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Natural variations in the zooxanthellae of temperate symbiotic Anthozoa.

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Natural variations in the zooxanthellae of temperate symbiotic Anthozoa

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Abstract

Few previous studies of zooxanthellae have considered temperate Anthozoan symbioses. The present study investigates how the characteristics of zooxanthellae symbiotic with temperate Anthozoa vary in response to natural variations in environmental parameters. Variations in the number (density), division rate, size and ultrastructure of zooxanthellae from the temperate anemones *Anemonia viridis* (Forskal) and *Anthopleura ballii* (Cocks) were examined in response to season, water depth and artificial irradiance (*A.viridis* in aquaria). In addition, variations in chlorophyll concentrations were considered in intertidal and laboratory-maintained *A. viridis*. Zooxanthellae from both intertidal and shallow subtidal *A. viridis* showed variations which correlated with seasonal variations in environmental parameters. Zooxanthella density in intertidal *A. viridis* showed an inverse relationship with temperature, daylength and sunshine. Higher zooxanthella density was observed in *A. viridis* from a shallow, subtidal habitat during February 1998 ($2.06 \pm 0.11 \times 10^8$ cells g^{-1} wet weight) than during July 1998 ($1.01 \pm 0.09 \times 10^8$ cells g^{-1} wet weight; $T= 7.67$, $p< 0.001$). Stereological analysis of transmission electron micrographs showed that zooxanthellae in intertidal *A. viridis* had significantly higher chloroplast volume fraction during February (32.1 ± 1.5 %) than July (21.8 ± 2.1 %; $T= 4.07$, $p<0.05$). The proportion of chlorophyll *a* per zooxanthella was significantly higher in December than all other months except January (ANOVA, $F= 5.62$ $p<0.05$). The zooxanthellae of *A. viridis* may thus photoadapt to low winter irradiances by increasing zooxanthellae density, chloroplast volume and the proportion of chlorophyll *a* per cell. By contrast, zooxanthellae from *A. viridis* maintained in artificial irradiances in the laboratory of 4 µmol m^{-2} s^{-1} and 20 µmol m^{-2} s^{-1} showed no variation in density or ultrastructure, due either to the low irradiances used or a lack of variation in other physical parameters compared to the field. *A. ballii* zooxanthella density responded to both depth and season and was lower at 6 m during summer than at 6 m during winter and at 18 m during both summer and winter. Chloroplast volume fractions in *A. ballii* was not affected by depth during winter, nor by season at 18 m. Starch and lipid stores in zooxanthellae from both *A. viridis* and *A. ballii* responded to seasonal fluctuations. Lipid was present in zooxanthellae during summer (intertidal *A. viridis*, volume fraction 19.8 $\pm$ 3.4 %) and absent during winter, and starch volume was significantly higher from zooxanthellae in *A. ballii* at 6 m in winter (14.3 ± 4.2 %) than 18 m in winter (4.7 ± 1.6 %) or summer (4.7 ± 1.1 %; ANOVA, $F= 6.04$ $p< 0.05$). It is concluded that the zooxanthellae of the temperate anemones *A. viridis* and *A. ballii* show variations in zooxanthellae characteristics which correspond to variations in day-to-day weather, season and water depth.

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CHAPTER 1

General introduction

The overall goal of the present study was to investigate photoadaptive variation in the zooxanthellae of temperate symbiotic Anthozoa. Many marine Cnidarians possess symbiotic dinoflagellates or 'zooxanthellae' from which they derive photosynthetic carbon (Muscatine 1990). Although the widely used term zooxanthellae lacks any taxonomic significance, it is used in the present study to describe symbiotic dinoflagellates of the genus *Symbiodinium*. The association formed between Anthozoa and dinoflagellates is particularly important as it is responsible for reef building in tropical corals. Among temperate anthozoans only 5 of the 74 British species of anemones possess endosymbiotic microalgae (Turner, 1988) suggesting a reduced selective pressure for symbiosis in eutrophic, temperate waters (Davy *et al.*, 1997). The two main carbon inputs to symbiotic cnidarians are photosynthesis by the zooxanthellae and heterotrophic feeding by the host. Carbon budget studies of symbiotic Anthozoa (Steen & Muscatine 1984, Edmunds & Davies 1986, Davies 1991, Davy *et al.* 1996) have shown that the need for heterotrophic feeding in temperate waters is far greater than in the oligotrophic waters of tropical latitudes, where photosynthesis by the zooxanthellae is sufficient to meet host metabolic requirements. Whilst temperate anthozoans appear to have a lesser energetic requirement for zooxanthellae than their tropical counterparts, zooxanthellae may provide a competitive advantage. Davy *et al.* (1997) observed that although the carbon requirements of the temperate anemones *Anthopleura ballii* (Cocks) and *Anemonia viridis* (Forskal) were largely met by heterotrophy, these anemones showed a depth distribution which corresponded to light availability. In addition, Davy *et al.*

(1997) reported that the symbiosis is maintained by maternal inheritance (Turner, 1988) and that both anemones occur at far higher densities than azooxanthellate species, hence excluding otherwise common azooxanthellate Anthozoa in Lough Hyne. Such observations suggest that the zooxanthellae are neither parasitic, nor of inconsequence, but provide an energetic advantage to the symbiosis. For example, even a small zooxanthellal contribution may enhance reproductive output. Thus, incorporation of an autotrophic partner by a heterotrophic anemone greatly increases the anemone's already considerable trophic flexibility (Shick, 1991).

Natural variation in zooxanthellae tends to be a response to variations in the prevailing light conditions, and may involve photoadaptation. The term photoadaptation is used throughout the present study to describe any light-related changes to the zooxanthellae and occurs on a time scale shorter than or comparable to a cell's generation (Falkowski & LaRoche, 1991). Symbiotic anthozoans show a remarkable range of adaptations to varying quantity and quality of solar radiation. Much of the capacity to adapt to light fluctuations resides in the flexibility of the photosynthetic apparatus of the zooxanthellae, to adjust to different radiation. Although there are a number of photoadaptory strategies, each achieves the same effect, which is to increase the light harvesting ability of the zooxanthellae. Hence, zooxanthellae may vary in number, size, division rate, spatial arrangement, pigment content (cellular and biochemical responses) and ultrastructure (morphological response) as well as adapting physiologically by altering rates of photosynthesis and respiration. Although a number of workers have investigated natural cellular and biochemical variations in the zooxanthellae of tropical Anthozoa (Drew 1972, Wethey & Porter 1976, Titlyanov *et al.* 1980, Falkowski & Dubinsky 1981, Muscatine *et al.* 1984, Porter *et al.* 1984, Berner *et al.* 1987, Muller-Parker 1987, Stimson 1997,

Fagoonee *et al.* 1999, Brown *et al.* 1999), few studies have examined these features in zooxanthellae of field populations of temperate Anthozoa (Dykens & Shick 1984, Bythell *et al.* 1997). Furthermore, whilst Muller-Parker (1987) considered the qualitative effects of natural light variations on the ultrastructure of zooxanthellae from the tropical sea anemone *Aiptasia pulchella*, no similar study on temperate Anthozoa exists.

In the field, several environmental variables exist which may influence the photoadaptive response. These include season, water depth, spectral composition of light, temperature and nutrient availability (Harland & Davies, 1994). Several studies have considered the effect of water depth (Wethey & Porter, 1976; Titlyanov *et al.* 1980; Dustan, 1982; Chalker *et al.* 1983; McCloskey & Muscatine, 1984) and sun- and shade-habitats (Wethey & Porter 1976, Titlyanov *et al.* 1980, Falkowski & Dubinsky 1981, Muscatine *et al.* 1984, Porter *et al.* 1984, Berner *et al.* 1987, Muller-Parker, 1987) on zooxanthellar variation in tropical Anthozoa, whilst only one previous study has considered such effects on a temperate anemone (Bythell *et al.*, 1997). Bythell *et al.* (1997) investigated the effects of intertidal and subtidal habitats and therefore depth, on zooxanthellar density and pigment variation in *Anemonia viridis* and observed no significant variation. In comparison, Dykens & Shick (1984) examined the effects of season on zooxanthellar density and pigment concentration in *Anthopleura elegantissima* and concluded that pigment concentration but not density showed seasonal variation.

In this thesis variation in zooxanthellar density, mitotic index (division rate), diameter, pigment content and ultrastructure were considered in temperate subtidal and intertidal *Anemonia viridis* (Forskal) during different seasons and in subtidal *Anthopleura ballii* (Cocks) during different seasons and at different water depths.

Thus photoadaptation of the zooxanthellae to natural variations in irradiance was considered.

The aim of the present study was to investigate variations in the number, division rate, size, pigment content and ultrastructure of the zooxanthellae of two temperate symbiotic anemones, *A. viridis* and *A. ballii* in response to natural variations in light. Thus strategies of zooxanthellae photoadaptation to a photic regime which varies enormously with day-to-day weather, season and water depth were considered. The thesis is divided into six chapters each one, except for chapters one and six, containing its own introduction and discussion. **Chapter one** provides general background knowledge to the thesis, whilst **chapter six** is a general discussion collating the most important aspects of each chapter and provides a focus for future research. **Chapter two** examines seasonal environmental change in temperate subtidal habitats. The importance of seasonality in two temperate sites Lough Hyne M.N.R., Ireland and Trearddur Bay, Anglesey, at which symbiotic Anthozoa occur, is considered by comparison with a tropical site in Mauritius, Indian Ocean. **Chapter 3** considers variation in the zooxanthellae characteristics density, mitotic index (MI) diameter and pigment content in subtidal and intertidal *A. viridis* during different seasons in Lough Hyne and Trearddur Bay. In addition zooxanthellae density, mitotic index and diameter are considered in subtidal *A. ballii* on Glannafeen cliff in Lough Hyne. Photoadaptation of zooxanthellae is discussed in terms of depth and season. **Chapter 4** investigates variations in the zooxanthellae density, mitotic index, diameter and pigment content in laboratory-maintained *A. viridis* in response to varying artificial irradiances and colour spectra. The effects of medium and low intensity artificial irradiance and red and green colour filters are considered. **Chapter 5** examines changes in zooxanthellae ultrastructure to both natural and artificial

variations in light. Season and depth responses are considered in zooxanthellae from subtidal *A. ballii*, whilst the effects of intertidal and subtidal habitats are examined in *A. viridis*. The volume of zooxanthellae chloroplasts, starch, lipid, pyrenoids, mitochondria, accumulation bodies and thylakoids are observed using TEM and photoadaptation is considered.

CHAPTER 2

Seasonality in tropical and temperate subtidal environments

2.1. Introduction

The aim of this chapter was to identify the physical factors that vary seasonally underwater, and emphasise their importance by comparing tropical and temperate marine environments using both published data and *in situ* measurements.

The position of the sun relative to the earth produces a continuum of change from one extreme to the other described as "seasons". Thus, the force driving seasonal environmental change is incoming radiation (Kain, 1989). The amount of radiation received at different latitudes varies according to season and the solstices. The solstice describes the point when the sun is farthest from the equator and at the summer solstice, when daylength is longest, the mean irradiance is similar for the whole hemisphere. By contrast, in winter mean irradiance is lower for higher latitudes. The result is a much steeper gradient in conditions in winter than summer, from the equator towards the poles. However, seasonal change is small only at latitudes less than 10° (Budyko & Miller, 1974).

Between incoming radiation and subtidal communities is the atmosphere which has variable weather, and seawater which varies in transparency. At higher latitudes there tends to be more cloud cover in winter, enhancing the latitudinal effect on incoming radiation. Water transparency shows considerable spatial and seasonal variation to different light spectra. Light penetration tends to be better in summer than in winter when storms increase the turbidity of the water. High turbidity exacerbates the

seasonal effect on incoming radiation and is another influence of weather (Kain, 1989).

Light is defined as the radiant energy capable of stimulating the human eye and covers the range 380 to 780 nm. The energy within specific wavelength intervals gives rise to colours: ultra-violet < 400 nm, violet 400-420, blue 420-480, green 500-560, yellow 580-600, red 620-780, infra-red >780. Plant physiologists are usually concerned with 'photosynthetically active radiation' (P.A.R.; 400 to 700 nm) which is able to be absorbed by the photosynthetic pigments of plants. However, solar ultraviolet radiation (UV) is also important due to its inhibitory effect on photosynthesis and growth (Calkins & Thordardottir 1980, Jokiel & York 1984) and potential to damage living tissue (Harm, 1980). As light passes through water it is subject to both absorption and scattering, and the overall reduction in irradiance is termed attenuation. The attenuation of total PAR with depth in a given water body is characterised by the vertical attenuation coefficient, K_d which provides a convenient and informative parameter for comparing the light attenuating properties of different water bodies (Kirk, 1994). Previous studies have considered vertical attenuation coefficients for different marine waters (reveiwed by Kirk (1994) chapter 6, table 6.1). However, none has considered the effect of season on K_d in a temperate sea loch.

The optical properties of sea water are determined by the water itself, dissolved organic matter (yellow substance) and particulate matter (Jerlov, 1976). Pure water is most transparent to blue light (475 nm) whereas red light undergoes strong absorption. The dissolved decomposition products of organic matter, generally termed yellow substance, give rise to significant absorption which starts in the yellow and increases

towards shorter wavelengths. Finally, particulate matter is responsible for both absorption and scattering. Fine particles, such as sediments, increase the scattering of light and since scattering is inversely proportional to the fourth power of the wavelength, the effect is most marked in the blue and UV regions of the spectrum. Larger particles, such as microorganisms and biological detritus both scatter and absorb light, and absorption is strongest in the blue region of the spectrum (Kain, 1989). The attenuation of the different wavebands of downward irradiance is determined by the behaviour of these different components, which affect the absorption spectrum of the aquatic medium (Kirk, 1994). Generally, in tropical and non-productive oceanic waters, water itself is the main absorber; blue and green light both penetrate deeply and to about the same extent. Conversely, red light is absorbed strongly and is attenuated much more rapidly. In coastal waters, which contain more yellow substance and phytoplankton than oceanic waters, green light is the most penetrating waveband. Only in the most coloured coastal waters influenced by major river discharge is blue light attenuated as strongly as red light (Jerlov, 1976). In addition, UV penetrates clear oceanic water nearly as well as visible light but is absorbed by dissolved and particulate organic material characteristic of coastal waters (Jokiel 1980, Fleischmann 1989).

