THE CONTRIBUTION OF ZOOXANTHELLAE TO THE ENERGY REQUIREMENTS OF THE SEA ANEMONE, *Anemonia sulcata* (Pennant)

THESIS

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CONTAINS A PULLOUT

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SUMMARY

The aim of this study was to estimate the contribution of zooxanthellae (Gymnodinium (=Symbiodinium) microadriaticum) to the energy requirements of the sea anemone Anemonia sulcata Pennant. A bioenergetic model of energy flow through this symbiosis was constructed for this purpose.

The energy inputs, the energy expenditure and the net energy retention of symbiotic and aposymbiotic (algal-free) anemones were measured in a series of laboratory experiments. Energy losses were calculated by subtraction.

Photosynthetic energy production (P) by the zooxanthellae was calculated from the gross oxygen production of the zooxanthellae in vivo. P increased in direct proportion to irradiance from 0 - 120 $\mu E.m^{-2}.sec^{-1}$. Above this range, the relationship becomes curvilinear. Saturation of photosynthesis was observed in some anemones at 160 $\mu E.m^{-2}.sec^{-1}$. Symbiotic anemones were either exposed to a 12h light/12h dark cycle at 70 $\mu E.m^{-2}.sec^{-1}$ or 140 $\mu E.m^{-2}.sec^{-1}$ or were maintained in darkness for 84 days. P at the higher irradiance was theoretically sufficient to allow this anemone to adopt a fully autotrophic existence.

Aposymbiotic anemones were maintained either in darkness or exposed to a 12h light/12h dark cycle to determine the extent to which light alone affected energy flow through the anemones.

Symbiotic and aposymbiotic anemones were either starved or fed twice weekly with squid mantle in excess of their respiratory energy expenditure (R). The carnivorous energy input was calculated from the weight of squid ingested.

R was calculated from the oxygen consumption ($\dot{M}_{O_2}$) of the anemone in darkness. The $\dot{M}_{O_2}$ of symbiotic anemones was dependent on the partial pressure of oxygen (PO_2) in the sea water. The shape of the curve relating $\dot{M}_{O_2}$ to PO_2 resembles a rectangular hyperbola. The effect of PO_2 on $\dot{M}_{O_2}$ may have been analagous to the effect of substrate concentration on the rate of reaction of enzymes obeying Michaelis-Menten enzyme kinetics. The $\dot{M}_{O_2}$ of symbiotic anemones increased in direct proportion to body organic weight which indicated that weight loss during starvation would be an exponential function of time. The $\dot{M}_{O_2}$ of symbiotic anemones was affected by both lighting and feeding regimes. The $\dot{M}_{O_2}$ of anemones maintained at 140 $\mu E.m^{-2}.sec^{-1}$ was higher than the $\dot{M}_{O_2}$ of those maintained in darkness or at 70 $\mu E.m^{-2}.sec^{-1}$. The preprandial $\dot{M}_{O_2}$ of fed anemones was not significantly different from the $\dot{M}_{O_2}$ of starved anemones maintained under the same lighting regime. A postprandial increase in $\dot{M}_{O_2}$ was observed in anemones fed with squid mantle. This Specific Dynamic Action (S.D.A.) typically reached a peak 1-8h after feeding and decayed to preprandial levels after at least 24h. 6.8% of the energy in a meal of squid was expended as S.D.A. in symbiotic anemones.

The $\dot{M}_{O_2}$ of fed aposymbiotic anemones was similar to that of symbiotic anemones. The $\dot{M}_{O_2}$ of starved aposymbiotic anemones was $\sim$ 50% lower than fed anemones. Light did not have a significant effect on the $\dot{M}_{O_2}$ of aposymbiotic anemones. 5.1% of the energy in a squid meal was expended as S.D.A. in aposymbiotic anemones.

The net energy retention of the anemones was calculated from changes in weight of the anemones. The body weight of symbiotic anemones which were starved in darkness or on a 12h light/12h dark cycle decreased exponentially with time over the 84 day period. Weight loss was significantly lower in anemones exposed to light than in those kept

in darkness. A. sulcata could not adopt a fully autotrophic existence under these laboratory conditions. The body weight of aposymbiotic anemones decreased exponentially with time during starvation. The weight loss was significantly greater in aposymbiotic anemones exposed to light than in aposymbiotic anemones in darkness. This difference could not be attributed to photosynthesis or related processes.

The body weight of symbiotic and aposymbiotic anemones fed with squid mantle twice weekly increased linearly with time over the 84 day period. The weight gain was greater in symbiotic anemones exposed to light than in those kept in darkness. The weight gain in aposymbiotic anemones was lower than in symbiotic anemones but there was no significant difference in weight gain between animals kept in darkness and animals exposed to light.

The net energy retention as biomass of zooxanthellae was calculated from changes in the size of the algal population. The numbers of zooxanthellae increased in symbiotic anemones maintained at 70 and 140 $\mu E.m^{-2}.sec^{-1}$. Since biomass of zooxanthellae increased, P was more than sufficient to meet the energy requirements of the zooxanthellae. The increase in biomass of zooxanthellae was greater at the higher irradiance and was also greater in anemones which were fed than in those which were starved. A mean of 11% of P was retained as biomass of zooxanthellae. Symbiotic anemones maintained in darkness lost zooxanthellae. This loss was greater in fed anemones.

It was estimated that 36 - 77% of P was translocated to the anemone. This met 38 - 41% of the R on maintenance of the anemone alone during starvation in light and met 52 - 67% of the total R on maintenance of the symbiotic organism. At 140 $\mu E.m^{-2}.sec^{-1}$, 45% of

P was lost from starved anemones, presumably as mucus and expelled zooxanthellae. It appears that the anemone can only utilise a certain amount of the energy translocated by the zooxanthellae.

The anemones required a carnivorous energy input to grow. The gross growth efficiency (K_1) was highest in symbiotic anemones exposed to light. These K_1 values were comparable with higher invertebrates. The K_1 values were lower in symbiotic anemones maintained in darkness and were lowest in aposymbiotic anemones. These differences may have been due to an ability to recycle nitrogenous wastes in symbiotic anemones.

The benefits to A. sulcata of harbouring zooxanthellae include

i) They provide a source of energy by translocating products of photosynthesis which meets some of the energy requirements for maintenance of the anemone.

ii) Their presence increases growth efficiency to a level comparable with higher invertebrates. This may allow A. sulcata to compete more effectively with higher invertebrates in the subtidal and intertidal waters of the west coast of Britain.

SECTION 1

Introduction

The presence of functional plant cells in invertebrates was first noted about a hundred years ago (Brandt, 1881; Geddes, 1882). Four types of plant bodies have been identified in association with invertebrates.

1) Zooxanthellae: Dinophyceae found in some marine Coelenterates, lamellibranch Molluscs, Platyhelminths and Protozoa.
2) Zoochlorellae: Chlorophyceae found in some fresh-water Coelenterates, Porifera and Protozoa and the marine intertidal platyhelminth Convoluta roscoffensis.
3) Cyanella: Cyanophyceae which are found in some fresh-water Protozoa (Droop, 1963), marine Porifera (Taylor, 1973) and also Prochloron found on marine Ascidians (Smith, 1979).
4) Chloroplasts: Chloroplasts originating from the sea weed Codium fragile which are found in the digestive cells of some marine saccoglossan gastropod Molluscs (Taylor, 1970).

These associations are found at their highest densities in tropical seas and oceans where zooxanthellae-invertebrate symbioses make up a large part of the standing crop of coral reef waters (Odum & Odum, 1955). However some species are found in sub-tropical and temperate waters (Droop, 1963).

Many theories have been put forward to explain the presence and the role of these plant bodies. It is generally agreed that these associations between plants and animals are symbioses, as defined by de Bary (1879), due to the intimate nature of the associations and that the associations are mutualistic, as the hosts obtain nutritional advantages from the associations (Smith, 1979). However the exact

nature of the nutritional advantages has been subject to controversy.

Boschma (1925) observed that zooxanthellae degenerated in the mesenteries of starved sea anemones, but remained healthy in fed animals. He concluded that the sea anemones fed on their zooxanthellae during starvation. Yonge (1936) proposed that clams of the family Tridacnidae "farmed" their zooxanthellae since amoebocytes in the circulation system of these molluscs were able to phagocytose zooxanthellae. Steele & Goreau (1977) have recently shown that extracts from the sea anemone Phyllactis flosculifera contained enzymes capable of digesting zooxanthellae.

Taylor (1969a) noted the removal and expulsion of degenerate zooxanthellae from the mesenteries of the sea anemone Anemonia sulcata. However, he concluded that the anemone was culling, and not consuming, the algal population, and that this culling was accelerated under stress, such as starvation, when the host could not support a large population of algae. Similarly Trench (1974) has shown that zooxanthellae degenerated but were not digested in the mesenteries of Zoanthus sociatus. Fankboner (1971) has also argued that Tridacnid clams did not digest healthy zooxanthellae but systematically removed and utilized degenerating zooxanthellae while Ricard & Salvat (1977) and Trench et al (1981) have found that intact zooxanthellae are expelled in the faeces of Tridacna. Although a mechanism for the digestion of zooxanthellae has been shown to exist (Steele & Goreau, 1977) there is no conclusive evidence which shows that an invertebrate host gains significant nutritional benefit from digesting its zooxanthellae (Trench, 1979).

Since the algal symbionts photosynthesise and produce oxygen under illumination, Smith (1939) and Shick & Brown (1977) have suggested

that zooxanthellae provide their hosts with a supply of oxygen. Shick & Brown (1977) have shown that oxygen produced by zooxanthellae allowed aerobic metabolism to continue in the sea anemone Anthopleura elegantissima exposed to hypoxic conditions in the light. However, it it not known whether A. elegantissima is exposed to these conditions in its natural habitat.

Droop (1963) has suggested that the algal symbionts may receive a supply of carbon dioxide from the respiration of the host. Since the concentration of CO_2 is a major factor limiting photosynthesis (Shelp & Canvin, 1980), algae in symbiosis with an invertebrate may be able to photosynthesise at elevated rates. Phipps & Pardy (1982) have shown that the rates of photosynthesis of zoochlorellae in symbiosis with Hydra were higher than those of isolated zoochlorellae, and they have postulated that this was due to a high level of CO_2 near the algae provided by respiration of the Hydra. Droop (1963) has also suggested that zooxanthellae regulate the pH in the host cells, which may be partly responsible for the light-enhanced calcification of reef-building corals Goreau (1959).

The ability of algal-invertebrate symbioses to recycle metabolic waste products was first demonstrated by Putter (1911) who found that the sea anemone Aiptasia diaphana was capable of extracting ammonia from the surrounding sea water. Kawaguti (1953), Szmant-Froelich & Pilson (1977) and Muscatine & D'Elia (1978) have shown that corals with zooxanthellae can take up ammonia from the surrounding sea water in the light. Yonge & Nicholls (1931), Yonge (1936), Smith (1939) and D'Elia (1977) have demonstrated that zooxanthellae-invertebrate symbioses can recycle phosphates and take up phosphates from the surrounding sea water. These recycling abilities may aid both host and symbiont to thrive in nutrient deficient tropical waters (Muscatine & Porter, 1976).

Muscatine & Hand (1958), Goreau & Goreau (1960), Taylor (1969b) and Trench (1971a) have shown that zooxanthellae exposed to light fix radioactively labelled NaH $^{14}CO_3$ *in vivo* and that some products of photosynthesis were translocated from the algae to their invertebrate host. Muscatine (1967) and Trench (1971b, c) have demonstrated with isolated zooxanthellae, that glycerol was the major product translocated. Lewis & Smith (1971) have shown that some of the ammonia taken up by corals was incorporated into alanine by the zooxanthellae and was translocated to the host. Glucose (Trench, 1971b) and fatty acids (Patton *et al*, 1977) may also be translocated. Muscatine (1967) and Muscatine *et al* (1972) have estimated that ~40% of the carbon fixed by zooxanthellae was translocated to their invertebrate hosts. This evidence raised the possibility that zooxanthellae-invertebrate symbioses could adopt fully autotrophic existences if the photosynthetic productivity of the zooxanthellae exceeded the energy requirements of the symbioses (Muscatine & Porter, 1976).

Kanwisher & Wainwright (1967), Roffman (1968), Davies (1977) and Kevin & Hudson (1979) have measured respiratory oxygen consumption (R) in darkness and photosynthetic oxygen production (P) under illumination of corals with polarographic oxygen electrodes in laboratory experiments. It has been predicted from the results of these studies that P during daylight hours *in situ* exceeded R in darkness. Kanwisher & Wainwright (1967) and Davies (1977) have calculated the rate of carbon fixation of corals from P by assuming that one mole of CO_2 was fixed for each mole of O_2 produced and they have predicted that zooxanthellae could fix enough carbon during daylight hours to meet the total carbon requirements of host and symbiont over a 24h period. Muscatine *et al*. (1931) have used a more elaborate mathematical model to predict that between 40 - 180% of

the carbon requirements of the corals Pocillopora damicornis and Fungia scutaria were met by photosynthesis of their zooxanthellae.

Franzisket (1969, 1970) and Johannes (1974) have demonstrated that the corals Fungia, Montipora, Pocillopora and Porites gained weight when illuminated. The growth rate of each species was similar in filtered and in unfiltered sea water, hence these corals appeared to be able to adopt a fully autotrophic existence. Similarly Gohar (1940, 1943) (cited by Goreau et al, 1971) has shown that Alcyonacean corals Xenia hicksoni and Clavularia hamra were almost completely dependent on their zooxanthellae.

In contrast, Goreau et al (1971) argued that most corals which harboured zooxanthellae could not be regarded as true autotrophs since they were highly adapted carnivores. Trench (1974) demonstrated that the zoanthid Zoanthus sociatus was polytrophic, i.e. it could adopt both autotrophic and heterotrophic modes of nutrition. Muscatine & Porter (1976) concluded that reef-building corals were also polytrophic and that the translocated products of photosynthesis were a major source of energy for the corals.

There is also some evidence of nutrient transfer from invertebrates to zooxanthellae. Cook (1971) has shown that labelled sulphur (^{35}S) in food ingested by the sea anemone Aiptasia pulchrella was transferred to its zooxanthellae. A similar transfer has been reported in Anthopleura elegantissima in which ^{35}S labelled material including cystine, methionine and cystathione were transferred to the zooxanthellae (Trench, 1979).

Although the energetic advantages to an invertebrate of harbouring zooxanthellae are well documented, the actual contribution

of zooxanthellae to the energy requirements of an invertebrate remains to be determined.

This may be determined by constructing a bioenergetic model of a zooxanthellae-invertebrate symbiosis. Bioenergetic models obey the laws of thermodynamics. The first law of thermodynamics states that the energy in a closed system is constant. When this law is applied to an open system, such as a living organism, it can be expressed in the form of the following equation (Wiegert, 1968).

1) Energy input = Energy Expenditure + Energy Retention + Energy Expulsion

When applied to a heterotrophic organism, equation (1) can be written as the energy equivalent of

2) Food Intake = Heat Production + Growth, Storage and Reproduction + Excretion and Secretion

When applied to an autotrophic organism, equation (1) can be written as the energy equivalent of

3) $h\nu \longrightarrow$ Photosynthesis = Heat Production + Growth, Storage and Reproduction + Excretion

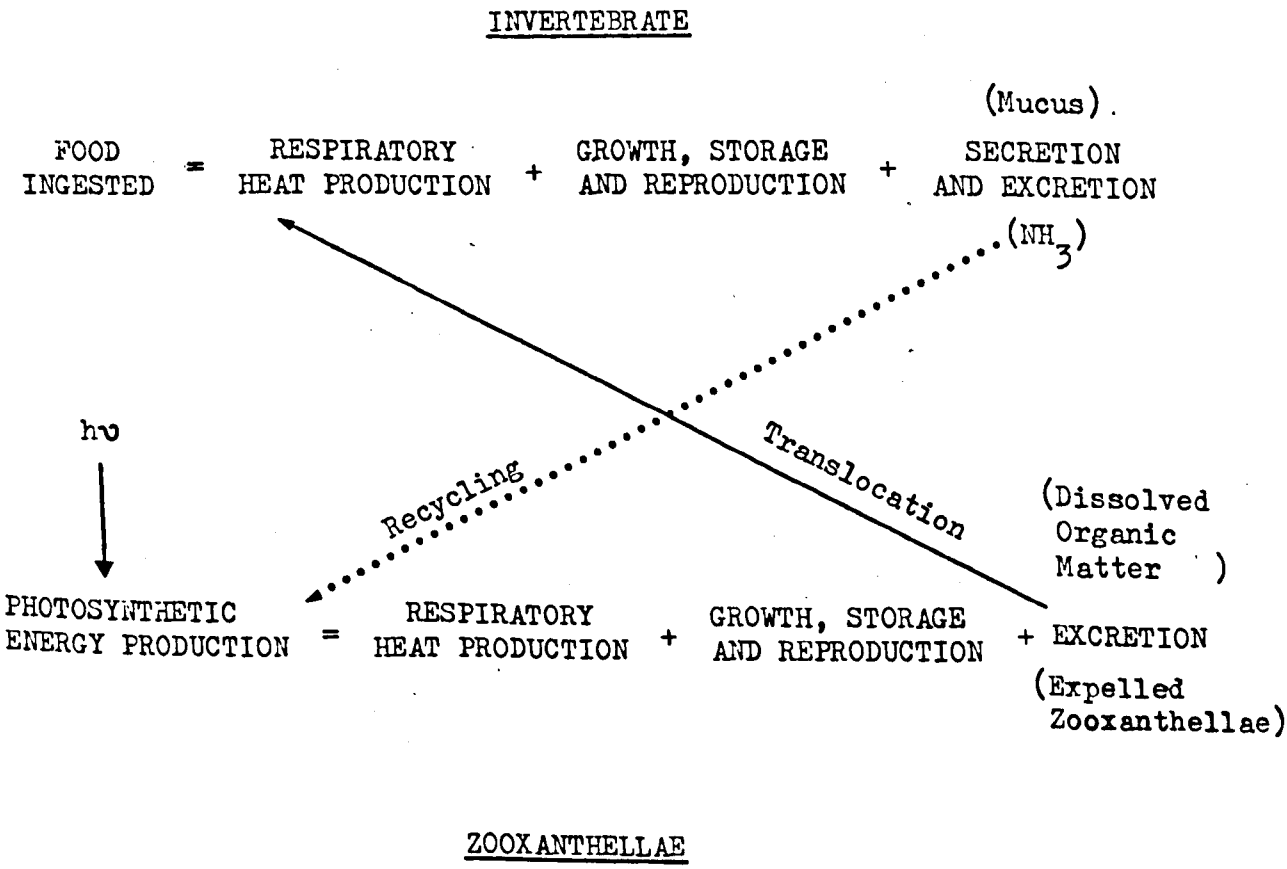
where h is Planck's Constant

ν is the frequency of light.

The input of energy is solar energy ($h\nu$). Some of this energy is trapped by light harvesting pigments and is used to produce ATP by photophosphorylation which is used in the dark reactions of photosynthesis to produce sugars and other compounds (Lehninger, 1971).

A bioenergetic model of a zooxanthellae-invertebrate symbiosis may take the form of two equations (Fig. 1); an equation for the heterotrophic invertebrate (c.f. equation 2) and an equation for the autotrophic zooxanthellae (c.f. equation 3). These equations are

Fig. 1. A model of energy flow through a zooxanthellae-Invertebrate symbiosis.



linked by the processes of translocation and recycling. Some of the losses from one symbiont become an energy input to the other, in both processes.

It is possible to estimate how much the zooxanthellae contribute to the energy requirements of the host by determining the size of the energy inputs and then by following the way in which this energy is partitioned into the different outputs of the system.

The sea anemone Anemonia sulcata Pennant which harbours zooxanthellae of the species Gymnodinium (=Symbiodinium) microadriaticum (Taylor, 1967b) was chosen as the experimental organism for this study as it has several advantages over other algal-invertebrate symbioses.

1) Each individual is a single large polyp.
2) Unlike hermatypic corals, it does not have a calcareous skeleton which may assimilate some of the carbon fixed by photosynthesis of the zooxanthellae (Young et al, 1971).
3) It is a sessile animal, therefore the energy it expends on muscular activity is likely to be small (Jones et al, 1977).
4) It is widespread and abundant in subtidal waters and in rock pools along the west coast of Britain (Taylor, 1967a).
5) Specimens are easy to collect and can be maintained under laboratory conditions for long periods of time (Taylor, 1967a)
6) The symbiosis is not obligate, viable aposymbiotic (algal-free) A. sulcata can be produced (Smith, 1939) and zooxanthellae can survive in isolation from the anemone (Taylor, 1969b).

A. sulcata is a Lusitanian species (Stephenson, 1935) which exists in two colour morphs, both of which harbour zooxanthellae (Taylor, 1967a). Smith (1939) has shown that the symbiotic anemones

can recycle inorganic phosphates. Trendelenburg (1909), Putter (1911) and Smith (1939) have shown that the photosynthetic O_2 production of this organism can exceed respiratory O_2 consumption. Taylor (1969b) has shown, using autoradiography, that zooxanthellae translocate products of photosynthesis to the anemone. Taylor (1969a) has demonstrated that weight loss was lower in anemones starved under illumination than in those starved in darkness, probably due to the photosynthesis of the zooxanthellae. He also found that the anemones gained weight when fed under illumination. Schlichter (1975) has shown that this anemone can take up significant amounts of dissolved organic material from the surrounding sea water. A. sulcata can therefore be regarded as polytrophic as defined by Trench (1974).

Factors in the model outlined in Fig. 1 were measured in a series of laboratory experiments on A. sulcata in this study, in order to construct a model of energy flow through hypothetical 'standard' A. sulcata. This model was then used to estimate the amount of photosynthetically produced energy available to the organism and how much this contributes to the energy requirements of the anemone and the zooxanthellae.

Section 2

Materials and Methods

1) a Source and maintenance of symbiotic Anemonia sulcata

Specimens of the brown colour morph only were collected from a subtidal population at the Island of Siel, Argyll and Bute and were maintained at a constant temperature of 10 ± 1°C in the recirculating sea water aquarium at the Department of Zoology, University of Glasgow. The specific gravity of the sea water was 1.022 ± 0.001 at 12 ± 2°C. The salinity was a constant 32°/oo. The anemones were kept in individually marked fish breeding traps in glass reinforced plastic tanks. The tanks and breeding traps were thoroughly cleaned every 21 days.

Anemones used in the experiments described in Section 3 were fed with meals of squid mantle every seventh day. The feeding regimes which the anemones used in Section 4 were maintained under are described in that section. Although squid mantle is not the natural food of this anemone, it was chosen as it was a marine food which has a relatively uniform water content and energy content and does not break up in sea water (Wallace, 1971). These properties allowed estimates of the energy input from carnivorous feeding to be made from the wet weight of squid ingested by each anemone. Mantles of frozen squid were obtained from the University Marine Station, Millport, Isle of Cumbrae, Firth of Clyde and were cut into meals with a 7mm cork borer, and were stored at -10°C before use.

Anemones used in Section 3 were exposed to an irradiance of 10 $\mu E.m^{-2}.sec^{-1}$. for 12h per day prior to the experiments. The lighting regimes which the anemones used in Section 4 were maintained under are described in that section.

1) b Production of Aposymbiotic Anemonia sulcata

Anemonia sulcata has a natural mechanism for culling its population of zooxanthellae (Taylor, 1969). The mechanism is not fully understood, but it may be accelerated by physical treatments (Smith, 1939; Taylor, 1969). An effective method of producing symbiont-free or aposymbiotic coelenterates is that of Pardy (1976) in which symbionts are photodestroyed at an irradiance of 620 $W.m^{-2}$ ($\sim$2800$\mu E.m^{-2}.sec^{-1}$) in the presence of 3- (3,4-dichlorophenyl)-1,1-dimethylurea (D.C.M.U.), an inhibitor of photosynthesis. This method was adapted for use with A. sulcata.

Anemones were kept in tanks of static sea water at 10°C containing 5×10^{-5}M D.C.M.U., 20 $units.ml^{-1}$ penicillin and 20$\mu g.ml^{-1}$ streptomycin. The anemones were exposed to an irradiance of $\sim$50 $\mu E.m^{-2}.sec^{-1}$ for 54 days with "Warm White" fluorescent tubes (Petcraft). Exposure to the irradiance used by Pardy (1976) caused the death of more than 75% of the anemones, and this was subsequently abandoned. Anemones were starved during the period of exposure. No algal pigments were visible in methanol-chloroform extracts of tissue precipitated with perchloric acid from a sample of 5 anemones sacrificed after this treatment.

2) Measurement of energy expenditure

There are two ways in which the energy expenditure of a living organism can be measured.

1) Measurement of heat production or direct calorimetry
2) Measurement of oxygen consumption or indirect calorimetry

Direct calorimetry is more accurate than indirect calorimetry. Heat is liberated by the hydrolysis of ATP and other high energy phosphates during energy expenditure. Additional heat is released as

substrates are catabolised to provide energy for the resynthesis of these high energy phosphates. Hence the rate of energy expenditure is directly related to the rate of heat production. Accurate direct calorimeters suitable for use with ectothermic organisms are relatively new tools used by an increasing number of workers including Spaargaren (1975), Pamatmat (1978) and Gnaiger (1980).

Indirect calorimetry is the more common method of estimating energy expenditure. The oxygen consumption of an aerobic organism can be directly related to its heat production if the substrates being metabolised are known (Brody, 1945; Kleiber, 1961 and Elliott & Davison, 1975). Indirect calorimetry was used to estimate the energy expenditure of Anemonia sulcata in this study for two reasons.

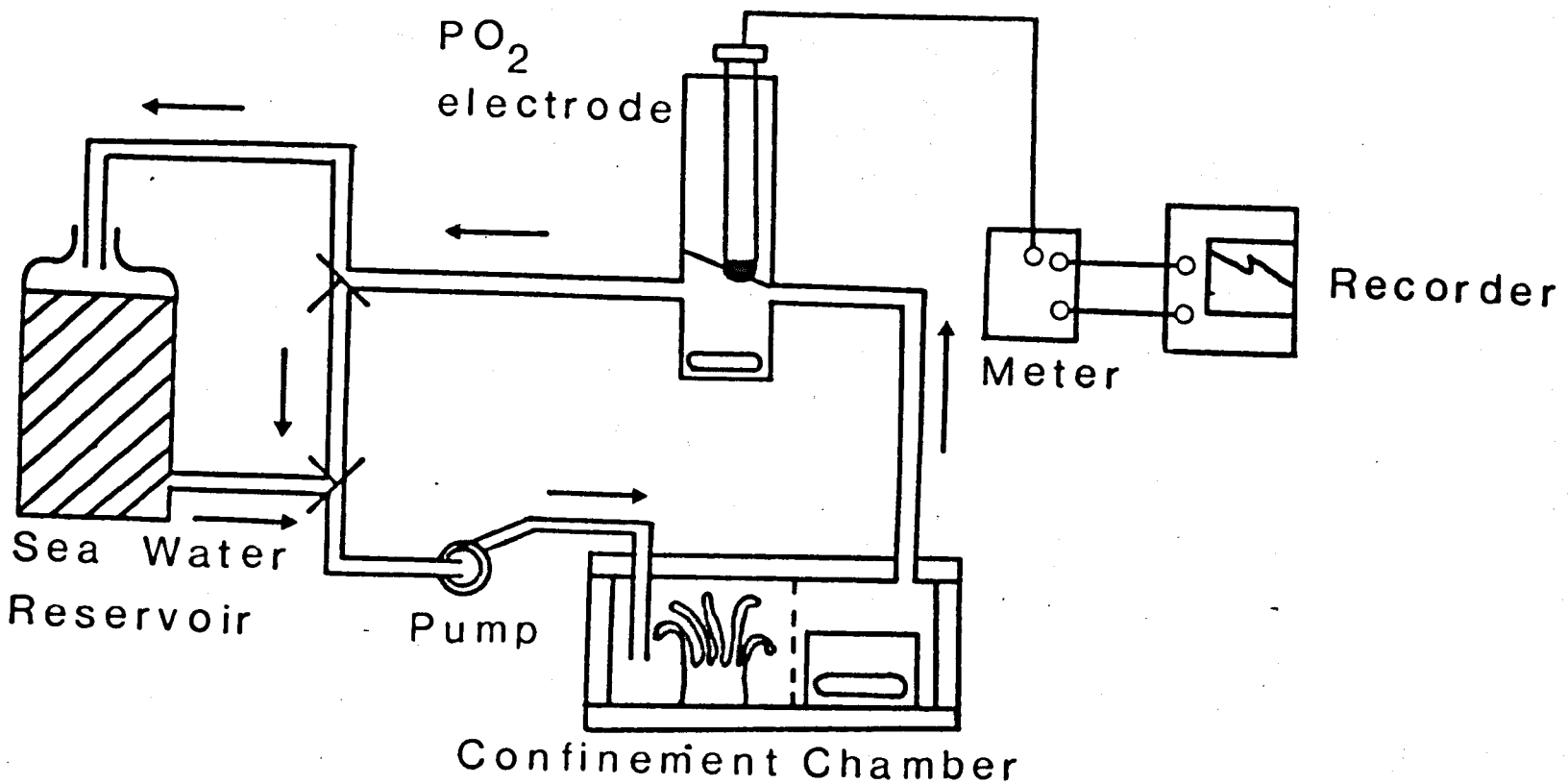
1) A suitable direct calorimeter was not available at the time of this study.
2) An indirect calorimeter can also be used to estimate energy production by photosynthesis.

Indirect calorimeters or respirometers are of two basic types

a) Open or continuous-flow respirometers
b) Closed or confinement respirometers

Initially a continuous flow respirometer similar to that of Jobling (1978) was built. This proved to be unsuitable for Anemonia sulcata, due to the lag between actual and recorded changes in oxygen partial pressure (see Fry, 1971 and Niimi, 1978). This lag was excessive as low water flow rates had to be used through large chambers since these anemones have low rates of oxygen consumption and relatively large volumes. This system was abandoned in favour of a manual confinement respirometer which allowed repeated measurement of oxygen consumed by a single animal. The respirometer in Fig. 2 consisted of four basic components.

Fig. 2 Manual Confinement Respirometer



i) A 2 l. glass aspirator used as a sea water (SW) reservoir.

ii) A centrifugal pump.

iii) A confinement chamber.

iv) A polarographic oxygen electrode.

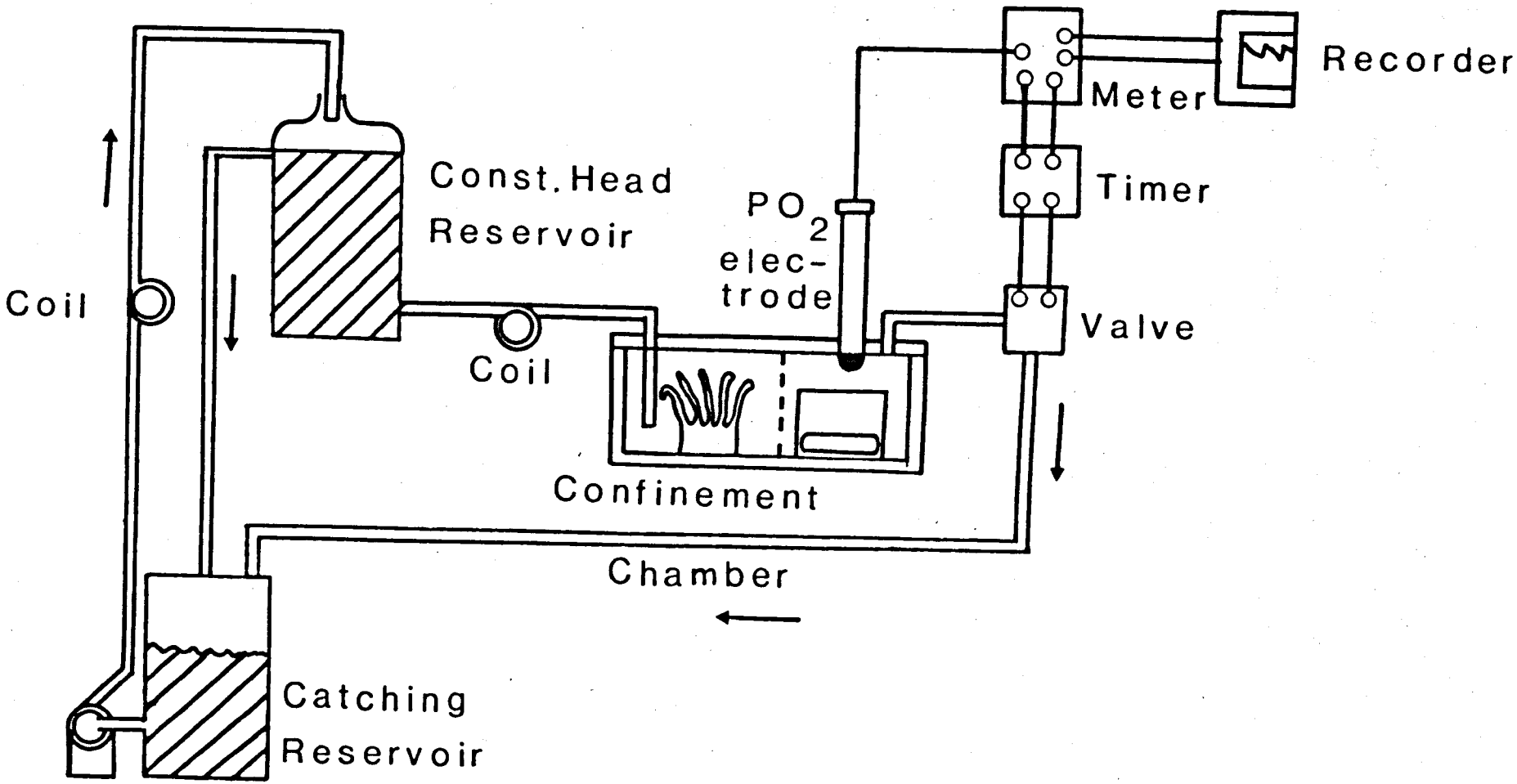
The centrifugal pump was a perspex cylinder containing a 40mm magnetic spinbar which propelled the SW. A collar was fixed to the base of the pump to fit an emersible magnetic stirrer (Rank Brothers) which rotated the spinbar. The inlet was situated in the centre of the pump top while the outlet was tangential to the pump body.

Two types of confinement chamber were used; a cylindrical chamber of 150ml capacity and a cuboidal chamber of 230ml capacity. Both were made of perspex. The cylindrical chamber had a detachable top which screwed down to provide a seal with a rubber 'O' ring. The cuboidal chamber was sealed with a rubber 'O' ring under a hinged lid retained by stainless steel clips. The cuboidal chamber was stirred by a 25mm spinbar enclosed within a perforated plastic tube and separated from the animal by a stainless steel grid. The cylindrical chamber was not stirred. A collar was fixed to the base of each chamber to fit a second emersible stirrer.

A polarographic oxygen electrode (YSI model 5311) was located in a glass chamber modified as in Jobling (1978), containing a spinbar driven by a third emersible stirrer. The electrode was connected to a polarising source (YSI model 53) and a chart recorder (Smith's Servoscribe S1 RE 541-20).

The components were connected in series by PVC tubing (Portex NT12) and the circuit was opened or closed with four way stopcocks (Baxter). The circuit was submerged in a water bath maintained at a

Fig. 3 Automated Confinement Respirometer



constant temperature of 10 ± 0.01°C by a thermostatic heater (Grant UX3) in conjunction with a flow refrigerator (Grant FC20T). The respirometer was filled with autoclaved SW as background respiration was excessive in stock SW. The SW was changed, and all tubing replaced every 14 days. All components were cleaned with 2% sterilising fluid (Milton 2). Electrodes were cleaned and membrane replaced every 4 to 7 days.

After obtaining preliminary data, the respirometer was automated to allow readings to be taken over a 24 to 96h period (Fig. 3).

Circulation of SW was provided by a constant head system by positioning an overflow pipe in the glass aspirator. SW overflowed through polythene tubing to an insulated plastic reservoir below the water bath, which was open to the atmosphere. A centrifugal aquarium pump (Eheim Model 1018) was used to return the SW, via a glass heat-exchange coil to the upper reservoir where it was gently aerated with a diffused stone. The SW from the upper reservoir passed through a second glass heat-exchange coil before entering the confinement chamber. The flow through the chamber was controlled by a single solenoid valve on the outlet side of the chamber. SW then drained into the lower reservoir. Cuboidal chambers of 230, 130 and 80ml capacity were used. An oxygen electrode was positioned above the spinbar in the chamber. The temperature in the chamber was a constant 10 ± 0.1°C.

