144

Proc 8th Int Coral Reef Sym 2:1307–1312. 1997

THE NATURE OF TEMPERATE ANTHOZOAN-DINOFLAGELLATE SYMBIOSES

S.K. Davy[1*], J.R Turner[1,2] and I.A.N Lucas[1]

[1]School of Ocean Sciences, University of Wales, Bangor, Marine Science Laboratories, Menai Bridge, Anglesey LL59 5EY, U.K.

[2]Department of Agricultural Sciences, University of Oxford, Parks Road, Oxford, U.K.

*Present address: Department of Symbiosis and Coral Biology, Harbor Branch Oceanographic Institution, 5600 U.S. 1 North, Fort Pierce, Florida 34946, U.S.A.

ABSTRACT

This study (i) characterised the algal symbionts of the temperate sea anemones *Cereus pedunculatus* (Pennant), *Anthopleura ballii* (Cocks) and *Anemonia viridis* (Forskål), and the temperate zoanthid *Isozoanthus sulcatus* (Gosse) (ii) investigated the nutritional inter-relationship between the algal symbionts and these Anthozoa. Light and electron microscopy demonstrated that the symbionts were intracellular dinoflagellates ("zooxanthellae") of the genus *Symbiodinium*. Cultured zooxanthellae from the various host species did not exhibit any ultrastructural differences, however their dimensions differed significantly. No motile zooxanthellae were observed in culture. Between 33.8% and 59.3% of ^{14}C fixed in photosynthesis was translocated to the Anthozoa, indicating that the zooxanthellae may be of nutritional significance to the host. However, when *A. ballii* and *A. viridis* were maintained in darkness and deprived of macroscopic (>200 µm) food for one month in the field, weight loss was negligible. This suggests that the anemones were satisfying their metabolic requirements by feeding on material of less than 200 µm in size. Temperate zooxanthellae therefore exhibit similar characteristics to their tropical counterparts, however the need for zooxanthellae in temperate waters does not appear to be great.

INTRODUCTION

Symbioses between Cnidaria and endosymbiotic dinoflagellates ("zooxanthellae") of the genus *Symbiodinium* are abundant in shallow, nutrient-poor tropical seas. Zooxanthellae translocate fixed carbon to their hosts, which may potentially be available for host respiration, growth and reproduction (Muscatine et al. 1983, 1984; Davies 1984; McCloskey et al. 1994). Zooxanthellae also promote the recycling and conservation of nutrients, such as nitrogen (Muscatine 1980; Cook 1983; Rahav et al. 1989). These nutritional interactions are believed to be of fundamental importance to the success of cnidarian-zooxanthella symbioses in an environment where the supply of zooplankton, bacteria and dissolved organic matter (DOM) is limited (Glynn 1973; Porter 1974; Sorokin 1990).

In contrast, cnidarian-zooxanthella symbioses are rare at temperate latitudes (Smith and Douglas 1987). That most temperate Cnidaria do not possess algal symbionts suggests a reduced selective pressure for symbiosis in eutrophic waters. Indeed, the conservation of nutrients is not thought to be a major feature of temperate associations (Jensen and Muller-Parker 1994), whilst carbon flux studies suggest that many species of temperate Cnidaria derive insufficient carbon from their zooxanthellae to support their metabolic requirements (Fitt et al. 1982; Shick and Dykens 1984; Farrant et al. 1987; Verde and McCloskey 1996b; Davy et al. in press). It has been suggested that the relatively high availability of exogenous organic carbon in temperate waters actually negates the requirement for zooxanthella-fixed carbon (Verde and McCloskey 1996b). However, there have been no attempts to address this matter quantitatively.

The symbiotic sea anemones *Cereus pedunculatus* (Pennant), *Anthopleura ballii* (Cocks) and *Anemonia viridis* (= *sulcata*) (Forskål), and the symbiotic zoanthid *Isozoanthus sulcatus* (Gosse) are found around the south-west coast of Europe where they are distributed between the mid-shore and a depth of 25 m (Manuel 1981; Turner 1988). Only the relationship between *A. viridis* and its zooxanthellae is well-documented (e.g. Taylor 1968; Tytler and Davies 1984; Stambler and Dubinsky 1987; Rands et al. 1993; Harland and Davies 1995). The zooxanthellae of *C. pedunculatus, A. ballii* and *I. sulcatus* have not been described, nor is it known whether they translocate photosynthetically-fixed carbon to their hosts.

In this paper, we describe the morphology of zooxanthellae from *C. pedunculatus, A. ballii, A. viridis* and *I. sulcatus*, and determine the extent to which the zooxanthellae release fixed carbon. We also determine whether the supply of symbiont-fixed carbon is essential to the survival of symbiotic Anthozoa (particularly *A. ballii* and *A. viridis*) in temperate waters, or whether the Anthozoa can survive via heterotrophy alone.