The decrease in overall transmittance which occurs during the progression from clear oceanic waters to turbid coastal waters is accompanied by a shift of the wavelength at which maximum transmission occurs, from 465 nm in clear waters to 575 nm in turbid waters (Dring, 1982). In addition, the spectral differences observed between different water types (i.e. oceanic *vs.* coastal) are intensified with depth. Light becomes increasingly monochromatic as it penetrates the sea, such that oceanic waters

can be broadly described as 'blue', whilst coastal waters are 'green'. Changes in spectral distribution that occur as light penetrates sea water are accompanied by changes in the overall intensity of light. Since the intensity of the more strongly attenuated wavelengths is reduced more rapidly than that of other wavelengths, the light becomes progressively richer in the wavelengths that penetrate furthest. Hence, as the light becomes more and more monochromatic, the overall transmittance gradually approaches that of the most penetrating wavelength.

Significant photosynthesis underwater takes place only down to that depth at which downwelling irradiance of photosynthetically active radiation (PAR) falls to 1 % of that just below the surface. The layer within which downwelling PAR falls to 1 % of the subsurface value is known as the euphotic zone and may differ spatially and seasonally (Kirk, 1994). Photosynthetic plant growth is restricted to the euphotic zone and any variation in the 1 % depth has physiological implications for sessile marine communities dependent on light for photosynthesis.

Change in radiation also has a direct effect on temperature due to the heating effects of infra-red wavebands, and greater seasonal changes in radiation are observed at higher latitudes. However, ocean currents, upwelling and ice complicate the effect of radiation in higher latitudes, such that the highest seasonal sea surface temperature ranges occur in temperate waters. Nutrients such as dissolved inorganic nitrogen are essential to microalgae, including symbiotic dinoflagellates, and their concentrations may change dramatically with season, due to phytoplankton activity (Blight *et al.* 1995, Fagoonee *et al.* 1999, Brown *et al.* 1999).

The hypothesis investigated states that seasonal variation and latitude have a marked effect on the under-water conditions experienced by temperate marine communities

including symbiotic Anthozoa. Hence, zooxanthellae in temperate anthozoans are exposed to photic environments which show considerable variation both spatially and seasonally. The objectives of the present study were firstly to collate information on seasonal variation in meteorological conditions for a tropical site and two temperate sites where symbiotic Anthozoa occurred. Previously published data and Meteorological Office data were used to compare seasonal cycles of temperature, rainfall, sunshine and daylength. Secondly, information was gathered on seasonal variation in water column parameters for the same sites. Previously published data were used to compare seasonal cycles of nitrate, phosphate and chlorophyll. Finally, underwater irradiance was measured at the tropical site and one temperate site using a spectroradiometer and an underwater quantum sensor. Daily cycles of irradiance were considered in different weather conditions and during different seasons, to assess how such variations effected the total amount of underwater light. In addition, attenuation of total downwelling irradiance with water depth was assessed for the tropical site, and both attenuation of total downwelling PAR and spectral irradiance were assessed at one temperate site. In addition, seasonal attenuation of downwelling PAR was considered in the temperate site only.

2.2. Methods

2.2.1. Study sites

Information on seasonal fluctuations in physical environmental parameters was collated for two temperate sites and a combination of two tropical lagoons. The temperate sites were selected due to the presence of symbiotic Anthozoa and the tropical sites selected for availability of data.

Site 1: Trearddur Bay, Anglesey, North Wales (53° 16' N, 4° 4' W; Figure 2.1); an exposed rocky shore with rock pools. Sources of information were Valley observatory, Weather log, Royal Meteorological Society 1998, Owen (1998) and Blight *et al.* (1995) for Menai Strait water column data and Laver's Liverpool tide tables.

Site 2: Lough Hyne Marine Nature Reserve, Ireland (Figure 2.2); a sheltered sea lough located in the south of Ireland (51° 29' N, 9° 18' W). Sources of information were Valentia observatory from the Irish Meteorological Office for air temperature, rainfall, sunshine and daylength data and the Aquatic Services Unit, Zoology Department, University College Cork for nitrate, phosphate, chlorophyll and water temperature data for Lough Hyne.

Site 3: Trou aux Biches lagoon, Mauritius, Indian Ocean (Figure 2.3) extends over 4 km in the north-south direction and measures 1.3 km across the widest point at Mont Choisy, and approximately 200 m at the narrowest point (between 57° 00' 28" S, 20° 33' 13" E and 57° 02' 27" S, 20° 32' 25" E; Daby, 1999). Sources of information were Daby (1999) and the Mauritian Meteorological Office.

Site 4: Trou D'Eau Douce lagoon, Mauritius, Indian Ocean (Figure 2.3) is 10 km long and 3 km wide and lies between 20° 13' 51" S, 57° 47' 33" E and 20° 19' 15" S, 57° 49' 30" E.

2.2.2. Seasonal fluctuations in meteorological conditions

Monthly air temperature (°C), rain fall (mm) and total sunshine (hours) data were obtained from previous studies and Meteorological Office publications as described in section 2.2.1. In addition mean monthly daylength was obtained from the Irish Meteorological office for Lough Hyne and Laver's Liverpool tide tables (1996) for

Trearddur Bay. Daylength is defined as the duration of day light in hours, and starts and ends when the centre of the sun is on the horizon.

2.2.3. Seasonal fluctuations in water column parameters

Monthly nitrate and phosphate concentrations and chlorophyll concentrations were taken from previous studies as described in section 2.2.1. Trou aux Biches data were obtained from Daby (1999). No information for Trearddur Bay was available, however data for the Menai Strait (53° 10' N, 04° 05' W; figure 2.1) were taken instead from Owen (1998) and Blight *et al.* (1995).

Total chlorophyll concentrations were available for the Menai Strait, whilst chlorophyll *a* concentrations were available for Lough Hyne. No chlorophyll data was available for Mauritius. Similarly, water temperature (°C) data were obtained for Lough Hyne and the Menai Strait.

2.2.4. Measurement of under water irradiance

2.2.4.1. The effect of weather and season on daily cycles of under water irradiance

Daily cycles of photosynthetically active radiation (PAR) at a particular depth were measured in Lough Hyne, Ireland and Trou D'Eau Douce, Mauritius to determine the effects of daily weather conditions on underwater light. Measurements of under water irradiance were not available for Trearddur Bay.

For Lough Hyne, daily cycles of light were also compared between seasons. Downwelling irradiance (photosynthetically active radiation, PAR) over a daily cycle was measured with a LI-COR underwater quantum sensor deployed at 6 m depth for 24 hours. Mean, maximum and minimum irradiance were recorded by a LI-1000

datalogger every 10 minutes. The data were subsequently downloaded to a PC using LiCor software and used to calculate total daily illumination.

2.2.4.2. The effect of season on attenuation of downwelling irradiance

Attenuation of total downwelling PAR and spectral irradiance with water depth during winter and summer were compared in Lough Hyne. Attenuation of light intensity and spectral composition was measured in Lough Hyne using the PRR600 radiometer (manufactured by Biospherical Instruments, San Diego, California). The radiometer comprised a surface unit and an underwater unit connected by cables to a data logger on the surface. Spectral composition and intensity of irradiance were recorded by 7 channels representing the PAR range (400-700 nm) at different water depths. Initiation of data logging was controlled by a lap-top P.C. on the surface to which data were downloaded from the datalogger. The underwater unit was lowered over the side of an inflatable boat to a maximium depth of 40 m in the Western Trough (Chapter 3, Figure 3.1). Downwelling irradiance and upwelling radiance were recorded at a frequency of 2 Hz for the duration of the descent to 40 m. Measurements were made in the middle of the day when the sun was directly overhead and care was taken not to shadow the underwater sensor with the boat. Profiles were recorded during winter (12/2/98) and summer (3/8/98) and the mean of two profiles plotted.

In addition, total downwelling irradiance (PAR) was measured over a depth range beyond the reef front at Trou aux Biches, Mauritius (1/1/96). Measurements were made using the LICOR underwater quantum sensor and LI-1000 datalogger. The sensor was attached to a weighted frame and lowered over the side of a boat; a 60 second average irradiance was logged at 3 m intervals to a depth of 30 m. Depth and irradiance measurements from both Lough Hyne and Mauritius were subsequently

used to obtain attenuation coefficients (K_d, m^{-1}) for tropical water during January 1996 and temperate water, during both February 1998 and August 1998. In addition, the depth at which downward irradiance reached 1 % of surface irradiance (z_{eu}, m) was determined for different wavelengths of PAR for Lough Hyne.

2.3. Results

2.3.1. Seasonal fluctuations in meteorological conditions

Figure 2.4 shows seasonal fluctuations in mean air temperatures (°C) at two temperate sites, Valley observatory (1998) and Valentia observatory (1998) and a tropical site, Mauritius (1996). Temperate mean air temperatures show a minimum value of 6.5 °C at Valley and 7.5 °C at Valentia in January and increase through the year to a maximum temperature of 15.6 °C at Valley and 15.5 °C at Valentia in August, whereafter temperature decreases towards January (Figure 2.4 a and b). Tropical air temperatures in Mauritius (Figure 2.4 c) are higher and show less seasonal variation; a maximum temperature of 28.0 °C was recorded in January (summer) compared with a minimum of 20.7 °C in July (winter).

Rainfall (mm) at Valley (Figure 2.5a) showed a seasonally varied pattern in 1998; the highest levels were recorded from October to January (range 90-126 mm), mid March to April (95 mm) and June (121 mm). Lowest rainfall was recorded during February (30 mm) and May (16 mm). Rainfall at Valentia showed a similar pattern in 1998 with highest levels recorded from October to January (range 193-221 mm) and in April (176 mm) and June (184 mm), and lowest levels recorded during May (31 mm) and February (41 mm; Figure 2.5b).

The rainfall pattern for tropical Mauritius was very different for 1995/96 (Figure 2.5c). Rainfall is high during January (110 mm) and December (240 mm) only, which

comprise the wet season, and minimal from May-November ranging from 5-32 mm (Figure 2.5 c).

Figure 2.6 shows seasonal variation in total sunshine (hours). At Valley maximum sunshine in 1998 was recorded from April-September (range 154-205 hours); maximum total sunshine of 228 hours was recorded for May and minimum of 25 hours was recorded for December (Figure 2.6 a). Similarly, at Valentia maximum sunshine in 1998 was also recorded from April-September (range 103-172 hours); maximum total sunshine was lower (172 hours for both April and May) and minimum sunshine was higher (37.5 hours for December) than at Valley (Figure 2.6 b). Sunshine in Mauritius (1995/96) was consistently high compared with temperate levels and showed a minimum of 100 hours in December and a maximum of 134 hours in February (Figure 2.6 c). However, the maximum total sunshine for tropical Mauritius was 134 hours (December) which was lower than the maximum of 228 hours total sunshine recorded at temperate Valley.

The pattern of seasonal daylength variation was the same for both temperate sites in 1998; daylength increased steadily from the Winter solstice on the 22nd December (shortest day, 6.5 hours) to the Summer solstice on 21st June (longest day, 16.5 hours), then decreased from June to December again (Figure 2.7a and b). Daylength in tropical Mauritius in 1998 (Figure 2.7 c) showed only slight seasonal variation with the shortest daylengths of 11 hours occurring April to July (winter) and the longest of 12.5 hours occurring November to January (summer). Temperate summer daylength was greater than tropical summer daylength.

2.3.2. Seasonal variations in water column parameters

Figure 2.8 shows seasonal variations in nitrate and phosphate concentrations. In the Menai Strait in 1994 (Figure 2.8a) nitrate showed a gradual rise from initially low

values (0.8 μmol l^{-1}) at the beginning of October to a peak value of 12 μmol l^{-1} in November. Concentrations remained high until the end of March. A rapid drop in nitrate concentration occurred in during April/May, falling by mid May to 0 μmol l^{-1}. Concentrations recovered only marginally in July to 1μmol l^{-1} and remained low until the end of October. Seasonal changes in phosphate were less pronounced. Phosphate concentrations remained stable at 0.8 μmol l^{-1} from January to May. A small fall in concentration to 0.3 μmol l^{-1} was observed at the beginning of May. Concentrations then rapidly returned to pre-May levels, rising again gradually at the end of August to October levels. Figure 2.9 shows seasonal variations in chlorophyll concentrations in the Menai Strait in 1994/95 and chlorophyll *a* concentrations in Lough Hyne for 1991 only. The seasonal cycle of chlorophyll in the Menai Strait showed low levels from November to March followed by a sharp rise in April to peak levels in May (5 μmol l^{-1}). Thereafter, concentrations fell but fluctuated between 1 and 2 μmol l^{-1} until October, whereafter chlorophyll fell to low winter levels (0.05 μmol l^{-1}). Similar variation was observed in chlorophyll *a* concentrations in Lough Hyne in 1991, with low levels from November to February (0.04-0.09 μmol l^{-1}), a peak during May (1.10 μmol l^{-1}) followed by a low in June and fluctuations between 0.16 and 0.54 μmol l^{-1} during the summer.

Water temperature fluctuations (Figure 2.10) reflect variations in seasonal air temperature (Figure 2.4). At the temperate sites water temperature is low during winter and high during summer with an annual range of the order 13 °C. For the Menai Strait, water temperature minimum and maximum values in 1994 were 6 °C and 19 °C respectively ie: a total range of 13 °C. The seasonal cycle of water temperature showed the expected sinusoidal pattern with maximum temperature in

August and minimum temperature during mid February to mid March (Figure 2.10a). By comparison, water temperature in Trou aux Biches lagoon remained consistently high through the seasons in 1995/96, with a maximum of 27.9 °C in January and a minimum value of 22.5 °C in July. The annual range in temperature in Trou aux Biches lagoon was of the order 5.4 °C.

2.3.3. Underwater irradiance

2.3.3.1. The effect of weather and season on daily cycles of underwater irradiance

Figure 2.11 shows the total mean downwelling irradiance over 24 hours at 6 m in Lough Hyne, during summer (7/98). Higher irradiance was recorded on a sunny day with intermittent cloud (Figure 2.11a) than on a dull, rainy day (Figure 2.11b). The maximum irradiance recorded over the whole 24 hour cycle was 3 times higher on a sunny day (497 μmol m^{-2} s^{-1}) than on a rainy day (149 μmol m^{-2} s^{-1}). The total daily illumination underwater in summer (7/98) on a sunny day was 5.63 mol m^{-2} day^{-1} compared with 2.90 mol m^{-2} day^{-1} on a dull, rainy day (Table 2.1). Thus, total irradiance was almost twice as high on a sunny day than on a rainy day in summer. During winter (2/98), data was only available for a dull, rainy day (Figure 2.12). The maximum irradiance recorded at 6 m over the whole 24 hour cycle, on a rainy day during winter was 70 μmol m^{-2} s^{-1}. Thus, at 6 m during winter on a rainy day the prevailing irradiance is less than half the irradiance experienced at 6 m during summer on a rainy day. Similarly, the total daily illumination on a dull, rainy day in winter (2.19 mol m^{-2} day^{-1}) was less than on a dull, rainy day in summer (2.90 mol m^{-2} day^{-1}; table 2.1).

