm.). Anemones were starved for up to 100 days in light at an irradiance approximating the maximum irradiance measured for a field collected population ($81 \ \mu E \ m^{-2}s^{-1}$) (Cook et al., in press). Zooxanthellae from anemones starved for 20–30 days showed the following characteristics of nutrient deficiency: algal cell mitotic rate decreased, chlorophyll a content decreased, and the ratio of carbon:nitrogen increased, suggesting that the zooxanthellae became in-

creasingly nitrogen limited. Over 100 days of starvation the total number of zooxanthellae per anemone decreased, indicating that zooxanthellae were lost from the host faster than they were replaced by mitosis. The mitotic index of zooxanthellae in starved anemones was stimulated by feeding the host or by addition of inorganic nitrogen or phosphorus to the incubation medium. The discrepancy in chlorophyll content between zooxanthellae isolated from starved and fed hosts indicates that host starvation influences the ability of zooxanthellae to synthesize chlorophyll. This important study shows that the nutritional history of the host can have a pronounced effect on the nutrient sufficiency of the zooxanthellae (Cook et al., in press), and suggests that zooxanthellae may usually be nutrient-limited within the host (G. Muller-Parker, pers. comm.). Furthermore it is a clear indication that under certain circumstances zooxanthellae can function as parasites.

Field populations of *A. pulchella* contain zooxanthellae which may be nitrogen-limited (Muller-Parker, 1987). The average C:N ratio of zooxanthellae isolated form lab cultures of fed *A. pulchella* was 5.12 (unpublished data cited in Muller-Parker, 1987), and those of cultured zooxanthellae from *A. pulchella* ranged from 5.35 to 6.07 (Chang et al., 1983). The C:N ratio of zooxanthellae isolated from field collected *A. pulchella* ranged from 8 to 13 (Muller-Parker, 1987). The *in situ* growth rate of zooxanthellae may be higher in hosts exposed to eutrophic water than in hosts in oligotrophic waters (Wilkerson et al., 1983). Zooxanthellae cell division is asynchronous and growth rate is generally low. This may be the result of growth constraints imposed by the intracellular habitat and by the low ambient nutrient regime to which the host is exposed (Wilkerson et al., 1983). It has been hypothesized that inhibition of symbiont growth rate results from nutrient limitation within the host or inhibitor secretion by the host (Muscatine and Pool, 1979).

The ratio of algal cell volume to host cell volume tends to be similar in a range of different symbioses, suggesting that algae in each association have a similar requirement for "living space" (Muscatine and Pool, 1979). The ratio of algal to animal cell volume may be regulated by expulsion or digestion of excess algal cells, or by inhibition of algal cell growth (Muscatine and Pool, 1979). Apparent synchrony between algal cell division and host cell division following feeding has been observed in *Hydra*, suggesting that host cells are able to control algal cell division so that an optimal algal population size is maintained (McAuley, 1985). Density of zooxanthellae within *A. tagetes* is greater in anemones maintained in high irradiance than in those under low irradiance (Steele, 1976). Algal density was hypothesized

to be determined by the interplay between increase in algal numbers, growth of host tissues, population of new host tissues by growing symbionts, and the extrusion of zooxanthellae by the host. Starved *A. tagetes* become smaller and lose zooxanthellae but the algal concentration does not change (Steele, 1976). However, these studies do not exclude the possibility that anemones lack any control over algal cell density; zooxanthellae may simply grow to the carrying capacity of the host cell. Anemones are capable of extrusion of zooxanthellae under a variety of circumstances (Steen and Muscatine, 1987; Schoenberg and Trench, 1980c; Steele, 1976), perhaps as a means of avoiding a lethal parasitemia.

C. Reproduction

Sexual reproduction

Optimum body size is the size at which the difference between energy intake and energy cost is maximized; this difference is the energy available for gonad production summed over the entire year and is thus directly related to reproductive success (Sebens, 1981a). However, anemones are unusual in that both sexual and asexual reproductive modes are commonplace and certain species have never been observed to reproduce sexually (Hunter, 1984). Asexual reproduction provides a mechanism for rapid colonization and proliferation of a successful genotype in a particular habitat, while sexual reproduction preserves genotypic variability through recombination (Hunter, 1984).

The energetic costs of sexual reproduction are almost completely unknown; that such costs exist is suggested by the observation that depletion of lipid storage tissue coincides with spawning of *A. elegantissima* in the field (Jennison, 1979). Energy diverted into production of planulae in the coral *Porites porites* was 0.4% of the total energy available for respiration and growth (Edmunds and Davies, 1986). However, the corresponding data are not yet available for anemones.