MATERIALS AND METHODS

Experimental organisms

Cereus pedunculatus, Anthopleura ballii, Anemonia viridis and *Isozoanthus sulcatus* were collected from Lough Hyne Marine Nature Reserve, Co. Cork, Eire (51° 29' N, 9° 19' W). Using snorkelling and SCUBA, anemones were collected from the sub-tidal zone at a depth of 1-3 m, whilst zoanthids were collected from 5-9 m. *A. viridis* were also collected from the mid-shore at Shell Island, Gwynedd, Wales (52° 47' N, 004° 06' W). All Anthozoa were maintained at 21°C in seawater from the Menai Strait, North Wales, illuminated at 80 µmol photons$\cdot m^{-2} \cdot s^{-1}$ on a 12 hour light:dark cycle and fed twice weekly with *Artemia* sp. nauplii. The light and temperature regimes were similar to those experienced in Lough Hyne during a warm summer.

Zooxanthellae were isolated from Anthozoa by homogenisation and centrifugation, and cultured in ASP8-A, artificial seawater medium (Blank 1987). Cultures were kept at 21°C and received 80 µmol photons m^{-2} s^{-1} on a 12 hour light:dark cycle. Subcultures were taken every 4 weeks.

Light and electron microscopy

Freshly isolated (FIZ) and cultured (CZ) zooxanthellae were examined under bright-field illumination using a Leitz Orthoplan microscope. Cultured zooxanthellae were sampled during the log-phase of growth. The dimensions of zooxanthella doublets were measured using an ocular micrometer. Distance parallel to the cell division plane was considered as doublet width, whilst distance perpendicular to the division plane was considered as doublet length. Light microscopy was also used to confirm the location of the zooxanthellae in tentacle squashes.

FIZ, CZ, excised anemone tentacles and zoanthid polyps were fixed for 30 minutes at 4°C and pH 7.3 in 3% glutaraldehyde (in 0.1 M phosphate buffer, 0.6 M sucrose solution). Samples were then post-fixed for 30 minutes at 4°C in 1% osmium tetroxide (in 0.1 M phosphate buffer), and dehydrated through an acetone series and 100% propylene oxide.

For SEM, dehydrated FIZ and CZ were dried using a critical point drier (CPD), coated with gold (thickness 675 Å) and observed using a Stereoscan S-120 scanning electron microscope (Cambridge Instruments). For TEM, dehydrated FIZ, CZ and tissue samples were infiltrated and embedded using Spurr's resin. 60-90 nm sections were stained with uranyl acetate and lead citrate, and observed using a Philips-200 transmission electron microscope. Structures were identified by comparison with published micrographs (e.g. Taylor 1968; Schoenberg and Trench 1980; Blank 1987; Trench and Blank 1987; Banaszak et al. 1993).

Table 1: Irradiance and potential food availability within cages at 2-3 m, 13-14 m, 15 m, and 20-21 m. Irradiance given is in cages with clear perspex tops on sunny and cloudy days. To control food availability, cages were surrounded with 1 cm mesh-size garden netting, with or without a layer of 200 µm mesh-size plankton netting. Available food is based on observations of organisms within cages and organisms in the vicinity of cages

Parameter	2-3 m	13-14 m	15 m	20-21 m
Irradiance (µmol photons m^{-2} s^{-1})				
Sunny day	308	36	33	11
Cloudy day	41	17	6	2
Food				
Food <200 µm: Dissolved organic matter (DOM); bacteria; fungi; flagellates; diatoms; Foraminifera; ciliates; rotifers; copepod nauplii; other small invertebrate larvae				
200 µm < Food < 1 cm: Cladocerans; copepods; large larvae (crustacean, echinoderm, molluscan and fish); pteropods; euphausiids; chaetognaths; cephalopods; sergestids; mycotophids; Scyphozoa; thaliaceans; decapod shrimps; amphipods; small fish (Gobiidae, Gasterosteidae)				

^{14}C translocation

Anthozoa of the following oral disc diameters were selected: *C. pedunculatus* 2.0 cm; *A. ballii* 3.5-4.0 cm; *A. viridis* 3.5-4.0 cm; *I. sulcatus* 1.0-1.25 mm. Anthozoa were settled in a 100 ml stoppered flasks containing 50 ml of UV-treated, 0.45 µm filtered seawater (FSW). 50% of the FSW was then renewed and the flasks placed in a glass sided water bath set to 21°C. The water bath was illuminated at saturating irradiance (Davy et al. in press) using a Thorn 150 W photosynthetically active radiation (PAR) 38 sealed-beam reflector lamp. 5 µCi of $NaH^{14}CO_3$ (Sigma) stock solution was added, and the anemones (1 in each of 5 flasks) and zoanthids (5 in each of 3 flasks) incubated for 3 hours. Control incubations were carried out in the dark.