Figure 2.13 shows total downwelling irradiance over a daily cycle at 0.5-1 m water depth in Trou D'Eau Douce (TDD) lagoon. On a bright, sunny day during April

irradiance reached a maximum of 2522 μmol m^{-2} s^{-1} at 10.30 hrs. By comparison, a maximum irradiance of only 1985 μmol m^{-2} s^{-1} was recorded at 11.30 hrs on a dull, overcast day in Mauritius (April 1997). Similarly, the total daily illumination on a sunny day in TDD lagoon was 47.73 mol m^{-2} day^{-1} compared with only 35.09 mol m^{-2} day^{-1} on a dull, overcast day (Table 2.1).

In temperate water daily cycles were measured at 6 m water depth compared with only 0.5-1 m in tropical water. To enable a comparison of the daily cycles of irradiance measured in tropical and temperate waters, knowledge of attenuation with depth was required. Figure 2.14 shows the attenuation profile for Mauritian coastal water during January 1996. At 6 m, irradiance was reduced to approximately half the sub-surface irradiance (eg: 891 μmol m^{-2} s^{-1} at 6 m compared with 1437μmol m^{-2} s^{-1} sub-surface); halving the irradiance measured over a daily cycle in Mauritius gave an approximate irradiance expected for a daily cycle at 6 m. Thus, the maximum mean irradiance of 2522 μmol m^{-2} s^{-1} over a daily cycle on a sunny day in Mauritius (4/98) was halved (2522/2 = 1261 μmol m^{-2} s^{-1}) and found to be 4 times higher than the maximum mean irradiance measured on a sunny day in Lough Hyne. Furthermore, maximum mean irradiance was higher on a dull, overcast day in Trou D'Eau Douce (TDD) lagoon (1167/ 2= 584 μmol m^{-2} s^{-1}) than on a sunny day during summer in temperate Lough Hyne (333 μmol m^{-2} s^{-1}). When a similar adjustment was made to the total daily illuminations for TDD lagoon, the sunny day total (47.73/2 = 23.87 mol m^{-2} day^{-1}) was more than 3 times higher than the Lough Hyne sunny day total for summer (5.63 mol m^{-2} day^{-1}). Similarly, total daily illumination for TDD on a dull, overcast day (35.09/2 = 17.55 mol m^{-2} day^{-1}) was more than 5 times higher than total daily illumination on a dull, rainy day in Lough Hyne in summer.

2.3.3.2. The effect of season on attenuation of downwelling irradiance

Figure 2.15 shows the effects of season on attenuation of downwelling irradiance underwater in Lough Hyne. Firstly, during both summer (Figure 2.15a) and winter (Figure 2.15b) diminution of downward irradiance was approximately exponential, showing rapid and increasing attenuation with depth. Secondly, the attenuation coefficient (K_d, m^{-1} ; table 2.2) was obtained using a computer programme (written and provided by Dr. D. Bowers, School of Ocean Sciences, University of Wales, Bangor) and was 0.239 m^{-1} during summer (July 1998) and 0.179 m^{-1} during winter (February 1998). Thus, attenuation of total downward irradiance (PAR) was greater in summer than winter. Figure 2.14 shows the attenuation of downwelling irradiance (PAR, μmol m^{-2} s^{-1}) in Mauritius which was also approximately exponential. Using regression analysis of the same data, the attenuation coefficient was obtained for coastal waters in Mauritius during January 1996 (Table 2.2) and found to be 0.072 m^{-1}. This value was much lower than the attenuation coefficient for Lough Hyne during both winter and summer.

In addition to total PAR attenuation, the constituent wavebands of PAR also demonstrated variation in attenuation with depth in summer and winter water in Lough Hyne. Figure 2.16 shows the spectral variation of attenuation coefficients in Lough Hyne. During summer the most penetrative wavelength was 555 nm (green light) with an attenuation coefficient of 0.178 m^{-1}. By comparison, the most penetrative wavelength during winter was 510 nm (blue-green light; 0.161 m^{-1}). The least penetrative wavelength was 665 nm (orange light) for both winter and summer. In addition, slightly greater attenuation of wave bands 412-490 nm was observed during summer (8/98).

The '1 % depth' (z_{eu}) was also calculated for Lough Hyne during summer and winter and for Mauritius (1/96) using the following equation:

$$z_{eu} = \frac{4.6}{K_d\,(PAR)}$$

where z_{eu} , is the depth at which downwelling PAR falls to 1% of sub-surface irradiance and K_d (PAR) is the attenuation coefficient (after Kirk, 1994). In Lough Hyne during winter, the 1 % depth was at 25.70 m compared with 19.25 m during summer, which directly reflects the greater attenuation of downwelling irradiance observed in summer (Table 2.2). By comparsion, the 1 % depth in Mauritius was 63.89 m (Table 2.2).

2.4. Discussion

Both tropical Mauritius and temperate sites in the UK showed seasonal variation in meteorological conditions. At the temperate sites during summer daylength and total sunshine or insolation were observed to be greater than in Mauritius. Kirk (1994) explained that at any given point on the Earth's surface the daylength and solar elevation reach their maximum values in summer and minimum values in winter. With increasing latitude, the solar elevation at noon, and therefore the maximum value of solar irradiance, decreases as does the daily insolation. In the summer, however, this effect is counteracted by the increase in daylength with increasing latitude, and the net result is that high latitude regions can have a greater daily insolation in mid-summer than tropical regions. In winter the high-latitude regions have shorter days as well as lower solar elevations, so their daily insolation is much less than at low latitudes, as demonstrated in the present study (Figures 2.6 and 2.7).

The air temperature variations also reflect these latitudinal differences with much lower and more varied temperatures occurring at the temperate sites. Rainfall showed a very different latitudinal effect with three peaks at the temperate sites corresponding to autumn, spring and one summer month. By contrast in Mauritius rainfall was consistently low during February-November (Figure 2.5) with two high rainfall peaks in December and January. The extremely high rainfall in December 1996 was due to cyclonic conditions over Mauritius (Daby, 1999).

Variations in meteorological conditions caused variations in physical parameters underwater. Water temperature fluctuations reflected seasonal fluctuations in air temperature, sunshine and daylength. Peak water temperatures occurred slightly later in the year than peak air temperaures due to the higher heat capacity of water than air (Tait, 1981). Important to consider here are reports that 1998 was the warmest year on record, and experienced the strongest El Nino ever recorded (Wilkerson *et al.* 1999). As a consequence high water temperatures were observed in many parts of the oceans, particularly the tropical Indian Ocean (Wilkerson *et al.* 1999), producing atypical conditions. In the present study, only daylength data for Mauritius from 1998 were considered; all other data for Mauritius were from 1995/1996. Daylength is a function of the relative position of the earth to the sun, and thus is not affected by climatic variation such as El Nino. Hence, 1998 daylength data for Mauritius were accepted as demonstrating typical annual variation. Meteorological data from 1998 was considered for the temperate sites. Although El Nino caused increases in sea water temperature in the tropics (Wilkerson *et al.* 1999) its effect on conditions in temperate waters is unknown. The data presented for temperate sites during 1998 must therefore be treated with some caution as the annual variations observed may have been altered by the El Nino event, and prove to be atypical of the sites.

In Trou aux Biches lagoon, Mauritius, the peak in nitrate level in August (Figure 2.8) coincided exactly with the highest rainfall recorded (45.3 mm) in the area. The high rainfall and peak in nitrate level were followed 2-3 weeks later by a phytoplankton bloom (Daby, 1999). The statistical correlation between phytoplankton abundance and nutrient concentration was weak in Trou aux Biches lagoon (Daby, 1999). However, the peak in phytoplankton density observed in September/October was preceeded by the peak in nitrate concentration in August, and the bloom subsided with depletion of nitrate, which attained the lowest level in September. Hence, Daby (1999) concluded that increased rainfall may result in the input of higher levels of nutrients, due to increased underground seepage as well as surface run-off, which may induce the formation of phytoplankton blooms.

In the Menai Strait a similar interaction of nutrients and algal abundance was observed by Owen (1995), such that chlorophyll and nutrient concentrations reflect the seasonal cycle of algal succession. The winter was characterised by low algal abundance (and therefore chlorophyll concentrations) and high nutrient concentrations, followed by a diatom bloom. This bloom was rapidly succeeded by an intense but short-lived bloom of several weeks duration, of *Phaeocystis pouchetti* in the spring (Owen, 1995). Accompanying these blooms was a large increase in chlorophyll concentrations, a rapid fall in nitrate concentrations and a small drop in phosphate concentration. In temperate waters in winter, the water column becomes vertically mixed (Tait 1981, Mann & Lazier 1996) due to more extreme meteorological conditions (wind and temperature). Nutrients from the seabed are mixed upwards and replenish water column concentrations, producing the observed high concentrations of nitrate in the Menai Strait and Lough Hyne in winter. The increased daylength and insolation that occur with the onset of Spring, caused the

surface water temperature to rise, and the water column to become stabilized by thermal stratification. Combined with high rainfall which further increased nutrient levels, optimal conditions were created for phytoplankton photosynthesis and growth. The result was a phytoplankton bloom and a peak in chlorophyll concentration observed in the Menai Strait and Lough Hyne (Figure 2.9). Subsequent depletion of nutrients occurs, whereafter conditions become nutrient limited, producing a rapid decline in phytoplankton abundance. Enhancing the decline in phytoplankton adundance there would have been a proliferation in herbivorous zooplankton (Tait, 1981). The second smaller peak in chlorophyll concentration observed in Lough Hyne and the Menai Strait in autumn represents a second bloom. This bloom occurs as a result of nutrient replenishment from deep vertical mixing and high rainfall in changing meteorological conditions. Immediately thereafter, nutrients become depleted and again become limiting. Although subsequent mixing restores nutrient levels, the water is more turbid and light levels are limiting to photosynthesis and leading to the observed low algal abundance in winter and a seasonal cycle which reflects meteorological conditions.

Underwater irradiance

Attenuation of total downwelling PAR was greater in summer than winter in Lough Hyne. This contradicts observations for the Irish Sea which demonstrates higher K_d (m^{-1}) in winter than summer (D. Bowers, pers. comms.). The 1 % depths reported for Lough Hyne illustrate how the lower vertical attenuation of PAR recorded during winter extended the lower limit of the euphotic zone to 25.7 m compared with only 19.3 m in summer. The results presented here represent the conditions during one summer month (7/98) and one winter month (2/98), and may require a full annual

cycle for adequate interpretation. Nevertheless, it is possible that the greater attenuation during July 1998 was caused by high phytoplankton abundance. In July 1991 the concentration of chl *a* in Lough Hyne was higher than in February indicating high phytoplankton abundance (Irish Wildlife Services). Alternatively, weather conditions may have been calm in the days or weeks preceeding measurment of underwater irradiance in February 1998, such that suspended sediments were low. Low turbidity combined with seasonally low plankton levels produced a lower K_d and deeper 1 % depth than in July.

Penetration of downward irradiance was greater in Mauritian coastal waters than in temperate Lough Hyne. In Mauritius a euphotic zone of more than 60 m was observed compared with 20-25 m in Lough Hyne. This supports the observations of Kirk (1994) who reported much lower vertical attenuation coefficients for tropical coastal waters than temperate coastal waters. The clarity of tropical waters in general may be attributed to the relatively stable vertical structure of the water column. In contrast to temperate waters, where the vertical structure of the water changes seasonally, tropical waters may be permanently stratified such that suspended sediment and nutrient levels are low (Mann & Lazier, 1996). The attenuance spectra for Lough Hyne showed variation with water depth. In both winter and summer the most penetrative wavelengths were in the blue-green colour waveband, and the least penetrative were in the red waveband. Thus, Lough Hyne may be described as 'green', typical of temperate coastal waters. Seasonal variation was small and restricted to the shorter wavelength wavebands (412-490 nm) which showed greater attenuation in summer. This may have been due to higher phytoplankton abundance in the summer and is discussed further in chapter 6.

Weather, season and latitude had a profound effect on total daily illumination underwater. During the summer in Lough Hyne, total illumination underwater was 61 % greater on a sunny day than on a dull, rainy day. Similarly, during April 1998 in Mauritius total illumination was 27 % greater on a sunny day with intermittent cloud, than on a dull, overcast day. The extent and type of cloud cover are of great importance in determining the amount of solar flux which penetrates to the Earth's surface (Kirk, 1994). For example, under a thin sheet of cirrus, total irradiance may be 70 % of that under a clear sky. In contrast, a deep layer of stratus cloud may transmit only 10 % of the solar radiation, with 70 % being reflected back to space by its upper surface and 20 % being absorbed within it. On a day with broken cloud, the irradiance is intermittently varying from the full sun value to perhaps 20-50 % of this as clouds pass over the sun (Monteith 1973, Kirk, 1994).

Seasonal variation in total daily illumination was only observed on a dull, rainy/overcast day in Lough Hyne. Total illumination underwater was 25 % greater on a dull day in July 1998 than in February 1998. This is directly related to shorter daylength (Figure 2.7) observed in winter than summer. Finally, latitudinal variation in total daily illumination underwater produced values which were at least 8 times higher in Mauritius than in Lough Hyne, on both sunny and dull days. Observation of daylength and total sunshine showed that both were greater at the temperate sites. Thus, it is likely that variation in total daily illumination underwater was caused by the higher intensity solar radiation experienced at low, tropical latitudes and the optical properties of the water. Finally, UV radiation varies between tropical and temperate latitudes and different water depths. The amount of UV reaching the surface of the sea increases with decreasing latitude, with highest levels recorded in the tropics (Jerlov 1950, Jokiel 1980). Thus, levels of UV are extremely high in

shallow, tropical waters and may severely inhibit photosynthesis (Jokiel 1980). In addition, UV radiation is able to penetrate as far as 20 m in clear oceanic waters (Chapter 2, p. 8) exposing organisms in deeper water habitats to its damaging effects.