The system was run in tandem with two separate respirometer circuits in the same water-bath. A simple time switch was fabricated from a strip of rubber taped to a rotating Kymograph drum (C.F. Palmer 12) which triggered two microswitches (Honeywell). One switched the solenoid valves while the other switched the electrode output on the polarising source. This automated confinement system was operated continuously. Readings were taken alternately from each circuit, when closed, over a 1h period using a single pen recorder.

A polarographic oxygen electrode measures changes in the partial pressure of oxygen (PO_2) in a solution. These changes were converted to rates of oxygen consumption ($\dot{M}_{O_2}$) with the following equation.

1) $\dot{M}_{O_2} = \Delta PO_2 \times \alpha \times (V_R - V_d)$ where $\dot{M}_{O_2}$ = oxygen consumption ($\mu mol\ O_2.h^{-1}$)

ΔPO_2 = changes in PO_2 ($mmHg.h^{-1}$)

α = Oxygen solubility coefficient ($\mu mol.\ mmHg^{-1}\ l^{-1}$)

V_R = Volume of the confinement chamber (l)

V_d = Theoretical dry volume of the animal (l) (Appendix 1)

In closed chamber respirometry, it is usual to subtract the volume of the animal from that of the chamber to obtain the volume of extracellular water from which the amount of oxygen in the system is calculated. However, coelenterates such as Anemonia sulcata are only two cells thick and the diffusion distance from the extracellular water to the mitochondria within the cells will therefore be very small. For this reason, it is likely that the intracellular PO_2 will be little different from the extracellular PO_2 and that the intracellular PO_2 will vary as the extracellular PO_2 varies in a closed system. For this reason the intracellular water should therefore be regarded as a part of the total volume of the system.

These assumptions have been made in the experiments, and the theoretical dry volume (calculated as in Appendix 1) has therefore been subtracted from the volume of the chamber to give the total volume of water in the system. This approach may lead to slight errors if the solubility of oxygen in the intracellular water was different from that in sea water or if the PO_2 in the intracellular water was different from

the PO_2 recorded in the extracellular water. These errors are probably smaller than the errors introduced by the more conventional method in which intracellular water is excluded from the system. Appropriate corrections were made for background oxygen consumption.

Rates of energy expenditure were calculated by multiplying the $\dot{M}_{O_2}$ values by the general oxy-calorific coefficient of Elliott & Davison (1975).

$$1\ \mu mol\ O_2 = 0.4525J$$

since respiratory quotients were not determined.

3) Sources of Irradiance

Two types of fluorescent striplight were used in this study; the Power-Drive "Tru-Light" (Durolite International) and the "Northlight" (Thorn). The spectral emission of these lights were measured with a spectroradiometer (ISCO Model SR) and was found to be similar to typical incident daylight. The "Northlight" was used as the light source in the aquarium (see Part 1 of this Section) as the "Tru-Light" was not available in a 1500mm length.

A bank of eight 20W, 600mm "Tru-Light" strip lights were suspended from a frame above the confinement chambers of the respirometers. The lights were triggered by waterproof starters (Arcadia) which allowed any number of lights from 1 to 8 to be used. This provided irradiance of up to $190\mu E.m^{-2}.sec^{-1}$ measured at the top of the confinement chambers with a quantum radiometer (Crump No. 550) with a submersible sensor (Crump 552-17-0980P). In some experiments the lights were connected to a time switch to give a 12h light/12h dark cycle. The framework was covered with black polythene sheet to provide darkness.

A bank of four and a bank of two 65 - 80W, 1500mm "Northlight"

striplights in waterproof LZ fitments (Thorn) were installed in the aquarium. These provided irradiances of 140 $\mu E.m^{-2}.sec^{-1}$ and 70 $\mu E.m^{-2}.sec^{-1}$ respectively at the top of the breeding traps in the centre of each tank. These lights were connected to a time switch to give a 12h light/12h dark cycle.

4) Measurement of Energy production of zooxanthellae in vivo

The rate of photosynthetic energy production can be calculated from the rate of CO_2 fixation. CO_2 fixation can be measured in three ways.

1) From the uptake of CO_2 using infra-red gas analysis appartus (Ludwig and Canvin, 1971).
2) From the uptake of radioactively labelled sodium bicarbonate ($NaH^{14}CO_3$) (Steeman Nielsen, 1952).
3) From the rate of gross O_2 production assuming a photosynthetic quotient (no. of moles of CO_2 fixed/no. of moles of O_2 produced) of 1 (Ryther, 1956).

Infra-red gas analysis is the most accurate of these methods, however the appropriate equipment was not available at the time of this study. The use of radioactively labelled bicarbonate has the disadvantage that compounds containing fixed labelled carbon may be respired during the course of the experiment (Ryther, 1954). The rate of CO_2 fixation of zooxanthellae in vivo was determined indirectly from the rate of gross O_2 production in this study. Net O_2 production was measured at set irradiances with the respirometers described in Part 3 of this Section. The gross O_2 production was calculated as the sum of the O_2 consumption in darkness and the net O_2 production at each irradiance (Ryther, 1956). It was assumed that the O_2 consumption in the light was the same as that recorded in darkness. O_2 consumption of plants may be elevated in light

by photorespiration, a process in which CO_2 fixation by the enzyme ribulose biphosphate carboxylase is inhibited by O_2 (Shelp & Canvin, 1980).

The evidence for photorespiration by zooxanthellae is equivocal (Trench, 1979). Inhibition of photosynthesis by O_2 has been observed in zooxanthellae isolated from Tridacnid clams and the coral Pocillopora (Black et al, 1976 and Downton et al, 1976). A post illumination increase in O_2 consumption, which is indicative of photorespiration, has been observed in zooxanthellae isolated from Pocillopora capitata (Burris, 1977) but was not observed in zooxanthellae isolated from the coral Acropora acuminata (Crossland & Barnes, 1977). Glycine and serine, two major products of photorespiration, are not accumulated in zooxanthellae (Burris, 1977 and Trench, 1979). Photorespiration by zooxanthellae in vivo has yet to be demonstrated.

The rate of energy production was calculated from the gross O_2 production by assuming that for every 6 moles of O_2 produced, 1 mole of glucose was synthesised and multiplying the rate of glucose synthesis by the heat of combustion of glucose, 2.816 $J.\mu mol^{-1}$ (Lehninger, 1971).

5) Treatment and preparation of tissue for determination of energy content and determination of the size of the zooxanthellae population.

The size of the zooxanthellae population and the energy content of each anemone was determined in this study. The energy content was determined by wet oxidation with dichromate which required correction for protein content (see Part 6a of this Section).

The procedure used to determine these values is outlined in Fig. 4. Each anemone was buoyant weighed(see Appendix 1), anaesthetised with magnesium chloride and cut longitudinally into 3 portions which were individually buoyant weighed. One portion was lyophilized with an

Fig. 4 Procedure for the fractionation of symbiotic Anemonia sulcata

Whole A. sulcata

Buoyant Weighed

Anaesthetised in 0.33M $MgCl_2$

Cut longitudinally into portions

1

Buoyant Weighed

Homogenised in distilled water

Freeze Dried

Energy content by wet oxidation with dichromate

Dry weight

500°C for 6h

Ash weight

2

Buoyant Weighed

Homogenised in 10ml TBSE Buffer pH7.8

Centrifuged at 1,300g for 10 min

Pellet and floc

Resuspended in 10ml TBSE Buffer

Centrifuged at 1,300g for 10min

Pellet and floc

Resuspended in 10ml TBSE Buffer

Centrifuged at 1,300g for 10min

Pellet and floc

Resuspended in 50ml TBSE Buffer

NUMBERS OF ZOOXANTHELLAE

Supernatant collected

Supernatant collected

Supernatant collected

Supernatants combined and made up to 30ml

NUMBERS OF ZOOXANTHELLAE

3

Buoyant Weighed

Homogenised in 10ml 0.6N P.C.A.

Centrifuged at 1,300g for 10min

Pellet

Resuspended in 10ml of 0.6N P.C.A. and made up to 16ml with distilled water

1ml Aliquots

3.75ml of $MeOH.CHCl_3$ (2:1) added

Centrifuged at 2,800g for 10min

Pellet

3.0ml of $MeOH:CHCl_3$

Centrifuged at 2,800g for 10min

Pellet

5.0ml of 1.0N NaOH added
Covered with Parafilm
Mixed and stored for 24h

PROTEIN

Supernatant discarded

Supernatant discarded

Supernatant discarded

Supernatant discarded

Edwards EF03 freeze drier and was used to determine the ash content and the energy content. Zooxanthellae were extracted from a second portion with autoclaved sea water or artificial saline by the method described in Part 7 of this Section.

The proteins were extracted from the third portion by a procedure based on that of Kochert (1978) using the solvent mixture of Bligh & Dyer (1959) to extract lipids. The protein content was determined by the method of Lowry et al (1951). (See Part 6b of this Section).

6) Determination of the energy content of tissue

There are three methods of determining the energy content of tissue.

1) Ballistic bomb calorimetry
2) Wet oxidation with Dichromate
3) Proximate Analysis.

A ballistic bomb calorimeter records the heat of combustion of dried material in an oxygen filled bomb. The adiabatic bomb calorimeter is the most accurate instrument available for determining the energy content of organic materials, but large amounts of material are required for each combustion (Paine, 1971). The non-adiabatic microbomb calorimeter requires less material but is less accurate, demanding replicate combustions (Phillipson, 1964).

Wet oxidation with dichromate is an indirect means of determining the energy content of tissue. Tissue is oxidised in a strongly acidic solution. The amount of oxygen used in this process is calculated from the amount of dichromate reduced to chromium ions. The energy content of the tissue is calculated by multiplying the amount of oxygen used by

oxycalorific coefficients (O'Shea & Maguire, 1962). This method is simple and reliable and requires very small amounts of tissue. However, chloride ions reduce dichromate (Strickland & Parson, 1968) and corrections must be made for proteins which are only partly oxidised in this process (O'Shea & Maguire, 1962).

Love (1970) defined proximate analysis as the determination of total nitrogen, lipid, water and ash. This classic approach has been expanded to include other organic compounds including carbohydrates (Craig et al, 1973). The energy content of the tissue is calculated by multiplying the amount of each organic compound by the corresponding energy coefficient determined for that compound (Brody, 1945).

The energy content of A. sulcata tissue was determined by wet oxidation with dichromate in this study as some anemones did not provide enough tissue for use for microbomb calorimetry.

6) a Wet Oxidation with Dichromate

The energy content of freeze-dried anemone and squid tissue was determined by a method of wet oxidation with dichromate based on that of Johnson (1949).

1ml of distilled water and 600µl of mercuric sulphate ($HgSO_4$) solution (Mackereth et al, 1978) was added to samples containing 2mg of tissue. The $HgSO_4$ was sufficient to stop oxidation of dichromate by up to 1.1mg of chloride ions (Golterman & Clymo, 1969). The samples were oxidised with 4ml of 0.1N potassium dichromate ($K_2Cr_2O_7$) sulphuric acid solution, containing 66.67 µmol of $K_2Cr_2O_7$, by heating for 1h in a boiling water bath. After diluting to 50ml with distilled water, the amount of $K_2Cr_2O_7$ reduced to Cr^{3+} was determined spectrophotometrically by reading

the absorbances at 440nm against standard containing 66.67 µmol $K_2Cr_2O_7$ and blanks reduced with sodium sulphite.

Calculation of energy content

The energy content per mg. dry weight of the tissue was calculated from the amount of $K_2Cr_2O_7$ reduced to Cr^{3+}. The reduction of 66.67 µmol $K_2Cr_2O_7$ releases 100µmol O_2 (Golterman & Clymo, 1969) which is equivalent to 45.25J using the general oxy-calorific coefficient of Elliott & Davison (1975). The energy content of the tissue per mg. dry weight was corrected for ash content to give the energy content in units of J.mg organic weight^{-1}. Forster & Gabbott (1971) have suggested that, typically 60% of proteins are oxidised in this process. A correction for the unoxidised protein was made with the following equation.

$$\frac{40}{100} \times 23.63 \times (P) = 9.454(P)J$$

where $23.63J.mg^{-1}$ is the energy content of a typical protein (Brody, 1945)

and (P) is the protein content in mg.mg organic weight^{-1} of each animal determined as described in Part 6b of this section.

6) b Determination of Protein Content

The most common method of determining protein content is that of Lowry et al (1951). The level of protein is determined spectrophotometrically from its reaction with the Folin-phenol reagent in an alkaline solution. This method is more accurate than the Biuret and Kjeldahl methods (Giese, 1967) but it is not as rapid and sensitive as the protein-dye binding method of Bradford (1976). The method of Lowry et al (1951) was used throughout this study in preference to the method of Bradford (1976) since trials with the latter method showed that the protein-dye complex adhered to glass, which distorted readings if glass cuvettes were used repeatedly.

Proteins were extracted from A. sulcata as described in Fig. 4. 0.5ml samples were used for determination of protein against standards of bovine serum albumin. Absorbance was read at 550nm instead of 750nm, because the absorbance is more linearly related to protein concentration at this wavelength.

This method gave highly repeatable results. However, subsequent comparison with proteins precipitated with 5% Trichloroacetic acid (T.C.A.) centrifuged and resuspended in 3% T.C.A. and washed twice with methanol:diethyl ether (1:1) showed that the results obtained by the latter method were higher by a factor of 1.92. All recorded values were multiplied by this amount. These results were used to calculate the protein content per mg organic weight from the buoyant weight of the portion of anemone used.

The protein content of the squid mantle tissue was determined from preweighed freeze-dried tissue, precipitated with 5% T.C.A., washed twice with methanol:diethyl ether (1:1) before addition of alkali. The results were corrected for ash content to give protein content per mg organic weight.

7) Estimation of the sizes of zooxanthellae populations

Zooxanthellae were separated from the anemone tissue by a method based on those of Franker (1970, 1971). They were partially purified by centrifugation and washing with saline. This allowed the size of the zooxanthellae population to be estimated from counts made using a haemacytometer.

Reagent: TBSE Buffer

NaCl	0.4M
$MgSO_4$	0.04M
KCl	0.01M
MgCl	0.003M
EDTA	0.005M
Tris HCl) Tris Base)	0.01M pH7.8

Procedure

Zooxanthellae were extracted by the procedure outlined in Fig. 4. Portions of A. sulcata were homogenised in cold TBSE buffer with a motor-driven, glass/PTFE potter homogeniser. After centrifugation at 1,300g some zooxanthellae were pelletised while the remainder congregated in a buoyant "floc" on the surface of the supernatant. The supernatant was carefully poured off and collected. The pellet and floc were resuspended in 4ml of TBSE buffer by repeated forcing through a 19g needle with a 5ml syringe and was then made up to 10ml. After two further resuspensions and recentrifugations, the supernatants were combined and made up to 30ml with TBSE buffer. The final pellet and floc were resuspended and made up to 25 or 50ml in a volumetric flask. Initially 5 replicate counts of samples and 3 counts of combined supernatants were made with an improved Neubauer haemacytometer. In later experiments, this was extended to 15 replicate counts of both samples and supernatants. In preliminary experiments, zooxanthellae were extracted with the NET Buffer of Franker (1970) or with autoclaved sea water, S.G. = 1.022 at $10 \pm 1^{\circ}C$. The total population of zooxanthellae (Z_{total}) was calculated with the equation

$$Z_{total} = \frac{W_w \text{ total}}{W_w \text{ portion}} \times 10^4 \left(\text{or}^{50}_{25} \; Z_s + 30 Z_L \right)$$

where

W_w total is the buoyant weight of the whole animal

W_w portion is the buoyant weight of the portion of the animal

Z_s is the mean no. of zooxanthellae per $0.1mm^3$ of sample

Z_L is the mean no. of zooxanthellae per $0.1mm^3$ of combined supernatants

8) Determination of Biomass

The body weight of each anemone had to be recorded in the experiments of this study since many of the processes investigated were dependent on the size of the anemone. The most useful measurement of biomass is the organic weight. This can be measured as the ash-free dry weight by sacrificing the animal. Some experiments in this study required the repeated measurement of the body weight. Since it has been shown in Appendix 1 that the weight of an A. sulcata in sea water, its so-called"buoyant weight", is directly related to its organic weight, measured as ash-free dry weight, the organic weight of each anemone was calculated from its buoyant weight, unless otherwise stated.

The weight of squid ingested by each anemone had also to be determined. Since the organic weight of the squid used was a relatively uniform percentage of its wet weight (Section 4B), the organic weight of the squid fed to each anemone was calculated from the wet weight of the meals. The organic weights of undigested or partly digested meals were determined as their ash-free dry weight and these were subtracted from the organic weight of the squid meals to give the organic weight of squid ingested by each anemone.

Section 3

Some factors affecting respiratory energy expenditure and photosynthetic energy production of symbiotic Anemonia sulcata

A) Factors affecting respiratory energy expenditure

Introduction

The energy expenditure on maintenance (R) is the work carried out to keep an organism functioning. This includes ionic and osmotic regulation, resynthesis of macromolecules and essential postural and muscular activity.

The R of Anemonia sulcata was assumed to be equivalent to the heat production due to standard metabolism of the anemone. Standard metabolism is the oxygen consumption ($\dot{M}_{O_2}$) during minimal functional activity when no food is being digested or absorbed (Krogh, 1916).

Many physical factors, including temperature, salinity and partial pressure of oxygen (PO_2) and biotic factors such as animal size, sex, activity level, stress and nutritional condition affect the $\dot{M}_{O_2}$ of marine invertebrates to varying degrees (Dejours, 1975). Temperature and salinity were kept constant throughout this study while the effects of PO_2 on $\dot{M}_{O_2}$ was investigated to determine a range of PO_2 over which the $\dot{M}_{O_2}$ would be recorded in subsequent experiments. The effect of the anemone body weight on $\dot{M}_{O_2}$ was investigated to determine a value of R for a standard sized anemone. The effect of feeding and light on the $\dot{M}_{O_2}$ of A. sulcata is investigated in subsequent sections (Section 4C and 5B).

i) The effect of PO_2 on $\dot{M}_{O_2}$

The $\dot{M}_{O_2}$ of many marine invertebrates is dependent on the PO_2 in the water surrounding the animal while in others the $\dot{M}_{O_2}$ can be regulated at a given level over a range of PO_2 down to a "critical" PO_2 below which

the animal switches to some form of anaerobic metabolism. The former has been given the term "oxygen dependent" while the latter are said to be "oxygen-independent" (Herreid, 1980).

Symbiotic A. sulcata were exposed to declining PO_2 in darkness to determine the relationship between $\dot{M}_{O_2}$ and PO_2.

Materials and Methods

Eight symbiotic Anemonia sulcata were allowed to reduce the PO_2 to less than 80mmHg within the manual confinement respirometer over a period of 4½h in darkness. Two of these animals were allowed to deplete the O_2 over a period of 7h. The $\dot{M}_{O_2}$ was calculated from the change in PO_2 over each 20 minute period.

Each animal had been starved for at least 3 days and were left undisturbed in darkness in the respirometers for 30 min before each experiment. The buoyant weight of each animal was measured after the experiment as described in Appendix 1.

Results

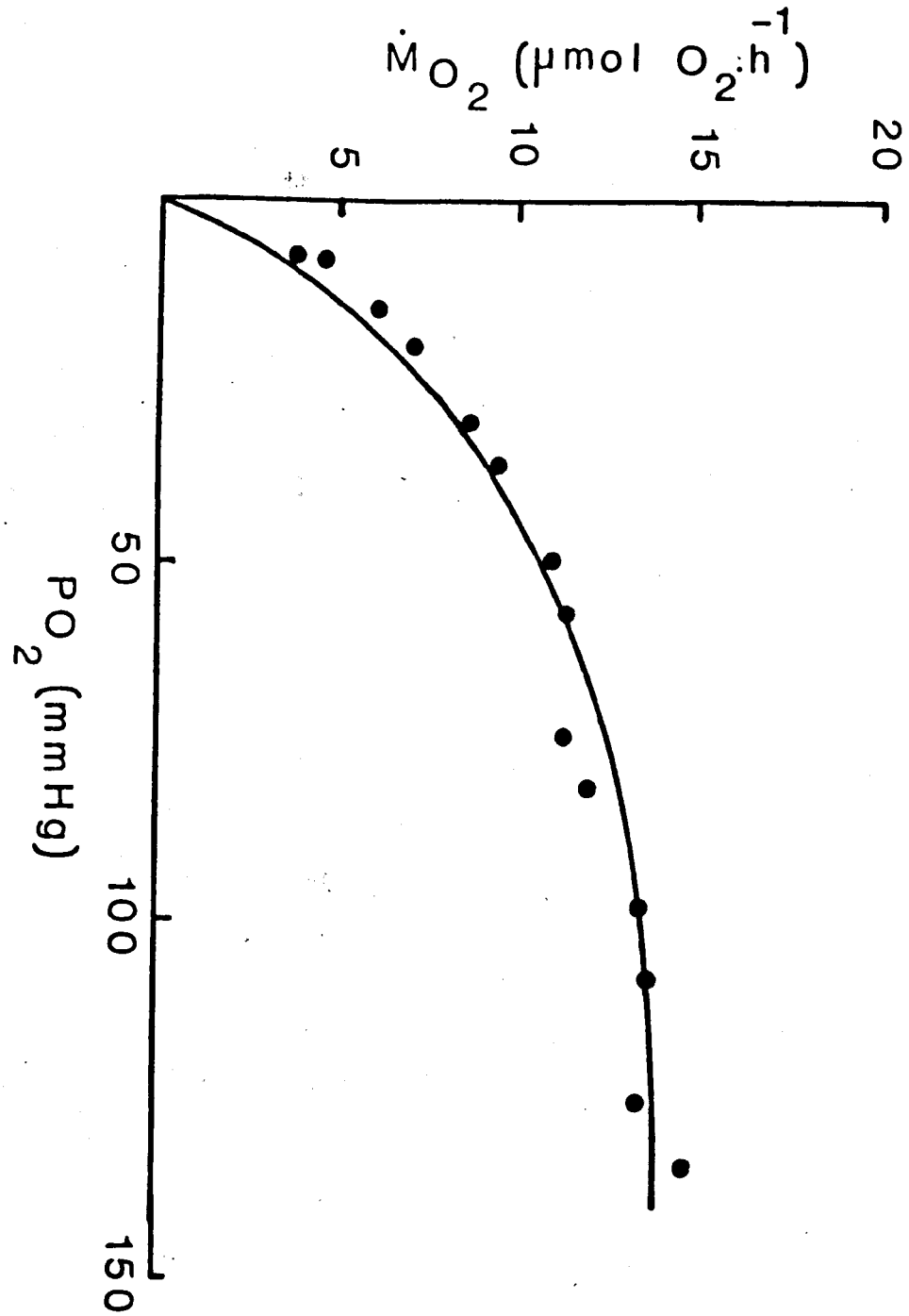
Fig. 5 shows the typical response of a symbiotic A. sulcata to declining PO_2. The $\dot{M}_{O_2}$ was wholly or partly dependent on PO_2 over the range tested. There was no clear critical PO_2.

Discussion

The relationship between $\dot{M}_{O_2}$ and PO_2 in A. sulcata is a curvilinear one, with no indication of a critical PO_2. Similar curves have been reported for other sea anemones including Metridium senile (Sassaman & Mangum, 1973;. Shumway, 1978).

Initial inspection of the curve obtained for A. sulcata suggested that the relationship was in the form of a rectangular hyperbola. In

Fig. 5 The effect of partial pressure of oxygen (PO_2) on the oxygen consumption ($\dot{M}_{O_2}$) of a symbiotic Anemonia sulcata (curve fitted by eye)



order to convert this into a linear form, the procedure of Tang (1933) has been adopted. If the equation for the hyperbola is given by

1) $A = \frac{P}{K_1 + K_2 P}$ where A is the $\dot{M}_{O_2}$, P is the PO_2, and K_1 and K_2 are constants

by rearrangement 2) $P/A = K_1 + K_2 P$

Thus in Fig. 6 $PO_2/\dot{M}_{O_2}$ is plotted against PO_2 and a regression line was fitted by the method of least squares. The significance of the fit of the regression line was tested by analysis of variance (Sokal & Rohlf, 1969). The F ratios given in Table 1 were highly significant which suggests that the initial supposition of a hyperbolic relationship was acceptable. $PO_2/\dot{M}_{O_2}$ v PO_2 plots may therefore be used for prediction of $\dot{M}_{O_2}$ with confidence limits at selected values of PO_2. However, in subsequent experiments, the PO_2 was not allowed to fall below 140 mmHg (~80% saturation) to minimise the effect of PO_2 on $\dot{M}_{O_2}$. This is consistent with the recommendation of Muscatine (1980).

Since the shape of the curve relating $\dot{M}_{O_2}$ to PO_2 resembles a rectangular hyperbola, the effect of PO_2 on $\dot{M}_{O_2}$ of A. sulcata may have been analagous to the effect of substrate concentration on the rate of reaction of enzymes obeying Michaelis-Menten enzyme kinetics.

A second degree polynomial function has been used by Mangum & Van Winkle (1973) and Sassaman & Mangum (1974) to relate $\dot{M}_{O_2}$ to PO_2 in other sea anemones but the theoretical justification for its adoption is not clear. Conversely Bayne (1971) and Taylor & Brand (1975) have used the rectangular hyperbolic function to relate $\dot{M}_{O_2}$ to PO_2 in some lamellibranch Molluscs.

ii) The effect of weight on $\dot{M}_{O_2}$

Introduction

The relationship between oxygen consumption and the body weight of an organism is a power function which can be described by the equation

Fig. 6 The relationship between PO_2 and $PO_2/\dot{M}_{O_2}$ in a symbiotic *A. sulcata*

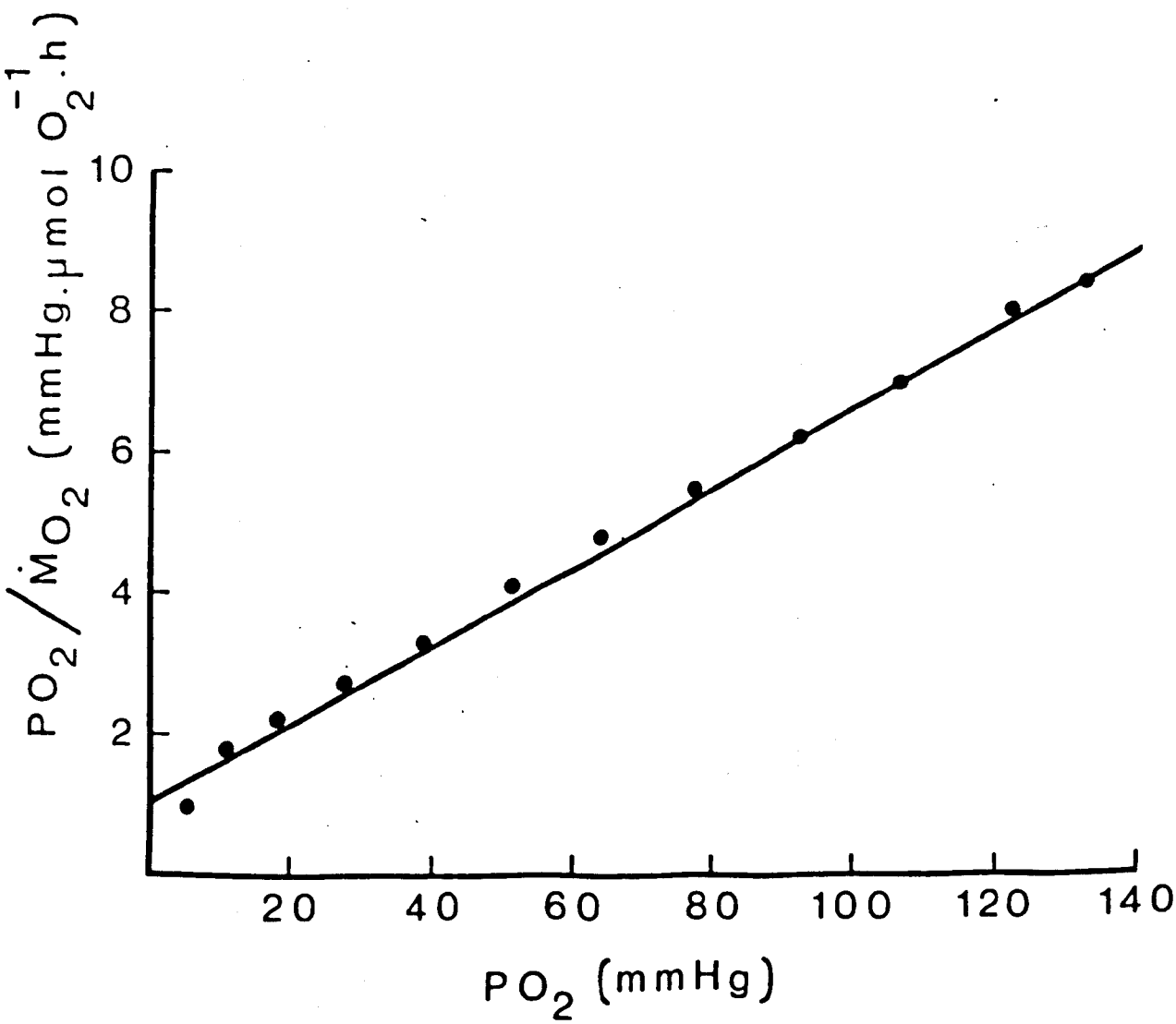


Table 1 Regression lines calculated by the method of least squares of PO_2 on $PO_2/\dot{M}_{O_2}$ in 8 symbiotic *Anemonia sulcata*

	Buoyant Weight (g)	n	Equation of regression line Y = a + b X	F	Degrees of freedom	
1)	0.146	10	Y = 9.840 + 0.0562X	18.379	1 & 8	$0.001 < P < 0.005$
2)	0.207	12	Y = 3.306 + 0.0570X	88.751	1 & 10	$P < 0.001$
3)	0.168	13	Y = 4.526 + 0.0853X	76.523	1 & 11	$P < 0.001$
4)	0.236	11	Y = 2.223 + 0.1077X	115.221	1 & 9	$P < 0.001$
5)	0.199	13	Y = 0.890 + 0.0930X	186.405	1 & 11	$P < 0.001$
6)	0.226	13	Y = 2.337 + 0.0473X	392.749	1 & 11	$P < 0.001$
7)	0.332	21	Y = 1.572 + 0.0630X	2446.628	1 & 19	$P < 0.001$
8)	0.167	12	Y = 1.071 + 0.0572X	2399.416	1 & 10	$P < 0.001$

$X = PO_2$ (mmHg)

$Y = PO_2/\dot{M}_{O_2}$ (mmHg.µmol O_2^{-1}h)

1) $Y = aX^b$ where Y is the oxygen consumed per unit time
X is the weight
a and b are constants

The logarithmic transformation of this equation yields the straight line relationship

2) $\log Y = \log a + b \log X$

Similarly the relationship between weight-specific oxygen consumption and weight can be described by the equation

3) $Y' = aX^{b'}$ where Y' is the weight-specific oxygen consumption $(\frac{Y}{X})$

$b' = b - 1$

A straight line relationship is also given by a logarithmic transformation of equation 3

4) $\log Y' = \log a + b' \log X$

The $\dot{M}_{O_2}$ of A. sulcata from a wide weight range was determined to establish the relationship between $\dot{M}_{O_2}$ and weight for this anemone.

Materials and Methods

The $\dot{M}_{O_2}$ of 12 symbiotic A. sulcata was measured with the manual confinement respirometer over a period of 1h under the conditions described previously. The animals were sacrificed after the experiment and their organic weights (W_d) were determined. The range of W_d was 0.316 - 1.082g.

Results

Fig. 7 shows the relationship between $\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$) and W_d (g). The equation of the regression line fitted by the method of least squares was

5) $\log \dot{M}_{O_2} = 1.038 + 0.966 \log W_d$

this has a correlation coefficient of +0.919 which was highly significant with $P < 0.001$.

Fig. 7 The relationship between oxygen consumption ($\dot{M}_{O_2}$) and organic weight (W_d) in symbiotic A. sulcata

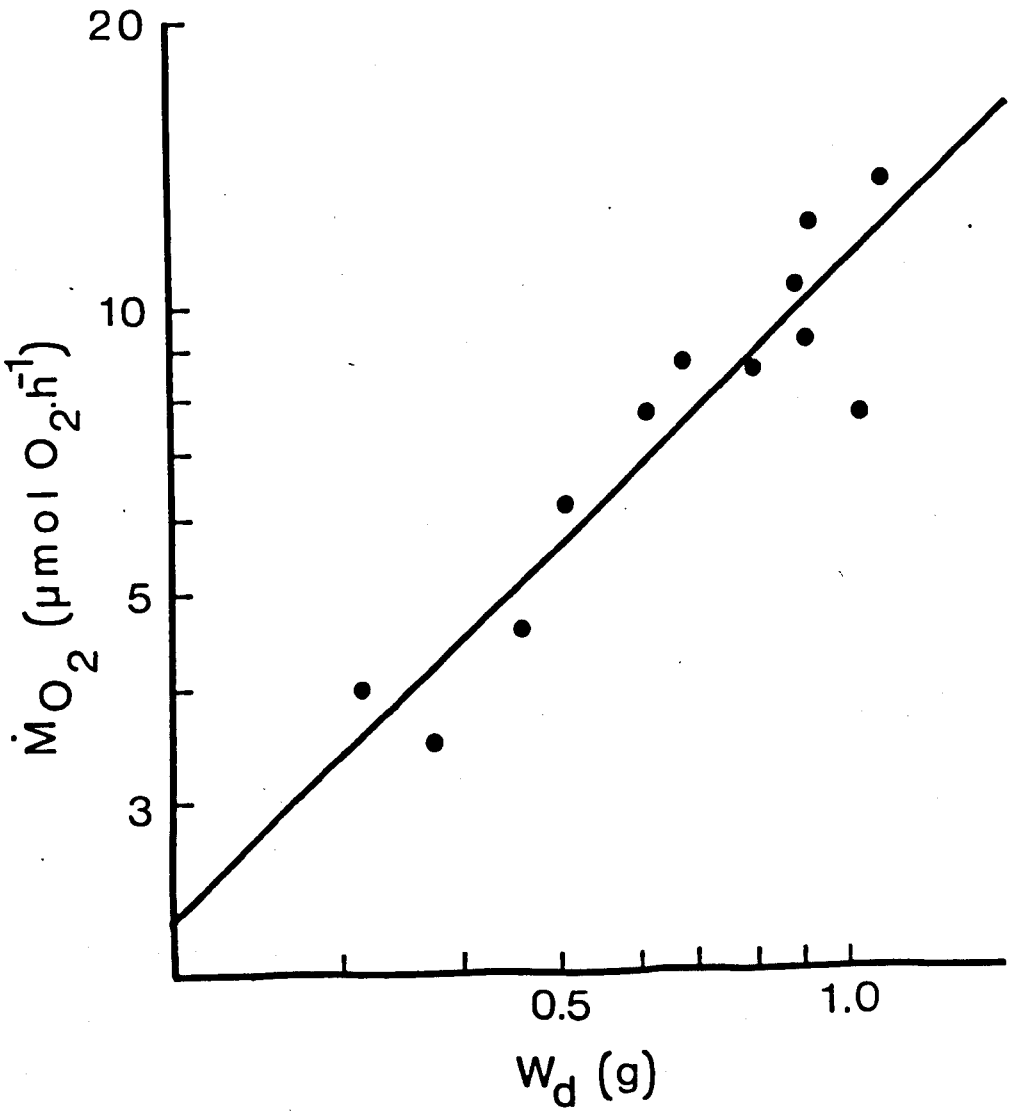


Fig. 8 shows the relationship between weight-specific $\dot{M}_{O_2}$ (μmol $O_2 \cdot g^{-1} \cdot h^{-1}$) and W_d (g). The equation of the regression line fitted by the method of least squares was

$$6) \quad \log \dot{M}_{O_2} = 1.038 - 0.034 \log W_d$$

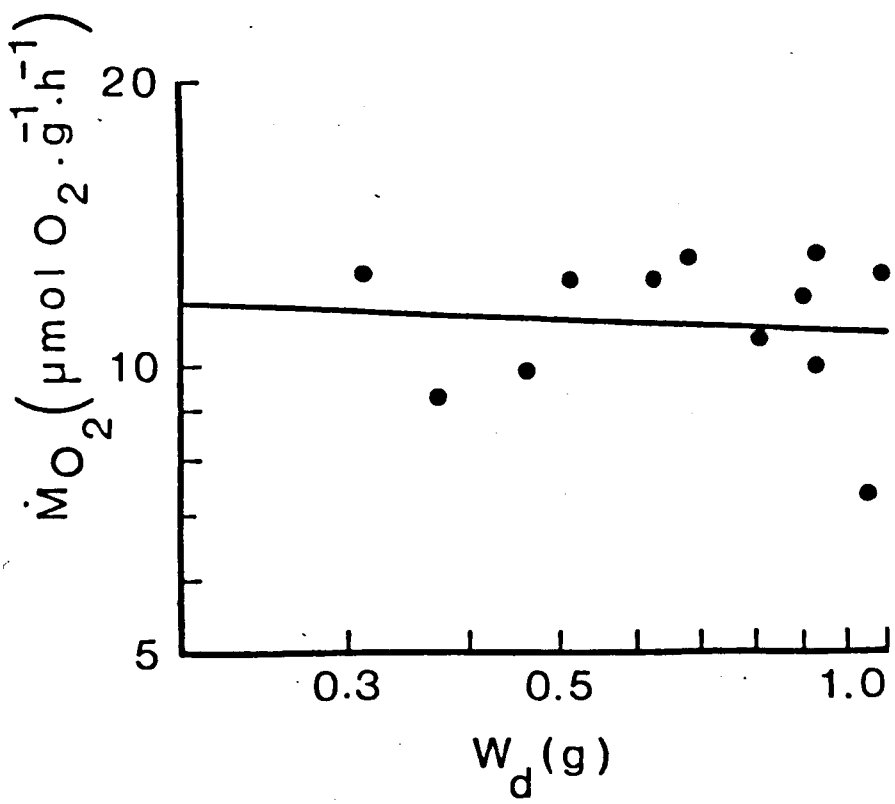
This has a correlation coefficient of -0.081 which was not significant with $P > 0.1$.