Asexual reproduction

The energetic costs of asexual reproduction by fission or by pedal laceration are somewhat better understood. Mathematical modeling of asexual reproduction predicts that energy surplus in anemones is maximized by the replication of organisms each slightly larger than is necessary to capture the most energetically important prey (Sabens, 1979). This leads to increased genotype fitness through the production of identical units of proven fitness

and by the maximization of reproductive output (Sebens, 1979). Populations of *Haliplanella luciae* reproduce exclusively by asexual reproduction and field sites are typically dominated by one or a few clones of anemones (Shick et al., 1979). Transplantation studies suggest that this population structure is achieved through differential mortality among colonizing clones, most of which are not adapted to local conditions. Asexual reproduction by surviving clone members leads to multiplication of one or a few genotypes which were fortuitously "preadapted" to the local environmental regimen (Shick et al., 1979). Environmental factors which have been shown to affect fission activity of *H. luciae* include temperature, food availability and feeding frequency, and cyclic periods of tidal emmersion (Minasian, 1979; Johnson and Shick, 1977).

Asexual reproduction can provide bioenergetic advantages to clone members as well as to the clone itself; living in dense clonal aggregations reduces the effective animal surface area, resulting in diminished desiccation during emersion and in reduced drag in wave-swept habitats (Shick and Lamb, 1977); Francis, 1979). Dense clonal aggregations can also exclude competitors and produce cooperativity among clone mates in food capture. However, there are disadvantages to the loss of genetic variability: field populations of *H. luciae* experience abrupt epidemic mortality perhaps as the limits of physiological tolerance are approached (Shick and Lamb, 1977).

Asexual reproduction of *A. elegantissima* is inhibited when the anemone is fed continuously, but starvation is not sufficient stimulus to initiate asexual fission (Sebens, 1980). In *A. pallida*, which reproduces by pedal laceration or fragmentation, anemone feeding regime had no significant effect on the total number of anemones produced after 8 weeks (Clayton and Lasker, 1985). However, starved anemones tended to reproduce sooner than fed anemones. Asexual reproduction by symbiotic anemones increased clonal biomass at a greater rate than individual biomass increased (Clayton and Lasker, 1985).

The energetic cost of reproduction has been quantified for *A. pulchella* using bomb calorimetry of whole anemones and new pedal lacerates (Hunter, 1984). The ratio of (energy allocated to reproduction) : (net assimilated energy) is defined as reproductive effort (RE). Not assimilated energy was calculated from food intake, algal photosynthesis, and catabolism of stored anemone reserves. The index of RE for pedal laceration was very low, ranging from 0.004 to 0.44, permitting a substantial rate of asexual reproduction with very little energetic cost to the reproducing organism (Hunter, 1984).

D. Export

A complete analysis of anemone bioenergetics is impossible without quantitative measures of the extent of loss of organic carbon or energy from the anemone. Such losses include host waste products, unassimilated food ejected from the coelenteron, unassimilated photosynthate translocated from zooxanthellae, mucus secreted by the anemone, expulsion of zooxanthellae, loss or autotomy of body parts (e.g., nematocysts, acontia), and loss of tissue to predators.

Host metabolic waste products and unassimilated food ejected from the coelenteron have often been treated as one under the rubric of waste. However, a distinction should be made between them in order to focus attention on the largely ignored category of host metabolic waste.

Zooxanthellae appear to have a strong impact on nutrient uptake or retention by the host. Since nutrient uptake by cells is an active process (Zubay, 1983) nutrient conservation may impact the host bioenergetic status. Symbiotic *Aiptasia pulchella* take up ammonia from nutrient enriched seawater, but anemones made aposymbiotic by maintenance in darkness typically release ammonium in the light (Wilkerson and Muscatine, 1984). Thirty aposymbiotic anemones released enough ammonium to make 200–400 ml of seawater approximately 4 μM (Wilkerson and Muscatine, 1984). Aposymbiotic *Anthopleura elegantissima* release 5.7 to 9.8 times as much ammonium as symbiotic congeners (Zamer and Shick, 1987). Average total release was 2.14 μmol $NH_4^+ \cdot g^{-1} hour^{-1}$ over a four hour incubation. The rate of ammonium excretion in aposymbiotic anemones appears to be an accurate indication of the level of protein catabolism in these animals (Zamer and Shick, 1987). Ammonium release in *Condylactus* is greater in light than darkness, and release is enhanced when the ameliorating influence of zooxanthellae is removed (Cates and McLaughlin, 1976).