Conclusions

Both tropical and temperate sites showed seasonal variation in meteorological conditions. In addition, latitudinal variation was observed. Daylength, sunshine and air temperature were greater in summer months than winter months at both tropical and temperate sites. Although daylength and total sunshine were greater at the temperate sites, total daily illumination measured underwater was greater in Mauritius. Air temperature was lower and more seasonally varied at the temperate sites. Furthermore, water temperature reflected seasonal variation in air temperature, sunshine and daylength.

Rainfall at the temperate sites showed 3 peaks corresponding to autumn, spring and one summer month, whilst rainfall was consistently low in Mauritius from February to November with 2 summer peaks in December and January (Figure 2.5). At both tropical and temperate sites peak rainfall coincided with peak nitrates, which produced a phytoplankton bloom and a peak in chlorophyll levels in subsequent months (Figure 2.9). Nitrates (Figure 2.8) and chlorophyll (Figure 2.9) showed a distinct pattern which was controlled by seasonal stratification of the water column, and hence seasonal variation in meteorological conditions.

The attenuation of downwelling PAR was greater in summer than winter in Lough Hyne. Thus, the 1 % depth was lower in summer than winter. Attenuation of PAR with depth in Mauritius was much less than in temperate waters, resulting in a 1 % depth which was more than 35 m deeper. Accompanying diminution of total PAR intensity with water depth in Lough Hyne was variation in the spectral attenuation;

blue-green wavebands were the most penetrative, whilst violet and red wavebands were attenuated the most. In addition, slight variation in spectral attenuation was observed between winter and summer months. Greater attenuation of violet and blue wavebands occurred during the summer, reflecting the higher algal abundance during summer. Finally, total daily illumination was lower on a dull day than on a sunny day in both Mauritius and Lough Hyne. Thus, poor weather conditions greatly reduced the irradiance available for photosynthesis underwater.

Table 2.1. Total daily illumination underwater calculated from mean downwelling irradiance over a daily cycle in Lough Hyne during summer and winter, and in Trou D'Eau Douce lagoon, Mauritius in April, on sunny and dull, rainy days. The units are moles m^{-2} day^{-1}.

Table 2.2. Attenuation coefficients (K_d, m^{-1}) and 1 % depths (z_{eu}, m) for temperate water during summer (8/98) and winter (2/98) and tropical water (1/1/96); values were obtained using measurements of downwelling irradiance over a depth range.

	Weather	Total daily illumination underwater (mol m^{-2} day^{-1})
Lough Hyne		
Summer (7/98)	Sunny	5.63
	Dull & rainy	2.90
Winter (2/98)	Dull & rainy	2.19
Trou D'Eau Douce lagoon		
April 1998	Sunny	47.7
	Dull & rainy	35.1

Site	K_d (m^{-1})	z_{eu} (m)
Lough Hyne, SUMMER	0.239	19.25
Lough Hyne, WINTER	0.179	25.70
Trou aux Biches, Mauritius	0.072	63.89

Figure 2.1. Location of Trearddur Bay (53° 16' N, 4° 4' W) and Valley on Anglesey, North Wales.

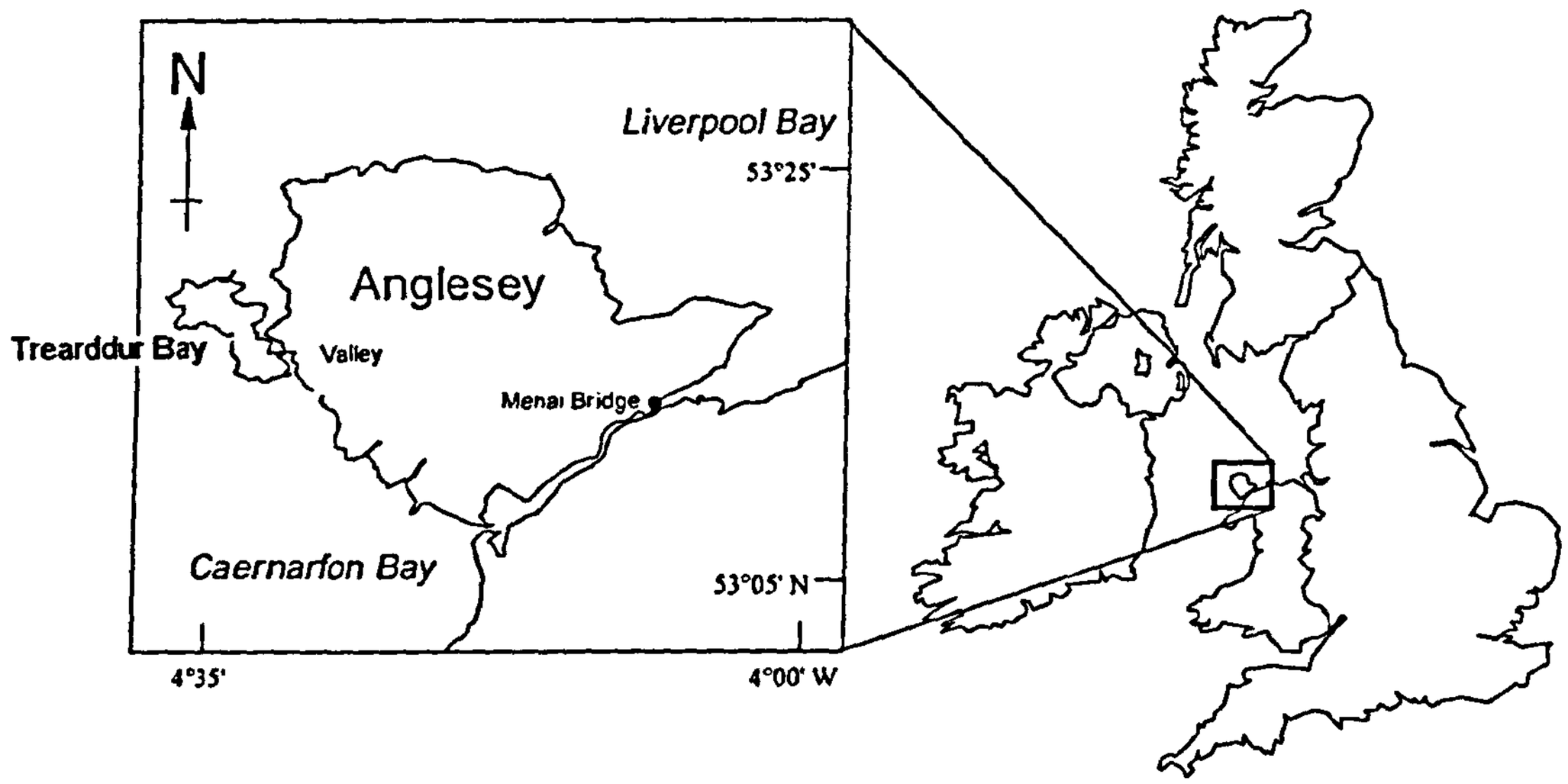

N
Liverpool Bay
53°25'
Anglesey
Trearddur Bay
Valley
Menai Bridge
Caernarfon Bay
53°05' N
4°35'
4°00' W

Figure 2.2. Location of Lough Hyne M.N.R. (51° 29' N, 9° 18' W)

in Co. Cork, Ireland.

IRELAND

Valencia Island

Cork

N

Bantry Bay

SOUTH WEST CO. CORK

51°30' S

LOUGH HYNE

Cape Clear

Atlantic Ocean

9°20' W

Figure 2.3. Location of Trou aux Biches lagoon (57° 00' 28" S, 20° 33' 13" E) and Trou D'Eau Douce lagoon (20° 13' 51" S, 57° 47' 33" E) on Mauritius, Indian Ocean.

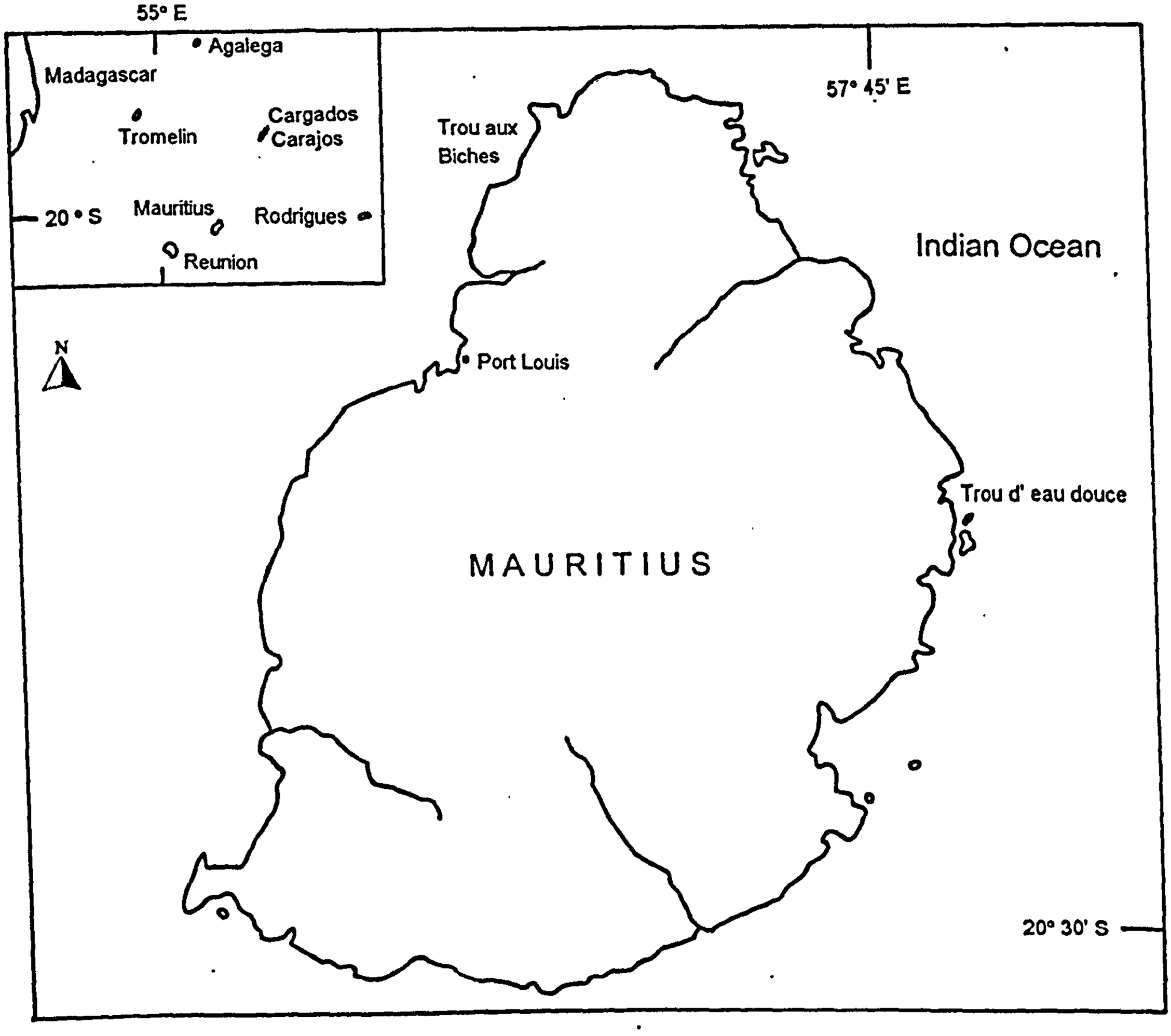
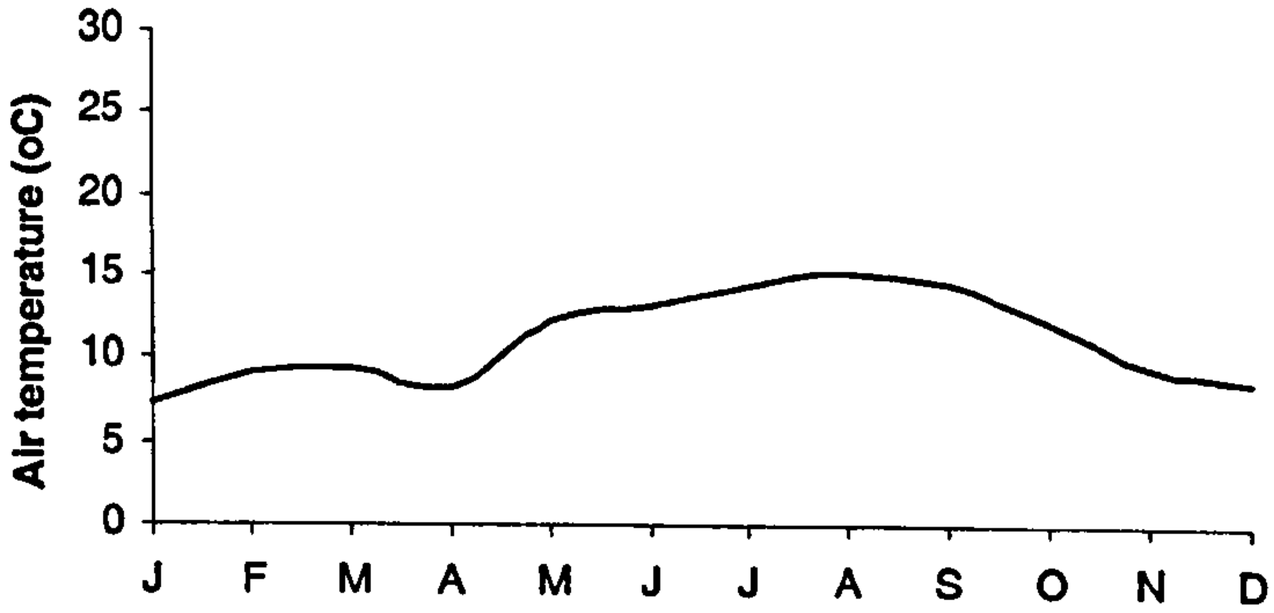
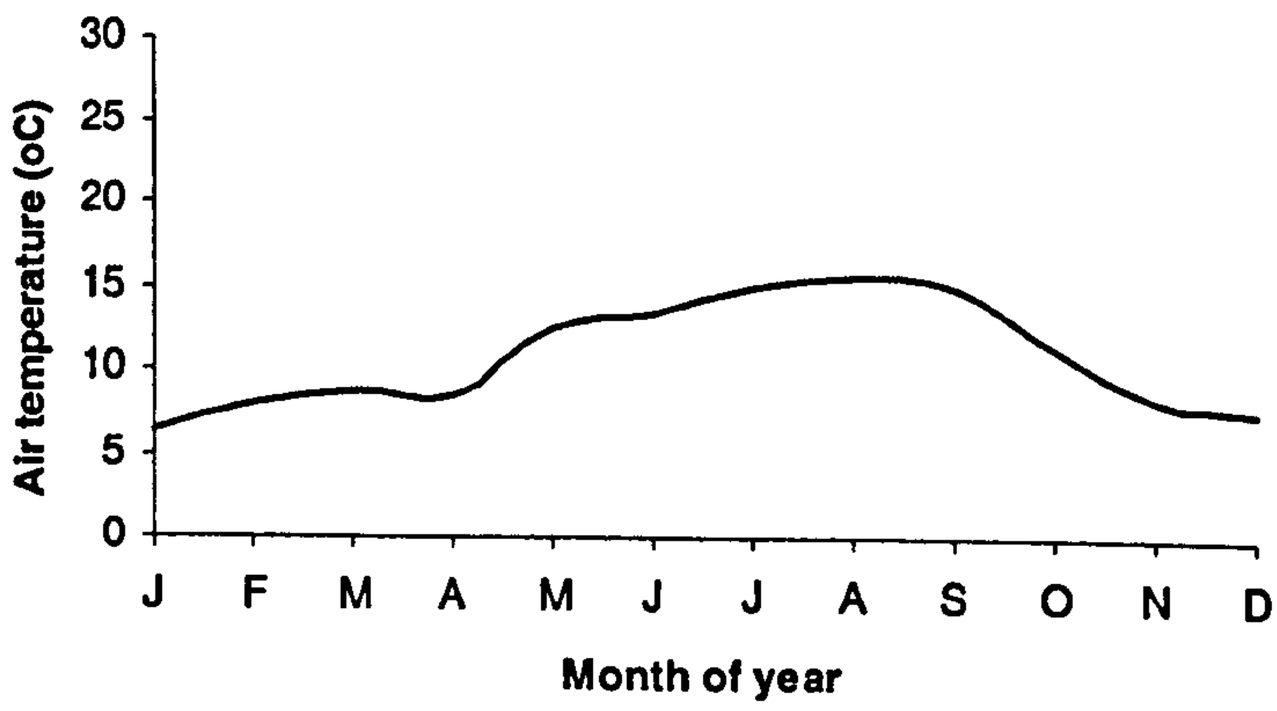