Discussion

Hemmingson (1960) has compiled data from many phyla which shows that, in general, the metabolic rate of an organism increased in proportion to body weight to the power of 0.75. The metabolic rate measured as $\dot{M}_{O_2}$ of this group of symbiotic A. sulcata increased in proportion to body weight to the power of 0.966. Student's 't' tests showed that the b value of 0.966 did not differ significantly from 1.0 with $0.8 < P < 0.9$ and from 0.75 with $0.1 < P < 0.2$. Although neither difference was significant, 0.966 is closer to 1.0 than to 0.75 and the value of P was much higher in the former test hence it appears that the $\dot{M}_{O_2}$ increased in direct proportion to body weight measured as W_d over the range tested. This phenomenon allowed the prediction of the pattern of weight loss during starvation (Appendix 2).

Similar b values have been reported for other anthozoans including 0.92 for Calliactis parasitica (Brafield, 1980), 0.89 for Metridium senile (Shumway, 1978) and 0.834 for Anthopleura elegantissima (Shick et al, 1979). Brafield (1980) has suggested that the surface area of anthozoa increases in direct proportion to increases in body weight due to their basic laminate plan and that this is partly responsible for the tendency of the $\dot{M}_{O_2}$ to increase in direct proportion to body weight.

Fig. 8 The relationship between oxygen consumption ($\dot{M}_{O_2}$) and organic weight (W_d) in symbiotic A. sulcata



The mean ($\pm$ 95% confidence limits) weight-specific $\dot{M}_{O_2}$ of a hypothetical 'standard' A. sulcata of 0.4g organic weight was 11.25 $\pm$ 5.09 µmol $O_2 \cdot g^{-1}$ organic weight h^{-1} at 10°C, 0.4g was chosen as the standard organic weight as this weight was within the weight range of most of the stocks of anemones used in this study. This is higher than the mean ($\pm$ S.D.) $\dot{M}_{O_2}$ of 3.43 $\pm$ 1.31 µmol $O_2 \cdot g^{-1} \cdot h^{-1}$ recalculated from the original data of Smith (1939) which was expressed in ml $O_2 \cdot gN^{-1} \cdot h^{-1}$, assuming that $Q_{10} = 2$ and that the organic weight was 6.25/0.53 of the total nitrogen content.

The values of $\dot{M}_{O_2}$ recorded in this study are similar to the mean ($\pm$ S.D.) M_{O_2} of 10.63 $\pm$ 0.74 µmol $O_2 \cdot g^{-1}$ dry wt. h^{-1} at 10°C for Calliactis parasitica (Brafield, 1980) and 11.11 $\pm$ 2.58 µmol $O_2 \cdot g^{-1}$ dry wt. h^{-1} at 10°C for Metridium senile (Shick et al, 1979) recalculated assuming that $Q_{10} = 2$.

A value of respiratory energy expenditure on maintenance (R) for a standard sized anemone was determined in Part B of this Section, using anemones from a narrower weight range.

B) The effect of irradiance on photosynthetic energy production

The photosynthetic energy production was calculated from the rate of gross O_2 production of the zooxanthellae in vivo (Section 2). The rate of photosynthesis of zooxanthellae is dependent on the total energy of the light quanta trapped by the light harvesting pigments. A maximum rate of photosynthesis is reached at high irradiance when the photosynthetic system becomes saturated (Halldal, 1968; Muscatine, 1980).

The relationship between photosynthesis measured as gross O_2 production, and irradiance was determined for zooxanthellae symbiotic with Anemonia sulcata in vivo to estimate the photosynthetic energy production

available to a 'standard' A. sulcata over a range of irradiances, with which the experiments in Section 4 would be designed.

Materials and Methods

Nine symbiotic anemones were exposed to 7 irradiances in the 34-190 $\mu E.m^{-2}.sec^{-1}$ range in manual confinement respirometers after a period of 12 hours in darkness, without disturbance. O_2 consumption($\dot{M}_{O_2}$) was recorded in darkness at the beginning of the experiment, while net O_2 production was recorded over a 30 min exposure period at each irradiance, in a sequence similar to that described by Davies (1977), in the 140-200 mmHg range of PO_2. After the experiment, each anemone was buoyant weighed, as described in Appendix 1 and sacrificed. The zooxanthellae were extracted with NET Buffer (Franker, 1970) by the method described in Section 2. The total population of zooxanthellae in each anemone was estimated from 5 replicate counts with a haemacytometer. Organic weights (W_d) were calculated from the buoyant weight as described in Appendix 1. Anemones in the 0.295-0.442g range of W_d, which had been starved for at least three days prior to the experiment, were used. Gross O_2 production was calculated by adding the net O_2 production at each irradiance to the $\dot{M}_{O_2}$ recorded in darkness (see Section 2).

Results

The rates of gross oxygen production at the seven irradiances were calculated and were expressed as oxygen production per 10^8 zooxanthellae and on a unit weight basis. Table 2 gives the raw data and shows that saturation of photosynthesis at an irradiance of 161 $\mu E.m^{-2}.sec^{-1}$ was observed in only two of the nine anemones used in this experiment. The mean $\pm$ S.D. values of gross oxygen production expressed in the two different ways mentioned above are shown in Table 3.

Table 2. Gross Oxygen Production by 9 symbiotic A. sulcata at 7 irradiances

Irradiance ($\mu E.m^{-2}.sec^{-1}$)		34	56	91	120	149	161	190
($\mu mol\ O_2 . 10^{-8}$ zoox. h^{-1})	1)	2.84	5.22	7.59	9.01	10.91	11.38	12.80
	2)	2.66	5.32	8.52	9.58	11.71	11.71	11.71
	3)	4.12	6.58	8.23	9.88	12.35	13.17	13.17
	4)	3.79	7.58	11.38	12.14	15.17	15.17	17.44
	5)	2.48	4.96	7.44	8.26	7.44	8.26	11.57
	6)	3.18	9.55	11.67	15.92	19.10	19.10	20.16
	7)	1.32	3.30	5.28	6.60	9.24	9.24	11.88
	8)	1.89	3.79	6.64	8.53	10.43	10.43	11.38
	9)	1.92	3.84	5.13	7.05	8.97	9.61	10.89

Table 3. Mean ± S.D. Gross Oxygen Production by 9 symbiotic *A. sulcata* at 7 irradiances

Irradiance ($\mu E.m^{-2}.sec^{-1}$)	34	56	91	120	149	161	190
$\mu mol\ O_2.g^{-1}.h^{-1}$	5.70 ± 1.82	11.58 ± 3.00	16.78 ± 3.98	20.17 ± 3.73	24.38 ± 4.74	25.07 ± 4.43	28.23 ± 4.18
$\mu mol\ O_2.10^{-8}$ zoox. h^{-1}	2.69 ± 0.91	5.57 ± 2.02	7.99 ± 2.32	9.66 ± 2.86	11.70 ± 3.56	12.01 ± 3.39	13.44 ± 3.19

Fig. 9 shows the effect of irradiance on mean gross O_2 production per 10^8 zooxanthellae. The gross O_2 production increased in direct proportion to irradiance from 34 to 120 $\mu E.m^{-2}.sec^{-1}$. The relationship becomes curvilinear between 120 and 190 $\mu E.m^{-2}.sec^{-1}$.

Discussion

Data relating photosynthesis to irradiance can be expressed as a mathematical function in order to make accurate estimates, with confidence limits, of the photosynthesis at given irradiances. The curve relating photosynthesis to irradiance in marine algae was best described by a hyperbolic tangent function (Jassby & Platt, 1976) and the relationship between gross O_2 production and irradiance of zooxanthellae in the corals Acropora cervicornis, A. formosa and Manicina areolata in vivo also approximates to this function (Chalker, 1981). The shape of the eye-fitted curve relating gross O_2 production to irradiance in Anemonia sulcata (Fig. 9) also resembles this function, but it was not possible to fit a hyperbolic tangent curve through the points since values of maximum rate of photosynthesis were not determined in all the anemones.

Saturation of photosynthesis was apparent in some anemones at irradiances higher than 150 $\mu E.m^{-2}.sec^{-1}$ (Table 2). Photosynthesis by zooxanthellae is saturated at a variety of irradiances in different species of host (Muscatine, 1980) and the variety of light sources and units of light measurement has made many published values difficult to compare. Saturating irradiances in vivo for zooxanthellae symbiotic with corals determined in recent studies are given in Table 4. Saturation of photosynthesis by zooxanthellae in Anemonia sulcata in vivo was apparent at the lower end of the range of published values for corals.

The photosynthetic energy production during a 12h exposure to different constant irradiances by a 'standard' A. sulcata of 0.4g organic

Fig. 9 The effect of irradiance on mean ± S.D. (n = 9) gross oxygen production in symbiotic A. sulcata (curve fitted by eye)

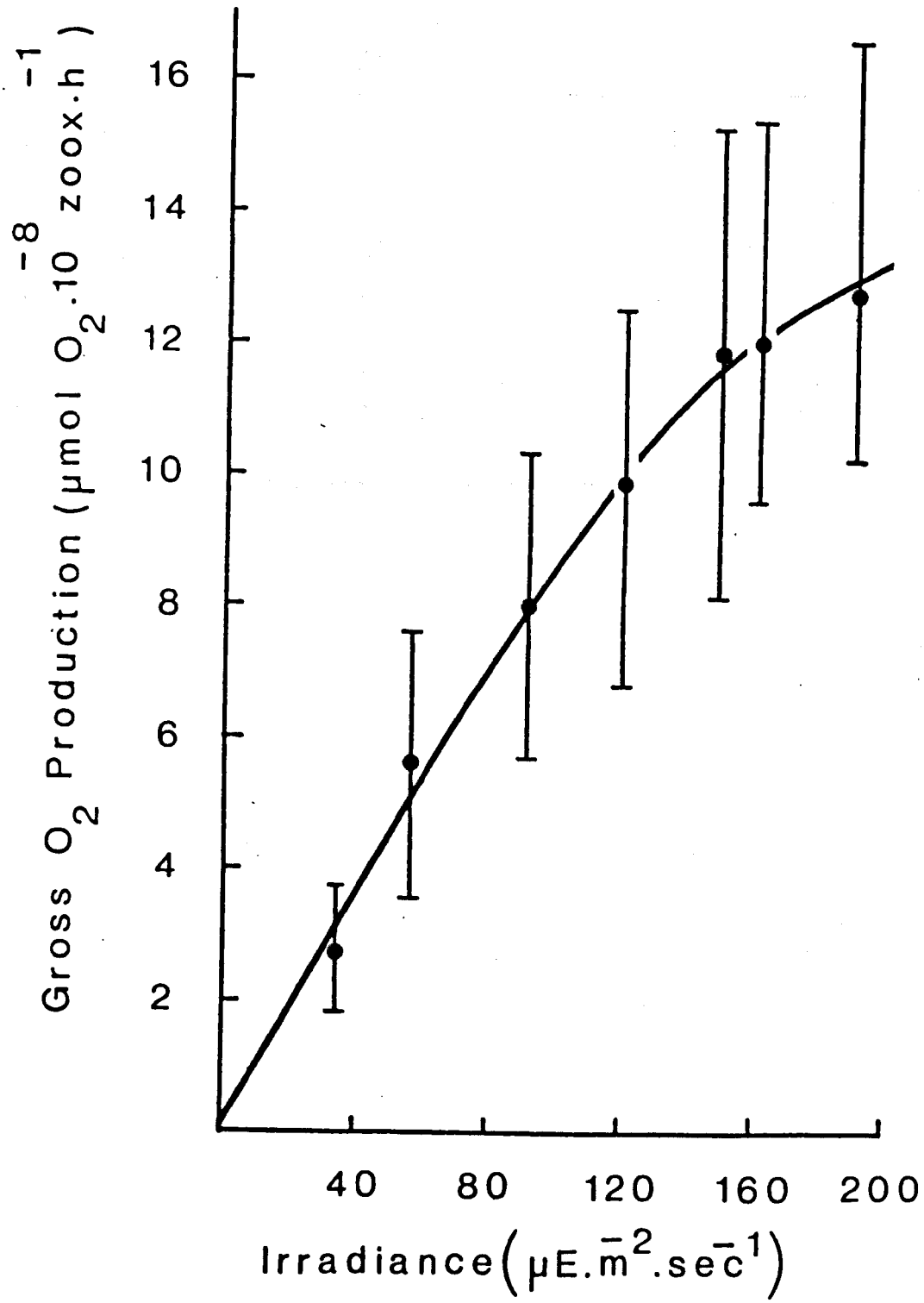


Table 4 Saturating irradiances for zooxanthellae symbiotic with some species of hermatypic corals *in vivo*

Species	Saturating irradiances ($\mu E.m^{-2}.sec^{-1}$)	Reference
Manicina areolata	700	Chalker (1981)
Plesiastria urvellei	690	Kevin & Hudson (1979)
Acropora cervicornis	500	Chalker & Taylor (1978)
Stylophora pistillata	210	Falkowski & Dubinsky (1981)
Acropora formosa	170	Chalker (1981)
Montastrea cavernosa	63-135 (302-643ft.ca.)	Davies (1977)

weight was calculated from the gross O_2 production (in μmol $O_2 . g^{-1} . h^{-1}$) given in Table 3 by assuming that 1 mole of glucose was formed for every 6 moles of O_2 produced (Section 2). The gross glucose synthesis of a 0.4g organic weight anemone was therefore

1) $\frac{\text{Gross } O_2 \text{ production}}{6} \times 12 \times 0.4$ μmol glucose

These values were multiplied by the heat of combustion of glucose 2.816 $J . \mu mol^{-1}$ (Lehninger, 1971) to give the photosynthetic energy production (P) as glucose given in Table 5.

The values of P were compared with the respiratory energy expenditure of (R)

i) to find the irradiance at which P over 12h would equal R over a 24h period.

ii) to give a measurement of the surplus energy available for growth, storage and reproduction when P over 12h is greater than R over a 12h period.

The value of R for a 'standard' A. sulcata of 0.4g organic weight was calculated from the $\dot{M}_{O_2}$. The equation of the regression line, fitted by the method of least squares, of $\dot{M}_{O_2}$ (μmol O_2 , h^{-1}) on W_d (g) for the group of anemones used in this experiment was

2) $\log \dot{M}_{O_2} = 1.128 + 1.484 \log W_d$.

The mean ($\pm$ 95% confidence limits) $\dot{M}_{O_2}$ of a 0.4g A. sulcata was 3.452 ± 5.623 μmol $O_2 . h^{-1}$. The value of R for 24h was calculated by multiplying the $\dot{M}_{O_2}$ by 24 and then by the general oxy-calorific coefficient of Elliott & Davison (1975) (see Section 2) to give 37.49 ± 61.07 $J . 24h^{-1}$.

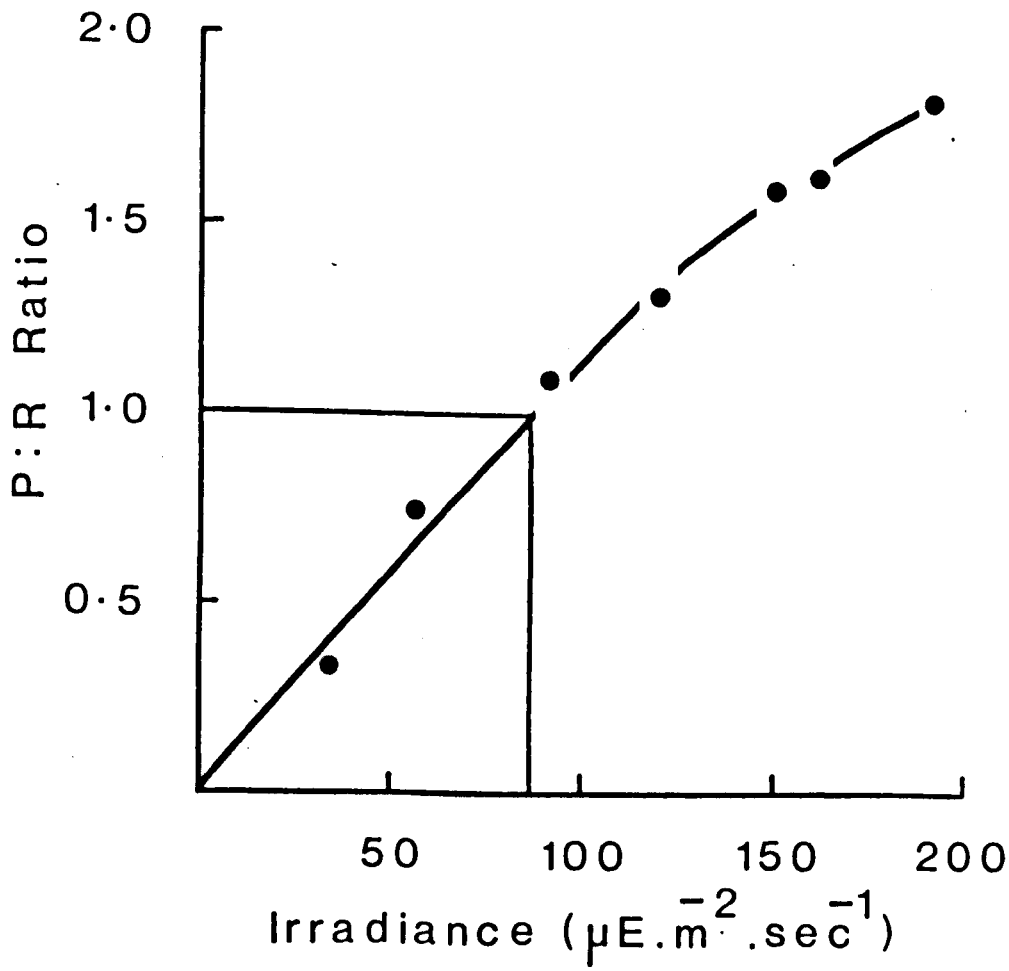
Fig 10 shows the effect of irradiance on the P:R ratio. From the eye fitted line, the P:R would be 1 at 93 $\mu E . m^{-2} . sec^{-1}$. In theory R would be met by P at this irradiance.

Table 5. Photosynthesis Energy Production (P) and Respiratory Energy Expenditure (R) of a hypothetical 'standard' *Anemonia sulcata* of 0.4g organic weight at 7 irradiances.

$\mu E.m^{-2}.sec^{-1}$	34	56	91	120	149	161	190
P ($J.24h^{-1}$)*	12.84 ± 3.15	26.10 ± 5.19	37.80 ± 6.90	45.43 ± 6.46	54.91 ± 8.21	56.48 ± 7.67	63.60 ± 7.24
R ($J.24h^{-1}$)*	37.49 ± 61.07						
P : R Ratio	0.342	0.696	1.008	1.212	1.465	1.506	1.697
P - R ($J.24h^{-1}$)	-24.65	-11.40	+ 0.312	+7.94	+17.42	+13.99	+26.11

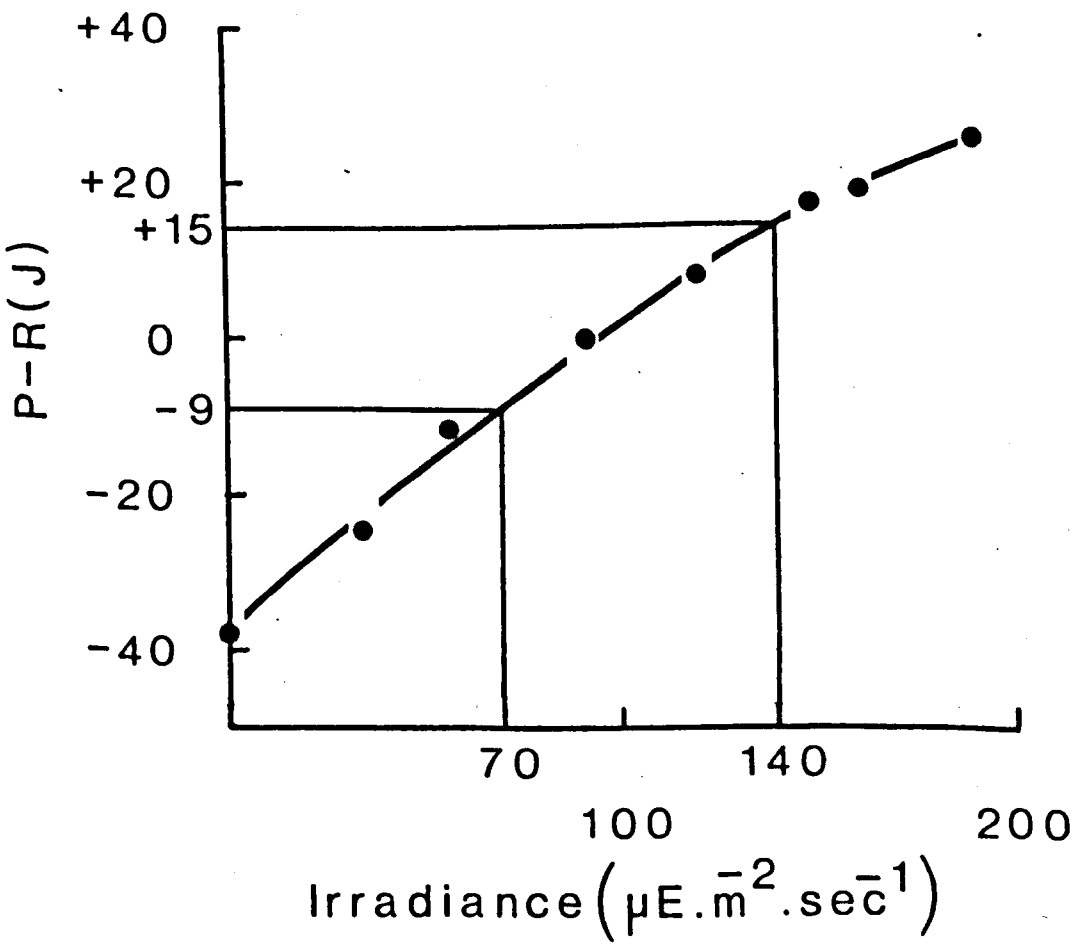
*Mean ± 95% Confidence limits

Fig. 10 The relationship between P : R ratio and irradiance in a 'standard' symbiotic A. sulcata of 0.4g organic weight (curve fitted by eye)



The amount of surplus energy available for growth, storage and reproduction at irradiances above 93 $\mu E.m^{-2}.sec^{-1}$ was estimated by subtracting R from the P values at each irradiance. The P - R values are given in Table 5 and are plotted against irradiance in Fig. 11. These values were used in the design of the energy balance experiments described in Section 4.

Fig. 11 The relationship between P-R and irradiance in a 'standard' symbiotic A. sulcata of 0.4g organic weight (curve fitted by eye)



Section 4

Energy Balance Experiments on Symbiotic Anemonia sulcata

Introduction

In order to construct the bioenergetic model described in Section 1, experiments were carried out on symbiotic Anemonia sulcata, to obtain values of the factors in the equations. Subsequently, in Section 5, experiments were carried out on aposymbiotic anemones to obtain equations for the animal alone.

Having determined the effect of weight on respiratory energy expenditure (R) and the effect of irradiance on photosynthetic energy production (P) in Section 3 it was possible to design energy balance experiments using this information.

A batch of symbiotic anemones were divided into stocks which were maintained under the conditions outlined in Table 6. Anemones were maintained either in total darkness or under a 12h light, 12h dark cycle. The irradiances of 70 and 140 $\mu E.m^{-2}.sec^{-1}$ were chosen as R over a 24h period would not be met by P alone at the lower irradiance, while P would exceed R at the higher irradiance, and surplus energy would be available for growth, storage and reproduction. The P:R ratio for a 'standard' anemone of 0.4g organic weight would be 0.75 and 1.3 at these two irradiances respectively (Fig. 11).

The difference between P and R (P-R) for a 0.4g animal at seven irradiance were given in Table 4 and were plotted against irradiance in Fig. 11. P would exceed R by $15J.24h^{-1}$ in anemones exposed to 140 $\mu E.m^{-2}.sec^{-1}$ for 12h per day while P would fall short of R by $9J.24h^{-1}$ in anemones exposed to 70 $\mu E.m^{-2}.sec^{-1}$ for 12h per day. Since R = 37.5 $J.24h^{-1}$, P would be 52.5 $J.12h^{-1}$ and 28.5 $J.12h^{-1}$ respectively at these

Table 6 Experimental Conditions under which symbiotic *Anemonia sulcata* were maintained

i) 12h Light / 12h Dark at 140 $\mu E.m^{-2}.sec^{-1}$

- a) Starved (HLS)
- b) Fed squid mantle twice weekly (HLF)

ii) 12h Light / 12h Dark at 70 $\mu E.m^{-2}.sec^{-1}$

- a) Starved (LLS)
- b) Fed squid mantle twice weekly (LLF)

iii) Total Darkness

- a) Starved (DS)
- b) Fed squid mantle twice weekly (DF)

7 anemones in each of the six stocks

two irradiances. The stocks of anemones were subdivided into those which were starved and those which were fed squid in excess of R.

Anemones were maintained under these conditions for 84 days in order to give sufficient time for the measurement of growth or weight loss. Initially there were 7 anemones in each stock, however deaths during the course of the experiment reduced the numbers. Two anemones from the HLF stock and an anemone from the LLF stock reproduced asexually. These anemones were excluded from the results.

As all the factors in the equations could not be determined simultaneously in the same anemones, a series of experiments were carried out on the 6 stocks of anemones in this section in which the following factors were measured: photosynthetic energy production calculated from the gross O_2 production rate; energy input from carnivorous feeding, calculated from the weight of squid consumed; energy expenditure, calculated from the O_2 consumption rate of the whole organism in darkness and the net energy retained as biomass of anemone and biomass of zooxanthellae, calculated from the change in weight of the anemone and the change in size of the zooxanthellae population. Values of these factors for hypothetical 'standard' anemones of 0.4g organic weight were extrapolated from the results and losses were estimated by subtraction in Section 6. 0.4g was chosen as the standard organic weight for the reasons given in Section 3.

The energy balance equations for each stock are given in Section 6 where they are used to estimate

1) The contribution of photosynthesis by zooxanthellae to the energy requirements of host and symbiont.

2) The contribution of carnivorous feeding by the anemone to the energy requirements of host.

A) Photosynthetic energy production of zooxanthellae *in vivo*

Introduction

The rates of oxygen production of symbiotic anemones were recorded at irradiances similar to those at which they were maintained to estimate the photosynthetic energy production of zooxanthellae *in vivo* in 'standard' anemones.

Materials and Methods

After at least 25 days acclimation to the experimental conditions outlined in Table 6 and after repeated measurements of oxygen consumption ($\dot{M}_{O_2}$) in darkness (see Part C of this Section), the net oxygen production of the LLS, LLF, HLS and HLF was recorded with automated confinement respirometers. Recordings were made three times on each anemone over intervals of 1h at either 61 or 152 $\mu E.m^{-2}.sec^{-1}$ between the hours of 0.600h and 13.00h in the 160-200 mmHg range of PO_2 in accordance with the recommendations of Muscatine (1980). The gross O_2 production was calculated by adding the net photosynthesis to the mean $\dot{M}_{O_2}$ recorded in darkness. The results were corrected using Fig. 9 in Section 3b to give the predicted photosynthesis at 70 and 140 $\mu E.m^{-2}.sec^{-1}$ respectively. The buoyant weight (W_w) of each anemone was recorded before the measurements of $\dot{M}_{O_2}$. The organic weight (W_d) was calculated from the W_w as described in Appendix 1. The values of gross O_2 production were divided by the W_d to give the gross O_2 production in units of $\mu mol\ O_2.g\ W_d^{-1}.h^{-1}$.

Results and Discussion

The mean $\pm$ S.D. gross O_2 production of the four stocks of anemones are given in Table 7. This table also shows the rates of glucose production and the rates of energy production in the form of glucose, calculated by the method described in Section 3b. A Student's

Table 7 Gross O_2 production, glucose synthesis and photosynthetic energy production (P) by four stocks of symbiotic Anemonia sulcata

Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	number of anemones	number of determinations (n)	Mean ± S.D. Gross O_2 production ($\mu mol\ O_2.g^{-1}.h^{-1}$)	Mean ± S.D. Glucose synthesis ($\mu mol.g^{-1}.h^{-1}$)	Mean ± S.D. P ($J.g^{-1}.h^{-1}$)
1) HLS	140	6	18	24.82 ± 8.67	4.137 ± 1.445	11.65 ± 4.07
2) LLS	70	4	12	10.03 ± 1.48	1.672 ± 0.247	4.71 ± 0.70
3) HLF	140	4	12	28.86 ± 6.20	4.477 ± 1.034	12.61 ± 2.91
4) LLF	70	3	9	22.43 ± 12.87	3.739 ± 2.144	10.53 ± 6.04

		t	degrees of freedom	
Students t values	1v3	0.700	28	$0.4 < P < 0.5$ n.s.
	2v4	3.338	19	$0.002 < P < 0.01$

n.s. = not significant

t test showed that there was no significant difference between the gross O_2 production of fed and starved anemones at 140 $\mu E.m^{-2}.sec^{-1}$ with $0.4 < P < 0.5$, However a t test showed that the gross O_2 production of fed anemones was significantly higher than starved anemones at 70 $\mu E.m^{-2}.sec^{-1}$ with $0.002 < P < 0.01$. This suggests that some factor related to the nutritional condition of the anemones had affected the photosynthesis at this irradiance, however the small size of the fed stock may in part account for this.

The photosynthetic energy production (P) of 'standard' anemones of 0.4g organic weight are given in Table 8. The P at 140 $\mu E.m^{-2}.sec^{-1}$ of both fed and starved stocks was similar to the initial prediction of 52.5 $J.12h^{-1}$ (see p34). The P of fed anemones at 70 $\mu E.m^{-2}.sec^{-1}$ was higher than the initial prediction of 28.5 $J.12h^{-1}$ (see p 34) while that of starved anemones was lower than this initial prediction.

These values of P are used in the energy balance equations in Section 6.

B) Energy intake from carnivorous feeding

Introduction

The amount of squid consumed by the three stocks of fed symbiotic anemones over the 84 day experimental period was measured to allow the calculation of the energy ingested by 'standard' anemones.

Materials and Methods

Anemones were individually fed twice weekly with meals of squid mantle (see Section 2) which they ingested voluntarily. The energy content of the squid ingested by the anemones was then estimated from the wet weight of the meals.

Table 8 Photosynthetic energy production (P) by 'standard' anemones of 0.4g organic weight from four stocks of symbiotic *Anemonia sulcata*

Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	P ($J.24h^{-1}$)
HLS	140	55.92
LLS	70	22.60
HLF	140	60.51
LLF	70	50.54

The organic weights of a sample of 12 wet meals was measured, as described in Appendix 1, to determine the amount of organic weight in a wet meal. The energy content per mg organic weight of 11 freeze dried samples of squid was determined by wet oxidation with dichromate. The results were corrected for ash content and for protein content determined by the method of Lowry et al (1951), as described in Section 2. Undigested and partly digested meals were collected from the breeding traps before the subsequent feed, and their organic weights were determined. The total organic weight of the undigested squid was subtracted from the total organic weight of squid ingested by each stock. The mean organic weights of squid consumed per anemone by each stock were calculated from these values.

Results and Discussion

The organic weight was (mean $\pm$ S.D.) 15.93 $\pm$ 0.63% of the wet weight and the mean $\pm$ S.D. energy content was 24.53 $\pm$ 0.63J.mg organic weight^{-1}. This is similar to the value of 25.03J.mg organic weight^{-1}, calculated from the original data of Wallace (1971) in cal.mg dry weight^{-1} determined by bomb calorimetry, assuming that, as in the squid used in this study, 9.5% of the dry weight of the squid was ash. This is higher than the 22.71 J.mg organic weight^{-1} for the squid Loligo pealei determined by bomb calorimetry (Krishnamoorthy et al, 1979).

The protein content of the squid was 76.87 $\pm$ 9.74% of the organic weight which was higher than the 51.87% for L. pealei determined by the microkjeldahl method (Krishnamoorthy et al, 1979).

The mean organic weights of squid consumed per anemone by each stock are given in Table 9, together with the percentage of the mean organic weight of the anemones that this represents.

Table 9 Weight and energy content of squid consumed by three stocks of symbiotic *A. sulcata*

Stock	Organic weight of squid consumed ($mg.24h^{-1}$)	Organic weight of squid consumed as a percentage of anemone organic weight	Energy intake as squid of 'standard' anemones ($J.24h^{-1}$)
HLF	5.41	0.999	132.63
LLF	5.44	1.141	133.53
DF	4.06	1.537	99.59

Since each anemone was fed with constant amounts of food over the experimental period, the weight of squid ingested by 'standard' anemones was assumed to be the same as the mean weight ingested by the experimental anemones in each stock (Table 9). These values were multiplied by the mean energy content per mg of squid, to give the energy ingested as squid (Table 9). These values are used in the energy balance equations in Section 6.

C) Energy expenditure of symbiotic Anemonia sulcata

i) Energy expenditure of maintenance: The effect of weight on the oxygen consumption ($\dot{M}_{O_2}$) of symbiotic Anemonia sulcata

Introduction

Values of energy expenditure on maintenance for each experimental stock of symbiotic anemone were required for the construction of energy balance equations for Anemonia sulcata. The relationship between oxygen consumption ($\dot{M}_{O_2}$) and organic weight (W_d) was determined for each stock and the energy expenditure in maintenance of 'standard' anemones was predicted from this relationship.

Materials and Methods

After at least 25 days acclimation to the experimental conditions outlined in Table 6, the $\dot{M}_{O_2}$ of each symbiotic anemone from the six experimental stocks was recorded in darkness over intervals of one hour between 18.00h and 06.00h in the 140-160mmHg range of PO_2 with automated confinement respirometers (Section 2). The $\dot{M}_{O_2}$ of fed stocks was measured 2-3 days after the previous feed by which time the postprandial increase in $\dot{M}_{O_2}$ had subsided (Part (ii) of this Section). The buoyant weight (W_w) of each anemone was recorded before the $\dot{M}_{O_2}$ measurements were made and values of W_d were calculated from the W_w as described in Appendix 1.