Similarly, inorganic phosphate is released to seawater by aposymbiotic *Condylactus* spp. in light or darkness over incubations of 18 to 72 hr (Cates and McLaughlin, 1979). Net excretion over 72 hr was 34–54 μg-atoms of phosphate phosphorus (g wet weight)$^{-1}$ (Cates and McLaughlin, 1979). Using NMR spectroscopy, inorganic phosphate loss from *Aiptasia pulchella* was observed directly for up to 6 days following feeding in dark-maintained symbiotic and aposymbiotic anemones (Steen, 1986b). Phosphate release probably should not be categorized as a host metabolic waste product, as it was hypothesized that most of this phosphate was never assimilated (Steen, 1986b).

Unassimilated food boli have been examined for energy content by bomb calorimetry in order to quantify anemone assimilation efficiency (Hunter, 1984). Assimilation efficiency was calculated as the ratio of ash free dry weight of net ingestion to total ingestion, the difference being represented by the ash free dry weight of ejected food boli. Average dry weight of the egested boli was 61% of the weight of the ingested food pellet indicating that the digestive efficiency was fairly low. However, the assimilation efficiency of digested food averaged 63% when calculated in energetic terms (Hunter, 1984).

Unassimilated photosynthate translocated from zooxanthellae and lost by the host can represent a substantial sink for algal productivity. This loss can be composed of photosynthetic products such as glycolate which the host does not metabolize (preliminary results reported in Steen, 1986b), or metabolic products of photosynthate such as mucus. Cooksey and Cooksey (1972) report that 50% of photosynthetically fixed ^{14}C is lost from the corals *Montastrea annularis* and *Siderastrea siderea* within 24 hr. Crossland et al. (1980a) estimate a 40% net loss of fixed carbon from the coral *Acropora acuminata*, largely in the form of mucus. In *Acropora formosa*, 50–60% of the total fixed ^{14}C is lost over a 40 hr period (Crossland et al., 1980b). Zooxanthellae from the coral *Stylophora pistillata* translocate 96.6% of the total carbon fixed in photosynthesis, but between 6% and 50% of this carbon is released by the coral (Falkowski et al., 1984). The efficiency of assimilation of translocated photosynthate by corals may be low because translocated products are deficient in nitrogen. Production of new host tissue may require nitrogen-rich nutritional sources such as zooplankton or dissolved inorganic nitrogen (Falkowski et al., 1984).

Mucus release has been measured in the coral *Porites porites* under stressed and unstressed conditions (Edmunds and Davies, 1986). Over incubations of 24 hr no mucus release could be detected, but field observations showed that coral nubbins produce mucus tunics which are shed after 5.2 days. These results suggest that there is a gradual accumulation of mucus in the tunics. Since these tunics are shed over a relatively short time, the assay used was unlikely to accurately quantify mucus release (Edmunds and Davies, 1986). Mucus production by a 1.0 g intertidal *A. elegantissima* was estimated to be between 1.8 and 2.4 mg of mucus per day at an assumed energetic cost of 5.2 calories (mg organic weight)$^{-1}$ (Zamer and Shick, 1987). Mucus from a variety of corals was found to contain wax ester (cetyl palmitate) and triglycerides; the avidity with which fish feed on artificially dispersed mucus

suggests that it may be an important energy source for reef fish (Benson and Muscatine, 1974).

Because purified mucus from the coral *Fungia scutaria* is low in caloric density, energy-rich lipids, and phosphorus, it has been suggested that corals minimize the energetic costs of mucus production by synthesizing an energy-poor mucus (Krupp, 1984). However, mucus production in *Acropora acuminata* has been estimated at 300 μg wax ester (mg animal protein)$^{-1}$day^{-1} (Crossland et al., 1980a). Mucus plays a role in feeding and digestion and in the reduction of growth by epibionts and bacteria (Cook, 1983).

Export of material from a symbiotic anemone can also take the form of expulsion of zooxanthellae. This can be energetically important not only as a loss of any animal tissue associated with the zooxanthellae, but also as a loss of any benefit from translocated photosynthate. Algal expulsion can be induced in anemones in the laboratory by stresses such as cold exposure (Steen and Muscatine, 1987), constant light, and elevated temperature or salinity (Steele, 1976, 1977). There are numerous reports of expulsion of zooxanthellae from corals in nature (Glynn, 1983, 1984; Egana and DiSalvo, 1982; Goreau, 1964; Jaap, 1979, 1985; Lasker et al., 1984). However, studies with unstressed corals suggest that expulsion of zooxanthellae is not a significant sink for fixed carbon (Hoegh-Guldberg et al., 1987). Expelled zooxanthellae did not exceed 0.1% of the total standing stock of zooxanthellae per day in a range of corals, and the carbon lost represented 0.01% of the total carbon fixed on a daily basis (Hoegh-Guldberg et al., 1987).