55° E
Agalega
Madagascar
Cargados
Carajos
Tromelin
20 ° S
Mauritius
Rodrigues
Reunion
57° 45' E
Trou aux
Biches
Indian Ocean
N
Port Louis
Trou d' eau douce
MAURITIUS
20° 30' S

Figure 2.4. Seasonal variations in air temperature at a) Valley weather station, Anglesey, North Wales during 1998 (data from Weather log, Royal Meteorological Society, UK); b) Valentia observatory, Ireland during 1998 (data from the Irish Meteorological Office); c) Mauritius during 1996 (data from the Mauritian Meteorological Office).

a)


Air temperature (oC)
30
25
20
15
10
5
0
J F M A M J J A S O N D
Month of year

b)


Air temperature (oC)
30
25
20
15
10
5
0
J F M A M J J A S O N D
Month of year

c)

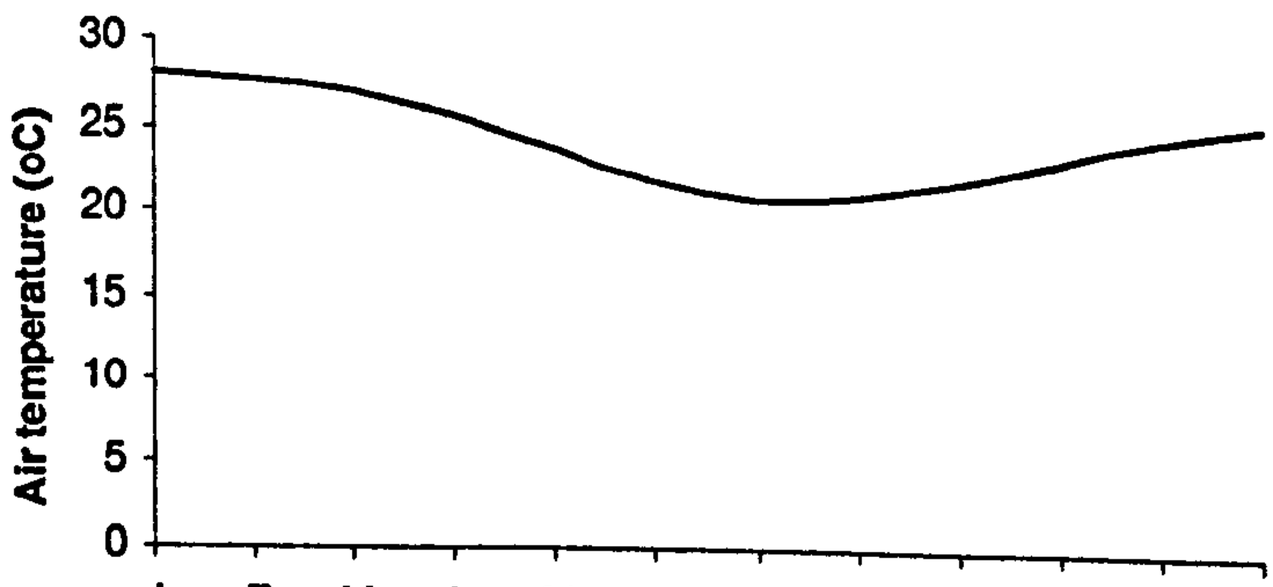
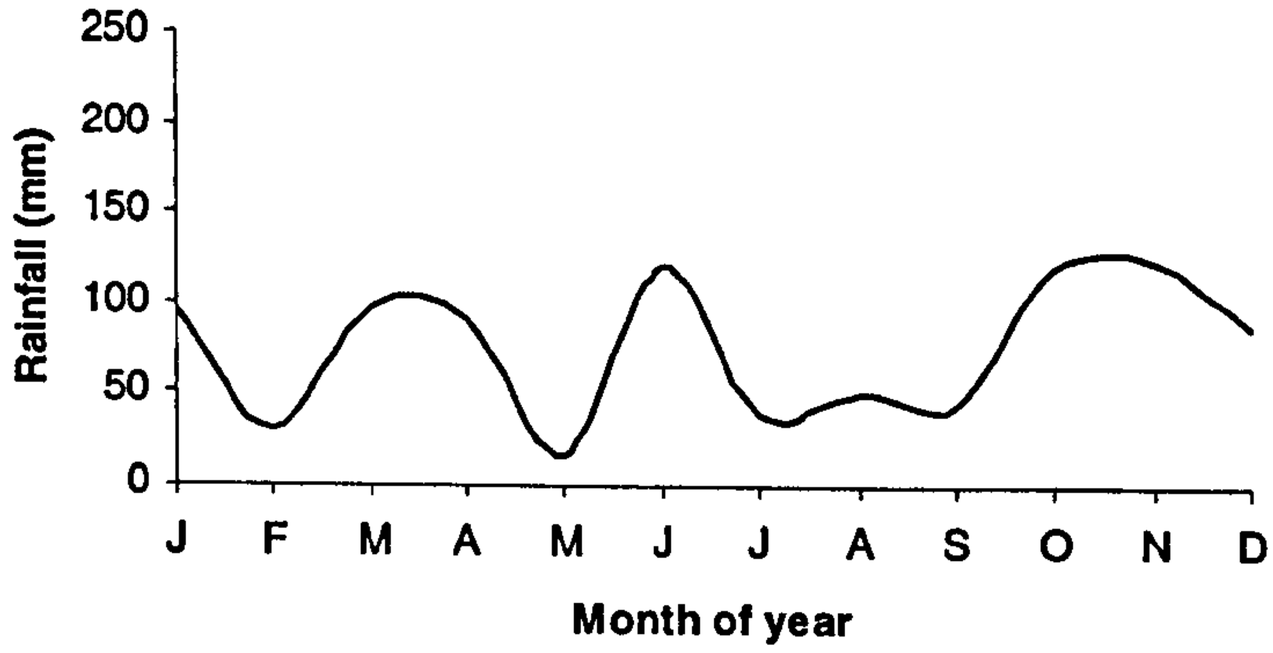
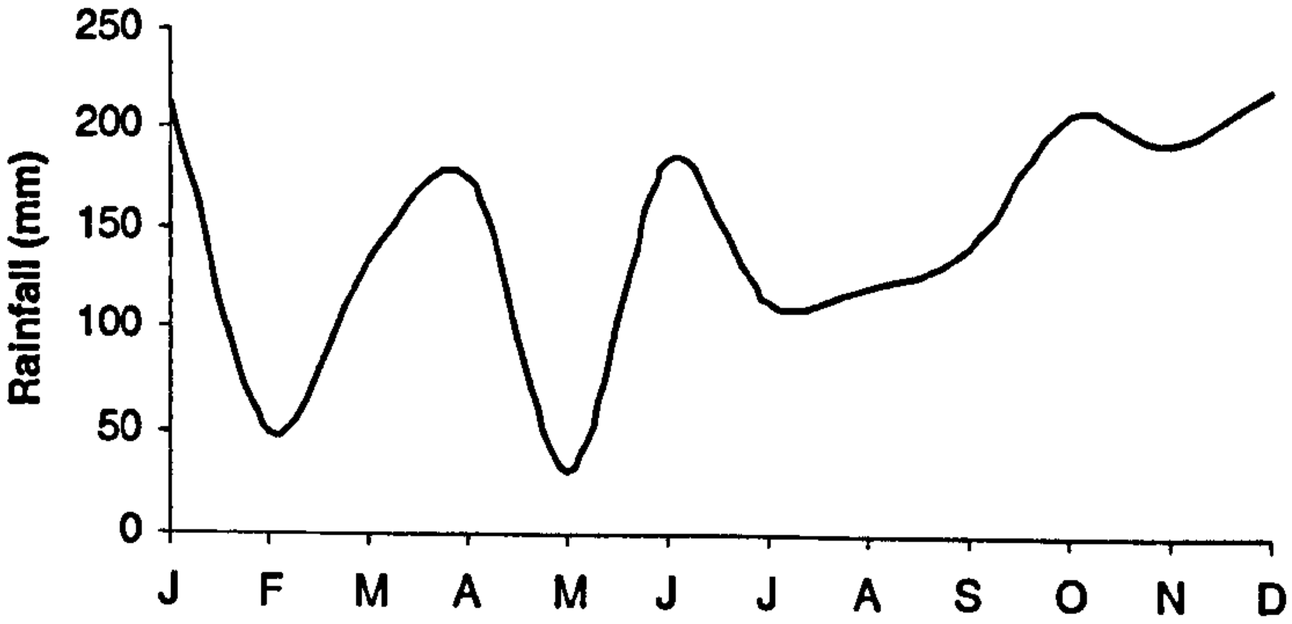

Air temperature (oC)
30
25
20
15
10
5
0
J F M A M J J A S O N D
Month of year

Figure 2.5. Seasonal variation in rainfall (mm) at a) Valley weather station, Anglesey, North Wales during 1998 (data from Weather log, Royal Meteorological Society, UK); b) Valentia observatory, Ireland during 1998 (data from the Irish Meteorological Office); c) Trou aux Biches lagoon, Mauritius during 1995/1996 (data from the Mauritian Meteorological Office).

a)


250
200
150
100
50
0
Rainfall (mm)
J F M A M J J A S O N D
Month of year

b)


250
200
150
100
50
0
Rainfall (mm)
J F M A M J J A S O N D
Month of year

c)