Following incubation, all procedures were performed in dim light. The incubation medium was decanted and saved. The Anthozoa were washed in 50 ml of unlabelled FSW, and the wash solution was combined with the incubation medium. The polyps were homogenised in a glass tissue grinder. Host and zooxanthella fractions were separated via a series of 3 washings and centrifugations, including one 10 minute wash in 0.1% sodium dodecyl sulphate (SDS) in FSW to strip host material from algal cells. All resultant supernatants were pooled and adjusted to a known volume. The final zooxanthella pellet was also adjusted to a known volume with FSW.

500 µl samples of homogenate and zooxanthellae were pipetted into glass scintillation vials and acidified overnight in 3 ml 20:1 methanol:glacial acetic acid. 10 ml Aquasol Universal Liquid Scintillation Cocktail (NEN Research Products) was then added to each vial and the samples counted in a Hewlett-Packard Canberra Tricarb-1900 CA liquid scintillation counter. Dark control counts for each fraction were subtracted from light incubation counts. The percentage of photosynthetically-fixed carbon was then expressed as the percentage of the total counts (seawater + host homogenate + zooxanthellae) appearing in the host homogenate + seawater.

Heterotrophy in the field

All field experiments were carried out using SCUBA in Lough Hyne Marine Nature Reserve, Eire. *A. ballii* were collected from a depth of 15 m and maintained unfed in the laboratory for 3 days. Buoyant weights were taken for each anemone and similarly sized individuals (average buoyant weight 91.0 mg) placed in cylindrical cages at a depth of 15 m (15 anemones per cage). Cages had either clear or black perspex tops, and were surrounded by garden netting (1 cm mesh size) with or without an additional layer of plankton netting (200 µm mesh size). Hence, 4 different treatments were created: (i) light and food freely available; (ii) reduced light with food freely available; (iii) light available with reduced food; (iv) reduced light and reduced food. Irradiance was 0 µmol photons·m^{-2}·s^{-1} in all reduced light treatments. Details of other PAR regimes and food availability are given in Table 1. Three replicates of each treatment were used (giving a total of 45 anemones per treatment), with 1 replicate being placed in each of 3 blocks of cages along the 15 m depth contour. This distribution accounted for any patchiness in food availability. Cages were cleaned daily to remove attached sediment and algae. After 33 days (the maximum period available in the field), anemones were retrieved and maintained unfed in the laboratory for 3 days. This 3 day period enabled digestion or egestion of any material in the enteron. Anemones were then buoyant weighed and the percentage change in weight over the trial period determined.

Table 2: Dimensions of freshly isolated and cultured zooxanthella doublets (means ± 1 SE) from temperate symbiotic Anthozoa. Anthozoa are *Cereus pedunculatus, Anthopleura ballii, Anemonia viridis* from Lough Hyne and Shell Island and *Isozoanthus sulcatus* (n = 40)

Host species	Freshly isolated doublets Length (µm)	Width (µm)	Cultured doublets Length (µm)	Width (µm)
Cereus pedunculatus	11.39±0.16	10.11±0.18	10.80±0.17	9.56±0.16
Anthopleura ballii	13.96±0.16	12.79±0.14	13.32±0.31	12.10±0.27
Anemonia viridis	11.61±0.18	10.45±0.17	11.83±0.14	10.86±0.14
(Lough Hyne)				
(Shell Island)	12.14±0.10	11.24±0.10	12.47±0.19	11.27±0.11
Isozoanthus sulcatus	13.43±0.21	12.22±0.16	13.53±0.28	12.60±0.2

Table 3 Translocation of photosynthetically-fixed carbon in temperate symbiotic Anthozoa. Anthozoa are *Cereus pedunculatus*, *Anthopleura ballii*, *Anemonia viridis* from Lough Hyne and Shell Island and *Isozoanthus sulcatus*. Percentages for zooxanthellae, host and seawater fractions are percentages of total radioactivity in each fraction. Values of percentage translocation are percentages of total radioactivity in host + seawater fractions (n = 5, except n = 3 for *I. sulcatus*; all values are means ± 1 SE)