4. Energy Budgets of Symbiotic Cnidarians

Energy budgets of high and low intertidal specimens of the anemone *Anthopleura elegantissima* were calculated from published values of oxygen flux (Zamer and Shick, 1987). The contribution of zooxanthellae to animal respiration (CZAR) was calculated from data on ingestion, absorption, and growth by the host and by assuming that 90% of the total algal photosynthate was translocated to the anemone. Calculations suggest that high and low intertidal anemones obtain 41 and 79% respectively of the respiratory carbon requirement from algal photosynthesis. Directly measured growth was not as large as the calculated scope for growth in either population of anemones, so it was hypothesized that the difference represents unmeasured costs such as mucus production or the enhanced metabolic rate associated with food intake. The low intertidal population actually lost biomass during the course of the experiment despite the calculated surplus of energy for

respiration (Zamer and Shick, 1987). This illustrates the difficulty of constructing integrated energy budgets at the present state of our knowledge.

Despite the manifest problems with estimating productivity of zooxanthellae *in situ*, an energy budget has been constructed for the coral *Porites porites* found at 10 m depth at Discovery Bay, Jamaica (Edmunds and Davies, 1986). Coral colony respiration and photosynthesis were measured by oxygen flux, algal productivity was calculated from net photosynthesis vs. irradiance data, and coral growth was measured as changes in reduced weight. All photosynthetic carbon in excess of that required for algal respiration or growth was assumed to be released to the coral host. This input of carbon to the coral, expressed in energetic terms, was then partitioned into coral respiration, growth, reproduction, and loss of energy from the colony. Nearly 50% of the energy fixed in algal photosynthesis was consumed in respiration by the zooxanthellae or the coral host, with about 7% of the total energy fixed going to growth of the algal or coral partner. The remaining 45% of energy fixed in algal photosynthesis could not be accounted for and was assumed to be lost from the colony as dissolved organic matter, mucus, and various cell debris (Edmunds and Davies, 1986). That such a large fraction of the total energy fixed could not be directly accounted for is disquieting, and may be due to the difficulty of measuring *in situ* photosynthesis by zooxanthellae.

These results are similar to several other energy budgets derived for corals. Zooxanthellae from the coral *Pocillopora eydouxi* were calculated to release to the host 90% of the energy fixed in photosynthesis, while the host respired 46% of the total carbon translocated (Davies, 1984). Of the energy translocated to the host, that which could not be accounted for as host respiration or growth was again assumed to be lost from the colony. A net loss of 48% of the photosynthetically fixed energy was calculated (Davies, 1984), which appears to be consistent with measurements of the export of radioactively labeled carbon from corals (see Section 3D).

5. Conclusions

This review has addressed the bioenergetics of symbiotic anemones and the energetic costs and benefits to anemones of symbiosis with zooxanthellae. Zooxanthellae impact upon host bioenergetics by affecting the balance between aerobic and anaerobic metabolism in the host, by acting as an organic carbon source through photosynthesis or a sink through heterotrophy, and by mediating the traffic of inorganic nutrients through the host. The cumulative effect of zooxanthellae on the energetic status of anemones is profound

because the interactions between host and symbiont are exceedingly complex. That there is benefit from such interactions seems evident because of the success of symbiotic anemones world-wide. However, much work remains to be done; elucidating the relative importance of photosynthetic oxygen and fixed carbon to the host; examining the impact of oxygen production on host metabolism under field conditions; accounting for the profligate manner in which algal fixed carbon is released by the host; clarifying the relative importance of phagotrophy, saprotrophy, and autotrophy to the host in nature; elucidating environmental conditions which affect whether zooxanthellae act as parasites or benefactors; and ascertaining net energy budgets for field populations of anemones.

In the past, symbiosis may have been seen as an arcane phenomenon of little general interest, but there is an increasing recognition that symbiosis can be found wherever one searches for it. Elucidation of some of the problems outlined above will provide understanding of one symbiosis which has achieved a striking degree of ubiquity on the rocky shores of the world. Furthermore the molecular biology of symbiosis has hardly been touched upon in any system except legumes and rhizobial bacteria. Zooxanthellae in symbiosis differ profoundly from zooxanthellae in culture; in morphology (Freudenthal, 1962), photosynthetic function (Muller-Parker, 1984a), expression of abundant cell proteins (Steen, 1987), and the translocation of photosynthate (Trench, 1971; Taylor, 1978). Therefore, this symbiosis may be an ideal system with which to examine regulatory mechanisms controlling photosynthesis and cellular physiology.

Acknowledgements

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