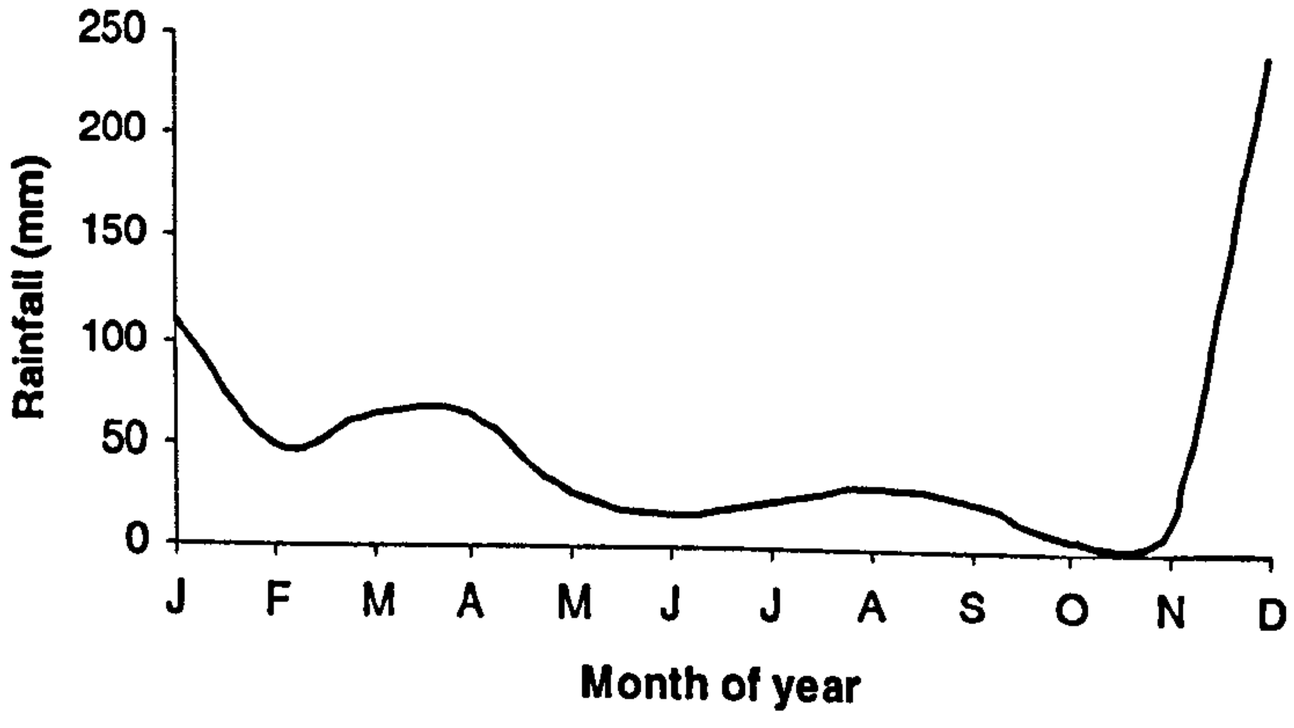
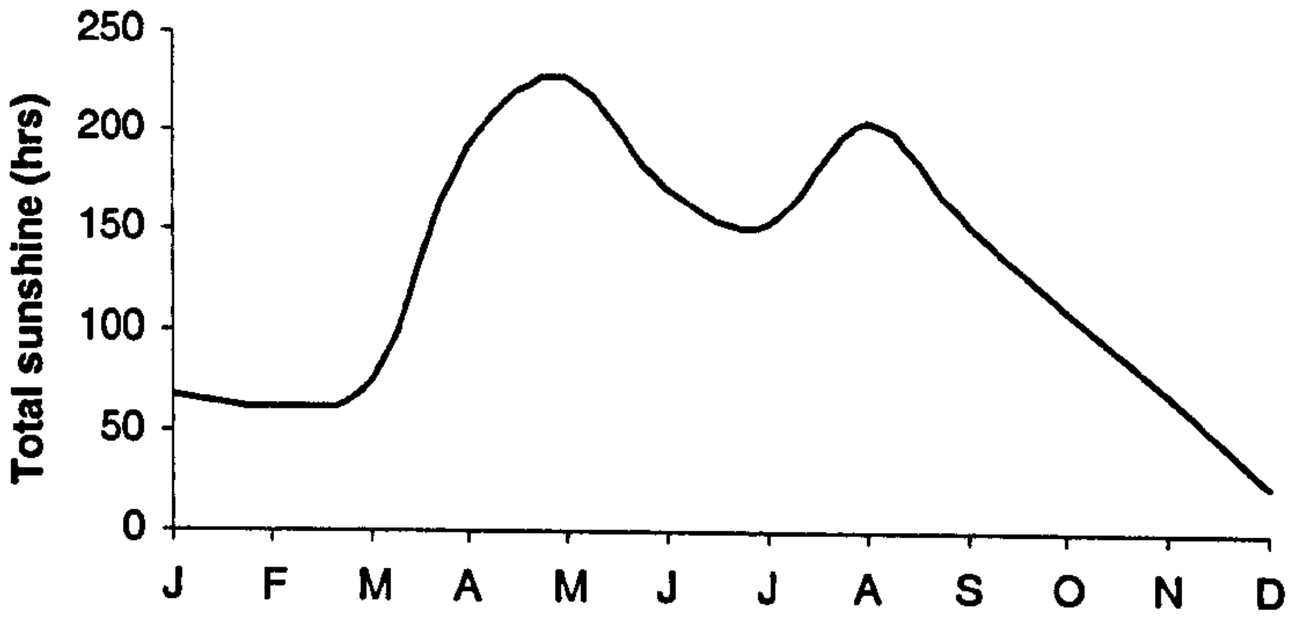
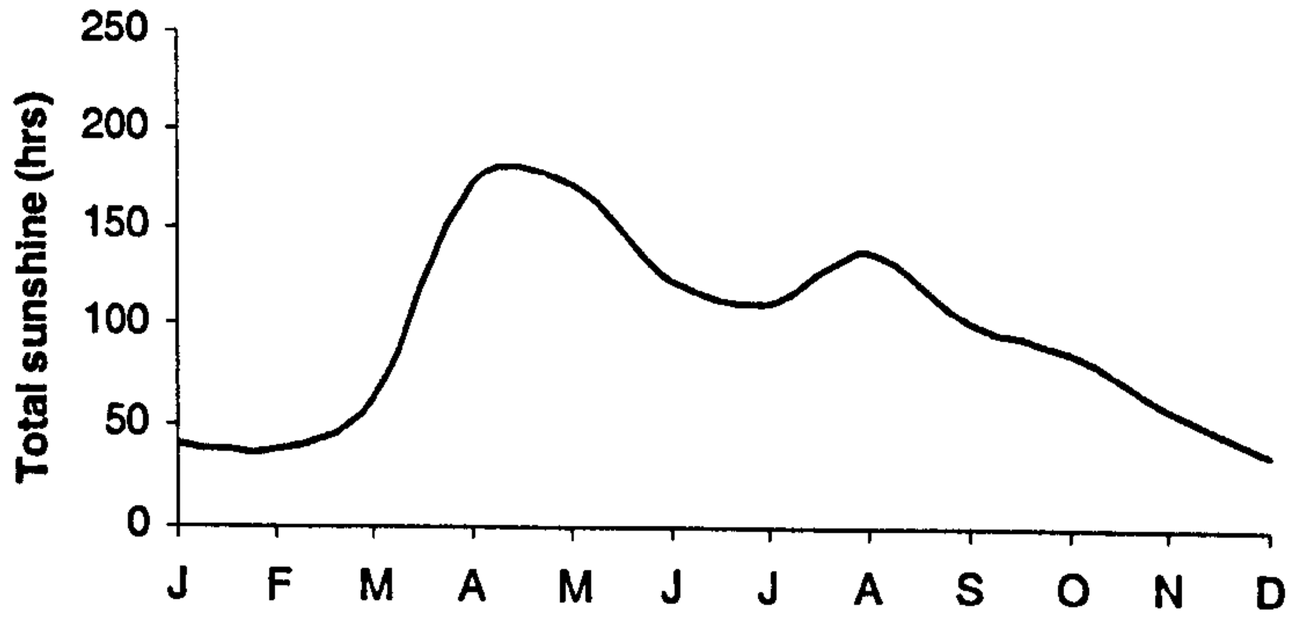

250
200
150
100
50
0
Rainfall (mm)
J F M A M J J A S O N D
Month of year

Figure 2.6. Seasonal variations in total monthly sunshine at a) Valley weather station, Anglesey, North Wales during 1998 (data from the Weather log, Royal Meteorological Society, UK); b) Valentia observatory, Ireland during 1998 (data from the Irish Meteorological Office); c) Trou aux Biches lagoon, Mauritius during 1995/1996 (data from the Mauritian Meteorological Office).

a)


Total sunshine (hrs)
250
200
150
100
50
0
J F M A M J J A S O N D
Month of year

b)


Total sunshine (hrs)
250
200
150
100
50
0
J F M A M J J A S O N D
Month of year

c)

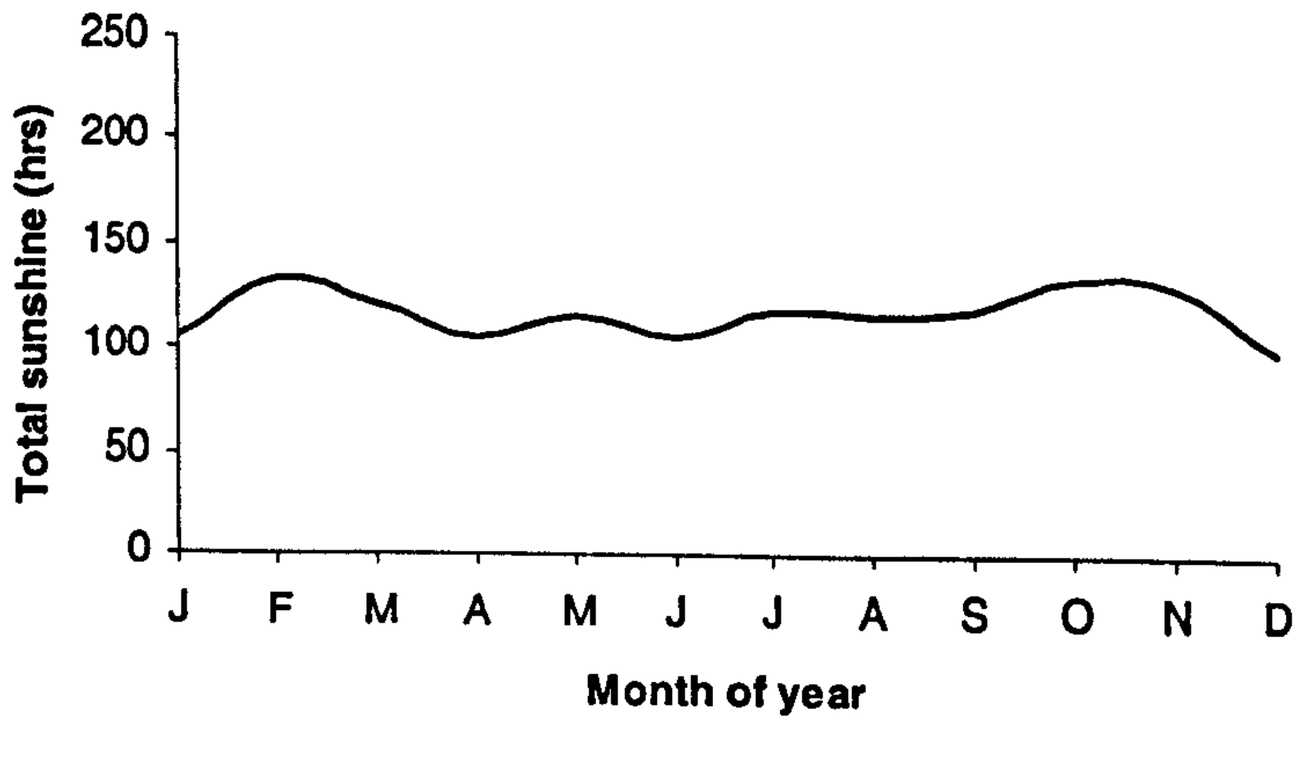
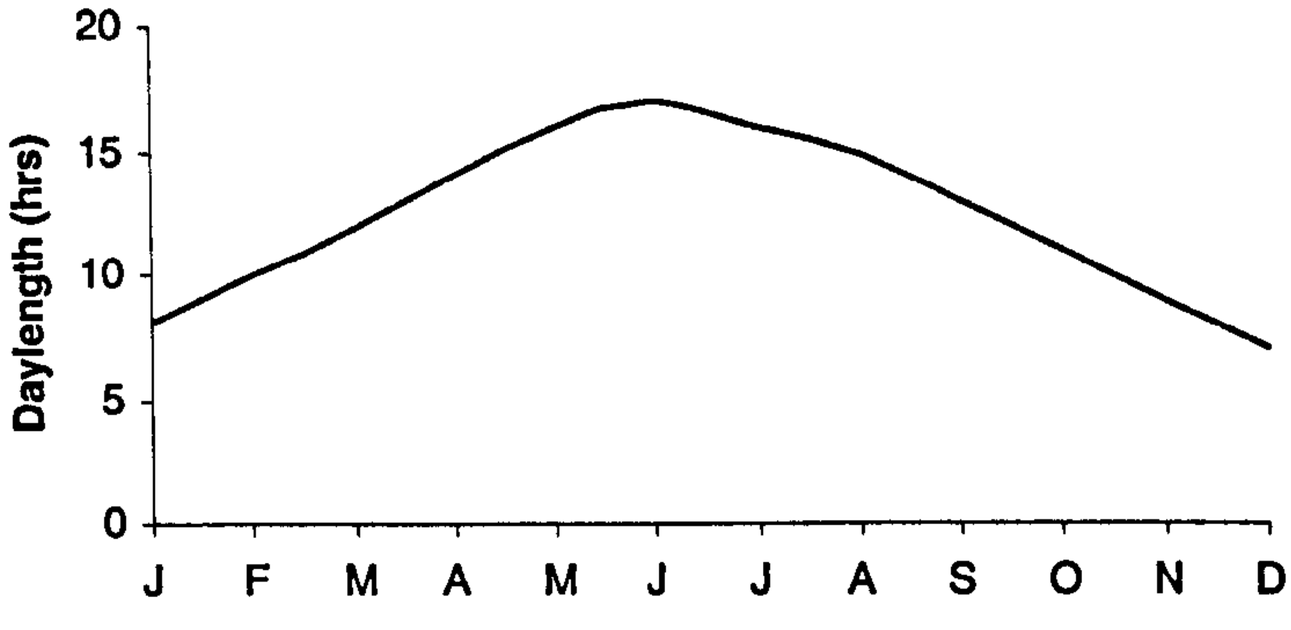
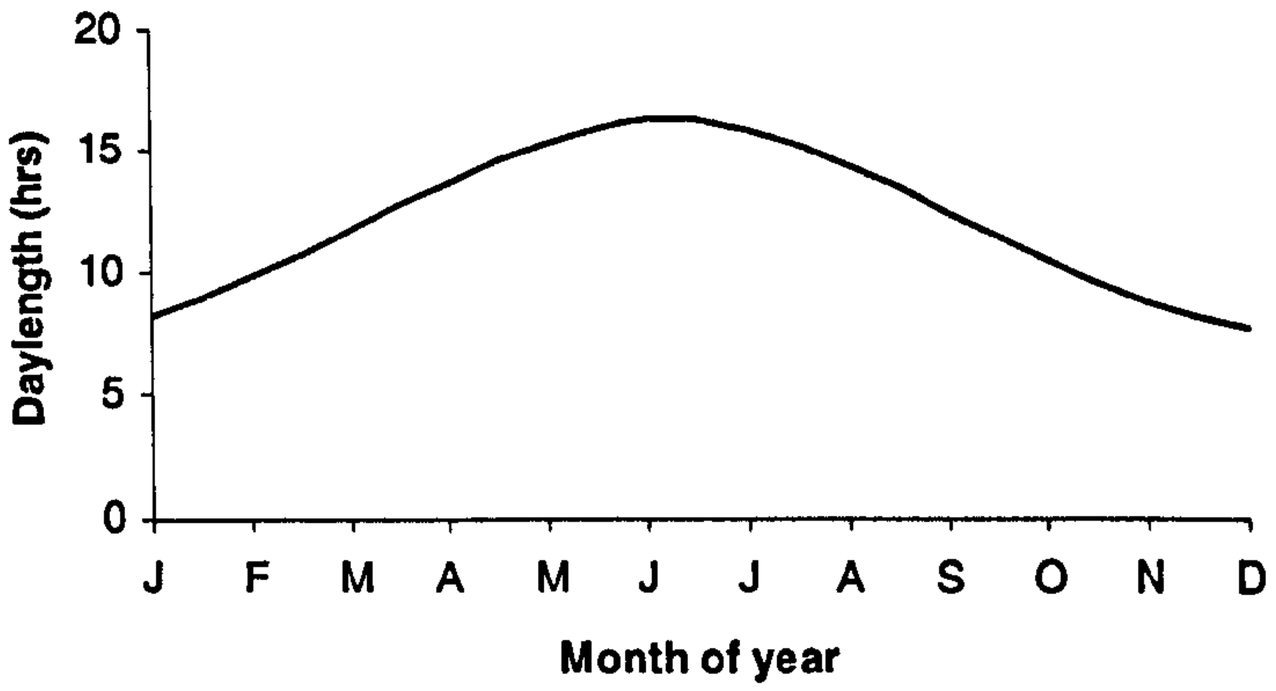