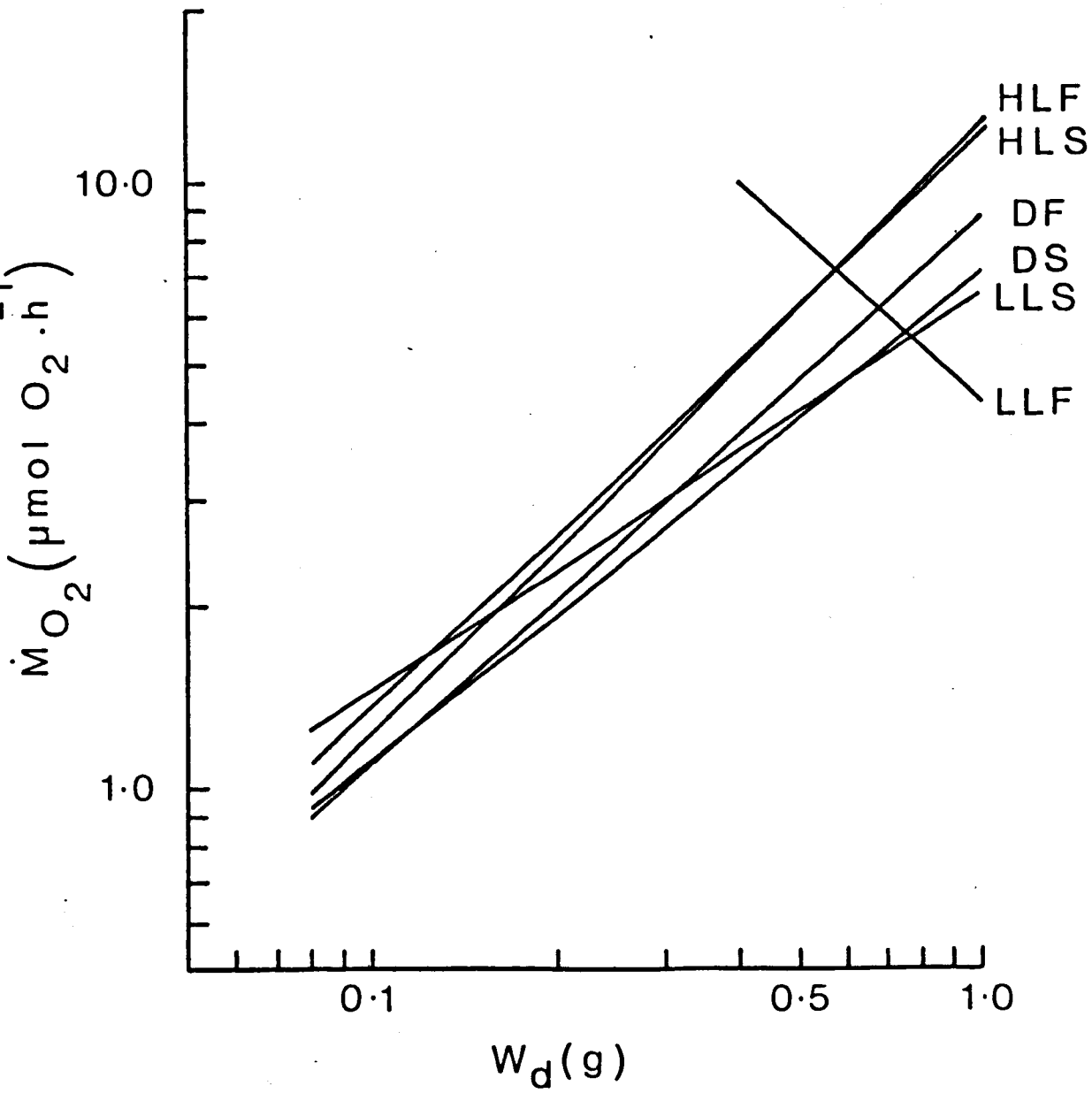
Results and Discussion

The regression lines fitted by the method of least squares relating log $\dot{M}_{O_2}$ to log W_d in the six stocks of symbiotic anemones are plotted in Fig. 12. The equation of the lines are given in Appendix 3, Table A. There was a negative regression coefficient in the LLF stock. This anomaly was probably due to the small sample size over a narrow weight range.

The slope of the lines were compared by analysis of covariance to test whether the lighting or feeding regime had a significant effect on the relationship between log $\dot{M}_{O_2}$ and log W_d. The F ratios given in Appendix 3, Table B show that there was a significant difference in slope among the 6 stocks. This was due to the LIF having a negative regression coefficient. There was no significant difference in slope among the other 5 stocks. Hence lighting and feeding regimes did not affect the relationship between log $\dot{M}_{O_2}$ and log W_d in the majority of stocks.

The elevation of the lines which were not significantly different in slope were compared by analysis of covariance to test whether the magnitude of the $\dot{M}_{O_2}$ recorded at a given W_d was affected by the lighting and feeding regime. The F ratios given in Appendix 3, Table C show there was a significant difference in elevation among the 5 slopes. The difference in elevation was also significant among the HLS, LIS and DS and between the HLF and DF lines. Hence the lighting regime significantly affected the magnitude of $\dot{M}_{O_2}$ recorded at a given W_d. There was no significant difference between the elevation of the HLS and HLF lines and the DS and DF lines. In these two pairs of lines, the feeding regime did not significantly affect the magnitude of the $\dot{M}_{O_2}$ recorded at a given W_d.

Fig. 12 Regression lines of log oxygen consumption ($\dot{M}_{O_2}$) on log organic weight (W_d) in six stocks of symbiotic A. sulcata



Since there were differences in elevation of the lines relating log $\dot{M}_{O_2}$ to log W_d, the $\dot{M}_{O_2}$ of 'standard' symbiotic anemones of 0.4g organic weight, given in Table 10, were calculated individually for each stock with the equation of the lines corrected to the combined regression coefficient (β) of 0.8859 (Appendix 3, Table B) with the formula

1) $\hat{Y} = \bar{Y} + \beta (X - \bar{X})$

where X = log W_d (log 0.4 = -0.39794)
$\bar{X}$ = mean log W_d
$\bar{Y}$ = mean log $\dot{M}_{O_2}$
$\hat{Y}$ = predicted log $\dot{M}_{O_2}$ at X = log W_d

The corrected lines are plotted in Fig. 13.

The values of $\dot{M}_{O_2}$ were used to calculate the energy expenditure on maintenance (R) over a 24h period by multiplying by 24 and then by the general oxy-calorific coefficient of Elliott & Davison (1975) (see Section 2). These values of R are also given in Table 10 and are used in the energy balance equations in Section 6. The value of R in most of the stocks was higher than the value of 37.5 J.24h^{-1} determined in Section 3.

ii) Specific Dynamic Action: The effect of feeding on the $\dot{M}_{O_2}$ of symbiotic Anemonia sulcata

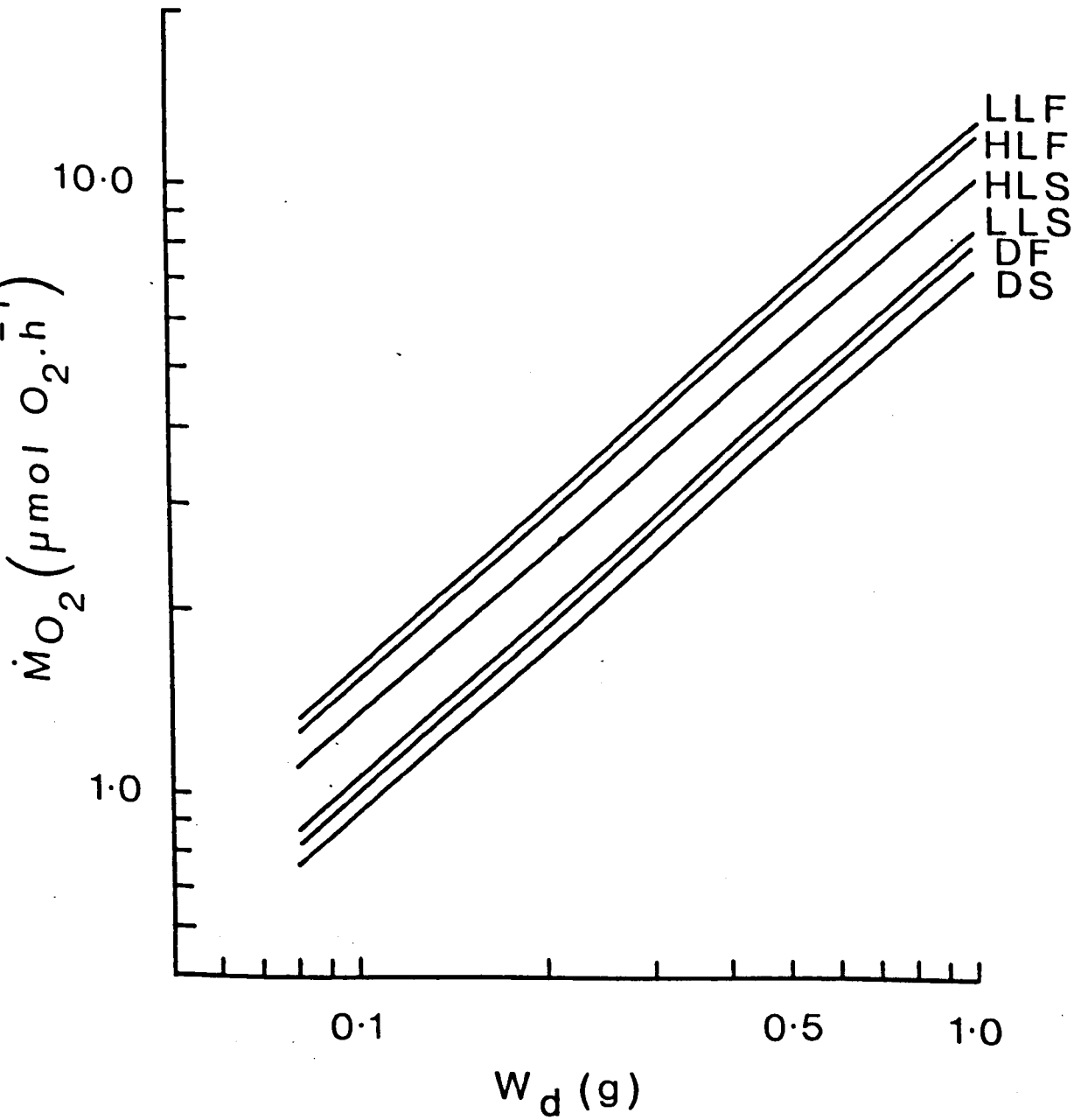
Introduction

Increases in metabolic rate after feeding have been observed in both endothermic and exothermic organisms. This phenomenon has been given the term specific dynamic action (S.D.A.)(Winberg, 1955; Kleiber, 1961 and Jobling, 1981). The biochemical basis of S.D.A. is not fully understood (Beamish, 1974). Krebs (1964) has concluded that S.D.A. is due to the degradation of nitrogenous compounds, however there is a larger body of

Table 10 Oxygen consumption ($\dot{M}_{O_2}$) and respiratory energy expenditure (R) of 'standard' anemones of 0.4g organic weight from six stocks of symbiotic Anemonia sulcata

Stock	$\dot{M}_{O_2}$ (μmol $O_2 . h^{-1}$)	R ($J . 24h^{-1}$)
HLS	4.372	47.48
LLS	3.448	37.45
DS	3.093	33.58
HLF	5.058	54.93
LLF	5.596	60.77
DF	3.567	38.74

Fig. 13 Regression lines of log oxygen consumption ($\dot{M}_{O_2}$) on log organic weight (W_d) in six stocks of symbiotic A. sulcata replotted to a combined regression coefficient (β) of 0.8859



evidence that suggests that S.D.A. represents the energy cost of growth (Jobling, 1981). This phenomenon is probably best regarded, at present, as an inescapable energy loss in food conversion (Ware, 1975).

Increase in oxygen consumption ($\dot{M}_{O_2}$) after feeding has been observed in the sea anemone Aiptasia diaphana (Svoboda & Poorman, 1980) and Actinia equina (Jones et al, 1977). As yet, no attempts have been made to quantify the increase in energy expenditure after feeding in coelenterates or any other invertebrate.

The effect of feeding on the $\dot{M}_{O_2}$ of symbiotic Anemonia sulcata was investigated to estimate how much of the energy provided by a meal of squid was expended during a postprandial increase in $\dot{M}_{O_2}$.

Materials and Methods

Five symbiotic anemones which had been fed squid mantle twice weekly were placed in automated confinement respirometers 2-3 days after their previous feed. The preprandial $\dot{M}_{O_2}$ was recorded every alternate hour in darkness for at least 12h prior to feeding. Each anemone was fed 2.3 - 6.5% of its body organic weight as squid mantle. The postprandial $\dot{M}_{O_2}$ was then recorded for 48 - 72h after feeding. Undigested squid and faeces were collected and their organic weights were determined. The buoyant weight (W_w) of each anemone was recorded before the measurement of $\dot{M}_{O_2}$. The organic weights (W_d) were calculated from the W_w as described in Appendix 1.

Two control experiments were performed on symbiotic anemones which had previously been starved in darkness. In one control the $\dot{M}_{O_2}$ of an anemone was recorded in darkness over 96h without disturbance. In the other control, the $\dot{M}_{O_2}$ of an anemone which was fed an indigestible

meal of non-toxic material (Blue-Tak) was recorded. The anemone captured the indigestible meal and transferred it to its coelenteron, subsequently rejecting it after a period of time.

Results

Fig. 14 shows the effect of a single meal on the $\dot{M}_{O_2}$ of a symbiotic anemone. The $\dot{M}_{O_2}$ increased immediately after feeding and remained at an elevated level which slowly fell to a value similar to the preprandial $\dot{M}_{O_2}$ after 24h.

Fig. 15a shows the changes in the $\dot{M}_{O_2}$ of the undisturbed anemone which may have been due to some rhythm in the activity of the anemone. The second control did not display a prolonged elevation in $\dot{M}_{O_2}$ after being disturbed and being fed an indigestible meal (Fig. 15b). This indicates that the postprandial increase in $\dot{M}_{O_2}$ of anemones fed with squid was not due to a mechanical effect of feeding, but was due to some biochemical effect of food conversion.

In accordance with Willhemj & Bollman (1928), the S.D.A. was calculated by plotting the curve relating postprandial $\dot{M}_{O_2}$ to time and integrating the area beneath it, taking the mean preprandial $\dot{M}_{O_2}$ as the base line (Fig. 14). The energy equivalent of the S.D.A. was calculated by multiplying the integrated increase in $\dot{M}_{O_2}$ by the general oxycalorific coefficient of Elliott & Davison (1975) (see Section 2). The integrated S.D.A. was calculated only in anemones in which the postprandial $\dot{M}_{O_2}$ had decreased to preprandial levels. The values of integrated S.D.A. are given in Table 11. The mean amount of squid expended as S.D.A. was 6.8%. This value was used to calculate the amount of energy expended as S.D.A. in 'standard' symbiotic anemones in Section 6.

Fig. 14 Specific Dynamic Action: The effect of a single meal on the oxygen consumption ($\dot{M}_{O_2}$) of a symbiotic A. sulcata (dashed line represents mean preprandial $\dot{M}_{O_2}$)

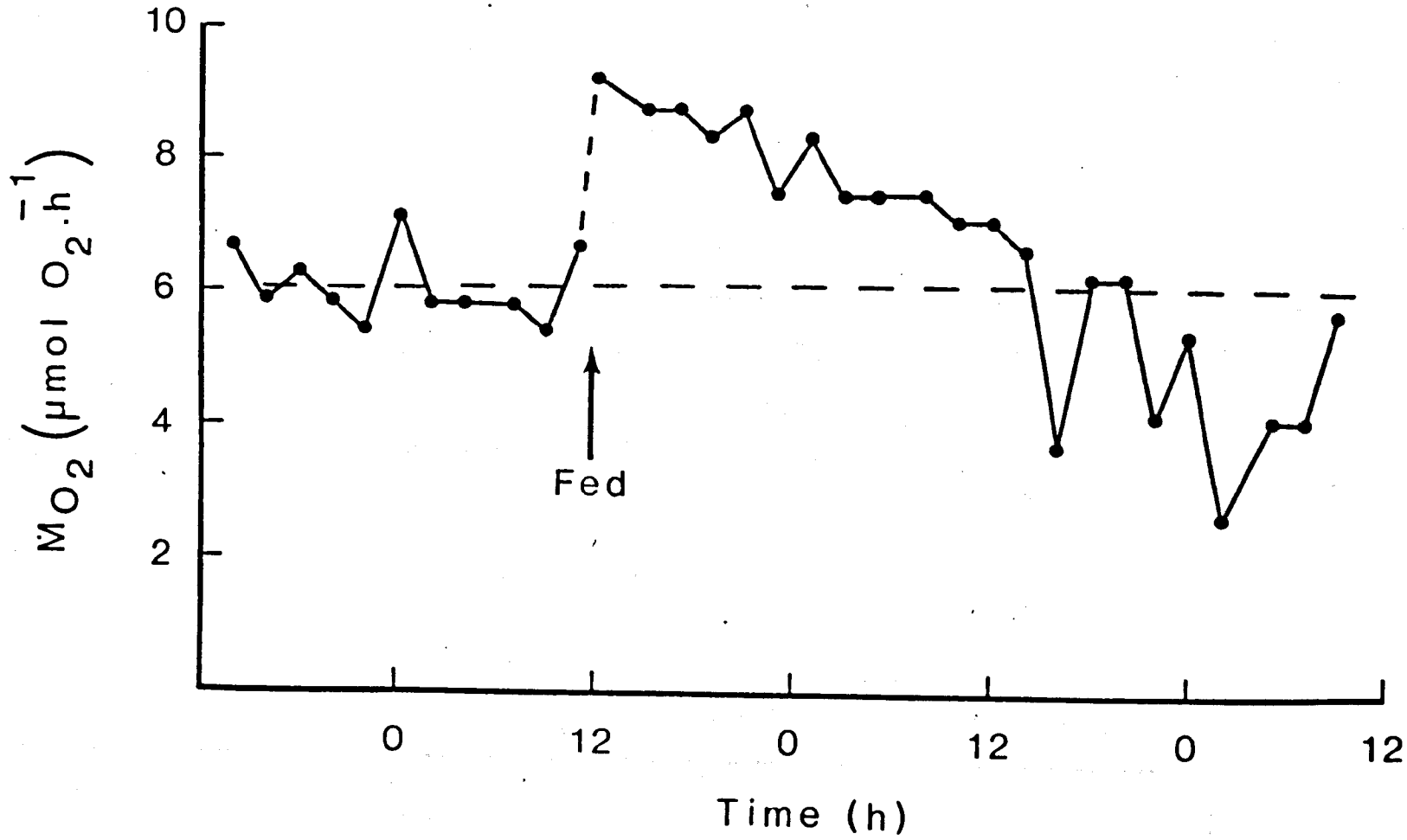


Fig. 15a The oxygen consumption ($\dot{M}_{O_2}$) of an undisturbed symbiotic A. sulcata

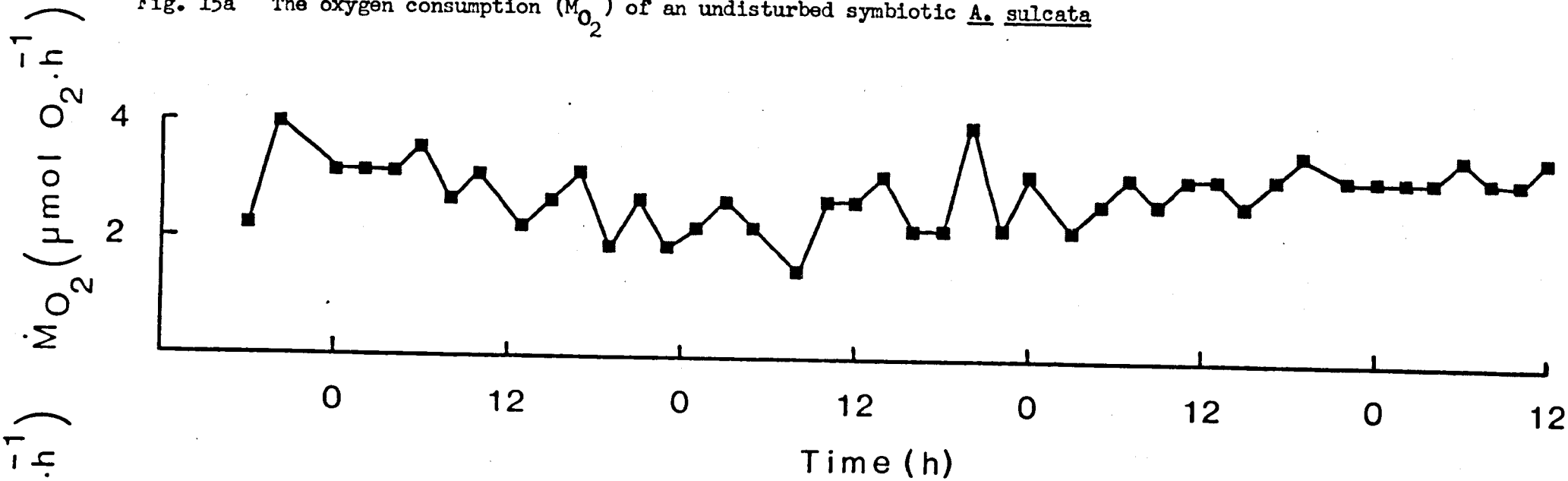


Fig. 15b The effect of an indigestible meal on the oxygen consumption ($\dot{M}_{O_2}$) of a symbiotic A. sulcata

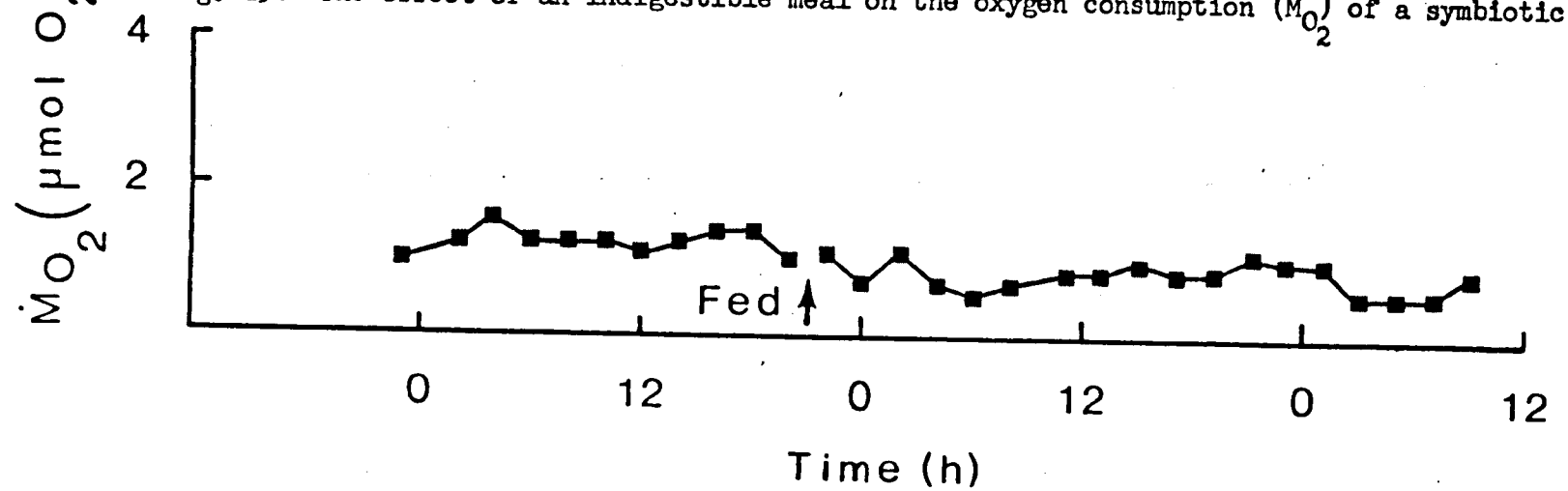


Table 11 Preprandial and postprandial O_2 consumption ($\dot{M}_{O_2}$) and integrated specific dynamic action (S.D.A.) of 5 symbiotic Anemonia sulcata fed in darkness

Mean ± S.D. (n = 6) Preprandial $\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$)	Duration of S.D.A.(h)	$\int$ S.D.A. (μmol O_2)	$\int$ S.D.A. (J)	S.D.A. as a percentage of digested squid	Postprandial peak $\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$)	Time of peak after feeding (h)	Postprandial peak $\dot{M}_{O_2}$ as a percentage of the preprandial $\dot{M}_{O_2}$
1) 2.125 ± 0.335	52	-	-	-	6.274	8	150.4
2) 6.158 ± 0.637	25	48.57	21.98	6.52	9.259	1	169.0
3) 2.756 ± 0.229	43	-	-	-	4.659	3	196.7
4) 2.222 ± 0.119	70	-	-	-	4.370	32	180.8
5) 8.318 ± 1.140	27	79.82	36.12	7.03	15.033	1	283.5
				Mean = 6.78%			Mean = 196.1%

The peak postprandial $\dot{M}_{O_2}$ of each anemone is given in Table 11 together with the time at which this peak occurred. This peak typically occurred just after feeding although in one anemone it occurred 32h after feeding. The value of the peak $\dot{M}_{O_2}$ ranged from 150-284% of the preprandial $\dot{M}_{O_2}$. The duration of the S.D.A. ranged from 25 to more than 70h.

Discussion

The results show that S.D.A. occurs in symbiotic Anemonia sulcata. However, it was a highly variable phenomenon. The pattern and duration of the S.D.A. was similar to that reported by Svoboda & Poorman (1980) for Aiptasia diaphana. The postprandial peak $\dot{M}_{O_2}$ was approximately twice that of the preprandial $\dot{M}_{O_2}$. This is comparable with values for teleosts reviewed by Jobling (1981).

The duration of the S.D.A. was highly variable. It will have depended on the rates of digestion and assimilation by the anemones, which remain to be determined. There have been few studies of the rates of digestion by sea anemones. Nicol (1959) has shown that 0.6 - 1.0g of gelatine was digested by Calliactis parasitica over 24h at 12°C. Observations during growth experiments indicated that all of the squid had been digested by most symbiotic A. sulcata within 72h after feeding.

The mean amount of a squid meal expended as S.D.A. was 6.8% in symbiotic A. sulcata. This is comparable with the 5.1-17.5% of artificial diets expended as S.D.A. by the teleost Micropterus salmoides (Tandler & Beamish, 1979) and an average of 10% of food expended as S.D.A. by humans (Garrow, 1978). The amount of ingested food expended as S.D.A. by the sea anemone Aiptasia diaphana could not be calculated from the results of Svoboda & Poorman (1980) for comparison since the size and energy content of the meals were not given.

D) Net energy retention by symbiotic Anemonia sulcata

i) Total net energy retention by symbiotic anemones

Introduction

Changes in body weight and energy content of stocks of anemones maintained under the conditions outlined in Table 6 were measured to estimate the following in 'standard' anemones.

1) The net energy retained from the input of photosynthesis
2) The net energy retained from the input of carnivorous feeding
3) The utilization of body energy reserves during starvation

Materials and Methods

The buoyant weights (W_w) of each anemone in the six stocks were recorded on every seventh day of a 84 day period. The W_w of fed anemones was recorded two days after their previous feed.

A sample of 3 anemones taken from the batch to be used in the experiment were sacrificed at the beginning of the experiment and all surviving experimental anemones were sacrificed at the end of the experiment. These were fractionated as described in Section 2. The energy content of the freeze dried tissue was determined by wet oxidation with dichromate and was corrected for ash and protein content as described in Section 2.

Results

a) Weight loss during starvation

The anemones in all three starved stocks lost weight. It was predicted that the weight loss during starvation would be an exponential function of time (Appendix 2). The following logarithmic transformation yields the straight line relationship

1) $\log_{10} W = \log_{10} W_o - \frac{k}{2.3026} t$

where t is time in days
W is the W_w at time t
W_o is the W_w at time t=0
k is the rate of weight loss

Regression lines relating $\log_{10} W_w$ to time for each anemone were fitted by the method of least squares and are given in Appendix 4, Table G. The lines for the DS stock are plotted in Fig. 16. Analysis of covariance showed no significant difference in the slopes of these lines (Appendix 4, Table H) which suggests that the weight loss in anemones from this stock had been at a uniform rate (k). This is shown in Fig. 17 where the lines have been replotted to the combined regression coefficient ($\beta = \frac{k}{2.3026}$) given in Appendix 4, Table H.

Analysis of covariance also showed no significant differences in the slope of the lines within the LLS stock and within the HLS stock (Appendix 4, Table H) hence the anemones were losing weight at uniform rates specific to their stock.

The sums of squares and sums of products were pooled and the slopes of the pooled lines for each stock (Fig. 18) were compared by analysis of covariance. There were significant differences in slope between the stock starved in darkness and the stocks starved on a 12h light/12h dark cycle at 70 and 140 $\mu E.m^{-2}.sec^{-1}$, therefore photosynthesis at these irradiances significantly reduced the rate of weight loss. However there was no significant difference in slope between the stocks starved at 70 and 140 $\mu E.m^{-2}.sec^{-1}$ (Appendix 4, Table H).

The weight loss of 'standard' symbiotic anemone of W_w = 0.0856g (= 0.4g organic weight) on day 0 (Fig. 19) were calculated using the combined regression coefficients for each stock (Appendix 4, Table H) with equation 1 above. The decrease in W_w over 24h of these anemones (Table 12) were calculated in the same way. These values were converted to changes in organic weight (W_d) as described in Appendix 1. These values were multiplied by the mean of the energy content per mg W_d of the day 0 anemones and the energy content of the experimental animals on day 84

Fig. 16 Regression lines of $\log_{10}$ buoyant weight (W_w) on time in seven symbiotic A. sulcata starved in darkness

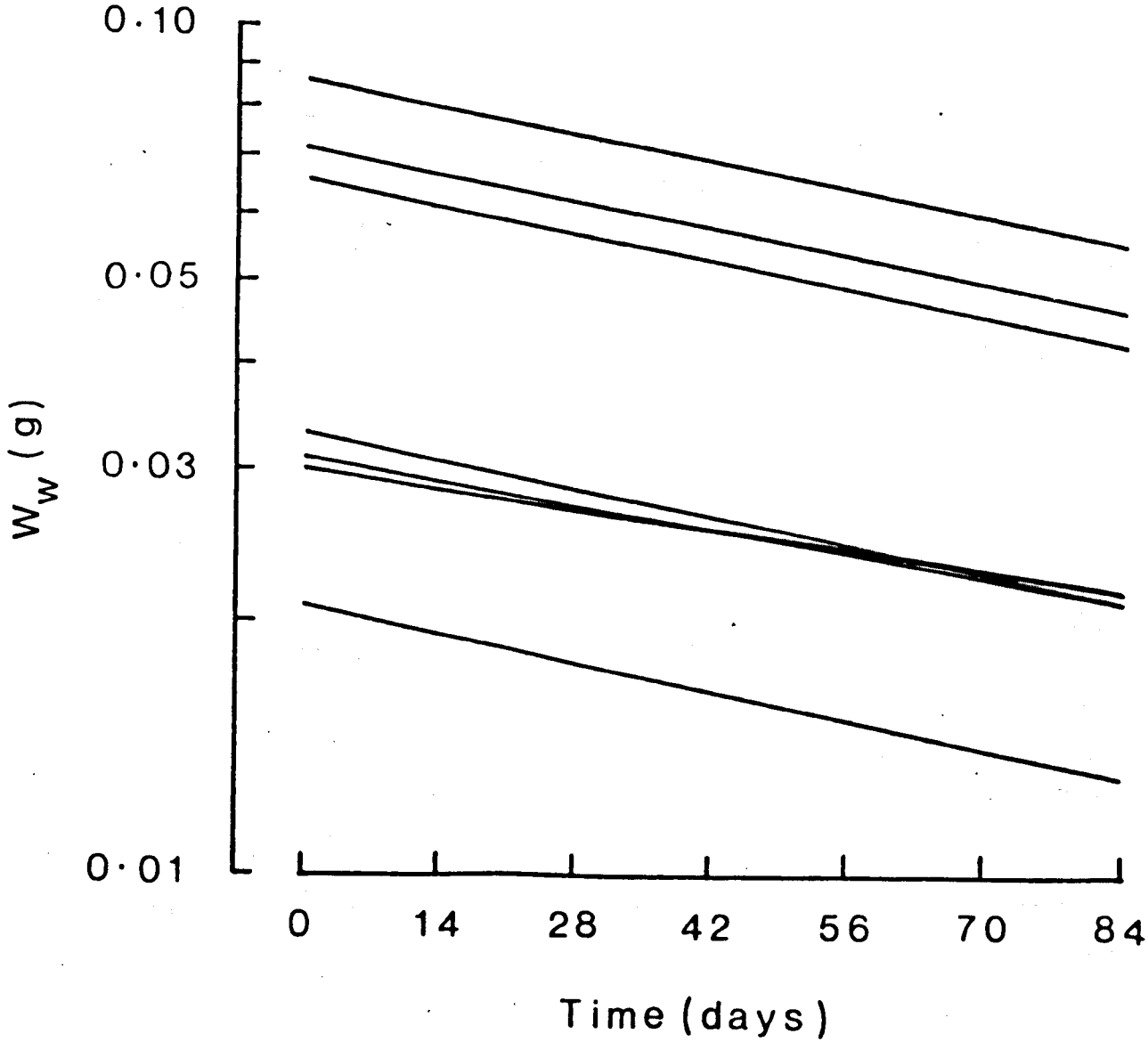


Fig. 17 Regression lines of $\log_{10}$ buoyant weight (W_w) on time in seven symbiotic A. sulcata starved in darkness replotted to a combined regression coefficient ($\beta = \frac{k}{2.3026}$) of -2.1949×10^{-3}

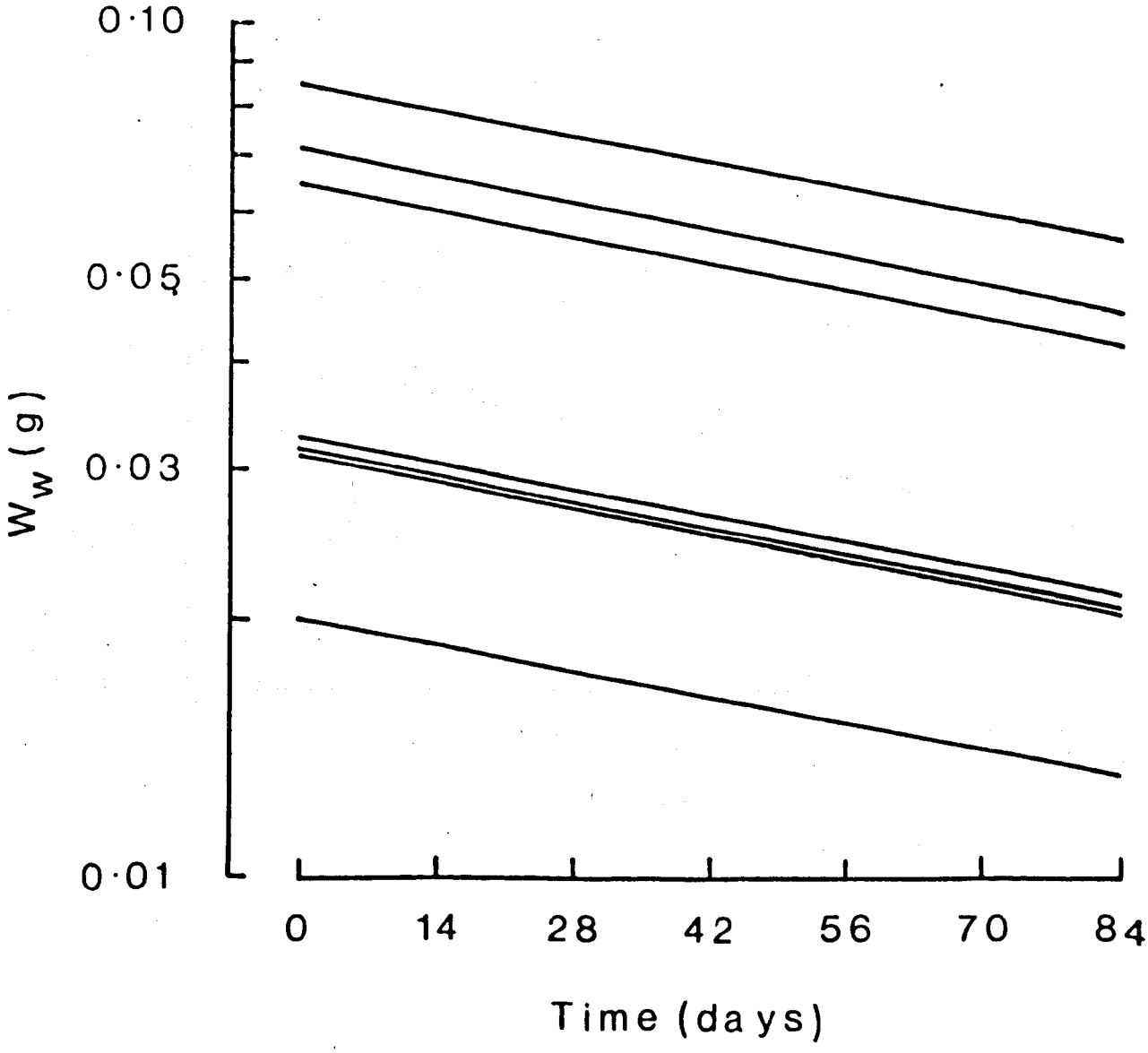


Fig. 18 Pooled regression lines of $\log_{10}$ buoyant weight (W_w) on time in three stocks of starved symbiotic *A. sulcata*