Host species	Zoox (%)	Host (%)	Seawater (%)	Translocation (%)
Cereus pedunculatus	51.4±5.4	48.0±4.9	0.6±0.6	48.6±5.4
Anthopleura ballii	66.2±2.7	32.5±2.8	1.3±0.9	33.8±2.7
Anemonia viridis				
(Lough Hyne)	54.6±2.8	45.2±2.7	0.2±0.2	45.4±2.8
(Shell Island)	58.1±2.6	40.0±2.9	1.9±1.3	41.9±2.6
Isozoanthus sulcatus	40.7±3.9	59.3±3.9	0.0±0.0	59.3±3.9

A similar experiment was performed with *A. viridis*. Specimens of *A. viridis* were collected from depths of 2-3 m, 13-14 m and 20-21 m. 10 anemones of average buoyant weight 93.8 mg were placed in cages (n = 3 cages per treatment; n = 30 anemones per treatment) at each of these depths. Only 2 treatments were used on this occasion: (i) light available with reduced food; (ii) reduced light and reduced food. Irradiance was again 0 µmol photons·m^{-2}·s^{-1} in reduced light treatments. Details of other PAR and feeding regimes are given in Table 1. The smaller number of treatments enabled all cages to be placed in just one block at each depth. Anemones were caged for 30 days before retrieval and weighing.

Statistical analyses

Significant differences (P<0.05) between zooxanthella dimensions were identified using one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Significant differences between weight changes in different treatments and at different depths were identified using 2-way ANOVA.

RESULTS

Light and electron microscopy

Zooxanthellae in *Cereus pedunculatus*, *Anthopleura ballii*, *Anemonia viridis* and *Isozoanthus sulcatus* were located in the endoderm only. Using light microscopy, zooxanthellae of all hosts were observed to be non-motile and coccoid; motile cells were not observed, even over a 24 hour period in culture. Zooxanthellae divided into 2 daughter cells during mitosis, both *in hospite* and in culture. The lengths and widths of freshly isolated and cultured zooxanthellae are given in Table 2. Comparison of cultured cell dimensions, which is probably more reliable than comparison of *in hospite* dimensions where the host environment may be a factor, revealed significant differences. Zooxanthella doublets from *C. pedunculatus* and *A. viridis* (Lough Hyne) were narrower and shorter than doublets from *A. ballii* and *I. sulcatus* (Tukey HSD *post-hoc* ANOVA, P<0.05).

SEM revealed that all zooxanthellae had smooth, unornamented surfaces. TEM demonstrated that zooxanthellae in all the host species were intracellular within the host's endoderm. Zooxanthellae from all host species possessed the following ultrastructural features: (i) a large "mesokaryotic-type" nucleus containing tightly-coiled chromosomes and a grainy nucleolus; (ii) a nuclear envelope consisting of 2 membranes; (iii) a large central pyrenoid with a stalk (or stalks?) attached to the inner face of the chloroplast, the lamellae of which did not invade the pyrenoid; (iv) a peripheral, multilobed chloroplast (or chloroplasts?), with thylakoids in stacks of 3, peripheral thylakoid lamellae and a pale stroma; (v) starch granules scattered throughout the cell; (vi) a membrane-bound, dark "accumulation body" which was separate from the pyrenoid; (vii) vacuoles containing crystals, possibly of calcium oxalate; (viii) a membrane en-

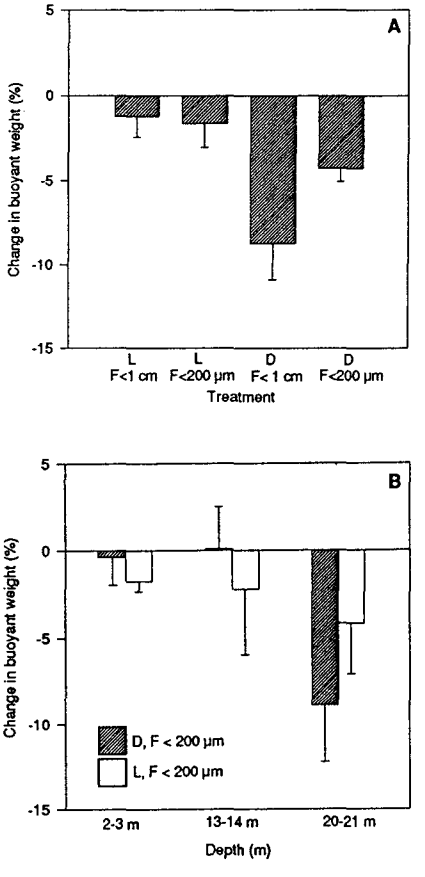

Figure 1: Percent changes in buoyant weight (means ± 1 SE) of temperate Anthozoa maintained under a range of light and feeding regimes in the field. Fig. 1A: *Anthopleura ballii* maintained at a depth of 15 m for 33 days (*L* light; *D* dark; *F < 1cm* food less than 1 cm in size available; *F < 200 µm* food less than 200 µm in size available; n = 3, with each replicate being average weight change of 15 anemones). Fig. 1B: *Anemonia viridis* maintained at a depth of 2-3 m, 13-14 m and 20-21 m for 30 days (abbreviations as in Fig. 1A; n = 3, with each replicate being average weight change for 10 anemones)