Total sunshine (hrs)
250
200
150
100
50
0
J F M A M J J A S O N D
Month of year

Figure 2.7. Seasonal fluctuations in daylength (hours) at a) Valley weather station, Anglesey, North Wales during 1998 (data from Lavers Liverpool tide tables); b) Valentia observatory, Ireland during 1998 (data from Irish Meteorological Office); c) Mauritius during 1998 (data from Mauritian Meteorological Office).

a)


Daylength (hrs)
20
15
10
5
0
J F M A M J J A S O N D
Month of year

b)


Daylength (hrs)
20
15
10
5
0
J F M A M J J A S O N D
Month of year

c)

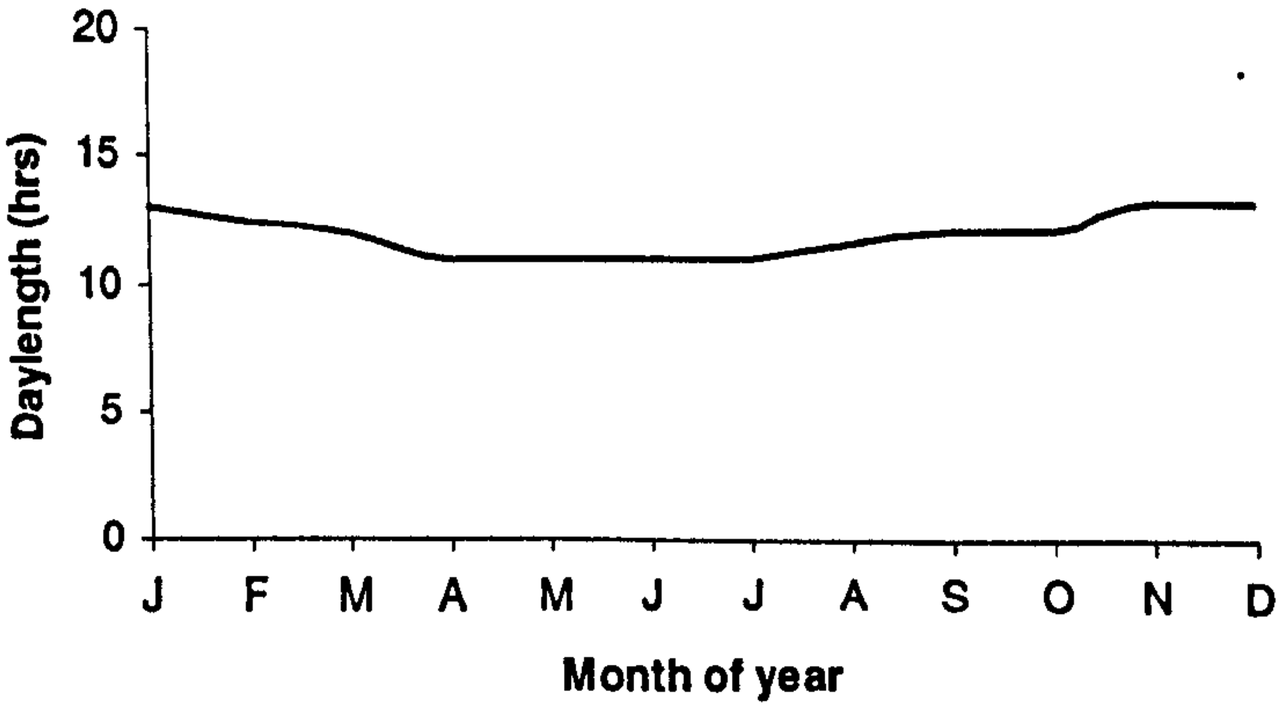
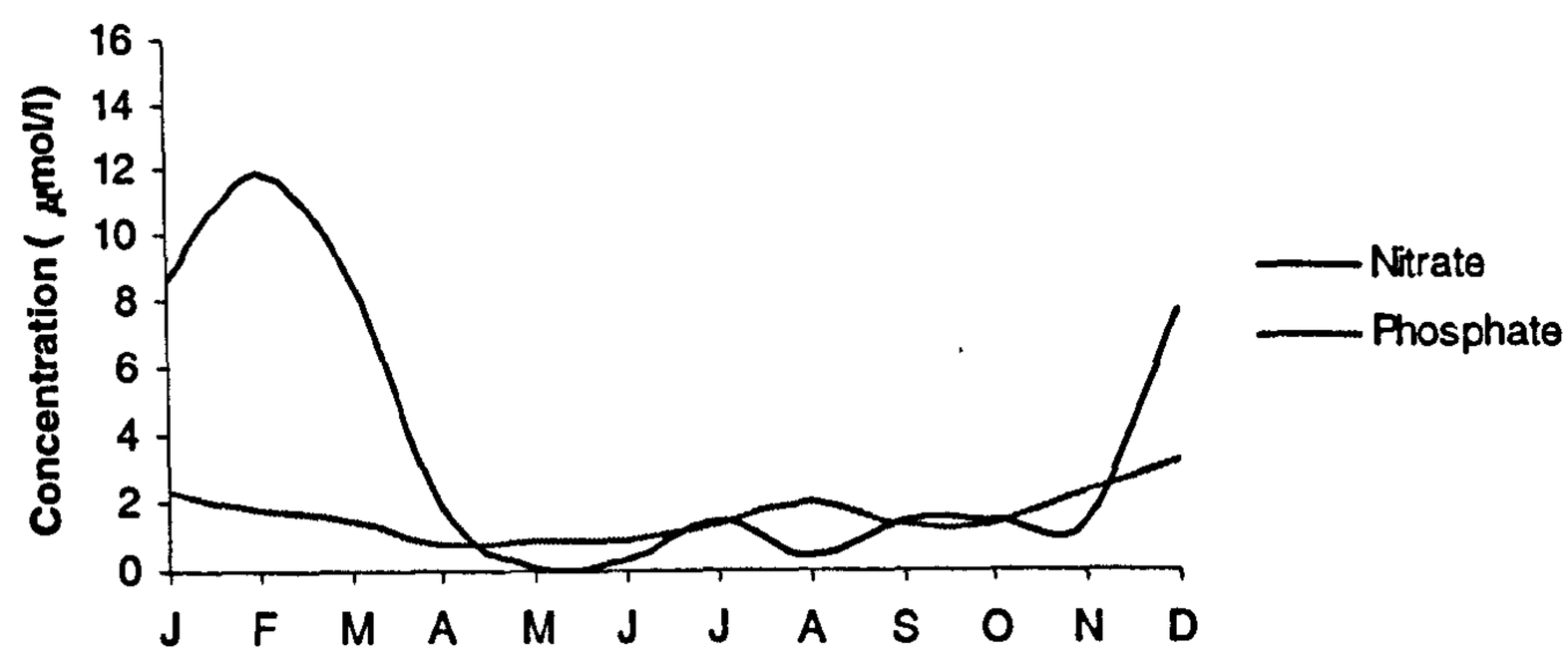

Daylength (hrs)
20
15
10
5
0
J F M A M J J A S O N D
Month of year

Figure 2.8. Mean monthly concentrations of nitrate and phosphate (μmol l^{-1}) in a) the Menai Strait, Anglesey, Wales; data for 1994 adapted from Owen (1998) and Blight *et al.* (1995); b) Lough Hyne M.N.R., Co. Cork; data for 1992 provided by the Aquatic Services, Zoology Department, University College Cork, Ireland; c) Trou aux Biches lagoon, Mauritius, Indian Ocean; data for 1995/1996 adapted from Daby (1999).

a)


Concentration (µmol/l)
16
14
12
10
8
6
4
2
0
J F M A M J J A S O N D
Month of year
Nitrate
Phosphate

b)


Concentration (µmol/l)
16
14
12
10
8
6
4
2
0
J F M A M J J A S O N D
Month of year
Nitrate
Phosphate

c)

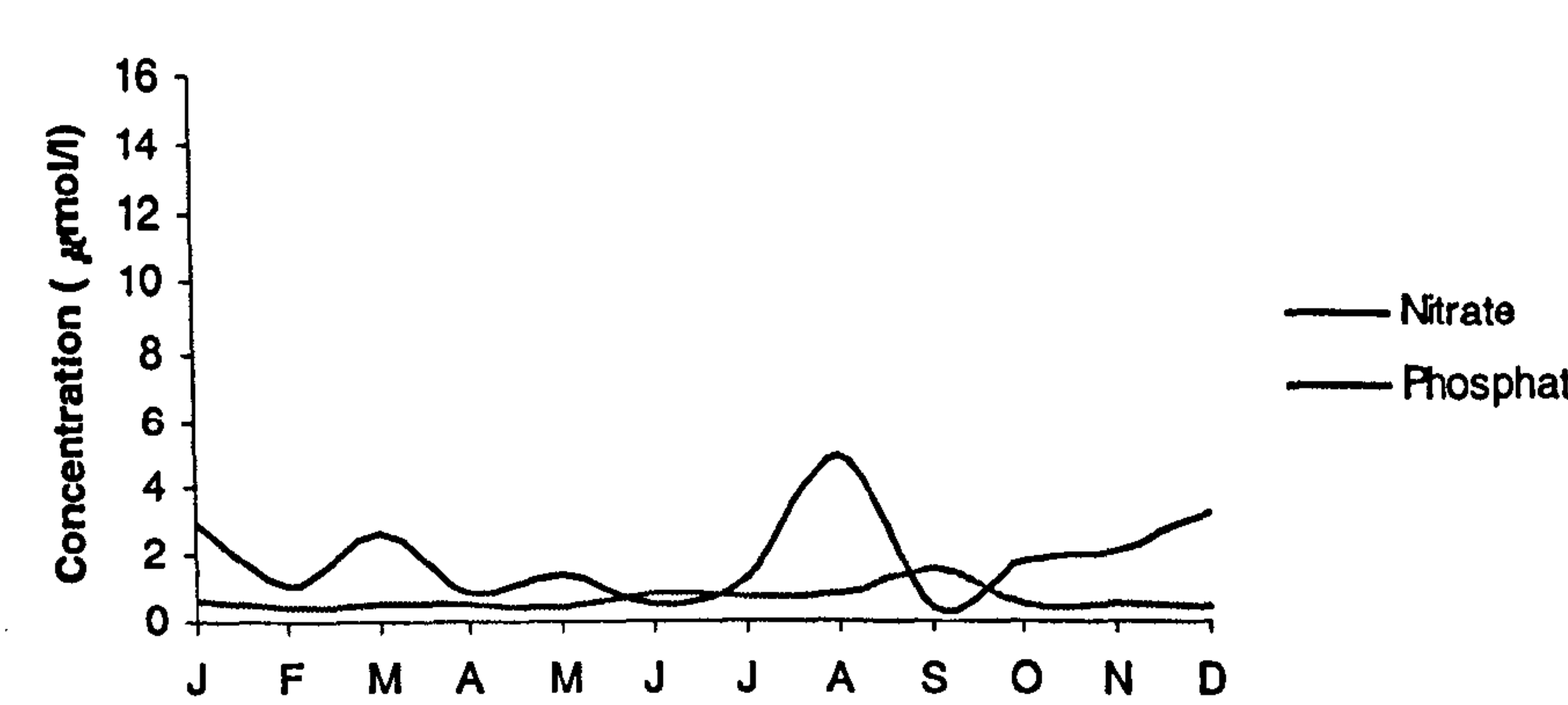
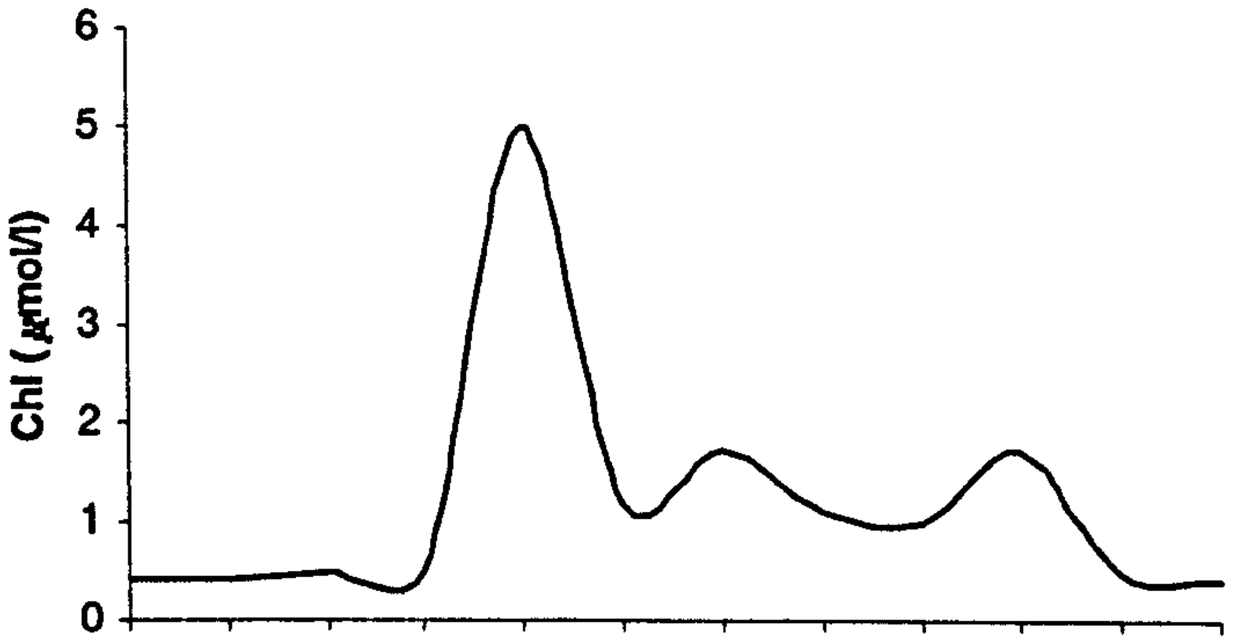