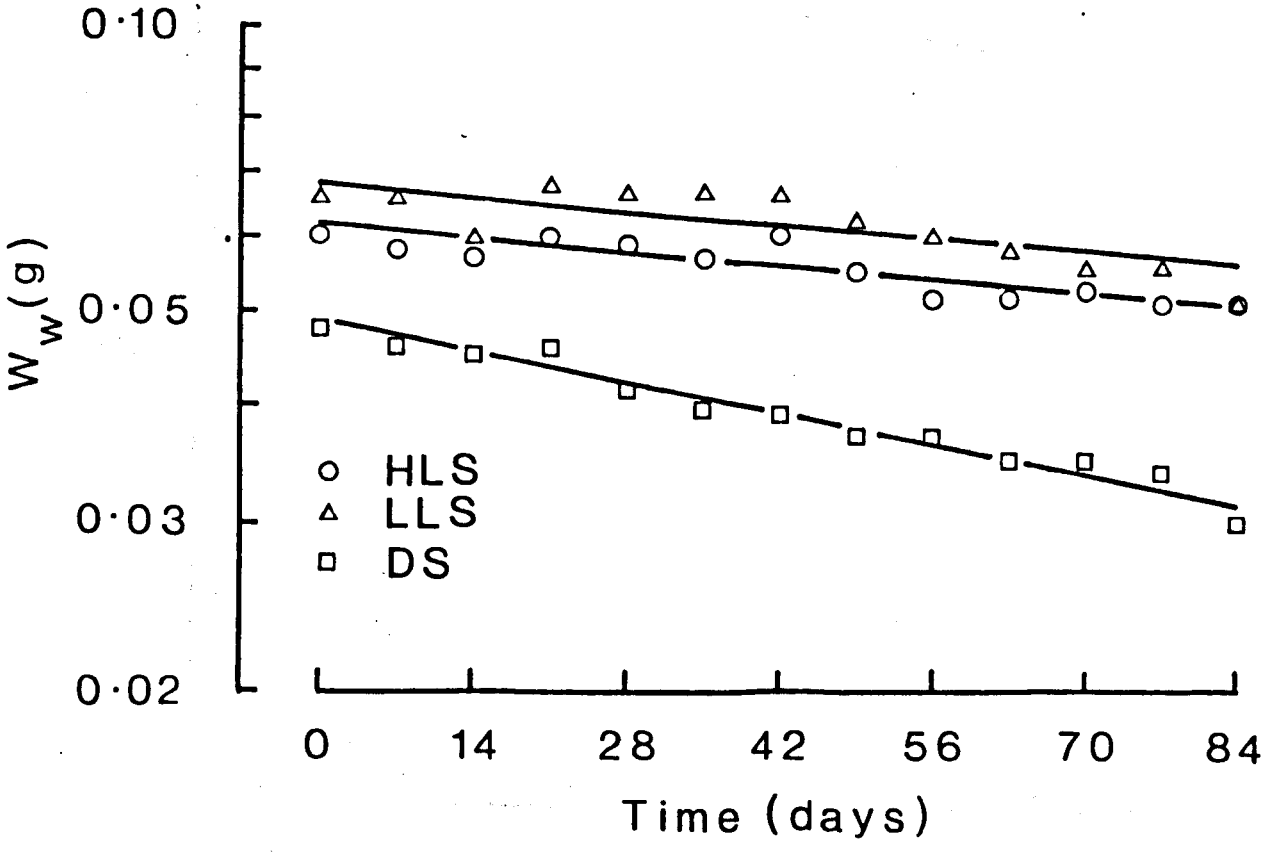


Fig. 19 Weight loss during starvation in 'standard' symbiotic *A. sulcata* of 0.0856g buoyant weight (W_w) (= 0.4g organic weight)

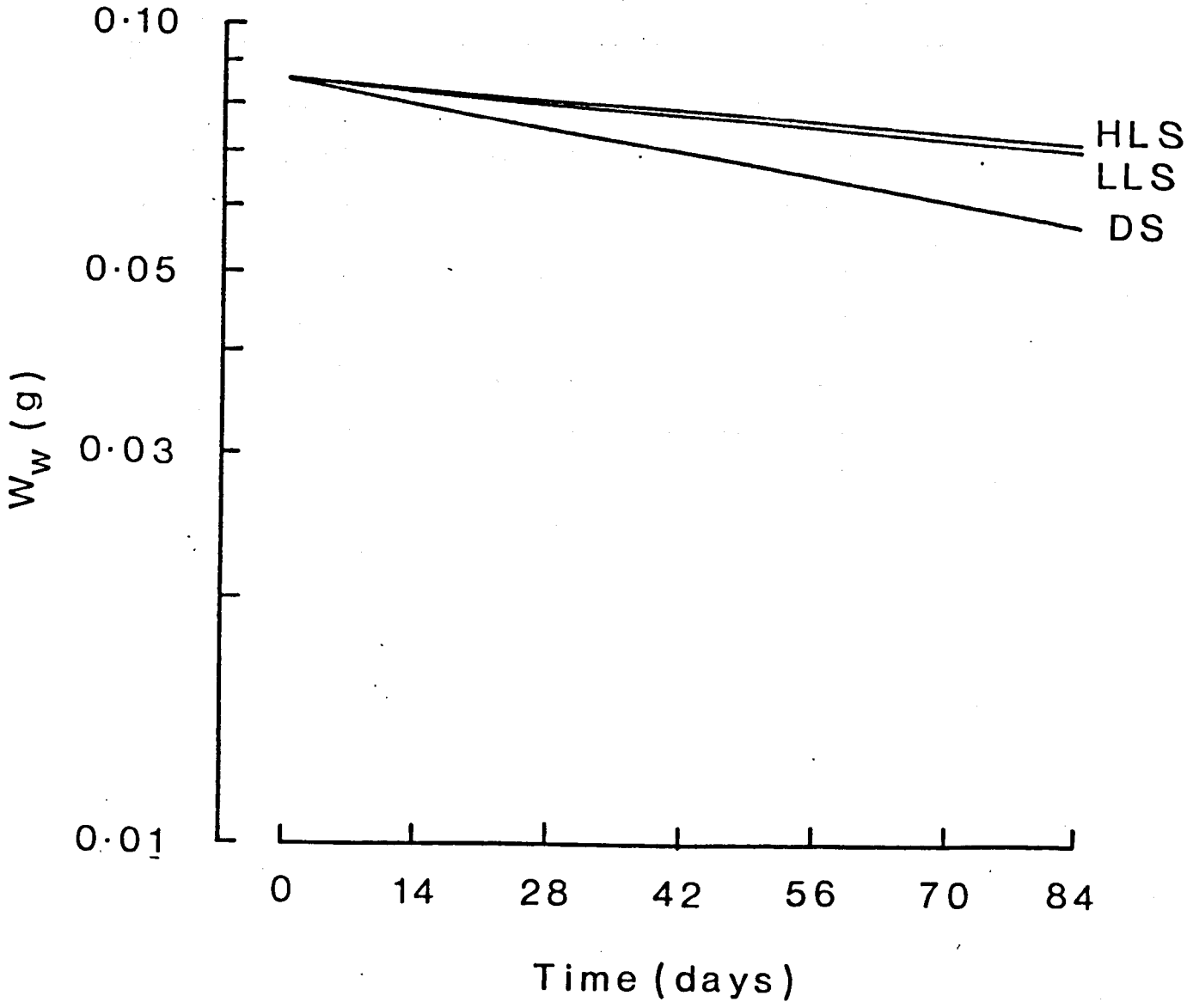


Table 12 Changes in buoyant weight (W_w), organic weight (W_d) and total energy content in 'standard' anemones of 0.4g organic weight from 6 stocks of symbiotic Anemonia sulcata

Stock	Change in W_w (mg. $24h^{-1}$)	Change in W_d (mg. $24h^{-1}$)	Change in total energy content (J. $24h^{-1}$)
HLS	- 0.1805	- 0.8073	- 16.91
LLS	- 0.1959	- 0.8881	- 18.09
DS	- 0.3908	- 1.7478	- 34.97
HLF	+ 0.6344	+ 2.8373	+ 59.27
LLF	+ 0.7287	+ 3.2589	+ 66.85
DF	+ 0.3121	+ 1.3960	+ 28.20

(Table 13) to give the net energy retention of 'standard' anemones from each stock.

b) Weight gain following carnivorous feeding

The anemones in all three fed stocks gained weight, except for one in the DF stock which was excluded from subsequent calculations. Graphs of weight gain versus time were plotted for each animal in two ways: Firstly as $\log_{10} W_w$ v time (assuming exponential growth) and secondly as a linear plot of W_w v time. Regression lines were fitted by the method of least squares. The significance of the fit of these lines was tested by analysis of variance (Sokal & Rohlf, 1969). The values of F which were obtained were highly significant for both statistical treatments, but were higher for the linear plots in most animals. Linear plots of W_w versus time were therefore adopted for simplicity in analysis. This is valid for dealing with the segment of the growth curve which these anemones form part of, but does not imply that the overall growth curve for A. sulcata is linear.

The regression equations of W_w on time are given in Appendix A, Table I. The lines for the HLF stock are plotted in Fig. 20. Analysis of covariance showed no significant difference in the slope of the lines (Appendix 4, Table J) which suggests that the anemones from this stock were gaining weight by a uniform amount per unit time. This is shown in Fig. 21 where the lines have been replotted to the combined regression coefficient from Appendix A, Table J.

Analysis of covariance showed no significant difference in the slope of the lines within the LLF stock, however there was a significant difference within the DF stock due to an anemone which had gained weight at a different rate from the others. This anemone was excluded from

Table 13 Energy content of symbiotic Anemonia sulcata, determined by wet oxidation with dichromate, at the beginning and end of the 12 week experimental period.

Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	number of anemones	number of determinations	Mean ± S.D. energy content ($J.mg^{-1}$)
Sample on Day 0	-	3	6	20.54 ± 0.61
HLS on day 84	140	5	10	21.34 ± 1.15
LLS " " "	70	7	14	20.16 ± 1.34
DS " " "	0	7	14	19.47 ± 0.57
HLF " " "	140	4	8	21.24 ± 0.91
LLF " " "	70	3	6	20.48 ± 1.00
DF " " "	0	5	10	19.85 - 0.30

Fig. 20 Regression lines of buoyant weight (W_w) on time in four symbiotic A. sulcata from the HLF stock

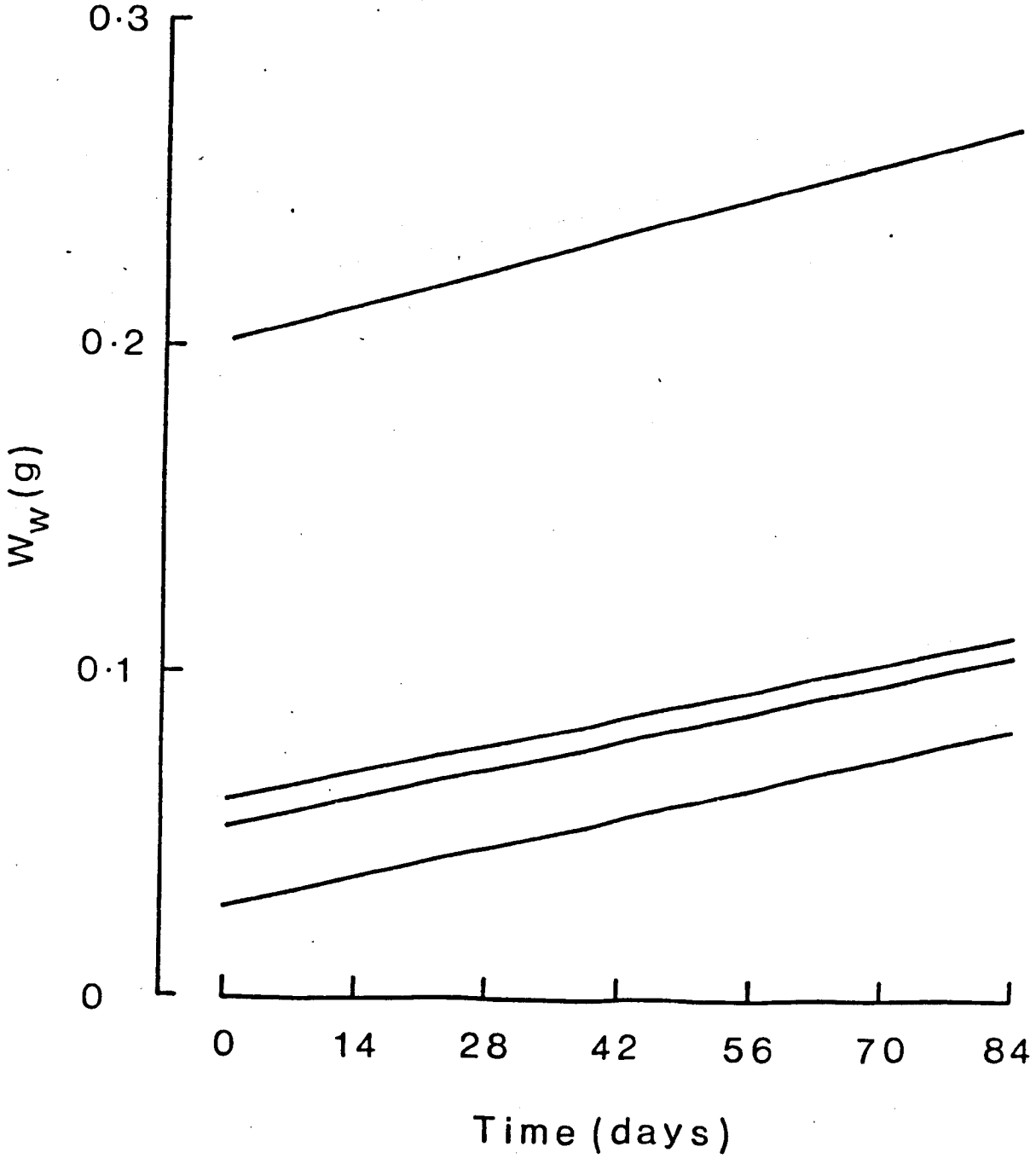
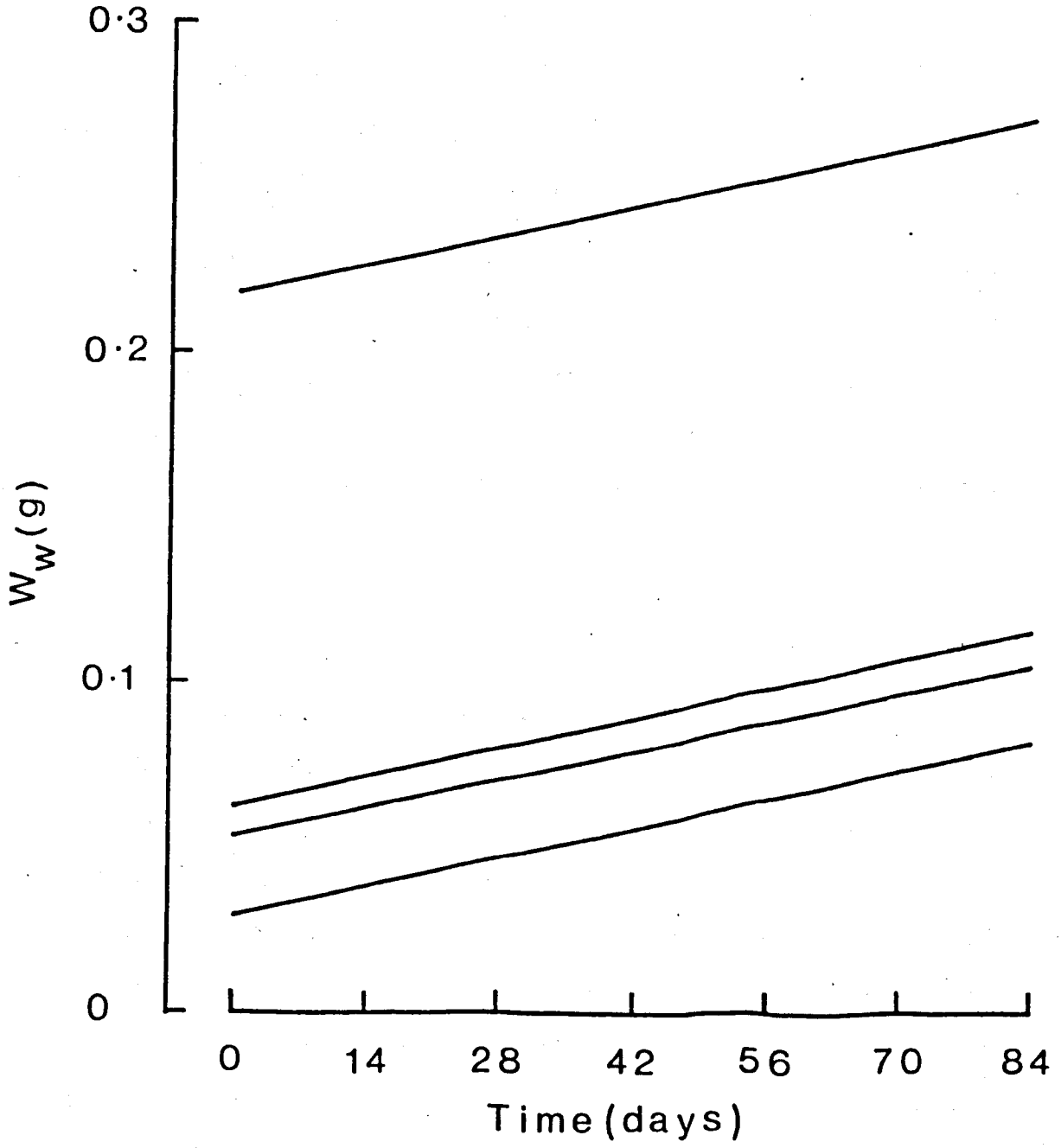


Fig. 21 Regression lines of buoyant weight (W_w) on time in four symbiotic A. sulcata from the HLF stock replotted to a combined regression coefficient (β) of 6.3442×10^{-4}



subsequent calculations. There was no significant difference in the slope of the lines for the three remaining anemones in this stock (Appendix 4, Table J). These results suggest that the anemones had gained weight by uniform amounts, specific to their stock, per unit time.

The sums of squares and sums of products were pooled and the slopes of the pooled lines (Fig. 22) for each stock were compared by analysis of covariance. There were significant differences in slope between the three fed stocks (Appendix 4, Table J) indicating that irradiance had a significant effect on the growth rate of these anemones.

The weight gain of 'standard' anemones (Fig. 23) were calculated using the combined regression coefficients given in Appendix 4, Table J. The increase in W_w over 24h of 'standard' anemones (Table 12) were calculated in the same way. These values were converted to changes in W_d and values of net energy retention (Table 12) by the method used for starved anemones.

Discussion

The results given in Table 12 show that although photosynthesis by zooxanthellae at 70 and 140 $\mu E.m^{-2}.sec^{-1}$ made a significant contribution to the net energy retained by starved anemones, this was insufficient to meet the total energy requirements of this symbiotic organism as the anemones lost weight at both irradiances. Since it had been predicted that photosynthesis would result in the fixation of energy in excess of respiratory energy expenditure at 140 $\mu E.m^{-2}.sec^{-1}$ (p34) these results suggest that a large amount of this energy input must be lost. The results also show that feeding with squid mantle was more than sufficient to meet the energy requirements of *A. sulcata* as fed anemones gained weight in darkness and in light. The energy gain was greater in light than in

Fig. 22 Pooled regression lines of buoyant weight (W_w) on time in three stocks of fed symbiotic A. sulcata

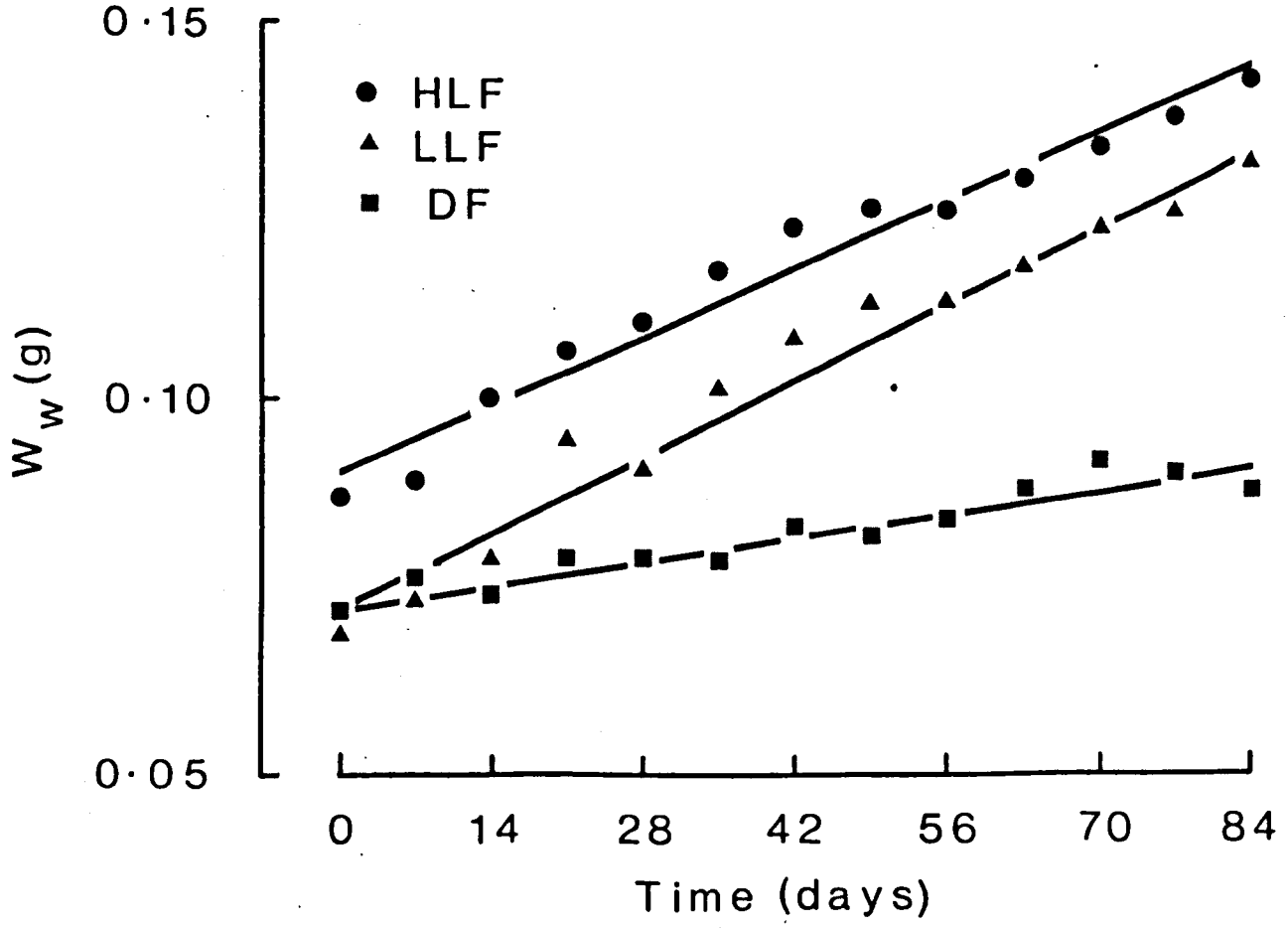
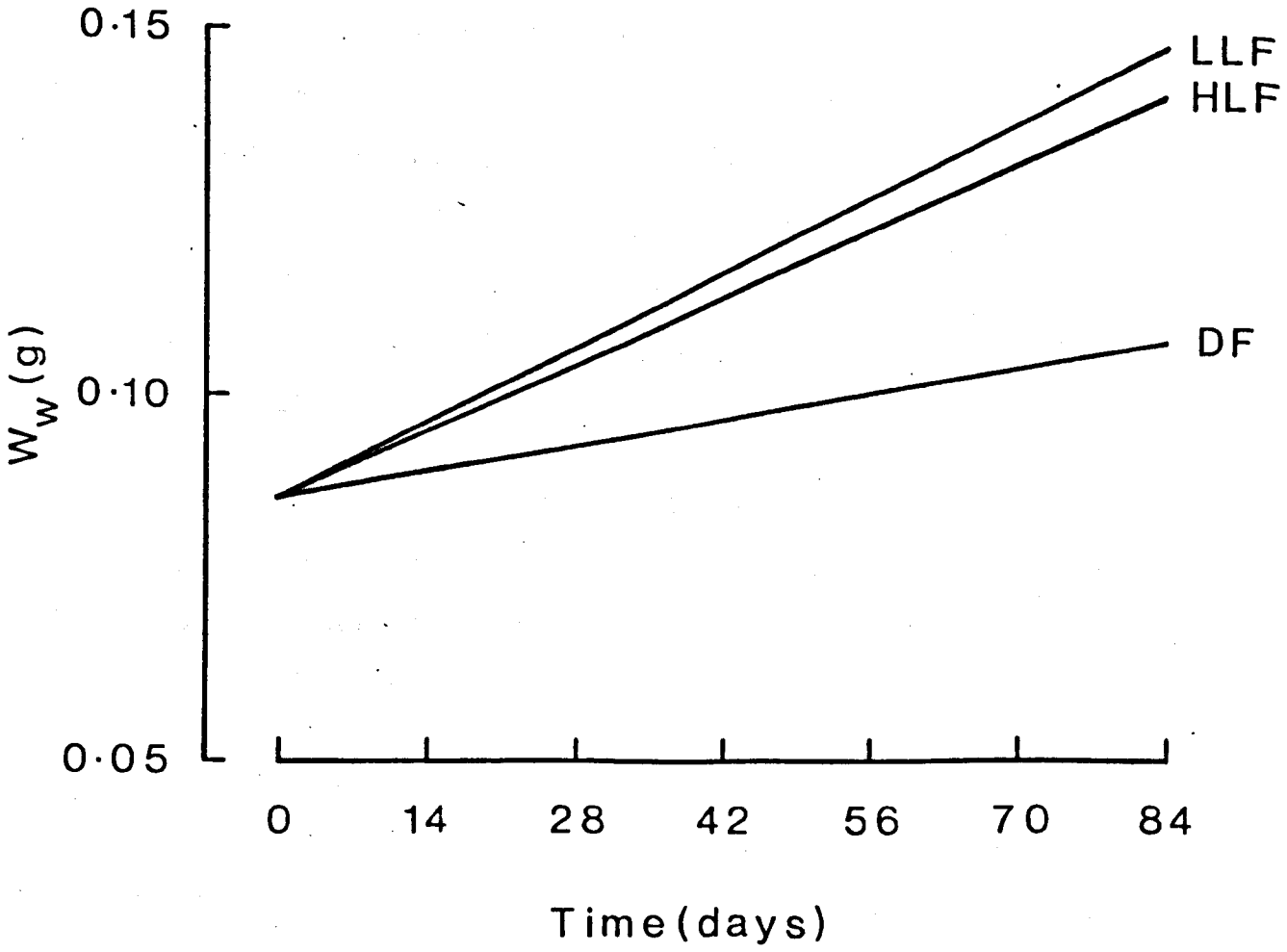


Fig. 23

Weight gain following carnivorous feeding in 'standard' symbiotic *A. sulcata* of 0.856g buoyant weight (W_w) (= 0.4g organic weight)



darkness, but the most energy was retained at the lower irradiance. This may have been partly due to differences in food intake. This is more fully discussed in Section 6.

These findings are similar to those of Taylor (1969a) who also found that Anemonia sulcata lost weight when starved both in light and in darkness. The weight loss was also greater in anemones starved in darkness. In contrast to this study, he found that A. sulcata fed in darkness also lost weight and only those fed in the light gained weight. A quantitative comparison with Taylor's study is not possible since the food type, its energy content and feeding frequency were not specified and his experiments were carried out at 15°C. Similar trends have been observed in the anemones Cereus pedunculatus (Taylor, 1967a) and Anthopleura elegantissima, where anemones starved under an irradiance of 200ft. ca ($\sim$42 $\mu E.m^{-2}.sec^{-1}$) lost weight more slowly than those kept in darkness (Muscatine, 1961).

It has been demonstrated that the weight loss of symbiotic Anemonia sulcata was an exponential function of time. Mayer (1914) observed an exponential decrease in the wet weight of the scyphozoan Cassiopea, which harbours zooxanthellae, during starvation in light and darkness.

The values of net energy retention in Table 12 do not reveal how much energy was retained as biomass of anemone and how much as biomass of zooxanthellae. This was estimated in part (ii) of this Section from changes in the size of the zooxanthellae populations which were monitored in the same animals.

ii) Energy retention as biomass of zooxanthellae

Introduction

Changes in the size of the zooxanthellae populations in the anemones exposed to the experimental conditions outlined in Table 6, were estimated to predict how much of the energy inputs to the anemones were retained as biomass of zooxanthellae and, by difference, how much energy was retained as biomass of anemone.

Materials and Methods

Since it was not possible to determine the number of zooxanthellae in an anemone without sacrificing it, the initial sizes of the zooxanthellae populations had to be predicted. This was done by fractionating a sample of 3 anemones from the batch to be used in the experiment and extracting the zooxanthellae with autoclaved SW using the method described in Section 2. The numbers of zooxanthellae were then estimated from 5 replicate counts with a haemacytometer and the densities of zooxanthellae per g organic weight (W_d) were calculated from these results. The W_d of each experimental anemone was determined on day 0 of the experiment and the size of their zooxanthellae populations were predicted from the mean density of zooxanthellae in the three anemones that were sacrificed.

At the end of the 84 day experimental period, all surviving anemones were sacrificed and the tissue was fractioned as described in Section 2. The size of their zooxanthellae populations were determined by 5 replicate counts with a haemacytometer and the densities of zooxanthellae per g W_d were calculated from these results.

Results

The mean size of the populations of zooxanthellae in each experimental stock at the beginning and end of the 84 day experimental

period are given in Table 14. The difference between the initial and final size of the zooxanthellae population was calculated and this was divided by 84 to give the change in numbers of zooxanthellae over a 24h period, and the percentage change in the zooxanthellae population of each stock over 24h (Table 14).

These results were used to predict the energy retained as biomass of zooxanthellae by 'standard' anemones of 0.4g W_d over a period of 24h. An anemone of this size will have harboured $(0.4 \times 2.495 \times 10^8) = 9.98 \times 10^7$ zooxanthellae on day 0. The changes in the size of the zooxanthellae populations of 'standard' anemones from each stock, given in Table 15 were calculated from the percentage change in the zooxanthellae populations of each stock. The changes in biomass of zooxanthellae (Table 15) were calculated by multiplying the change in population size by 3.02×10^{-7}mg, the W_d of a zooxanthella (Appendix 5). The energy retained as biomass of zooxanthellae (Table 15) was calculated by multiplying the change in biomass of zooxanthellae by 21.27 $J.mg^{-1}$, the mean energy content of zooxanthellae (Appendix 5).

Since a 'standard' anemone of 0.4g W_d will have harboured 9.98×10^7 zooxanthellae on day 0, the organic weight of the zooxanthellae was $\left(\frac{9.98 \times 10^7 \times 3.02 \times 10^{-7}}{1000}\right) = 0.03$g, therefore the remaining 0.37g was composed of anemone tissue alone.

Discussion

The results in Table 14 show that the size of the zooxanthellae populations increased in all anemones exposed to light. These increases were greater in anemones maintained at the higher irradiance than those maintained at the lower irradiance. This indicates that the reproduction rate of the zooxanthellae was dependent on the irradiance to which the

Table 14 Mean sizes of the zooxanthellae populations in 6 stocks of symbiotic anemones at the beginning and end of an 84 day experimental period

Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	Mean initial density of zooxanthellae (10^8.g organic weight^{-1})	Predicted Day 0	Day 84	Mean final density of zooxanthellae (10^8.g organic weight^{-1})	Change in numbers of zooxanthellae per 24h	Percentage change in the zooxanthellae population per 24h
HLS	140	2.495	7.19×10^7	1.276×10^8	5.688	$+ 6.63 \times 10^5$	+ 0.92
LLS	70		7.98×10^7	1.059×10^8	4.733	$+ 3.11 \times 10^5$	+ 0.39
DS	0		5.75×10^7	4.96×10^7	3.432	$- 9.41 \times 10^4$	- 0.16
HLF	140		1.052×10^8	2.335×10^8	3.535	$+ 1.53 \times 10^6$	+ 1.45
LLF	70		8.52×10^7	1.595×10^8	2.598	$+ 8.85 \times 10^5$	+ 1.04
DF	0		5.13×10^7	3.56×10^7	1.103	$- 1.87 \times 10^5$	- 0.83

Table 15 Changes in number, biomass and energy content of zooxanthellae in 'standard' anemones of 0.4g organic weight, harbouring 9.98×10^7 zooxanthellae on Day 0, from six stocks of symbiotic Anemonia sulcata

Stock	Percentage change in zooxanthellae population per 24h	Change in numbers of zooxanthellae per 24h	Change in biomass of zooxanthellae ($mg.24h^{-1}$)	Change in energy content of zooxanthellae ($J.24h^{-1}$)
HLS	+ 0.92%	$+ 9.20 \times 10^5$	+ 0.28	+ 5.91
LLS	+ 0.39%	$+ 3.89 \times 10^5$	+ 0.12	+ 2.50
DS	- 0.16%	$- 1.63 \times 10^5$	- 0.05	- 1.05
HLF	+ 1.45%	$+ 1.448 \times 10^6$	+ 0.44	+ 9.30
LLF	+ 1.04%	$+ 1.036 \times 10^6$	+ 0.31	+ 6.66
DF	- 0.83%	$- 8.26 \times 10^5$	- 0.25	- 5.30

anemones were exposed. The increase in numbers of zooxanthellae was greater in anemones which were fed than in those which were starved at both irradiances. Since the fed animals were increasing in weight while the starved anemones were decreasing in weight (Part(i) of this Section), the density of zooxanthellae was highest in the starved anemones at the end of the experimental period (Table 14). It appears that during starvation the reproduction rate of the zooxanthellae was restricted by the size of the animal, measured as bodyweight, while in a growing anemone the zooxanthellae were multiplying at a rate which kept pace with animal growth and cell division (Muscatine & Pool, 1979). However the rates of cell division of the anemones and the change in numbers of zooxanthellae in each gastrodermal cell are not known. The density of the zooxanthellae was probably controlled by inhibition of mitosis as in Hydra viridissima (McAuley, 1981) or by expulsion of zooxanthellae as in Aiptasia tagetes (Steele, 1976).

These findings are consistent with those of Steele (1976) that the reproduction rate of zooxanthellae in the sea anemone Aiptasia tagetes was dependent on the irradiance at which they were maintained. Johannes (1974) has also shown that the density of zooxanthellae in the coral Fungia increased in laboratory maintained specimens, compared with controls on the reef. The range of irradiance which the A. sulcata were exposed to in the field prior to this study is not known. It appears that the conditions in the field had maintained the density of zooxanthellae at a level below the maximum.

The size of the zooxanthellae population fell in the two stocks maintained in darkness. Since the anemones which were fed in darkness increased in overall weight (Part (i) of this Section), the density of zooxanthellae in these anemones was very low at the end of the experimental period (Table 14). This loss of zooxanthellae in darkness is consistent with

electron microscopy observations on A. sulcata (Taylor, 1969b) and field observations on corals (Yonge & Nichols, 1931) maintained in darkness.

It was assumed that the numbers of zooxanthellae increased as a linear function of time over the experimental period. A similar assumption has been made by Steele (1976), although Pardy (1974) has shown that the numbers of zoochlorellae in Hydra increased exponentially with time. The adoption of a linear instead of an exponential model of zooxanthellae reproduction is likely to have led to only small differences in the predicted changes in the zooxanthellae population over the relatively short experimental period in this study.