closing the cell contents and overlaid by a variable (1-4) series of wavy membranes in an apparently amorphous layer, and one tightly fitting outer membrane; (ix) an unidentified 'coat' (perhaps glycoprotein) on the outer surface of the outermost cell membrane. The only difference noted was the apparent lack of a pellicle by zooxanthellae *of C. pedunculatus* when *in hospite*. All zooxanthellae in culture possessed pellicles. Electronmicrographs of all the features described are given in Davy (1994).

^{14}C translocation

Zooxanthellae in all Anthozoa translocated a substantial portion of photosynthate to the host. The percentages of the total fixed carbon present in the zooxanthella, host and seawater (DOC) fractions, and the percentages of carbon translocated are given in Table 3.

Heterotrophy in the field

The average weight of both *A. ballii* and *A. viridis* changed by no more than ± 5% in most treatments over one month (Fig. 1). From Figure 1, it is evident that there was little relationship between light/food availability and weight change. Indeed, weight changes were not significantly different under the various light and feeding regimes. (2-way ANOVA, $P>0.05$).

DISCUSSION

The results demonstrate that zooxanthellae in *Cereus pedunculatus, Anthopleura ballii, Anemonia viridis* and *Isozoanthus sulcatus* are intracellular dinoflagellates of the genus *Symbiodinium*. The zooxanthellae translocate photosynthetically-fixed carbon to the hosts. These temperate associations therefore appear similar to their tropical counterparts. However, the necessity of this carbon to the host is questionable. It is tentatively suggested that *Anemonia viridis* and *Anthopleura ballii* can satisfy most, or all of their metabolic energy requirements via heterotrophic feeding.

Morphology of zooxanthellae

The ultrastructural features of coccoid zooxanthellae *in C. pedunculatus, A. ballii, A. viridis* and *I. sulcatus* are consistent with the characteristics of the genus *Symbiodinium* (Trench and Blank 1987). Observations of zooxanthellae from *A. viridis* revealed similar features to those previously reported for zooxanthellae from this host species (Taylor 1968; Dodge 1973). However, the failure to observe motile zooxanthellae in culture was unexpected. The lack of motile cells may simply reflect unsuitable culture conditions. However, it is also conceivable that these zooxanthellae do not have a motile stage during their life history. Given that *A. ballii* and *A. viridis* acquire their zooxanthellae via maternal inheritance (Turner 1988), the need of motility for symbiont transmission may not be great.

Differences in the average size of both freshly isolated and cultured zooxanthellae (Table 2) indicate that zooxanthella populations in *C. pedunculatus, A. ballii, A. viridis* and *I. sulcatus* are genetically different. The consistently greater average size of zooxanthellae in *A. viridis* (Shell Island) than in *A. viridis* (Lough Hyne) also hints that zooxanthella populations may vary between conspecific host populations. Conspecific variation in zooxanthella populations has been reported previously in scleractinian corals (Rowan and Knowlton 1995) and fire corals (Banaszak et al. 1993). However, it is not known whether the zooxanthella populations *in C. pedunculatus, A. ballii, A. viridis* and *I. sulcatus* are genetically homogeneous or mixed. Further genetic analyses of these zooxanthellae awaits the application of molecular techniques.

Carbon translocation

The translocation of photosynthetically-fixed carbon from symbiont to host is a general feature of algal-invertebrate symbioses. It is therefore not surprising that a substantial portion of photosynthate is translocated in *C. pedunculatus, A. ballii, A. viridis* and *I. sulcatus* (Table 3). Presumably, as in other tropical and temperate cnidarian-zooxanthella symbioses (Muscatine 1990; Verde and McCloskey 1996b), this carbon is available for host respiration, growth and reproduction, or is lost from the association. The presence of labelled dissolved organic carbon (DOC) in the seawater fraction (Table 3) certainly suggests the latter. The translocation rates measured here (33.8%-59.3%) are within the range measured using ^{14}C for other zooxanthellate Cnidaria (5.0%-82.0%) (von Holt and von Holt 1968; Muscatine and Cernichiari 1969; Trench 1971; Clark and Jensen 1982; Muscatine et al. 1984; Farrant et al. 1987). A translocation rate of 40.2%-50.0% has been reported previously *for A. viridis* (Taylor 1969a; Stambler and Dubinsky 1987). It should be noted, however, that the use of ^{14}C probably underestimates translocation (Muscatine et al. 1984).