The net energy retained as biomass of zooxanthellae for each group given in Table 15 was subtracted from the net energy retention of the whole A. sulcata (from Part (i) of this Section , Table 12) to give the net energy retention as biomass of anemone alone (Table 16).

The values of net energy retention as biomass of anemone and as biomass of zooxanthellae are used in the energy balance equations in Section 6.

Table 16 Changes in energy content of the zooxanthellae and anemone tissue in 'standard' anemones of 0.4g organic weight, harbouring 9.98×10^7 zooxanthellae on Day 0, from six stocks of symbiotic *Anemonia sulcata*

Stock	Change in total energy content of *A. sulcata* ($J.24h^{-1}$)	Change in energy content of zooxanthellae ($J.24h^{-1}$)	Change in energy content of anemone alone ($J.24h^{-1}$)
HLS	-16.91	+5.91	-22.82
LLS	-18.09	+2.50	-20.59
DS	-34.97	-1.05	-33.92
HLF	+59.27	+9.30	+49.97
LLF	+66.85	+6.66	+60.19
DF	+28.20	-5.30	+33.50

Section 5

Energy Balance Experiments on Aposymbiotic Anemonia sulcata

Introduction

Experiments were carried out on aposymbiotic Anemonia sulcata to measure factors used to construct energy balance equations. These equations are used in Section 6 to compare energy flow through the animal alone with energy flow through anemones harbouring zooxanthellae.

The energy input of food ingestion, the energy expenditure and the net energy retention were measured in aposymbiotic anemones maintained under different conditions. Values of these factors for hypothetical 'standard' anemones were extrapolated from the results. Losses were calculated by subtraction in Section 6 .

In order to make true comparisons with the symbiotic anemones, (Section 4), it was necessary to derive a standard weight which was similar to the animal component of the symbiotic association. It was shown in Section 4D that in a 'standard' 0.4g organic weight symbiotic anemone, 0.37g was animal tissue. This latter value was therefore used as the standard organic weight of an aposymbiotic anemone.

Aposymbiotic anemones were maintained under the conditions outlined in Table 17. The anemones were either maintained in darkness or under a 12h light, 12h dark cycle, to determine whether light alone affected energy flow through the animal. The anemones were either starved or were fed squid mantle in excess of energy expenditure to determine the contribution of carnivorous feeding to the energy requirements of the anemone. Aposymbiotic anemones were maintained in tanks separate from symbiotic anemones to reduce chances of reinfection with zooxanthellae.

Table 17 Experimental Conditions under which aposymbiotic *Anemonia sulcata* were maintained

i) 12h Light / 12h Dark at 140 $\mu E.m^{-2}.sec^{-1}$

a) Starved (LSA)

b) Fed squid mantle twice weekly (DFA)

ii) Total Darkness

a) Starved (DSA)

b) Fed squid mantle twice weekly (DFA)

5 animals in each of the four stocks

A) Energy intake from carnivorous feeding

Introduction

The energy content of the squid consumed by the two fed stocks of aposymbiotic anemones over the experimental period was measured to allow the calculation of the energy ingested by 'standard' animals.

Materials and Methods

The materials and methods used are as described in Section 4B.

Results

The mean organic weights of squid consumed per animal are given in Table 18 along with the percentage of the mean organic weight of the animals that this represents. The amounts of energy ingested by 'standard' anemones of 0.37g organic weight given in Table 18 were calculated as described in Section 4B. These values are used in the energy balance equations in Section 6.

B) Energy expenditure of aposymbiotic Anemonia sulcata

i) Energy expenditure on maintenance: The effect of weight on the oxygen consumption of aposymbiotic Anemonia sulcata

Introduction

In order to determine values of the energy expenditure of the animal alone, the relationship between oxygen consumption ($\dot{M}_{O_2}$) and organic weight (W_d) was determined for each stock of aposymbiotic Anemonia sulcata so that the energy expenditure on maintenance of 'standard' anemones could be calculated from this relationship.

Materials and Methods

After at least 37 days acclimation to the experimental conditions outlined in Table 17, the $\dot{M}_{O_2}$ of each aposymbiotic anemone was measured

Table 18 Weight and energy content of squid consumed by three stocks of aposymbiotic *A. sulcata*

Stock	Organic weight of squid consumed ($mg.24h^{-1}$)	Organic weight of squid consumed as a percentage of anemone organic weight	Energy intake as squid of 'standard' anemones ($J.24h^{-1}$)
LFA	4.20	2.525	102.92
DFA	3.86	2.450	94.67

using the methods described in Section 4C(i). The W_d of each anemone was calculated for its buoyant weight, measured before the $\dot{M}_{O_2}$ measurements, as described in Appendix 1.

Results

The regression lines relating log $\dot{M}_{O_2}$ to log W_d in the four stocks of aposymbiotic Anemonia sulcata are plotted in Fig. 24. The equations of the lines are given in Appendix 3, Table D.

The slopes of the lines were compared by analysis of covariance to test whether the lighting or feeding regime had a significant effect on the relationship between log $\dot{M}_{O_2}$ and log W_d. The F ratio given in Appendix 3, Table E shows that there was no significant difference in the slope of the four lines proving that the lighting and feeding regime did not have a significant effect on the relationship between log $\dot{M}_{O_2}$ and log W_d. This is illustrated in Fig. 25 where the lines have been replotted to the combined regression coefficient (β) given in Appendix 3, Table E.

The elevations of the four lines were compared by analysis of covariance to test whether the lighting or feeding regime had a significant effect on the magnitude of the $\dot{M}_{O_2}$ recorded at a given W_d. The F ratios given in Appendix 3, Table F show that there was a significant difference in elevation within the four lines. There was no significant difference in elevation between the two starved stocks, and the two fed stocks. However there was significant differences between fed and starved stocks, maintained in either light or darkness. Hence the lighting regime had no significant effect on the magnitude of the $\dot{M}_{O_2}$ recorded at a given W_d while feeding had a significant effect on the magnitude of $\dot{M}_{O_2}$ recorded at a given W_d.

Since feeding had a significant effect on the magnitude of $\dot{M}_{O_2}$ recorded at a given W_d, the $\dot{M}_{O_2}$ of 'standard' aposymbiotic anemones given

Fig. 24 Regression lines of log oxygen consumption ($\dot{M}_{O_2}$) on log organic weight (W_d) in four stocks of aposymbiotic A. sulcata

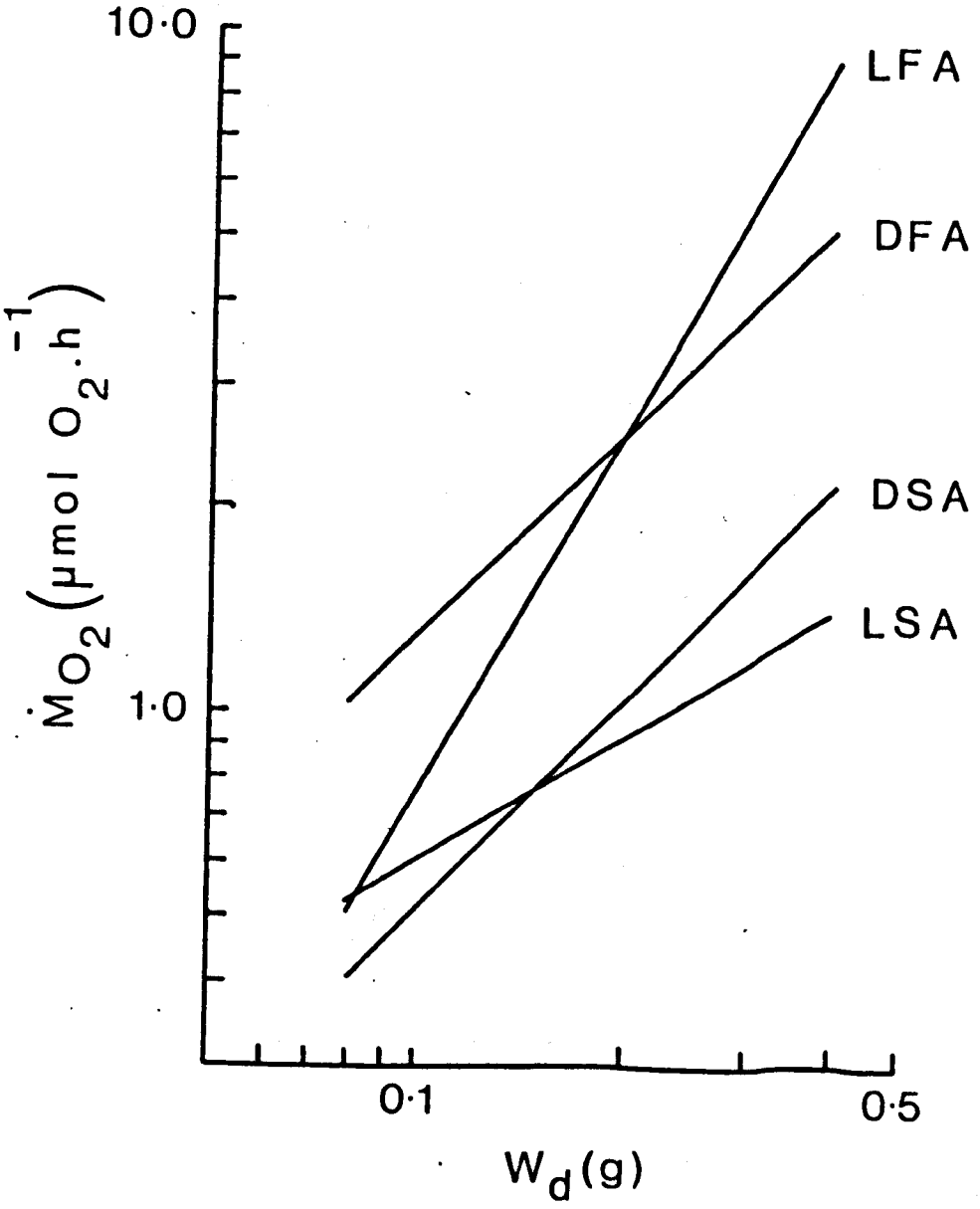
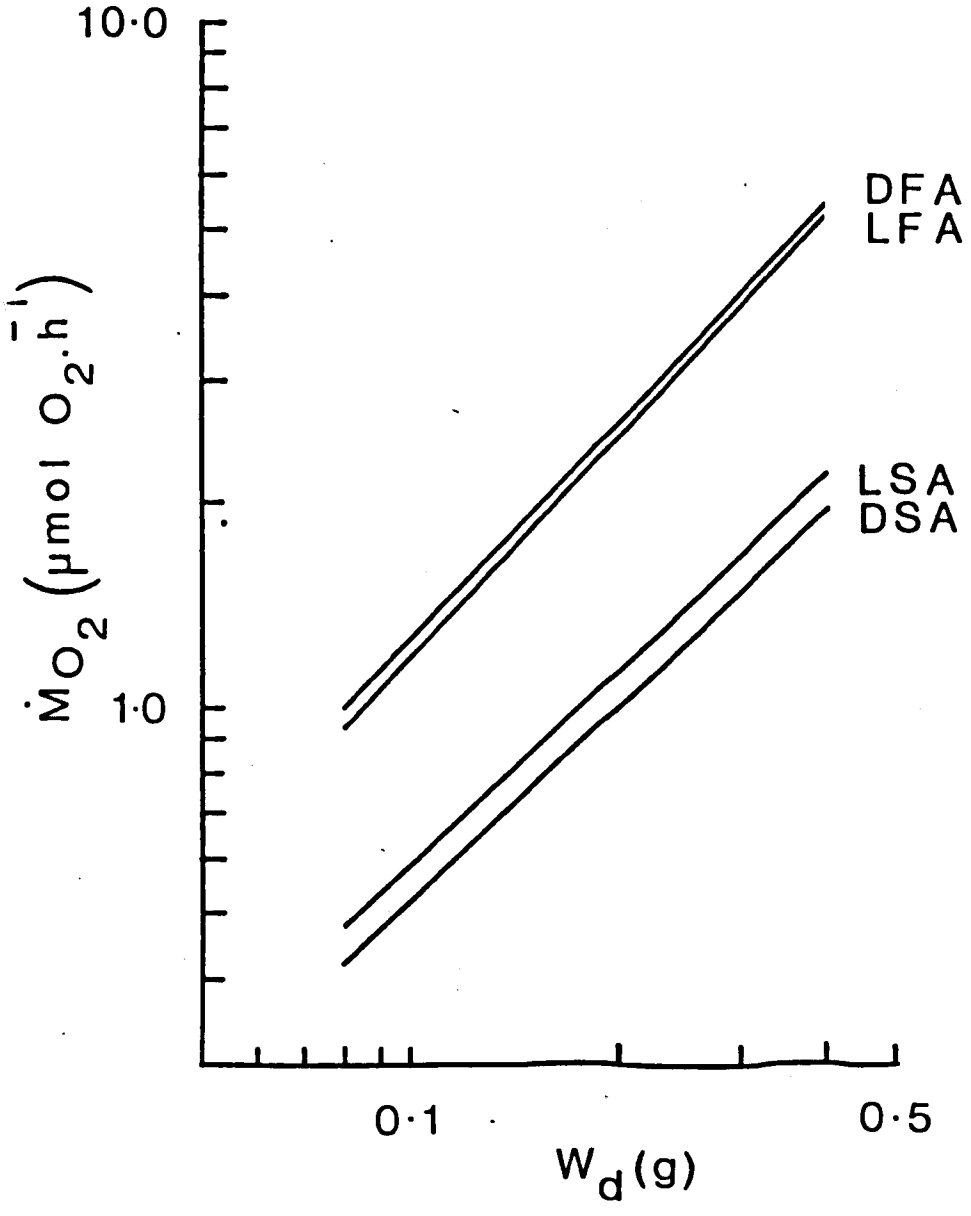


Fig. 25 Regression lines of log oxygen consumption ($\dot{M}_{O_2}$) on log organic weight (W_d) in four stocks of aposymbiotic A. sulcata replotted to a combined regression coefficient (β) of 0.9009



in Table 19 were calculated individually for each stock from the raw data using the combined regression coefficient (β) of 0.9009 given in Appendix 3, Table E with the equation given in Section 4C and using 0.37g as the standard W_d. The energy expenditure on maintenance (R) of these animals over 24h, calculated as in Section 4C are also given in Table 19 and are used in the energy balance equations in Section 6.

Discussion

The $\dot{M}_{O_2}$ of the 'standard' fed aposymbiotic anemones were similar to the $\dot{M}_{O_2}$ of the symbiotic anemones given in Section 4C, Table 10 while the $\dot{M}_{O_2}$ of the starved aposymbiotic anemones were at least 50% lower than the $\dot{M}_{O_2}$ of the symbiotic and fed aposymbiotic anemones.

These results differ from those of Smith (1939) who found that aposymbiotic A. sulcata consumed more oxygen per gN than symbiotic anemones. However the nutritional history of the anemones was not defined by Smith (1939). Fitt & Pardy (1981) have shown that the $\dot{M}_{O_2}$ of aposymbiotic Anthopleura elegantissima was similar or higher that symbiotic specimens, however the $\dot{M}_{O_2}$ of aposymbiotic Anthopleura elegantissima fed shrimp food was higher than aposymbiotic anemones which were starved, which is in agreement with the findings of this study. Pardy & White (1977) have shown that aposymbiotic green Hydra have similar oxygen consumption rates to symbiotic Hydra maintained in darkness.

ii) The effect of light on the $\dot{M}_{O_2}$ of aposymbiotic anemones

Anemonia sulcata expands in response to light, presumably due to the presence of symbiotic algae. In darkness, the anemones relax and contract (Smith, 1939), This behaviour also occurs in aposymbiotic anemones (pers. obs.). Decreased $\dot{M}_{O_2}$ during contraction has been recorded in other sea anemones (Shick et al, 1979). The effect of light on the

Table 19 Oxygen consumption ($\dot{M}_{O_2}$) and respiratory energy expenditure (R) of 'standard' anemones of 0.37g organic weight from four stocks of aposymbiotic Anemonia sulcata

Stock	$\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$)	R ($J.24h^{-1}$)
LSA	1.835	19.93
DSA	1.725	18.73
LFA	4.890	53.11
DFA	4.147	45.04

$\dot{M}_{O_2}$ of aposymbiotic A. sulcata was investigated to test whether

i) This behaviour significantly affected the $\dot{M}_{O_2}$

ii) The $\dot{M}_{O_2}$ recorded in darkness was representative of the $\dot{M}_{O_2}$ under illumination.

Materials and Methods

The $\dot{M}_{O_2}$ of eight aposymbiotic anemones, which had been maintained under a 12h light/12h dark cycle at 140 $\mu E.m^{-2}.sec^{-1}$. was recorded every alternate hour in darkness between 18.00h and 06.00h and then at 152 $\mu E.m^{-2}.sec^{-1}$. between the hours of 06.00h and 18.00h in automated confinement respirometers. The buoyant weight (W_w) of each anemone was recorded before the measurements of $\dot{M}_{O_2}$. The organic weight(W_d) was calculated from the W_w as described in Appendix 1. The $\dot{M}_{O_2}$ values were divided by the W_d to give the weight-specific $\dot{M}_{O_2}$ in units of $\mu mol\ O_2.g W_d^{-1}.h^{-1}$.

Results

The weight specific $\dot{M}_{O_2}$ of most aposymbiotic anemones was lower in light than in darkness (Table 20). Students t tests showed that this difference was significant in only 2 of the 8 anemones used. The $\dot{M}_{O_2}$ of anemone 3 was significantly higher in light than in darkness, while there was no significant difference between the $\dot{M}_{O_2}$ recorded in darkness and that recorded in light in the remaining 5 anemones. The $\dot{M}_{O_2}$ of aposymbiotic A. sulcata was not significantly affected by exposure to light in the majority of anemones.

Discussion

The results of this experiment are similar to those of Smith (1939) who found that the $\dot{M}_{O_2}$ of aposymbiotic A. sulcata in light was

Table 20 Weight specific oxygen consumption ($\dot{M}_{O_2}$) of aposymbiotic Anemonia sulcata in darkness and at 156 $\mu E.m^{-2}.sec^{-1}$. ($\mu mol\ O_2.g^{-1}.h^{-1}$)

	n	Mean ± S.D. $\dot{M}_{O_2}$ in Darkness	n	Mean ± S.D. $\dot{M}_{O_2}$ at 156 $\mu E.m^{-2}.sec^{-1}$	t	
1)	5	8.368 ± 2.163	6	7.924 ± 2.219	0.334	0.7 < P < 0.8 n.s.
2)	5	11.932 ± 2.481	5	11.355 ± 1.880	0.414	0.6 < P < 0.7 n.s.
3)	4	20.630 ± 2.319	5	28.351 ± 2.340	4.938	0.001 < P < 0.002
4)	5	5.400 ± 1.675	5	4.110 ± 1.679	1.216	0.2 < P < 0.3 n.s.
5)	6	6.217 ± 1.893	5	4.375 ± 1.158	1.892	0.05 < P < 0.1 n.s.
6)	4	5.961 ± 0.791	6	4.775 ± 0	3.795	0.002 < P < 0.01
7)	5	5.306 ± 0.839	5	4.304 ± 1.302	1.446	0.1 < P < 0.2 n.s.
8)	4	7.198 ± 2.639	5	4.044 ± 1.302	2.365	P = 0.05

n.s. = not significant

similar to, or lower than that in darkness. Although aposymbiotic A. sulcata expand in response to light, this activity does not significantly affect the $\dot{M}_{O_2}$. These results suggest that the assumption made in the calculation of energy expenditure over 24h that the $\dot{M}_{O_2}$ of A. sulcata is similar in light to that recorded in darkness (Section 2) is acceptable for aposymbiotic anemones.

Contraction reduces the surface area of a sea anemone, which reduces the area available for gas exchange, and increases the distance for diffusion of oxygen (Shick et al, 1979). Reduced $\dot{M}_{O_2}$ in contracted anemones has been observed in Actinia equina (Smith, 1939; Jones et al, 1977), Metridium senile (Shumway, 1978) and Anthopleura elegantissima (Shick et al, 1979). Unlike these anemones, Anemonia sulcata cannot fully contract (Stephenson, 1935; Smith, 1939) which may account for the small differences between the $\dot{M}_{O_2}$ recorded in darkness and that recorded in light.

iii) The effect of feeding on the $\dot{M}_{O_2}$ of aposymbiotic A. sulcata

Introduction

The effect of feeding on the $\dot{M}_{O_2}$ of aposymbiotic anemones was investigated to estimate how much of the energy provided by a meal of squid was expended as specific dynamic action (S.D.A.) during a post prandial increase in $\dot{M}_{O_2}$ (see Section 4C(ii)).

Materials and Methods

Experiments were carried out on 3 aposymbiotic anemones which had previously been maintained in darkness using the methods described in Section 4C(ii) with the exception that they were fed 6.2-7.7% of their body organic weight as squid mantle.

Results and Discussion

Fig. 26 shows the effect of a single meal on the $\dot{M}_{O_2}$ of an aposymbiotic anemone. The pattern of the S.D.A. is similar to that in symbiotic anemones (Section 4C, Fig. 14).

The integrated S.D.A. was calculated as described in Section 4C in the anemones in which the $\dot{M}_{O_2}$ had decreased to preprandial levels. The values of integrated S.D.A. are given in Table 21. The mean amount of digested squid expended as S.D.A. was 5.1%. This is slightly smaller than that in symbiotic anemones (Section 4C(ii)). This value was used to calculate the amount of energy expended as S.D.A. in 'standard' aposymbiotic anemones in Section 6.

The peak postprandial $\dot{M}_{O_2}$ of each anemone is given in Table 21. together with the time at which this peak occurred. This peak occurred within 15h after feeding. The value of the peak $\dot{M}_{O_2}$ ranged from 141-177% of the preprandial $\dot{M}_{O_2}$. The duration of the S.D.A. ranged from 26 to more than 35h. These results are similar to those obtained from symbiotic anemones (Section 4C, Table 11).

C) Net energy retention by aposymbiotic Anemonia sulcata

Introduction

Changes in body weight and energy content of stocks of anemones maintained under the lighting and feeding conditions outlined in Table 17 were measured to estimate the following in 'standard' anemones.

1) The net energy retained from the input of carnivorous feeding.

2) The utilization of body energy reserves during starvation.

3) The effect of light on these processes.

Fig. 26 Specific Dynamic Action: The effect of a single meal on the oxygen consumption ($\dot{M}_{O_2}$) of an aposymbiotic A. sulcata (dashed line represents mean pre-prandial $\dot{M}_{O_2}$)

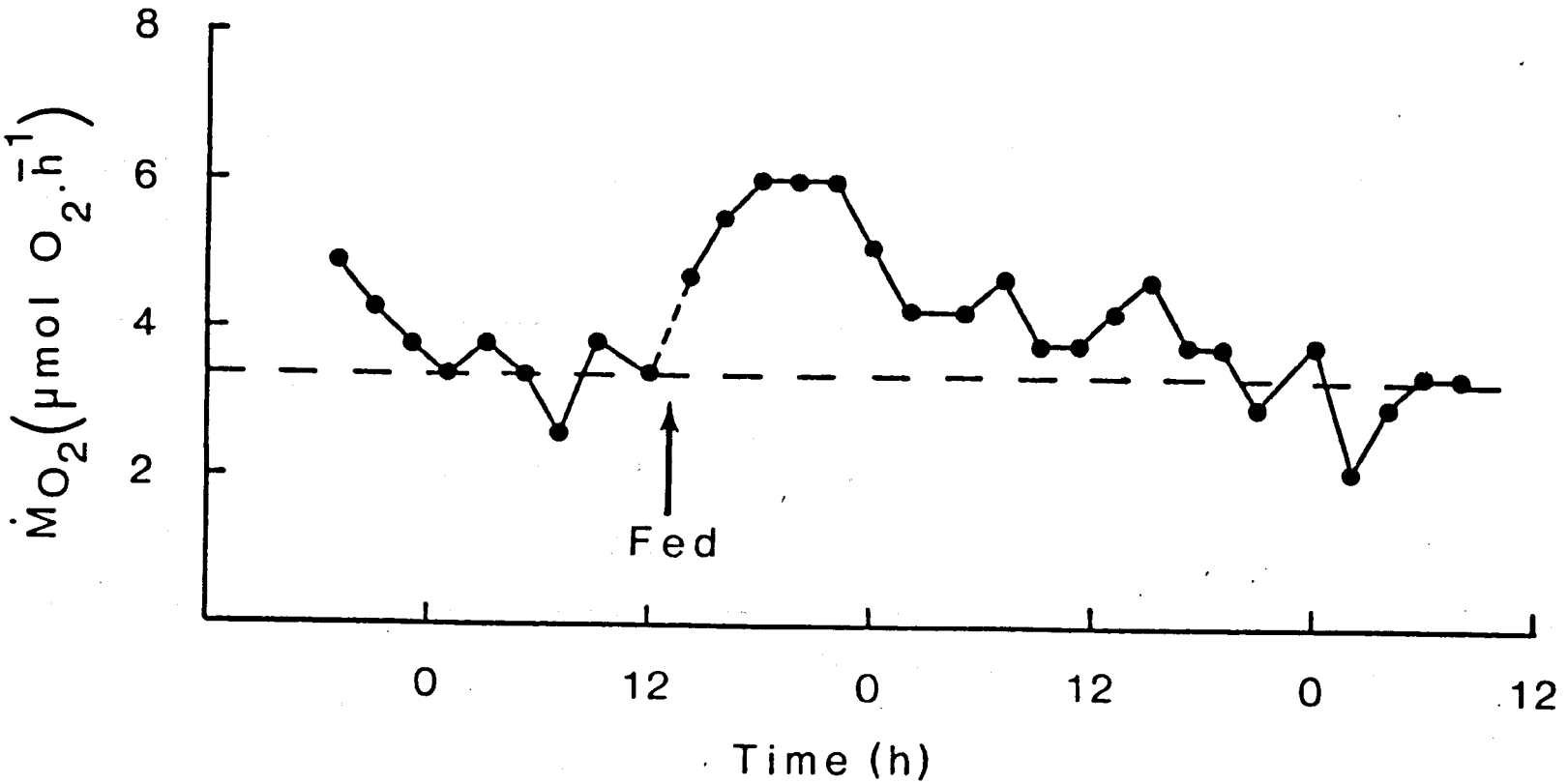


Table 21 Preprandial and postprandial O_2 consumption ($\dot{M}_{O_2}$) and integrated specific dynamic action (S.D.A.) of 3 aposymbiotic Anemonia sulcata fed in darkness

Mean ± S.D. (n=6) Preprandial $\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$)	Duration of S.D.A. (h)	$\int$ S.D.A. (μmol O_2)	$\int$ S.D.A. (J)	S.D.A. as a percentage of digested squid	Postprandial peak $\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$)	Time of peak after feeding (h)	Postprandial peak $\dot{M}_{O_2}$ as a percentage of the preprandial $\dot{M}_{O_2}$
1) 2.114 ± 0.382	35	-	-	-	3.745	12	177.2
2) 3.407 ± 0.466	31	39.82	18.02	4.12	5.962	5	175.0
3) 3.985 ± 0.327	26	26.62	12.04	6.06	5.601	15	140.6
				Mean = 5.09%			Mean = 164.3

Materials and Methods

The buoyant weight (W_w) of each anemone in the four stocks were recorded on every seventh day of an 84 day period. The W_w of fed anemones was recorded three days after their previous feed.

A sample of 5 anemones, from the batch of animals to be used in the experiment were sacrificed at the beginning of the experiment and all surviving experimental animals were sacrificed at the end of the experiment. These were fractionated as described in Section 2. The total energy content of the tissue was determined by wet oxidation with dichromate and was corrected for ash and protein content as described in Section 2. Terminal energy contents for starved anemones were not determined due to an accidental fracture of the freeze-drying ampoules.

Results

1) Weight loss during starvation

The anemones in both starved stocks lost weight over the experimental period. It had been predicted that the weight loss during starvation would be an exponential function of time (Appendix 2). The logarithmic transformation given in Section 4D yields a straight line relationship. Regression lines relating $\log_{10} W_w$ to time were fitted and the equations are given in Appendix 4, Table K.

Analysis of covariance showed no significant difference in the slope of the lines within each of the stocks (Appendix 4, Table L) which suggests that the animals in each stock had lost weight at a uniform rate (k). The sums of squares and sums of products of each stock were pooled and the slopes of the pooled lines for the two stocks (Fig. 27) were compared by analysis of covariance. There was a significant difference in slope between the two stocks (Appendix 4, Table L). The weight loss

Fig. 27 Pooled regression lines of $\log_{10}$ buoyant weight (W_w) on time in two stocks of starved aposymbiotic A. sulcata

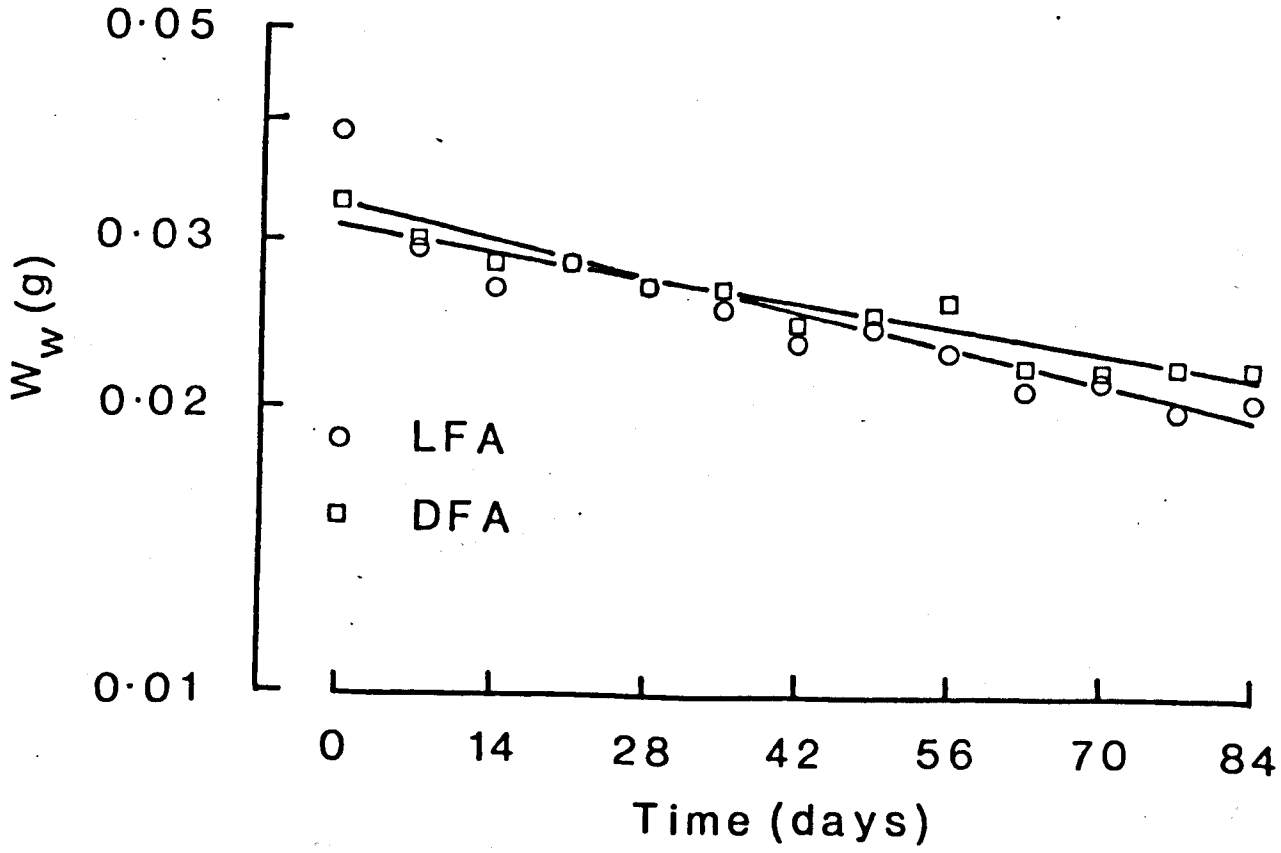
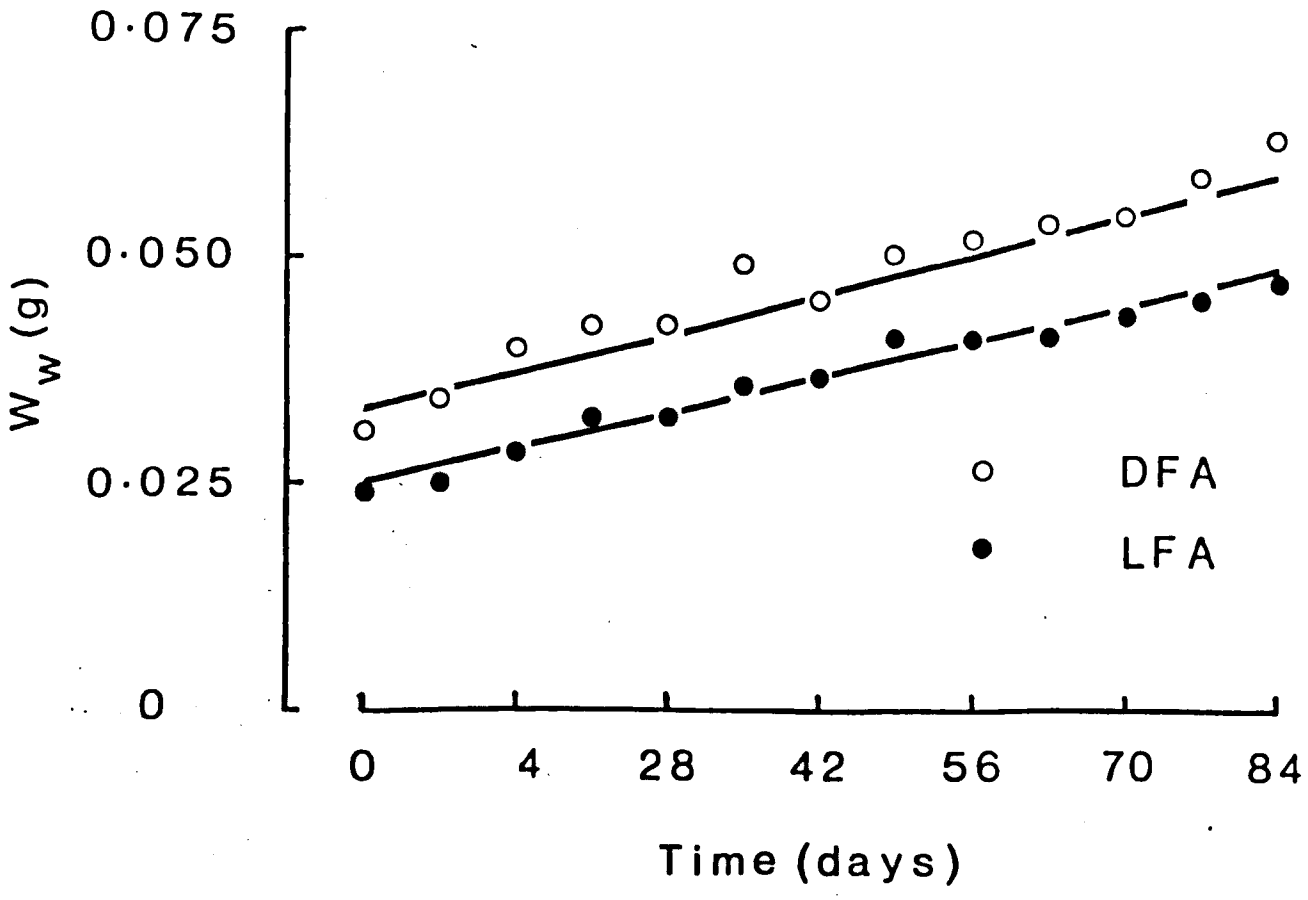


Fig. 28 Pooled regression lines of buoyant weight (W_w) on time in two stocks of fed aposymbiotic *A. sulcata*



was greater in starved animals which were exposed to light (Fig. 27).

The decrease in W_w over 24h of 'standard' aposymbiotic anemones of W_w = 0.0788g (= 0.37g organic weight) given in Table 22 were calculated using the combined regression coefficients ($\beta = \frac{k}{2.3026}$) for each stock given in Appendix 4, Table L with the equation given in Section 4D. The changes in organic weight (W_d) and net energy retention (Table 22) were calculated from these values by the method used in symbiotic anemones (Section 4D) with the exception that net energy retention was calculated using the Day 0 values (Table 23) alone since the terminal energy contents were not available. The values of net energy retention are used in the energy balance equations in Section 6.

ii) Weight gain following carnivorous feeding

The anemones in both fed stocks gained weight over the experimental period. As with the symbiotic anemones (Section 4D) the weight gain was treated as a linear function of time. Regression lines relating W_w to time were fitted and the equations are given in Appendix 4, Table M. Analysis of covariance showed no significant difference in slope within each of the stocks (Appendix 4, Table N) which suggests that the anemones from each stock were gaining weight by a uniform amount per unit time. The sums of squares and sums of products were pooled and the slopes of the pooled lines for the two stocks (Fig. 28) were compared by analysis of covariance. There was no significant difference in slope between the two fed stocks (Appendix 4, Table N). The increase in W_w over 24h of 'standard' anemones (Table 22) was calculated using the combined regression coefficients given in Appendix 4, Table N. The changes in W_d and the values of net energy retention (Table 22) were calculated by the method described in Section 4D using the energy contents given in Table 23. The values of net energy retention are used in the energy balance equations in Section 6.

Table 22 Changesin buoyant weight (W_w), organic weight (W_d) and total energy content in 'standard' anemones of 0.37g organic weight from four stocks of aposymbiotic Anemonia sulcata

Stock	Change in W_w ($mg.24h^{-1}$)	Change in W_d ($mg.24h^{-1}$)	Change in total energy content ($J.24h^{-1}$)
LSA	-0.5032	-2.250	-46.41
DSA	-0.3479	-1.556	-32.09
LFA	-0.2904	+1.299	+26.16
DFA	-0.3304	+1.478	+29.63

Table 23 Energy content of aposymbiotic Anemonia sulcata determined by wet oxidation with dichromate at the beginning and end of the 84 day experimental period

Stock	number of anemones	number of determinations (n)	Mean $\pm$ S.D. Energy content ($J.mg^{-1}$)
Sample on Day 0	5	10	20.63 ± 1.83
LFA on Day 84	3	6	19.66 ± 0.95
DFA on day 84	3	6	19.54 ± 0.42

Discussion

As predicted in Appendix 2, aposymbiotic anemones lost weight during starvation and the weight loss was an exponential function of time as in starved symbiotic anemones (Section 4D). The decrease in energy content of the starved aposymbiotic anemones given in Table 22 was similar to the decrease in energy content of the animal portion of the symbiotic anemones during starvation in darkness (Section 4D, Table 1).

The decrease in energy content of aposymbiotic anemones starved in light was greater than that of anemones starved in darkness. This effect of light cannot be attributed to algal photosynthesis which would have reduced the weight loss during starvation. In contrast, Muscatine (1961) found that aposymbiotic Anthopleura elegantissima lost weight at similar rates in light and in darkness.

The input of energy from squid mantle was more than sufficient to meet the energy requirements of the aposymbiotic anemones as both fed stocks gained weight. The net energy retained by the fed aposymbiotic anemones was lower than the net energy retained as biomass of anemone alone in symbiotic anemones fed in darkness (Section 4D, Table 16) which suggests that the conversion of the energy intake of squid to anemone tissue was more efficient in symbiotic anemones, however this is more fully discussed in Section 6.

Section 6 Energy flow through symbiotic and aposymbiotic Anemonia sulcata

A) Energy flow through symbiotic Anemonia sulcata

Introduction

In order to estimate the contribution of zooxanthellae to the energy requirements of A. sulcata using the bioenergetic model described in Section 1, the values of the energy inputs from photosynthesis and carnivorous feeding, the energy expenditure and the net energy retention as biomass of anemone and biomass of zooxanthellae in 'standard' symbiotic anemones determined in Section 4 are compiled in this section in the form of energy balance equations. Losses were calculated by subtraction.

Energy losses from algal-coelenterate symbiosis may be in the form of mucus (Benson & Muscatine, 1974), expelled zooxanthellae (Steele, 1976) ammonia and other nitrogenous compounds (Szmant-Froelich & Pilson, 1977). It is also possible that there are losses in the form of gametes.

Energy losses from A. sulcata were not measured in this study as there was insufficient time to develop the appropriate techniques. However, expulsion of zooxanthellae and secretion of copious amounts of mucus were observed in anemones kept in the aquarium.

There were additional losses from anemones which were fed with squid mantle. There were losses in the form of faeces, made up of indigestible material, most of which was collected (Section 4B). Winberg (1955) has suggested that typically 85% of food is digestible. Since the digestive processes of anthozoa are both extracellular and intracellular (Tiffon et al, 1973), it is probable that there were losses of digested material and digestive enzymes from the coelenteron

of the anemones. Nicol (1959) has suggested, from observations made on Calliactis parasitica, that losses of this kind are likely to be minimal due to the close contact between food bolus and the mesenterial filaments. However, Szmant-Froelich & Pilson (1977) have found a surge of nitrogenous excretion from the coral Astrangia danae after feeding. Approximately half of this loss was in the form of amino acids, presumably from the digestion of protein in the food.

Values for the energy expenditure of the zooxanthellae (R_z) and of the anemone alone (R_a) were required to partition the energy expenditure (R) between host and symbiont. There are three possible ways of calculating R_z.

i) from the oxygen consumption of zooxanthellae in vitro.

ii) from the ratio of zooxanthellae : animal biomass assuming that the R_z is the same as the R_a per unit biomass (Muscatine et al, 1981).

iii) by taking the difference between the R of symbiotic and aposymbiotic anemones.

Unfortunately, the oxygen consumption of zooxanthellae isolated from A. sulcata was not determined. The oxygen consumption of zooxanthellae isolated from the coral Montastrea cavernosa was 11.64 $\pm$ 1.83 μmol $O_2.10^{-8}$ zoox.h^{-1} at 28°C (Mean $\pm$ S.D., n = 4) (Davies, unpublished) and that of zooxanthellae isolated from the clam Tridacna maxima was 16-23 μmol $O_2.10^{-8}$zoox.h^{-1} at 25°C (Deane & O'Brien, 1978). Assuming that Q_{10} = 2 and using the general oxycalorific coefficient of Elliott & Davison (1975) (see Section 2), these values are equivalent to 35.1 $\pm$ 5.5 J.10^{-8}zoox.$24h^{-1}$ at 10°C for M. cavernosa and 57.9-83.3 J.10^{-8}zoox.$24h^{-1}$ at 10°C for T. maxima.

The R_z values are similar to the total R of 'standard' A. sulcata harbouring 9.98×10^7 zooxanthellae and therefore cannot be representative of the R_z of A. sulcata. This anomaly may either be due to the R_z of A. sulcata being lower than that of corals and clams or due to the oxygen consumption of zooxanthellae in vivo being lower than that recorded in vitro since Deane & O'Brien (1978) have shown that the oxygen consumption of zooxanthellae freshly isolated from T. maxima was lower than that of cultured zooxanthellae.

It was possible to calculate the R_z of A. sulcata by the method of Muscatine et al (1981) since the biomass of zooxanthellae and anemone in a 'standard' A. sulcata was known (Section 4D(ii)). However, it is not known whether the R_z was the same as R_a per unit biomass in A. sulcata.

Since values of R_a had been determined in aposymbiotic anemones, the R_z of A. sulcata was calculated as the difference between the R of a 0.4g symbiotic and a 0.37g aposymbiotic anemone as the zooxanthellae in a 'standard' symbiotic anemone on day 0 weight 0.03g (Section 4D(ii)). $34.2\ J.24h^{-1}$, the mean R of the 4 stocks of aposymbiotic anemones was used as the 'standard' value of R_a. This method of calculation had the disadvantage that it gave rise to a wide range of values of R_z.

i) Symbiotic anemone starved in darkness.

	Input		Expenditure		Retention		Losses
Overall Equation ($J.24h^{-1}$)	0	=	33.58	-	34.97	+	1.39*
Partitioned Equation							
Anemone	0	=	34.20	-	34.37*	+	0.17*
Zooxanthellae	0	=	-0.62*	-	(0.60)	+	1.22*

* Values calculated by subtraction

The partitioned equation shows that the energy expenditure of the anemone was very similar to the utilization of the anemone tissue. Losses were negligible. The negative energy expenditure of the zooxanthellae is an anomaly due to the low energy expenditure of the whole anemone. There was a net loss of zooxanthellae from the anemone. These expelled zooxanthellae were assumed to represent a loss of energy from this organism since there is no conclusive evidence that invertebrate hosts obtain significant nutritional benefit from digesting their zooxanthellae (Trench, 1979).

Schlichter (1975) has calculated that A. sulcata can obtain 0.0319-0.0374 cal.g wet weight^{-1}.h^{-1} from 90µg l^{-1} of glucose dissolved in sea water. Assuming that 10% of the wet weight of A. sulcata is organic weight, this amounts to 12.8-15.0 J.24h^{-1} for an anemone of 0.4g organic weight. Since the levels of organic matter dissolved in the sea water in which these animals were maintained is not known, the amount of organic matter taken up and utilized by the anemones cannot be estimated, however, these results suggest that A. sulcata can meet most of its energy requirements from catabolism of its own body reserves.

ii) Symbiotic anemones starved in light.

	Input		Expenditure		Retention		Losses
Overall Equations							
HLS (J.24h^{-1})	55.92	=	47.48	-	16.91	+	25.35*
LLS	22.60	=	37.45	-	18.09	+	3.24*
Partitioned Equations							
HLS Anemone	0	=	34.20	-	21.17*	+	25.35*
HLS Zooxanthellae	55.92	=	13.28*	38.38* +	4.26		
LLS Anemone	0	=	34.20	-	20.09*	+	3.24*
LLS Zooxanthellae	22.60	=	3.25*	17.35* +	2.00		

* Values calculated by subtraction

The experiments carried out on symbiotic A. sulcata starved in light were designed to determine how much photosynthesis by zooxanthellae contributed to the energy requirements of host and symbiont.

The partitioning of the photosynthetic energy production (P) is illustrated in Fig. 29. 14.4% and 23.8% of P was expended by the zooxanthellae while 7.6 and 8.9% of P was retained as biomass of zooxanthellae respectively at 70 and 140 $\mu E.m^{-2}.sec^{-1}$ (Table 24). P at both these irradiances was more than sufficient to meet the energy requirements of the algal symbionts since the energy retained as biomass of zooxanthellae increased in both anemones.

The amount of energy translocated to the anemones was calculated by assuming that the P which was not expended or retained by the zooxanthellae was translocated to the anemone. However this does not include energy lost in the form of expelled zooxanthellae. 68.6% and 76.8% of P was translocated by these anemones (Table 24). This is higher than the estimations of translocation, made from incubation of zooxanthellae in the presence of host homogenates, of Taylor (1969b) that 58% of the carbon fixed by zooxanthellae was translocated to A. sulcata and those of Muscatine (1967) and Muscatine et al (1972) that $\sim$40% of the carbon fixed by zooxanthellae was translocated to coelenterate and mollusc hosts.

P met 41.3% of the energy expenditure of the anemone alone (R_a) at 70 $\mu E.m^{-2}.sec^{-1}$ and 38.1% of this at 140 $\mu E.m^{-2}.sec^{-1}$. (Table 24). A. sulcata could not adopt a fully autotrophic existence, although P over 12h per day at 140 $\mu E.m^{-2}.sec^{-1}$ was theoretically sufficient to allow this. The percentage contributions to R_a in A. sulcata (Table 24) are lower than the estimations of Muscatine et al (1981) that typically 63-68% of carbon requirements of the corals Pocillopora damicornis and

Fig. 29 Partitioning of the photosynthetic energy input in 'stardard' symbiotic A. sulcata

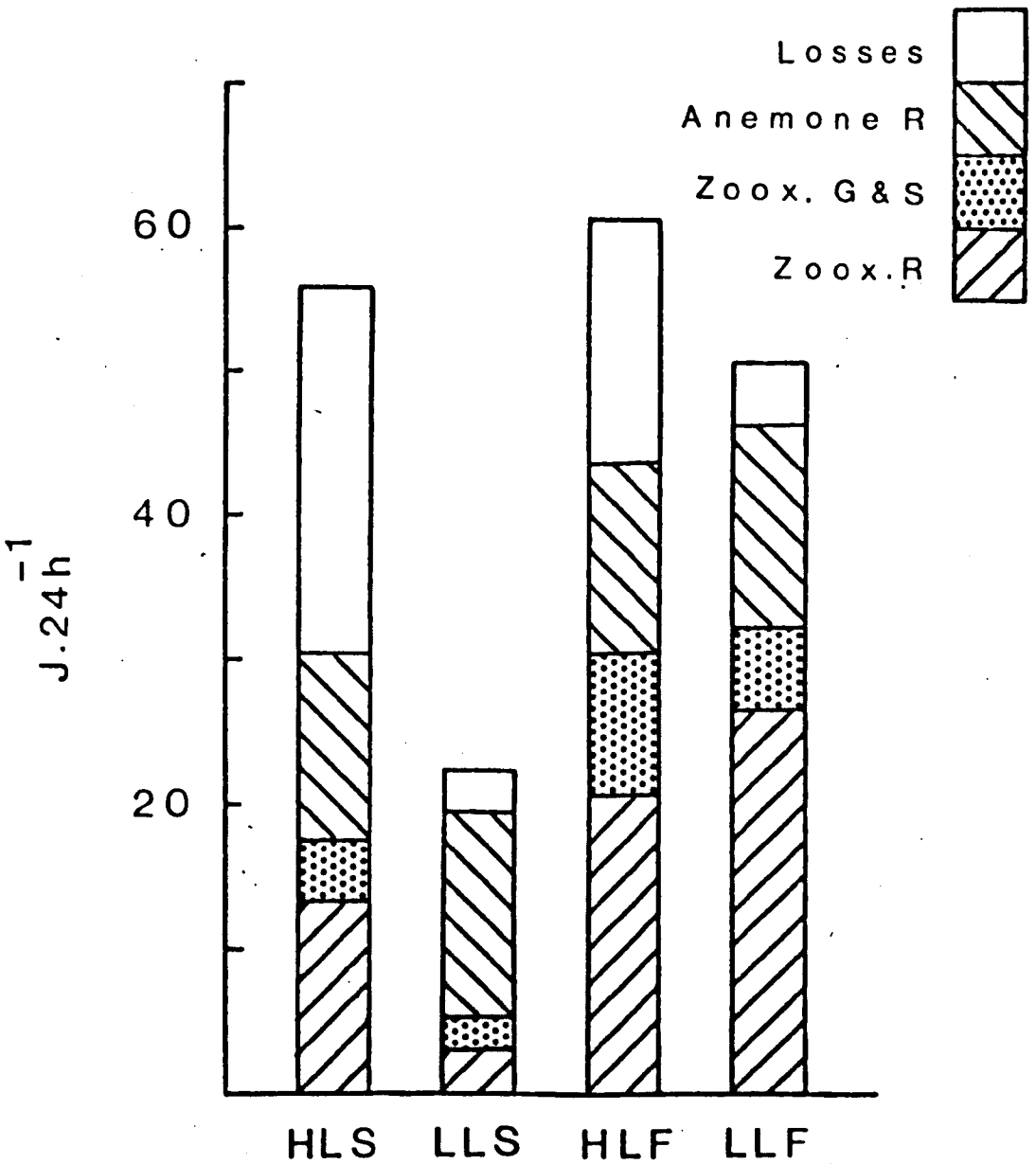


Table 24 Partitioning of the Photosynthetic energy input (P) in 'standard' symbiotic *Anemonia sulcata* expressed as a percentage of P

Stock	Energy expenditure of zooxanthellae (R_z)	Growth and storage of zooxanthellae	Energy expenditure of the anemone (R_a)	Losses	Translocation	Percentage contribution to total R	Percentage contribution to R_a
HLS	23.8	7.6	23.3	45.3	68.6	55.4	38.1
LLS	14.4	8.9	62.4	14.3	76.8	51.7	41.3
HLF	34.3	16.2	21.5 *	28.0 *	49.5	61.5	38.1
LLF	52.6	11.2	27.9 *	8.3 *	36.2	66.9	41.3
Mean	31.2	11.0	33.8	20.4	57.8	58.9	39.7

* Predicted values

Fungia scutaria were met by translocated products of photosynthesis and that of Trench et al (1981) that 62-84% of the carbon requirements of the clam Tridacna maxima was met by translocated products of photosynthesis. However, the methods of calculation used by Muscatine et al (1981) and Trench et al (1981) did not account for losses of photosynthetically produced carbon.

14.3% and 45.3% of P was lost from A. sulcata starved at 70 $\mu E.m^{-2}.sec^{-1}$ and 140 $\mu E.m^{-2}.sec^{-1}$ respectively (Table 24). The latter value is similar to the estimation of Crossland et al. (1980) that 40% of the carbon fixed by the coral Acropora acuminata was secreted as mucus. They have suggested that the corals could utilise only a certain amount of the carbon translocated by the zooxanthellae and that surplus carbon is secreted as mucus. It appears that A. sulcata could utilise only a certain amount of the photosynthetically produced energy, the surplus energy may have been secreted as mucus, as in A. acuminata.

iii) Symbiotic anemone fed in darkness

Overall Equation

	Input		Expenditure maintenance		S.D.A.		Retention		Losses
($J.24h^{-1}$)	99.59	=	38.74	+	6.75	+	28.20	+	25.90*

Partitioned Equation

Anemone	99.59	=	34.20	+	6.75	+	33.50*	+	25.14*
Zooxanthellae	0	=	4.54 *	+		-	(5.30)	+	0.76

*Values calculated by subtraction

The experiments carried out on symbiotic anemones fed in darkness were designed to determine how much of the energy requirements of host and symbiont were derived from carnivorous feeding.

The partitioning of the carnivorous energy input is illustrated in Fig. 30. 34.3% of the energy was expended on maintenance of the anemone (Table 25). 6.8% of the ingested squid was assumed to have been expended as specific dynamic action (S.D.A.) (Section 4C(ii)). It was assumed that S.D.A. increased in direct proportion to the energy intake, which has been observed in most species of fish (Jobling, 1981).

As the biomass of the anemone tissue increased, this input was more than sufficient to meet the energy requirements of the anemone. The gross growth efficiency (K_1) (Ivlev, 1939, 1939a, cited by Winberg, 1955) given in Table 25 was calculated with the equation;

1) $$K_1 = \frac{G_a \& S_a}{C} \times 100$$

where G_a & S_a = energy retained as growth and storage of the anemone alone

C = energy ingested

33.5% of the ingested squid was retained as growth and storage of the anemone (Table 25). This is higher than the 3-11% of ingested copepod retained as growth by the Ctenophores Pleurobrachia and Mnemiopsis (Reeve et al, 1978) but is similar to the gross growth efficiency of 37% for Hydra pseudologactis fed with Artemia nauplii (Schroeder, 1969) and for the Scyphozoans Cyanea capillata and Aurelia aurita (Fraser, 1969).

25.4% of this energy input was lost from the anemone (Table 25). The squid mantle used as food in this study had a high protein content (Section 4B). The utilization of this predominantly proteinaceous energy input may have resulted in large losses in the form of ammonia and other nitrogenous compounds in the process of food conversion as in the coral Astrangia danae (Szmant-Froelich & Pilson, 1977).

Fig. 30 Partitioning of the carnivorous energy input in 'standard' symbiotic and aposymbiotic A. sulcata

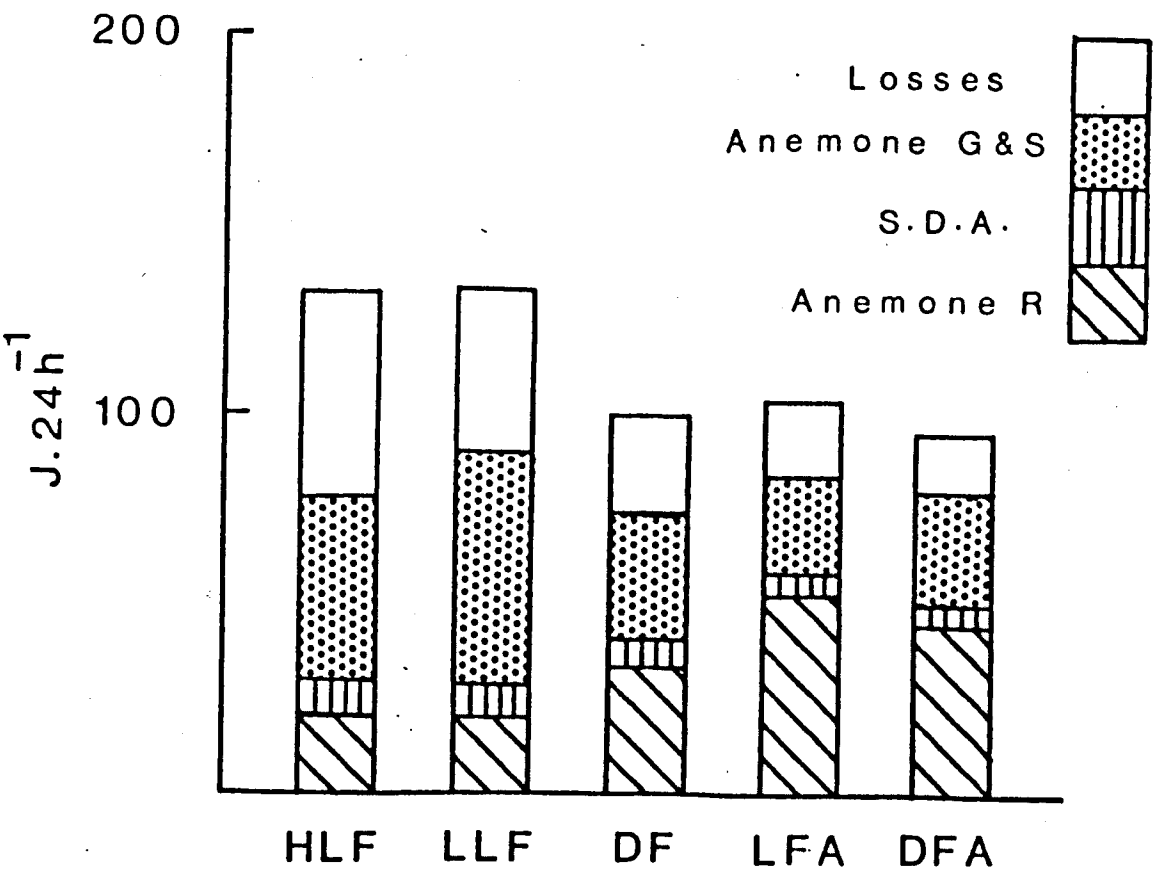


Table 25 Partitioning of the carnivorous energy input (C) in 'standard' symbiotic and aposymbiotic Anemonia sulcata expressed as a percentage of C

Stock	Energy Expenditure (R_a)	S.D.A.	Growth and storage of anemone (Gross growth efficiency, K_1)	Losses
HLF	16.0	6.8	37.3	40.0
LLF	15.0	6.8	45.8	32.4
DF	34.3	6.8	33.5	25.4
LFA	51.6	5.1	25.4	17.9
DFA	47.6	5.1	31.4	16.0

The energy retained as biomass of zooxanthellae decreased and this decrease was greater than that in symbiotic anemones starved in darkness, which indicates that the zooxanthellae derived little or no energy from this input, although it has been reported that ^{35}S in food ingested by the sea anemone Aiptasia pulchrella and Anthopleura elegantissima was transferred to their zooxanthellae (Cook, 1971 and Trench 1979) and the ^{35}S was transferred to the zooxanthellae of A. elegantissima in organic compounds (Trench, 1979).

iv) Symbiotic anemones fed in the light.

Overall equations

	Inputs		Expenditure: Maintenance		S.D.A.		Retention		Losses
HLF ($J.24h^{-1}$)	193.14	=	54.93	+	8.98	+	59.27	+	69.96*
LIF	184.07	=	60.77	+	9.05	+	66.85	+	47.40*

Partitioned Equations

HLF Anemone	132.63	=	34.20	+	8.98	+	49.97*	+	69.96*
HLF Zooxanthellae	60.51	=	20.73*	(29.98*)		+	9.80		
LLF Anemone	133.53	=	34.20	+	9.05	+	61.13*	+	47.40*
LLF Zooxanthellae	50.54	=	26.57*	(32.25*)		+	5.68		

* Values calculated by subtraction.

The experiments carried out on symbiotic anemones fed in light were designed to measure the contribution of photosynthesis and carnivorous feeding to the energy requirements of the symbionts.

The partitioning of the photosynthetic energy production (P) is illustrated in Fig. 29. 34.3% and 52.6% of P was expended by the zooxanthellae while 16.2% and 11.2% was retained as biomass of zooxanthellae respectively at 140 and 70 $\mu E.m^{-2}.sec^{-1}$ (Table 24). The amount of energy retained as biomass of zooxanthellae was higher

than in anemones starved at these irradiances (Table 24). 49.5% and 36.2% of P was translocated to the anemone in each stock. This is lower than the percentage translocated by starved anemones but is comparable with the estimates of percentage translocation of Taylor (1969b), Muscatine (1967) and Muscatine et al (1972). The percentage contribution of P to R_a was assumed to have been the same as that in the anemones starved at the same irradiance (Table 24). This energy was assumed to have been expended on maintenance of the anemone, rather than retained as growth and storage. This assumption is supported by the observations of Fitt & Pardy (1981) that the respiratory quotients of the sea anemone Anthopleura elegantissima, fed with brine shrimp, indicated metabolism of photosynthetically produced carbohydrate in symbiotic anemones maintained in light. The remainder of P was assumed to have been lost.

These anemones also received an input of energy from carnivorous feeding. Since some of the energy expenditure of the anemone (R_a) was assumed to have been met by translocated products of photosynthesis, these amounts had to be subtracted from R_a in the partitioning of the carnivorous energy input. The partitioning of this input is illustrated in Fig. 30. 16.0% and 15.0% of this input was expended on maintenance respectively at the two irradiances. S.D.A. was assumed to be the same percentage of this input as that in symbiotic anemones fed in darkness (Table 25).

More energy was retained by the anemone maintained at 70 $\mu E.m^{-2}.sec^{-1}$ and its gross growth efficiency (K_1) was higher than the anemone maintained at 140 $\mu E.m^{-2}.sec^{-1}$ (Table 25). Both these K_1 values were higher than that of the symbiotic anemone maintained in darkness (Table 25) and were comparable with K_1 values of higher invertebrates (Calow, 1977). This increased efficiency may have been achieved by recycling in the light of nitrogenous wastes from compounds catabolised during digestion, as in

the coral Astrangia danae (Szmant-Froelich & Pilson, 1977).

However the estimated losses from this input were greater in these anemones than in the symbiotic anemone maintained in darkness (Table 25). If it is assumed that the assimilation efficiency (Klekowski & Duncan, 1975) of symbiotic anemones was the same in light and darkness, then more assimilated energy must have been lost from the anemones maintained in light, perhaps as mucus. This suggests that the anemones could utilise only a certain amount of the energy input.

The contribution of this energy input to the zooxanthellae is not known. The increase in biomass of zooxanthellae was greater in these fed anemones than in the starved anemones, hence it is possible that the zooxanthellae may have obtained energy from the squid (c.f. Cook, 1971 and Trench, 1979) however the larger increase in biomass of zooxanthellae in fed anemones may simply have been due to the zooxanthellae having more space in which to reproduce in the growing fed anemone (Section 4D(ii)).

As the biomass of anemone and biomass of zooxanthellae both increased the combined inputs of photosynthesis and carnivorous feeding was more than sufficient to meet the energy requirements of both symbionts.

Photoadaptation

Experiments were carried out at two irradiances to determine whether the zooxanthellae in A. sulcata photoadapted under laboratory conditions. Photoadaptation or "sun and shade" adaptation involves elevation of rates of photosynthesis at low irradiances (Ryther & Menzel, 1959). Photoadaptation has been observed in reef corals in relation to depth and shading. "Shade adapted" Stylophora pistillata from deeper waters photosynthesised at a higher rate than "sun adapted" specimens from shallow waters at any given irradiance. This difference may have been

partly due to specimens from deeper waters having more chlorophyll a per zooxanthella than those from shallower waters (Falkowsky & Dubinsky, 1981).

Davies (1977) has observed saturation of photosynthesis at lower irradiances in corals from deeper waters than in corals from shallow waters. Wethey & Porter (1976), Davies (1977) and Falkowski & Dubinsky (1981) have found that respiratory oxygen consumption of corals from deeper waters was lower than specimens from shallow waters. Wethey & Porter (1976) have found that the ratio of photosynthetic oxygen production to respiratory oxygen consumption is constant regardless of depth and have suggested that translocation of products of photosynthesis is increased with depth or shade. Muscatine (1980) has concluded from these observations that the net nutritional benefit of translocation to an invertebrate host could be invariate with depth and irradiance once the zooxanthellae had adapted to the light conditions.

There is some evidence of photoadaptation in Anemonia sulcata under the laboratory conditions of this study. The energy expenditure of anemones starved at 70 $\mu E.m^{-2}.sec^{-1}$ was lower than that of anemones starved at 140 $\mu E.m^{-2}.sec^{-1}$, however the opposite was true in fed anemones. The rate of photosynthesis was elevated at 70 $\mu E.m^{-2}.sec^{-1}$ in fed anemones but was not elevated in starved anemones at this irradiance. The contribution of the translocated products of photosynthesis to the energy requirements for maintenance (R_a) of starved anemones, measured as percentage contribution to R_a (Table 24) were very similar at both irradiances.

These results are similar to those of Svoboda & Poorman (1980) who have shown that the oxygen consumption ($\dot{M}_{O_2}$) of Aiptasia diaphana harbouring zooxanthellae was higher in anemones maintained at 86 $\mu E.m^{-2}.sec^{-1}$

than in those which were maintained at 15 $\mu E.m^{-2}.sec^{-1}$. However these authors have suggested that the elevated $\dot{M}_{O_2}$ in anemones maintained at 86 $\mu E.m^{-2}.sec^{-1}$ was due to an effect of photosynthesis on the $\dot{M}_{O_2}$ similar to that caused by carnivorous feeding, i.e. there was a "specific dynamic action" associated with the utilization of the translocated products of photosynthesis. They also found that photosynthetic oxygen production per µg chlorophyll was higher in animals adapted to the lower irradiance. This could not be attributed to changes in algal density or concentration of chlorophyll per zooxanthella.

B) Energy flow through aposymbiotic Anemonia sulcata

Introduction

The experiments carried out on aposymbiotic anemones were designed to compare energy flow through aposymbiotic anemones with energy flow through symbiotic anemones maintained in darkness. The values of carnivorous energy input, energy expenditure and energy retention of 'standard' aposymbiotic anemones determined in Section 5 are compiled in this section in the form of energy balance equations. Losses were calculated by subtraction as in symbiotic anemones.

i) Aposymbiotic anemones starved in light and in darkness

	Input		Expenditure		Retention		Losses
LSA $(J.24h^{-1})$	0	=	19.93	-	46.41	+	26.48*
DSA	0	=	18.73	-	32.09	+	13.36*

*Values obtained by subtraction.

The decrease in energy content of the tissue of aposymbiotic anemones was greater than the amount used for energy expenditure. The predicted losses of the starved aposymbiotic were much higher than the losses calculated for symbiotic anemones maintained in darkness. This

suggests that A. sulcata obtained some energetic benefit from harbouring zooxanthellae, even in darkness.

The ability of corals with zooxanthellae to take up and utilise ammonia from sea water continues in darkness (Lewis & Smith, 1971). Prolonged exposure of the coral Pocillopora damicornis to darkness led to a net excretion of ammonia, however the ammonia excretion of P. damicornis was lower than that of the non-symbiotic coral Tubastrea aurea (Muscatine & D'Elia, 1978). Kawaguti (1953) and Szmant-Froelich & Pilson (1977) have also shown that corals with zooxanthellae excreted less ammonia in darkness than corals without zooxanthellae, while Cates & McLaughlin (1976) have shown that specimens of the sea anemone Condylactus with zooxanthellae excrete less ammonia in darkness than aposymbiotic specimens. Similar recycling abilities may have reduced losses from symbiotic A. sulcata during starvation in darkness.

The small differences between the energy flow through aposymbiotic anemones maintained in light and those maintained in darkness could not have been due to photosynthesis or related processes. Photosynthesising organisms in the sea water may have enriched the energy sources of dissolved and particulate organic matter in illuminated tanks. However weight loss was greater in the starved anemone exposed to light.

ii) Aposymbiotic anemones fed in light and in darkness

	Input	Expenditure			Retention		Losses
		Maintenance		S.D.A.			
LFA	102.92	53.11	+	5.24	+ 26.16	+	18.41*
DFA	94.67	45.04	+	4.82	+ 29.63	+	15.13*

($J.24h^{-1}$)

* Values calculated by subtraction

The partitioning of the carnivorous energy input is illustrated in Fig. 30. 51.6% and 47.6% of this input was expended on maintenance

respectively in the two stocks (Table 25). This is higher than the values for symbiotic anemones (Table 25). Values of S.D.A. were calculated at 5.1% of the ingested energy, the mean value determined from aposymbiotic anemones fed in darkness.

As the biomass of the anemones increased, this input was more than sufficient to meet the energy requirements of the aposymbiotic anemones. The gross growth efficiencies (K_1) were lower than symbiotic anemones fed in darkness (Table 25). The K_1 value for the aposymbiotic anemone exposed to light is lower than the aposymbiotic anemone maintained in darkness. This difference cannot be attributed to photosynthesis or related processes. Since the squid used as food for the anemones had a high protein content (Section 4B), the recycling of nitrogenous wastes may have contributed to the higher K_1 values of the symbiotic anemones.

However, the predicted losses from this input were lower in aposymbiotic anemones than in symbiotic anemones (Table 25). If it is assumed that the assimilation efficiency (Klekowski &Duncan, 1975) of aposymbiotic anemones was the same as that of symbiotic anemones, then less assimilated energy must have been lost from the aposymbiotic anemones.

Most of the differences between energy flow through aposymbiotic anemones exposed to light and those maintained in darkness were negligible.

Section 7 General Discussion: The contribution of zooxanthellae to the energetic requirements of the sea anemone Anemonia sulcata

The advantages to an alga and an invertebrate of establishing and maintaining a symbiotic association are reviewed in Section 1. The energetic advantages of the symbiotic association found in A. sulcata over non symbiotic sea anemones is assessed in this discussion.

Symbiotic A. sulcata have two energetic advantages from harbouring zooxanthellae over non symbiotic anemones: an ability to photosynthesise and an ability to recycle nutrients (Smith, 1939).

The contribution of photosynthesis by the zooxanthellae to the energy requirements of host and symbiont was investigated in this study. Photosynthetic energy production met between 42 - 67% of energy expenditure on maintenance of the whole organism (Table 24) hence the anemones could not adopt a fully autotrophic existence under the experimental conditions of this study.

The anemones required a carnivorous energy input to grow. The gross growth efficiency was highest in symbiotic anemones maintained in light. This efficiency was lower in symbiotic anemones maintained in darkness and was lowest in aposymbiotic anemones. These differences may have been due to an ability to recycle nitrogenous wastes in symbiotic anemones.

The gross growth efficiencies of symbiotic anemones maintained in light were similar to that of higher invertebrates reviewed by Calow (1977). Since this anemone is a highly adapted carnivore which probably feeds on herbivorous zooplankton, it is at a relatively high position in the marine food web. The presence of zooxanthellae has been shown to increase growth efficiency, which may allow it to compete more successfully with

other carnivores including higher invertebrates in subtidal and intertidal waters. Unfortunately there is no published data available on the gross growth efficiency of non-symbiotic sea anemones for comparison.

It appears that the assumed ability to recycle nitrogenous wastes is just as important to the association as the contribution of photosynthesis by the zooxanthellae. The most important contribution of the photosynthetic energy production may be to meet some of the energy requirements of the anemone when zooplankton is unavailable or available in limited amounts.

In order to determine how representative the findings and conclusions of this study are of the energy flow through A. sulcata in its natural habitat, experiments could be carried out in situ, in which the following parameters are measured.

1) Photosynthetic oxygen production
2) Plankton and dissolved nutrient availability
3) Respiratory oxygen consumption
4) Growth of the anemone
5) Reproduction of the zooxanthellae
6) Rates of mucus secretion
7) Excretion of nitrogenous wastes

There is also a need for parallel studies on non-symbiotic sea anemones such as Actinia, Tealia and Calliactis.