Heterotrophy in the field

The buoyant weights of *A. viridis* and *A. ballii* changed by less than ± 5% in most treatments. This change is very small compared to observations of laboratory investigations run over a comparable period of time. Over 30 days, *Anthopleura elegantissima* and *A. viridis* changed weight (buoyant or wet) by -35% to 95% depending upon light and feeding regime (Muscatine 1961; Taylor 1969b; Sebens 1980; Janssen and Möller 1981). Over a period of 14 weeks, Tsuchida and Potts (1994) measured a change in wet weight of *A. elegantissima* of -30% and 200%, when unfed and fed three times per week respectively. In part, the differences between the results of the present study and these published values may result from the use of different sizes of anemones. However, Tsuchida and Potts (1994) found that percent weight loss during starvation was the same in small and large specimens of *A. elegantissima*. It is also possible that, had the experiments been run for a longer period of time, weight changes would have been more obvious and differences between treatments may have become apparent.

As *A. viridis* and *A. ballii* maintained their weight even when the only food available was <200 µm in size, it appears that these anemones can satisfy most, if not all of their metabolic energy requirements via feeding on microscopic particulate matter or DOM. DOM is believed to be an important source of nutriment for *A. viridis* (Schlichter 1975, 1978; Janssen and Möller 1981). The reason why anemones with ready access to food were not observed to grow during the experiment is unknown. However, it is possible that the cages did not give the animals access to all available food. Alternatively, as the anemones were large adults, nutrients that were excess to metabolic requirements may have been used for reproduction rather than growth.

Therefore, temperate hosts may not be dependent upon their symbionts to any significant degree (Verde and McCloskey 1996b). This may explain how *A. viridis* and *A. ballii* survive at depths in excess of 20 m, where irradiance is minimal (Turner 1988). It may also explain how *A. ballii* is able to survive for long periods of time when buried several inches below the sediment surface (Davy, pers. obs.). Why then do temperate Anthozoa harbour zooxanthellae? Perhaps the zooxanthellae are parasitic, exploiting the host for protection and nutrients. Alternatively, food may be so abundant that the presence of zooxanthellae is of no consequence to the host. However, over the long-term, it seems far more likely that zooxanthellae provide a competitive advantage to temperate Anthozoa. This is supported by three observations. Firstly, the depth distribution of *A. viridis* and *A. ballii* in Lough Hyne is determined by light availability, suggesting that these anemones actively seek out exposed, well-illuminated positions (Turner 1988). Secondly, both *A. viridis* and *A. ballii* acquire their zooxanthellae via maternal inheritance (Turner 1988). It seems improbable that, if exploited by zooxanthellae, Anthozoa would go to such extremes to maintain the symbiosis. Thirdly, *A. viridis and A. ballii* may occur at far higher densities than comparable, azooxanthellate invertebrates. In Lough Hyne, *A. viridis*

occurs at a maximum density of 185 per m^2, whilst *A. ballii* occurs at a maximum density of 40 per m^2. At these densities, *A. viridis* and *A. ballii* exclude otherwise common azooxanthellate Anthozoa such as *Metridium senile* and *Corynactis viridis* (Turner 1988). Davy et al. (in press) demonstrated that zooxanthellae contribute little carbon to *A. viridis and A. ballii* under cloudy conditions and at depths of 9 m or more. However, if carbon requirements can largely be met via heterotrophy, then even a small zooxanthellal contribution may enhance the reproductive output of these anemones. Also, zooxanthellae may supplement the host's metabolic needs during occasional periods of food deprivation (Van-Praët 1985). In both these cases zooxanthellae would increase the rate of colonisation by the largely asexual *A. viridis* and sexual *A. ballii*. Of course, the advantage conveyed by zooxanthellae may not necessarily be a nutritional one. For example, zooxanthellae could be distasteful to some predators.

The lesser requirement for zooxanthellae in temperate waters than in the tropics may be one reason for the scarcity of temperate Cnidaria-zooxanthella symbioses. Other reasons may relate to the temperature tolerance of symbiotic algae (O'Brien and Wyttenbach 1980; Trench, this volume) or the resistance of temperate Cnidaria to infection by algae. A reduced selective pressure for symbiosis may also explain why temperate hosts receive a smaller proportion of their metabolic carbon requirements from their zooxanthellae than do tropical hosts (Verde and McCloskey 1996a; Davy et al. in press). Presumably, this is indicative of a lower level of host-symbiont integration in temperate symbioses than in tropical ones. However, the competitive advantage provided by zooxanthellae may drive the evolution of cnidarian-algal symbioses in temperate waters, albeit at a slower rate than occurs in the tropics. This may steadily increase both the diversity of cnidarian-zooxanthella symbioses at temperate latitudes and the degree of host-symbiont integration in existing associations.