In addition, further laboratory experiments could be carried out to determine the accuracy of some of the predicted values in

Section 6 including

1) Rates of mucus secretion
2) Rates of expulsion of zooxanthellae
3) Excretion and recycling of nitrogenous wastes
4) CO_2 fluxes from which respiratory and photosynthetic quotients may be determined
5) Photosynthesis, oxygen consumption, reproduction and excretion of isolated zooxanthellae

A more thorough investigation of specific dynamic action in symbiotic and aposymbiotic anemones is required in which this phenomenon is investigated in relation to animal size, meal size, meal composition, feeding frequency, rates of growth, rates of digestion and rates of nitrogenous excretion to determine whether this represents the energy cost of growth.

The principles used originally by Odum and Odum (1955) have been used to construct a descriptive bioenergetic model of an algal-invertebrate symbiosis. Models of this type will be improved by the development and use of more refined techniques including the use of automated systems under microprocessor control. In future, predictive models of this type should be constructed for algal-invertebrate symbioses of commercial importance such as corals and Tridacnid clams, with which predictions of the effect of changes in energy availability, irradiance or some other changes in the environment on the standing crop of these organisms and their contribution to other organisms such as invertebrates and fish (Benson & Muscatine, 1974).

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Appendix 1

The use of the Principle of Archimedes for determining biomass

An accurate, repeatable method of measuring the biomass of the sea anemone Anemonia sulcata was necessary for experiments in this study. The most useful measurement of biomass is the organic weight which can be determined as the ash-free dry weight by sacrificing the anemone. The buoyant weight technique, which is based on the Principle of Archimedes (Jokeil et al, 1978), has been used to determine the weight of sea anemones by Muscatine (1961), Taylor (1969a) and Fitt and Pardy (1981).

The principle of Archimedes states that: "The weight of an object in water (W_w) is equal to its weight in air (W_a) minus the weight of water (W_{H_2O}) it displaces".

that is (1) $W_w = W_a - W_{H_2O}$

W_{H_2O} is equal to the volume of water displaced times the density of the water (D_w).

Since the volume of a submerged object (V_a) is equal to the volume of water it displaces it follows that

$$(2) \quad W_w = W_a - V_a D_w$$

since $V_a = W_a D_a^{-1}$ (where D_a is the density of the object)

it follows that

$$(3) \quad W_w = W_a - D_w W_a D_a^{-1}$$

$$\therefore (4) \quad W_w = W_a (1 - D_w D_a^{-1})$$

The buoyant weight (W_w) of a marine invertebrate, such as Anemonia sulcata, recorded in sea water consists of two parts; that due to organic matter and that due to intracellular water and salt. Hence equation 4 can be expanded to

$$(5)\quad W_w = W_d\,(1-D_w\,D_d^{-1}) + W_i\,(1-D_w\,D_i^{-1})$$

(organic matter) (intracellular water and salt)

where W_d = dry weight of organic matter in air

D_d = density of the dry organic matter

W_i = weight of intracellular water and salt

D_i = density of intracellular water and salt

The intracellular water and salt will not contribute to the W_w if it has the same density as the surrounding water, i.e. $D_i = D_w$. In this case, the organic matter alone contributes to the W_w and equation 5 simplifies to

$$(6)\quad W_w = W_d\,(1-D_w\,D_d^{-1})$$

or

$$(7)\quad W_d = W_w \times \frac{1}{(1-D_w\,D_d^{-1})}$$

Equation 7 shows that it is theoretically possible to establish a relationship between W_d measured as the ash-free dry weight and W_w measured as the buoyant weight in sea water of density D_w for any one organism. This relationship can then be used to calculate the W_d from the W_w recorded in subsequent experiments.

Materials and Methods

The buoyant weights in sea water (SW) and ash-free dry weights in air of 18 symbiotic Anemonia sulcata were measured to establish the relationship between these two variables over a range of buoyant weights.

1) Buoyant Weight

The weights of whole A. sulcata submerged in 1 l. of SW of specific gravity of 1.022 $\pm$ 0.001 at 12 $\pm$ 2°C were recorded to the

nearest 1.0mg with a torsion balance (Gallenkamp Model 0) zeroed in SW. The anemones were attached to the balance by a metal clip on a length of nylon thread. The clip was attached to the base of the animals to minimise the damage inflicted.

2) Dry weight

Whole A. sulcata were dried on preweighed aluminium trays at 110°C for 24h in an oven. Dry weights were recorded to the nearest 0.1mg after the material had cooled for 24h over silica gel.

3) Ash weight

After weighing, predried tissue was ashed by heating at 500°C for 6h in a muffle furnace. Samples were allowed to cool to 100-200°C before transferring them to a desiccator. Weights were recorded to the nearest 0.1mg after 24h storage over silica gel. Ash weight was subtracted from oven-dried weight to give ash-free dry weight.

Fig. A shows the relationship between buoyant weight (W_w) and ash-free dry weight (W_d). This had a highly significant correlation coefficient of +0.995 with 16 degrees of freedom for which $P < 0.001$. The equation of the line was

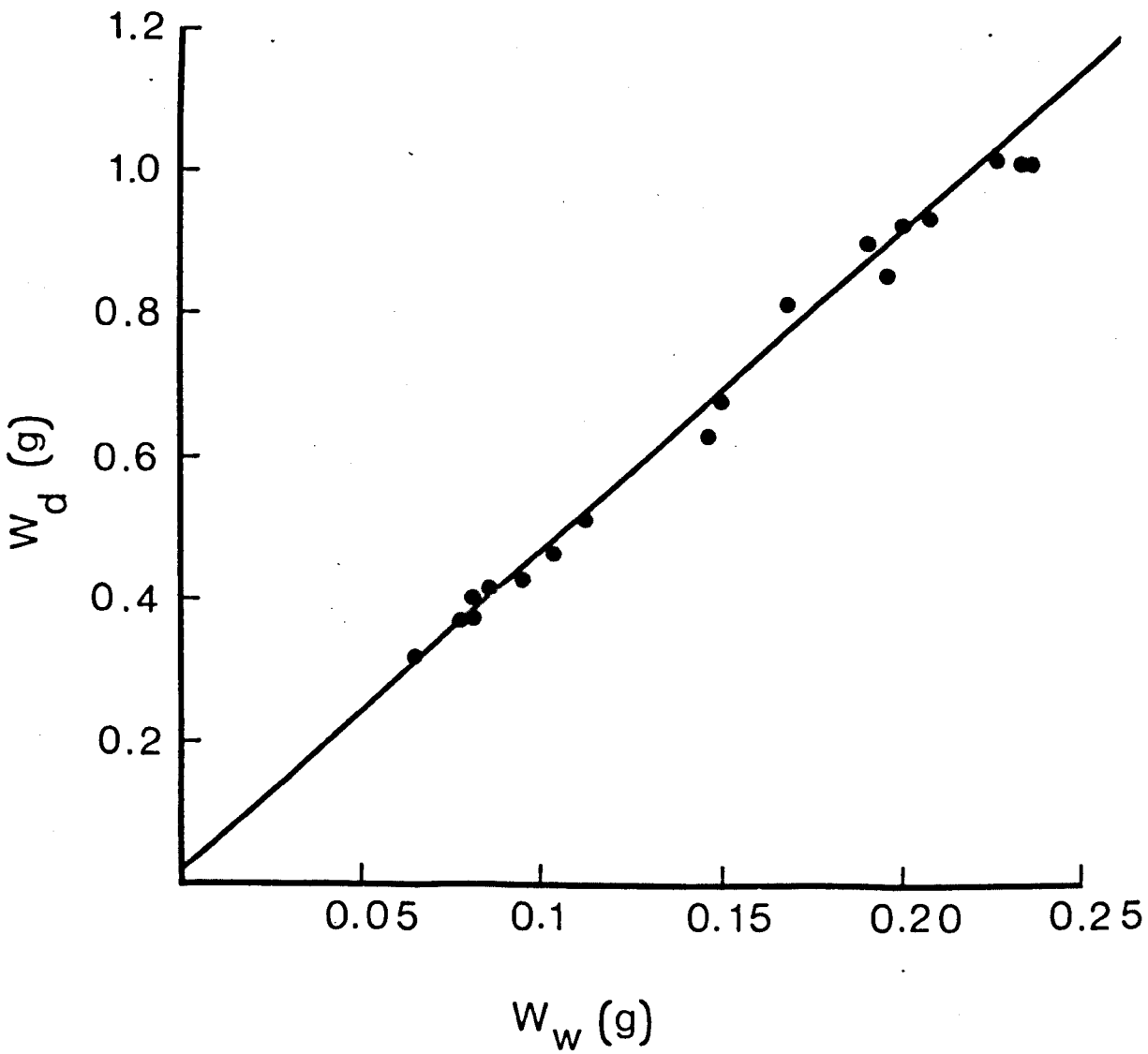
(8) $W_d = 4.472\ W_w + 0.017g$ where W_d is in g
W_w is in g

This equation was used for the calculation of the W_d of anemones from their W_w in all subsequent experiments.

The theoretical dry volume (W_d) of the anemones was used in the calculation of oxygen consumption (Section 2). Since $V_d = W_d\ D_d^{-1}$ and the mean ($\pm$ S.D.) D_d of A. sulcata, calculated with equation 7, was 1.305 ± 0.015, the values of V_d were calculated from the W_d with the equation

9) $$V_d = \frac{W_d}{1.305}$$

Fig. A The relationship between buoyant weight (W_w) and organic weight (W_d) in symbiotic *A. sulcata*



Appendix 2

The relationship between oxygen consumption and weight loss during starvation

The relationship between oxygen consumption ($\dot{M}_{O_2}$) and body weight (W) is a power function described by the equation

1) $\dot{M}_{O_2} = aW^b$

In the sea anemone Anemonia sulcata b≈1 (Section 3), therefore the $\dot{M}_{O_2}$ increases in direct proportion to W

2) $\dot{M}_{O_2} = aW$

The rate of change in $\dot{M}_{O_2}$ $\left(\frac{d\dot{M}_{O_2}}{dt}\right)$ during starvation is some function of $\dot{M}_{O_2}$

3) $\frac{d\dot{M}_{O_2}}{dt} = -k\,\dot{M}_{O_2}$ where k is a constant

by rearrangement 4) $\frac{d\dot{M}_{O_2}}{\dot{M}_{O_2}} = -k\,dt$

5) $\left(\frac{d\dot{M}_{O_2}}{\dot{M}_{O_2}}\right) = -k\,dt$

6) $\ln \dot{M}_{O_2} = -kt + C$ initial conditions at $t = 0$ $\dot{M}_{O_2} = (\dot{M}_{O_2})_o$

$\therefore\ C = \ln (\dot{M}_{O_2})_o$

7) $\ln \dot{M}_{O_2} = -kt + \ln (\dot{M}_{O_2})_o$

8) $\ln \frac{\dot{M}_{O_2}}{(\dot{M}_{O_2})_o} = -kt$

9) $\dot{M}_{O_2} = (\dot{M}_{O_2})_o e^{-kt}$

from equation 2 10) $W = W_o e^{-kt}$ where $W_o = \frac{(\dot{M}_{O_2})_o}{a}$

$\therefore$ Weight will decrease exponentially with time during starvation

The logarithmic transformation of equation 10 yields the straight line

$$11)\quad \ln W = \ln W_o - kt$$

since $\ln = 2.3026 \log_{10}$

$$12)\quad \log_{10} W = \log_{10} W_o - \frac{k}{2.3026}\, t$$

This equation was used to determine values of k in Section 4D and 5C

Appendix 3

Regression lines relating log oxygen consumption ($\dot{M}_{O_2}$) to log organic weight (W_d) in symbiotic and aposymbiotic Anemonia sulcata and comparison of the slopes and elevations of these lines by analysis of covariance.

Table A Regression equations, calculated by the method of least squares, of log $\dot{M}_{O_2}$ on log W_d in six stocks of symbiotic Anemonia sulcata

Stock	Range of W_d(g)	numbers of animals	number of determinations (n)	Equation of regression line
1) HLS	0.116 - 0.487	6	24	Y = 1.0766 + 1.0221X
2) LLS	0.106 - 0.634	6	24	Y = 0.7972 + 0.7384X
3) DS	0.088 - 0.334	7	28	Y = 0.7837 + 0.8082X
4) HLF	0.419 - 1.068	4	16	Y = 1.1111 + 1.1549X
5) LLF	0.478 - 0.665	3	12	Y = 0.6846 - 0.7385X
6) DF	0.245 - 0.919	5	20	Y = 0.9557 + 1.0111X

$Y = \log \dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$) $\quad X = \log W_d$ (g)

Table B Comparison of slopes of lines relating log $\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$) to log W_d (g) in six stocks of symbiotic Anemonia sulcata by analysis of covariance

Lines Compared (from Table A)	F Ratio	degrees of freedom		Combined Regression Coefficient (β)
1v2v3v4v5v6	2.286	5 & 112	P = 0.005	-
1v2v3v4v6	1.244	4 & 102	0.25 < P < 0.5 n.s.	0.8859

n.s. = not significant

Table C Comparison of elevations of lines relating log $\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$) to log W_d (g) in six stocks of symbiotic Anemonia sulcata by analysis of covariance.

Lines Compared (from Table A)	F Ratio	degrees of freedom	
1v2v3v4v6	6.099	4 & 106	$P < 0.001$
1v2v3	7.254	2 & 72	$0.001 < P < 0.005$
4v6	6.785	1 & 33	$0.01 < P < 0.025$
1v4	0.041	1 & 37	$P > 0.75$ n.s.
3v6	1.071	1 & 45	$0.25 < P < 0.5$ n.s.

n.s. = not significant

Table D Regression equations, calculated by the method of least squares, of log $\dot{M}_{O_2}$ on log W_d in four stocks of aposymbiotic Anemonia sulcata

Stock	Range of W_d(g)	number of animals	number of determinations (n)	Equation of regression line Y = a + b X
1) LSA	0.089 - 0.1426	5	20	Y = 0.3590 + 0.5983X
2) DSA	0.089 - 0.1426	5	20	Y = 0.7801 + 1.0668X
3) IFA	0.2097 - 0.2320	3	12	Y = 1.2483 + 1.1606X
4) DFA	0.2097 - 0.3260	3	12	Y = 1.1182 + 1.0927X

$Y = \log \dot{M}_{O_2}$ (μmol $O_2 . h^{-1}$) $\quad X = \log W_d$ (g)

Table E Comparison of slopes of lines relating log $\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$) to log W_d (g) in four stocks of aposymbiotic Anemonia sulcata

Lines Compared (from Table D)	F Ratio	degrees of freedom		Combined Regression Coefficient (β)
1v2v3v4	0.345	3 & 56	P > 0.75 n.s.	0.9009

n.s. = not significant

Table F Comparison of elevations of lines relating log $\dot{M}_{O_2}$ (μmol $O_2 \cdot h^{-1}$) to log W_d (g) in four stocks of aposymbiotic Anemonia sulcata

Lines Compared (from Table D)	F Ratio	degrees of freedom	
1v2v3v4	11.837	3 & 59	P < 0.001
1v2	0.527	1 & 37	0.25 < P < 0.5 n.s.
3v4	1.214	1 & 21	0.25 < P < 0.5 n.s.
1v3	11.524	1 & 29	0.001 < P < 0.005
2v4	11.425	1 & 29	P < 0.001

n.s. = not significant

Appendix 4

Regression lines relating to buoyant weight (W_w) and log W_w to time in symbiotic and aposymbiotic Anemonia sulcata and comparison of the slopes of these lines by analysis of covariance.

Table G Regression equations of $\log_{10} W_w$ on time calculated by the method of least squares in three stocks of starved symbiotic Anemonia sulcata

Animal No.	Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	n	Equation of regression line $Y = a + b X$
1)	HLS	140	13	$Y = -0.9397 - 1.1730 \times 10^{-3}X$
2)	"	"	13	$Y = -1.3191 - 7.8940 \times 10^{-4}X$
3)	"	"	13	$Y = -1.1478 - 3.1683 \times 10^{-4}X$
4)	"	"	13	$Y = -1.2651 - 1.5320 \times 10^{-3}X$
5)	"	"	13	$Y = -1.5572 - 9.9605 \times 10^{-4}X$
6)	"	"	13	$Y = -1.3168 - 6.9677 \times 10^{-4}X$
7)	LLS	70	13	$Y = -1.3596 - 1.5118 \times 10^{-3}X$
8)	"	"	13	$Y = -1.2971 - 1.4582 \times 10^{-3}X$
9)	"	"	13	$Y = -0.7606 - 9.3543 \times 10^{-4}X$
10)	"	"	13	$Y = -1.0793 - 1.0235 \times 10^{-3}X$
11)	"	"	13	$Y = -1.2644 - 1.0072 \times 10^{-3}X$
12)	"	"	13	$Y = -1.3041 - 1.2431 \times 10^{-3}X$
13)	"	"	13	$Y = -1.6928 - 1.1421 \times 10^{-4}X$
14)	DS	0	13	$Y = -1.5166 - 1.8163 \times 10^{-3}X$
15)	"	"	13	$Y = -1.1820 - 2.2904 \times 10^{-3}X$
16)	"	"	13	$Y = -1.1459 - 2.2862 \times 10^{-3}X$
17)	"	"	13	$Y = -1.6829 - 2.4379 \times 10^{-3}X$
18)	"	"	13	$Y = -1.0655 - 2.2616 \times 10^{-3}X$
19)	"	"	13	$Y = -1.5095 - 2.0202 \times 10^{-3}X$
20)	"	"	13	$Y = -1.4813 - 2.2515 \times 10^{-3}X$

$Y = \log_{10} W_w$ (g) $\quad X$ = Time (days)

Table H Comparison of slopes of lines relating $\log_{10} W_w$ (g) to time (days) in three stocks of starved symbiotic Anemonia sulcata by analysis of covariance. For comparison of stocks, sums of squares and sums of products of deviations from regression were pooled.

Lines Compared (from Table G)	Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	F Ratio	Degrees of freedom		Combined Regression Coefficient (β)
1v2v3v4v5v6	HLS	140	1.456	5 & 66	$0.1 < P < 0.25$ n.s.	-9.173×10^{-4}
7v8v9v10v11v12v13	LLS	70	1.246	6 & 77	$0.25 < P < 0.5$ n.s.	-1.009×10^{-3}
14v15v16v17v18v19	DS	0	0.512	6 & 77	$P > 0.75$ n.s.	-2.195×10^{-3}
Stocks Compared						
DS v HLS	-	-	72.607	1 & 154	$P < 0.001$	-
DS v LLS	-	-	11.553	1 & 166	$P < 0.001$	-
HLS v LLS	-	-	1.547	1 & 154	$0.1 < P < 0.25$ n.s.	-

n.s. = not significant

Table I Regression equations of W_w on time calculated by the method of least squares in three stocks of fed symbiotic Anemonia sulcata

Animal No.	Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	n	Equation of regression line Y = a + b X
1)	HLF	140	13	$Y = 0.06185 + 6.4992 \times 10^{-4}X$
2)	"	"	13	$Y = 0.05269 + 6.3736 \times 10^{-4}X$
3)	"	"	13	$Y = 0.02839 + 6.4992 \times 10^{-4}X$
4)	"	"	13	$Y = 0.21908 + 6.0047 \times 10^{-4}X$
5)	LLF	70	13	$Y = 0.09772 + 6.8053 \times 10^{-4}X$
6)	"	"	13	$Y = 0.04523 + 7.2527 \times 10^{-4}X$
7)	"	"	13	$Y = 0.07353 + 7.8022 \times 10^{-4}X$
8)	DF	0	13	$Y = 0.05249 + 2.8493 \times 10^{-4}X$
9)	"	"	13	$Y = 0.05607 + 3.3516 \times 10^{-4}X$
10)	"	"	13	$Y = 0.01763 + 3.1633 \times 10^{-4}X$
11)	"	"	13	$Y = 0.02956 + 1.6797 \times 10^{-4}X$

$Y = W_w$ (g)

X = Time (days)

Table J Comparison of slopes of lines relating W_w (g) to time (days) in three stocks of fed symbiotic Anemonia sulcata by analysis of covariance. For comparison of stocks, sums of squares and sums of products of deviations from regression were pooled.

Lines Compared (from Table I)	Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	F Ratio	Degrees of freedom		Combined Regression Coefficient (β)
1v2v3v4	HLF	140	0.166	3 & 44	$0.5 < P < 0.75$ n.s.	$+6.344 \times 10^{-4}$
5v6v7	LLF	70	0.859	2 & 33	$0.25 < P < 0.5$ n.s.	$+7.297 \times 10^{-4}$
8v9v10v11	DF	0	3.122	3 & 44	$0.025 < P < 0.005$	-
8v9v10	DF	0	0.297	2 & 33	$0.5 < P < 0.75$ n.s.	$+3.121 \times 10^{-4}$
Stocks Compared						
HLF v LLF	-	-	4.926	1 & 82	$0.025 < P < 0.05$	-
HLF v DF	-	-	66.564	1 & 82	$P < 0.001$	-
LLF v DF	-	-	104.957	1 & 70	$P < 0.001$	-

n.s. = not significant

Table K Regression equations of $\log_{10} W_w$ on time calculated by the method of least squares in two stocks of starved aposymbiotic Anemonia sulcata

Animal No.	Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	n	Equation of regression line Y = a + b X
1)	LSA	140	13	$Y = -1.4307 - 2.7815 \times 10^{-3}X$
2)	"	"	13	$Y = -1.4953 - 1.7537 \times 10^{-3}X$
3)	"	"	13	$Y = -1.5484 - 3.7905 \times 10^{-3}X$
4)	"	"	13	$Y = -1.4083 - 2.3004 \times 10^{-3}X$
5)	"	"	13	$Y = -1.5599 - 3.2821 \times 10^{-3}X$
6)	DSA	0	13	$Y = -1.6262 - 1.9164 \times 10^{-3}X$
7)	"	"	13	$Y = -1.4704 - 1.9914 \times 10^{-3}X$
8)	"	"	13	$Y = -1.4066 - 2.4524 \times 10^{-3}X$
9)	"	"	13	$Y = -1.5898 - 1.2608 \times 10^{-3}X$
10)	"	"	13	$Y = -1.4751 - 1.9859 \times 10^{-3}X$

$Y = \log W_w$ (g)

X = Time (days)

Table L Comparison of slopes of lines relating $\log_{10} W_w$ (g) to time (days) in two stocks of starved aposymbiotic Anemonia sulcata by analysis of covariance. For comparison of stocks, sums of squares and sums of products of deviations from regression were pooled.

Lines Compared (from Table K)	Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	F Ratio	degrees of freedom		Combined Regression Coefficient (β)
1v2v3v4v5	LSA	140	1.967	4 & 55	$0.1 < P < 0.25$ n.s.	-2.782×10^{-3}
6v7v8v9v10	DSA	0	1.867	4 & 55	$0.1 < P < 0.25$ n.s.	-1.921×10^{-3}
Stocks Compared						
LSA v DSA	-	-	8.234	1 & 118	$0.001 < P < 0.005$	-

n.s. = not significant

Table M Regression equation of W_w on time calculated by the method of least squares on two stocks of fed aposymbiotic Anemonia sulcata

Animal No.	Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	n	Equation of regression line Y = a + b X
1)	LFA	140	13	$Y = 0.02408 + 2.9827 \times 10^{-4}X$
2)	"	"	13	$Y = 0.02697 + 3.0848 \times 10^{-4}X$
3)	"	"	13	$Y = 0.03158 + 2.6452 \times 10^{-4}X$
4)	DFA	0	13	$Y = 0.02307 + 3.0063 \times 10^{-4}X$
5)	"	"	13	$Y = 0.04404 + 3.5243 \times 10^{-4}X$
6)	"	"	13	$Y = 0.03287 + 3.3830 \times 10^{-4}X$

$Y = W_w$ (g)

X = Time (days)

Table N Comparison of slopes of lines relating W_w (g) to time (days) in two stocks of fed aposymbiotic *Anemonia sulcata* by analysis of covariance. For comparison of stocks, sums of squares and sums of products of deviations from regression were pooled.

Lines Compared (from Table M)	Stock	Irradiance ($\mu E.m^{-2}.sec^{-1}$)	F Ratio	degrees of freedom		Combined Regression Coefficient (β)
1v2v3	LFA	140	2.266	2 & 33	$0.1 < P < 0.25$ n.s.	$+2.904 \times 10^{-4}$
4v5v6	DFA	0	0.670	2 & 33	$0.5 < P < 0.75$ n.s.	$+3.305 \times 10^{-4}$
Stocks Compared						
LFA v DFA	-	-	3.696	1 & 70	$0.05 < P < 0.1$ n.s.	-

n.s. = not significant

Appendix 5

Isolation and purification of zooxanthellae by density gradient centrifugation with the silica sol Percoll

Introduction

The energy retained as biomass of zooxanthellae could not be measured directly in this study on Anemonia sulcata. This was estimated by multiplying the change in the number of zooxanthellae in 'standard' anemones by the organic weight of a zooxanthella and then multiplying the change in organic weight by the energy content per mg. organic weight of zooxanthellae (Section 4D (ii)).

This required the development of methods of isolating zooxanthellae from the anemone tissue. Several methods were already in existence, including those of McLaughlin & Zahl (1959) and Muscatine (1967). In these methods, tissue containing zooxanthellae is homogenised in sea water or an artificial saline. Zooxanthellae are then extracted from the homogenate by repeated centrifugation and washing with saline.

Samples of zooxanthellae obtained from A. sulcata, using the method of Muscatine (1967), were heavily contaminated with animal debris and nematocyst cells. The mucus secreted by the anemone in copious amounts was largely responsible for this, causing frothing of solutions and clumping of cells.

Isolation of the zooxanthellae was improved by the removal of calcium ions, and the addition of EDTA to the tris buffered artificial saline, used to homogenise and wash the material. Isolation was not improved by repeated resuspension and centrifugation or by the addition of bovine serum albumin. These modifications permitted the quantitative

recovery of zooxanthellae by the method described in Section 2. The samples of zooxanthellae recovered were pure enough for counting total numbers with a haemacytometer but not pure enough for determining organic weight or energy content of the zooxanthellae.

Samples of pure isolated zooxanthellae were recovered using density gradient centrifugation with sucrose by the method of Franker (1970) but this method did not give consistent results.

Recovery of samples of pure isolated zooxanthellae was consistently achieved using density gradient centrifugation with the silica sol "Percoll" (Pharmacia) by a method based on that of Price et al (1978).

Materials and Methods

Reagents 1) Tris buffered Sorbitol Artificial Saline (TBSAS)

Sorbitol	0.5M
NaCl	0.15M
KCl	0.01M
EDTA	0.005M
Tris HCl)	0.05M
Tris Base)	pH 7.8 at 20°C

Specific Gravity = 1.05

2) Tris buffered Sorbitol Percoll Saline (TBSPS)

Sorbitol	0.5M
NaCl	0.15M
KCl	0.01M
EDTA	0.005M
Tris HCl)	0.05M
Tris Base)	pH 7.8 at 20°C
Distilled Water	10%
Percoll	90%

Specific Gravity = 1.14

Procedure

The procedure used is outlined in Fig. B. Tentacles dissected from A. sulcata were homogenised in 10ml of cold TBSAS with a motor driven glass/PTFE potter homogeniser. The crude homogenate was centrifuged at 1,300g at 10°C for 10 min. The supernatant and buoyant floc were poured off and collected. The pellet was resuspended in 2ml of TBSAS and was carefully added to 30ml of TBSPS in a stoppered graduated cylinder and made up to 36ml with TBSAS to give a 75% Percoll solution of starting density 1.124.

The solution was mixed thoroughly and centrifuged at 70,000 g_{av} at 10°C in a fixed angle 8 x 50 rotor, for 30 min with an ultracentrifuge (MSE Prepsin 50).

Zooxanthellae congregated in bands at the middle of the self generated density gradient while animal debris and contaminated zooxanthellae formed bands above the zooxanthellae.

Aliquots of purified zooxanthellae were carefully syphoned off with a syringe through a 19g needle. Zooxanthellae extracted in this way were repeatedly centrifuged at 1,300g and washed with TBSE buffer (see Section 2). The zooxanthellae were resuspended in 10ml of TBSE after three washes and the number of cells recovered was determined from 15 replicate counts with a haemocytometer. $10^6 - 10^7$ zooxanthellae per gradient were recovered by this method. Cell lysis was not apparent during this procedure. The viability of the cells was tested with an oxygen electrode apparatus (YSI model 53) which showed that suspensions of cells still respired and photosynthesised. Fig. C shows a sample of zooxanthellae isolated with Percoll, which shows that contamination has been reduced to the occasional nematocyst and piece of animal debris.

Fig. B Procedure for isolating and purifying zooxanthellae from Anemonia sulcata

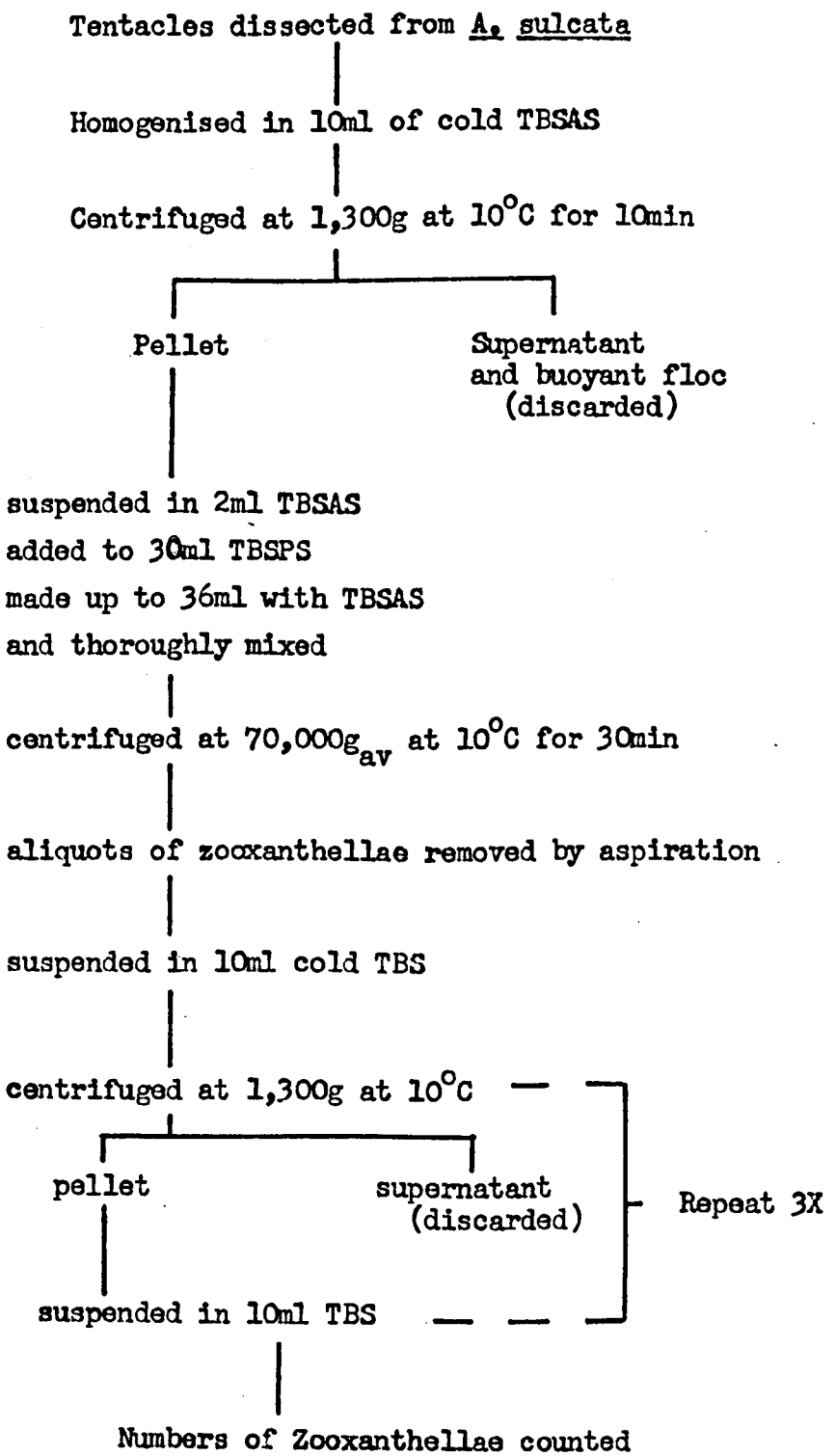
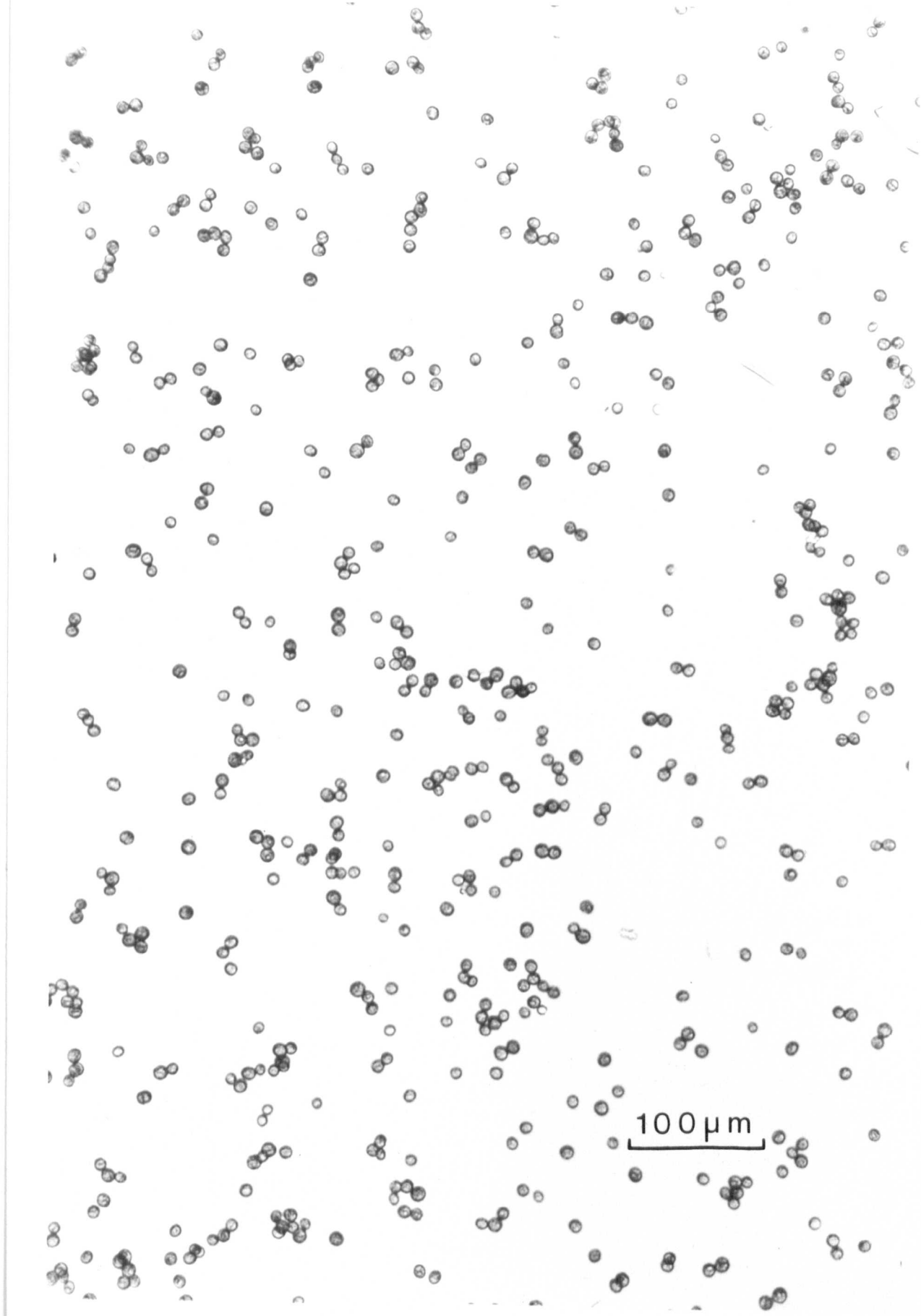


Fig. C A sample of zooxanthellae isolated by ultracentrifugation down a density gradient of the silica sol "Percoll".


100μm

Samples were recentrifuged and lyophilised for determination of organic weight and energy content. The organic weight of a zooxanthella was 3.02×10^{-7} mg. The mean ($\pm$ S.D. n = 3) energy content determined by wet oxidation with dichromate was 21.27 ± 1.05 J.mg organic weight, assuming that 60% of the organic weight was protein and that 40% of this protein was not oxidised (see Section 2).