ACKNOWLEDGEMENTS

SKD wishes to thank Dr. Claire Goiran for assistance during preparatory stages of the ^{14}C work, Mrs. Ann Buckland for sectioning of TEM samples and Dr. Gisèle Muller-Parker for helpful discussions. JRT wishes to thank Professor Sir David Smith FRS FRSE for advice and support, and the Lough Hyne Marine Nature Reserve authorities for permission to use the site for field work. This work was funded by a SERC award to SKD and a NERC award to JRT. This paper is Contribution # 1144 of the Harbor Branch Oceanographic Institution.

REFERENCES

Banaszak AT, Iglesias-Prieto R, Trench RK (1993) *Scrippsiella velellae* sp nov (Peridiniales) and *Gloeodinium viscum* sp nov (Phytodiniales), dinoflagellate symbionts of two hydrozoans (Cnidaria). J Phycol 29: 517-528

Blank RJ (1987) Cell architecture of the dinoflagellate *Symbiodinium* sp inhabiting the Hawaiian stony coral *Montipora verrucosa*. Mar Biol 94: 143-155

Clark KB, Jensen KR (1982) Effects of temperature on carbon fixation and carbon budget partitioning in the zooxanthellal symbiosis of *Aiptasia pallida* (Verrill). J Exp Mar Biol Ecol 64: 215-230

Cook CB (1983) Metabolic interchange in algae-invertebrate symbiosis. Int Rev Cytol supplement 14: 177-210

Davies PS (1984) The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs 2: 181-186

Davy SK (1994) The specificity of temperate anthozoan-dinoflagellate symbioses. PhD thesis, University of Wales

Dodge JD (1973) The Fine Structure of Algal Cells. Academic Press

Farrant PA, Borowitzka MA, Hinde R, King RJ (1987) Nutrition of the temperate Australian soft coral *Capnella gaboensis*. II. The role of zooxanthellae and feeding. Mar Biol 95: 575-581

Fitt WK, Pardy RL, Littler MM (1982) Photosynthesis, respiration, and contribution to community productivity of the symbiotic sea anemone *Anthopleura elegantissima* (Brandt, 1835). J Exp Mar Biol Ecol 61: 213-232

Glynn PW (1973) Ecology of a Caribbean coral reef, the *Porites* reef flat biotope. Part II. Plankton community with evidence for depletion. Mar Biol 22: 1-21

Harland AD, Davies PS (1995) Symbiont photosynthesis increases both respiration and photosynthesis in the symbiotic sea anemone *Anemonia viridis*. Mar Biol 123: 715-722

Janssen HH, Möller H (1981) Effects of various feeding conditions on *Anemonia sulcata*. Zool Anz 206: 161-170

Jensen SL, Muller-Parker G (1994) Inorganic nutrient fluxes in anemone-dominated tide pools. Pacific Science 48: 32-43

Manuel RL (1981) British Anthozoa. Academic Press, London

McCloskey LR, Muscatine L, Wilkerson FP (1994) Daily photosynthesis, respiration and carbon budgets in a tropical marine jellyfish (*Mastigias* sp.). Mar Biol 119: 13-22

Muscatine L (1961) Symbiosis in marine and freshwater coelenterates. In Loomis WF (ed) The Biology of Hydra. University of Miami Press, pp 255-268

Muscatine L (1980) Uptake, retention, and release of dissolved inorganic nutrients by marine algae-invertebrate associations. In CB Cook, PW Pappas, ED Rudolph (eds) Cellular Interactions in Symbioses and Parasitism, Columbus, Ohio State University Press, pp 229-244

Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In Dubinsky Z (ed) Coral Reefs, Elsevier, Amsterdam, pp 75-87

Muscatine L, Cernichiari E (1969) Assimilation of photosynthetic products of zooxanthellae by a reef coral. Biol Bull 137: 506-523

Muscatine L, Falkowski PG, Dubinsky Z (1983) Carbon budgets in symbiotic associations. In W. Schwemmler, H.E.A. Schenk (eds) Endocytobiology II, Walter de Gruyter and Co, Berlin, pp 649-658

Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc R Soc Lond B 222: 181-202

O'Brien TL, Wyttenbach CR (1980) Some effects of temperature on the symbiotic association between zoochlorellae (Chlorophyceae) and the sea anemone *Anthopleura xanthogrammica*. Trans Amer Micros Soc 99: 221-225

Porter JW (1974) Zooplankton feeding by the Caribbean reef-building coral *Montastrea cavernosa*. Proc 2nd Int Coral Reef Symp 1: 111-125Schlichter D (1975) Die Bedeutung in Meerwasser gelöster Glucose für die Ernährung von *Anemonia sulcata* (Coelenterata: Anthozoa). Mar Biol 29: 283-293

Rands ML, Loughman BC, Douglas AE (1993) The symbiotic interface in an alga-invertebrate symbiosis. Proc R Soc Lond B 253: 161-165

Rahav O, Dubinsky Z, Achituv Y, Falkowski PG (1989) Ammonium metabolism in the zooxanthellate coral *Stylophora pistillata*. Proc R Soc Lond B 236: 325-337

Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis. Proc Natl Acad Sci USA 92: 2850-2853

Schlichter D (1975) Die Bedeutung in Meerwasser gelöster Glucose für die Ernährung von *Anemonia sulcata* (Coelenterata: Anthozoa). Mar Biol 29: 283-293

Schlichter D (1978) On the ability of *Anemonia sulcata* (Coelenterata: Anthozoa) to absorb charged and neutral amino acids simultaneously. Mar Biol 45: 97-104

Schoenberg DA, Trench RK (1980) Genetic variation in *Symbiodinium* (= *Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. II. Morphological variation in *S. microadriaticum*. Proc R Soc Lond B 207: 429-444

Sebens KP (1980) The regulation of asexual reproduction and indeterminate body size in the sea anemone *Anthopleura elegantissima* (Brandt). Biol Bull 158: 370-382

Shick JM, Dykens JA (1984) Photobiology of the symbiotic sea anemone *Anthopleura elegantissima*: photosynthesis, respiration, and behaviour under intertidal conditions. Biol Bull 166: 608-619

Smith DC, Douglas AE (1987) The Biology of Symbiosis. Edward Arnold, London

Sorokin YI (1990) Plankton in the reef ecosystems. In Dubinsky Z (ed) Coral Reefs, Elsevier, Amsterdam, pp 291-327

Stambler N, Dubinsky Z (1987) Energy relationships between *Anemonia sulcata* and its endosymbiotic zooxanthellae. Symbiosis 3: 233-248

Taylor DL (1968) In situ studies on the cytochemistry and ultrastructure of a symbiotic marine dinoflagellate. J Mar Biol Ass UK 48: 349-366

Taylor DL (1969a) The nutritional relationship of *Anemonia sulcata* (Pennant) and its dinoflagellate symbiont. J Cell Sci 4: 751-762

Taylor DL (1969b) On the regulation and maintenance of algal numbers in zooxanthellae-coelenterate symbiosis, with a note on the nutritional relationship in *Anemonia sulcata*. J Mar Biol Ass UK 49: 1057-1065

Trench RK (1971) The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. II. Liberation of fixed ^{14}C by zooxanthellae in vitro. Proc R Soc Lond B 177: 237-250

Trench RK, Blank RJ (1987) *Symbiodinium microadriaticum* Freudenthal, *S. goreauii* sp nov, *S. kawagutii* sp nov and *S. pilosum* sp nov: Gymnodinioid dinoflagellate symbionts of marine invertebrates. J Phycol 23: 469-481

Tsuchida CB, Potts DC (1994) The effects of illumination, food and symbionts on growth of the sea anemone *Anthopleura elegantissima* (Brandt, 1835). I. Ramet growth. J Exp Mar Biol Ecol 183: 227-242

Turner JR (1988) The ecology of temperate symbiotic Anthozoa. DPhil thesis, University of Oxford

Tytler EM, Davies PS (1984) Photosynthetic production and respiratory energy expenditure in the anemone *Anemonia sulcata* (Pennant). J. Exp Mar Biol Ecol 81: 73-86

Van-Praët M (1985) Nutrition of sea anemones. Adv Mar Biol 22: 65-99

Verde EA, McCloskey LR (1996a) Carbon budget studies of symbiotic cnidarian anemones-Evidence in support of some assumptions. J Exp Mar Biol Ecol 195: 161-171

Verde EA, McCloskey LR (1996b) Photosynthesis and respiration of two species of algal symbionts in the anemone *Anthopleura elegantissima* (Brandt) (Cnidaria; Anthozoa). J Exp Mar Biol Ecol 195: 187-202

von Holt C, von Holt M (1968) Transfer of photosynthetic products from zooxanthellae to coelenterate hosts. Comp Biochem Physiol 24: 73-81