Concentration (µmol/l)
16
14
12
10
8
6
4
2
0
J F M A M J J A S O N D
Month of year
Nitrate
Phosphate

Figure 2.9. Total chlorophyll (Chl) and chlorophyll *a* (Chl *a*) concentrations (µmol/l) in a) the Menai Strait, North Wales during 1994/95 (adapted from Owen, 1998); b) Lough Hyne M.N.R., Co. Cork, Ireland during 1991 (data from the Aquatic Services Unit, Zoology Department, University College Cork).

a)


Chl (µmol/l)
0
1
2
3
4
5
6
J F M A M J J A S O N D
Month of year

b)

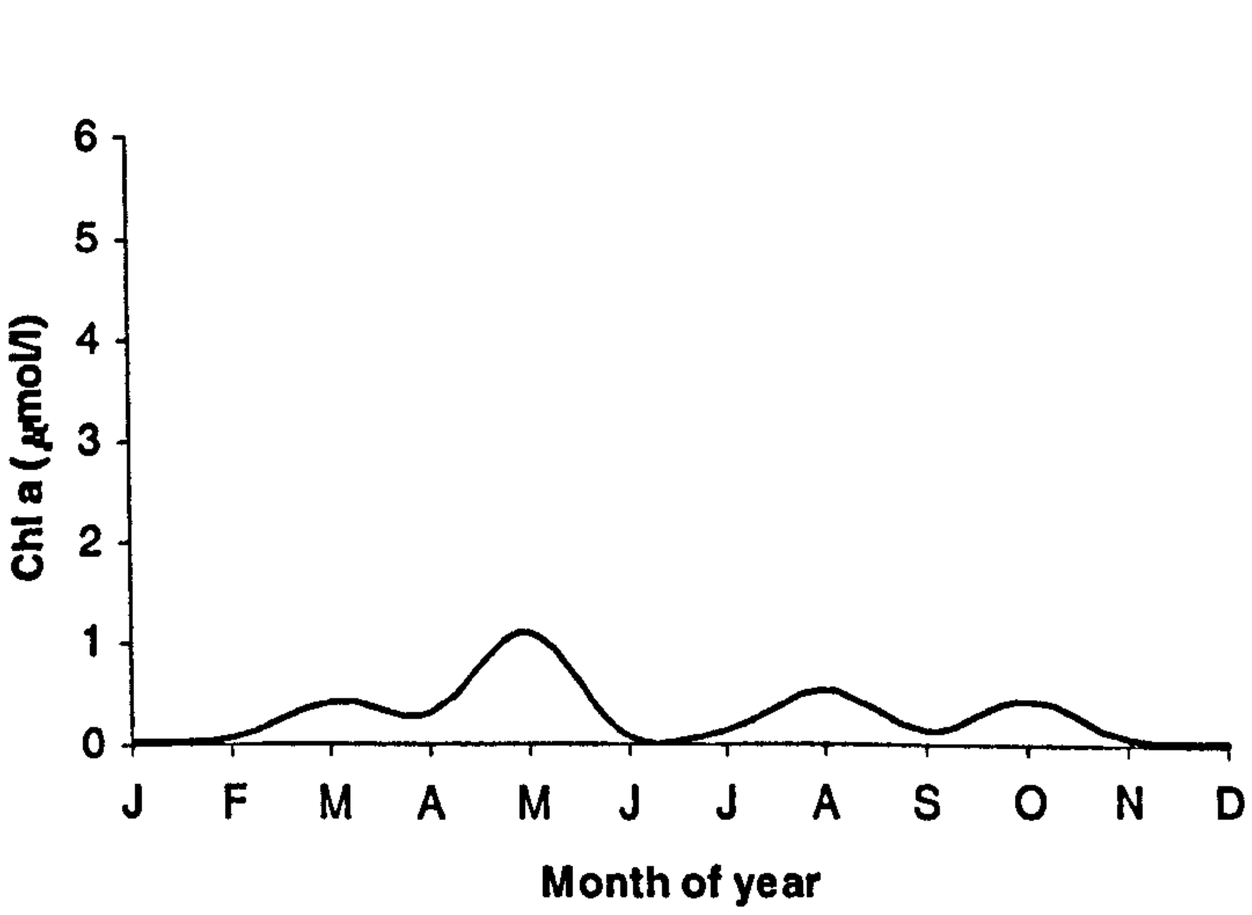
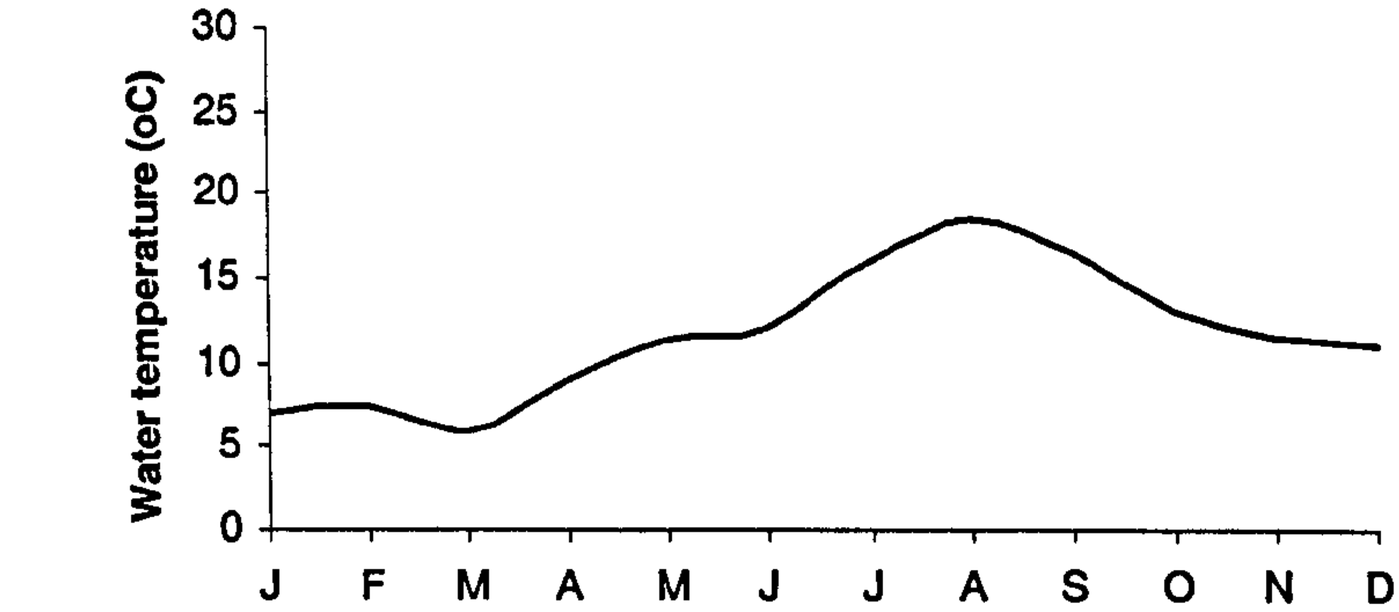
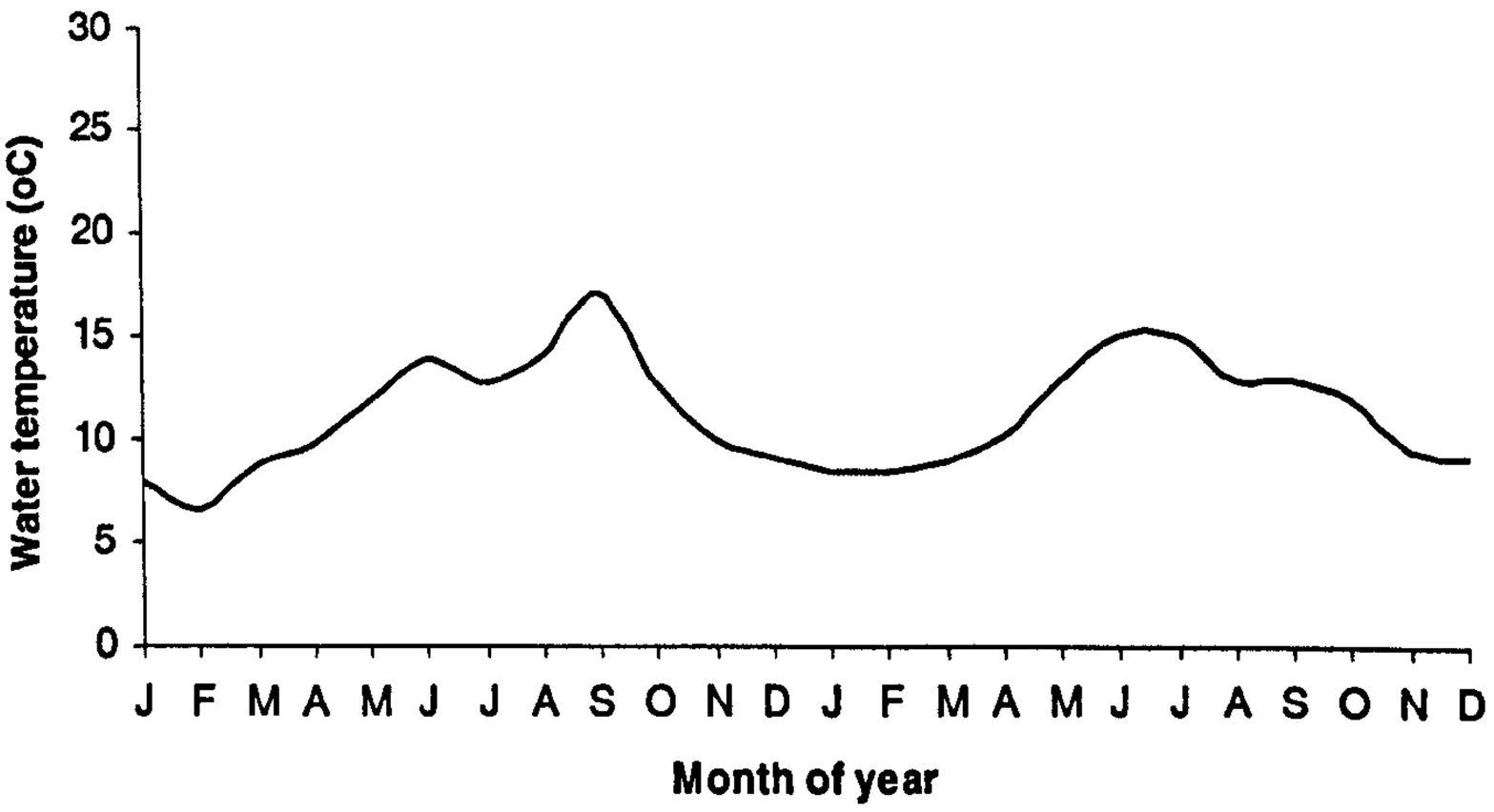

Chl a (µmol/l)
0
1
2
3
4
5
6
J F M A M J J A S O N D
Month of year

Figure 2.10. Seasonal variations in mean water temperature (°C) for a) Menai Strait, North Wales during 1994; b) Lough Hyne, Co. Cork, Ireland during 1991 and 1992; c) Trou aux Biches lagoon, Mauritius, Indian Ocean during 1995/1996. Lough Hyne data was provided by the Aquatic Services Unit, University College Cork, and Menai Strait and Trou aux Biches data were adapted from Owen (1998) and Daby (1999) respectively.

a)



b)



1991 1992

c)

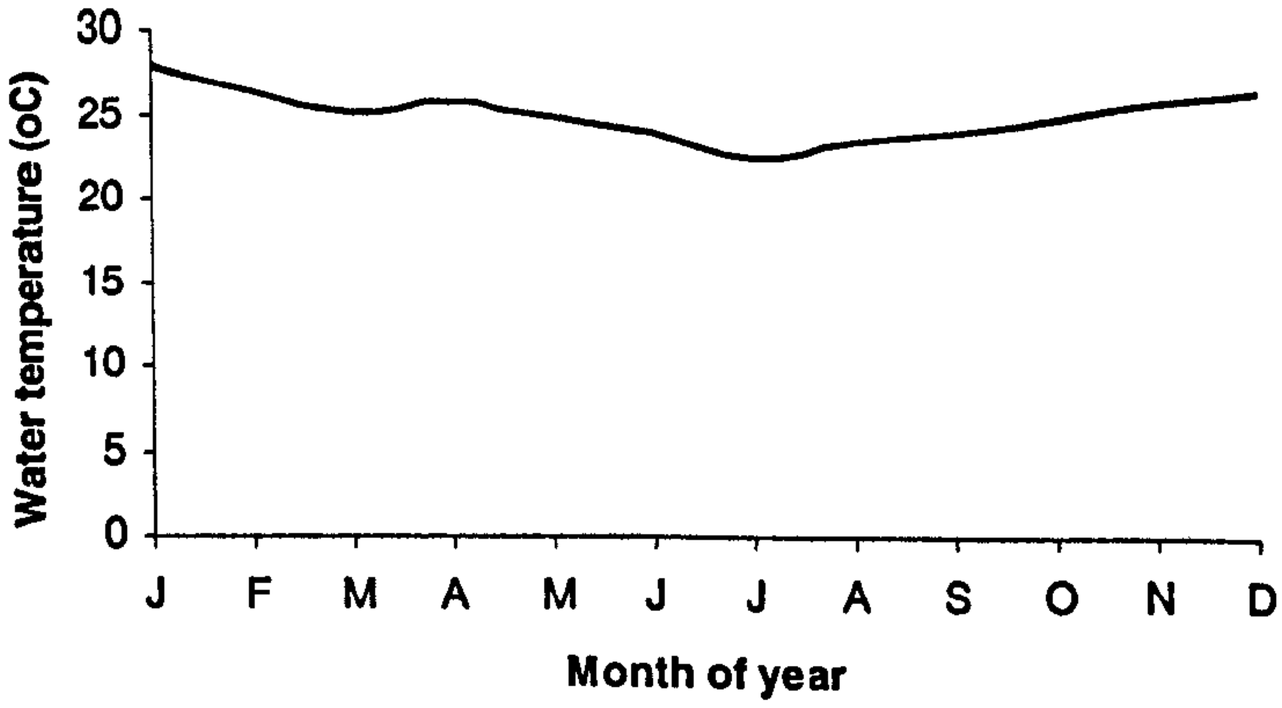
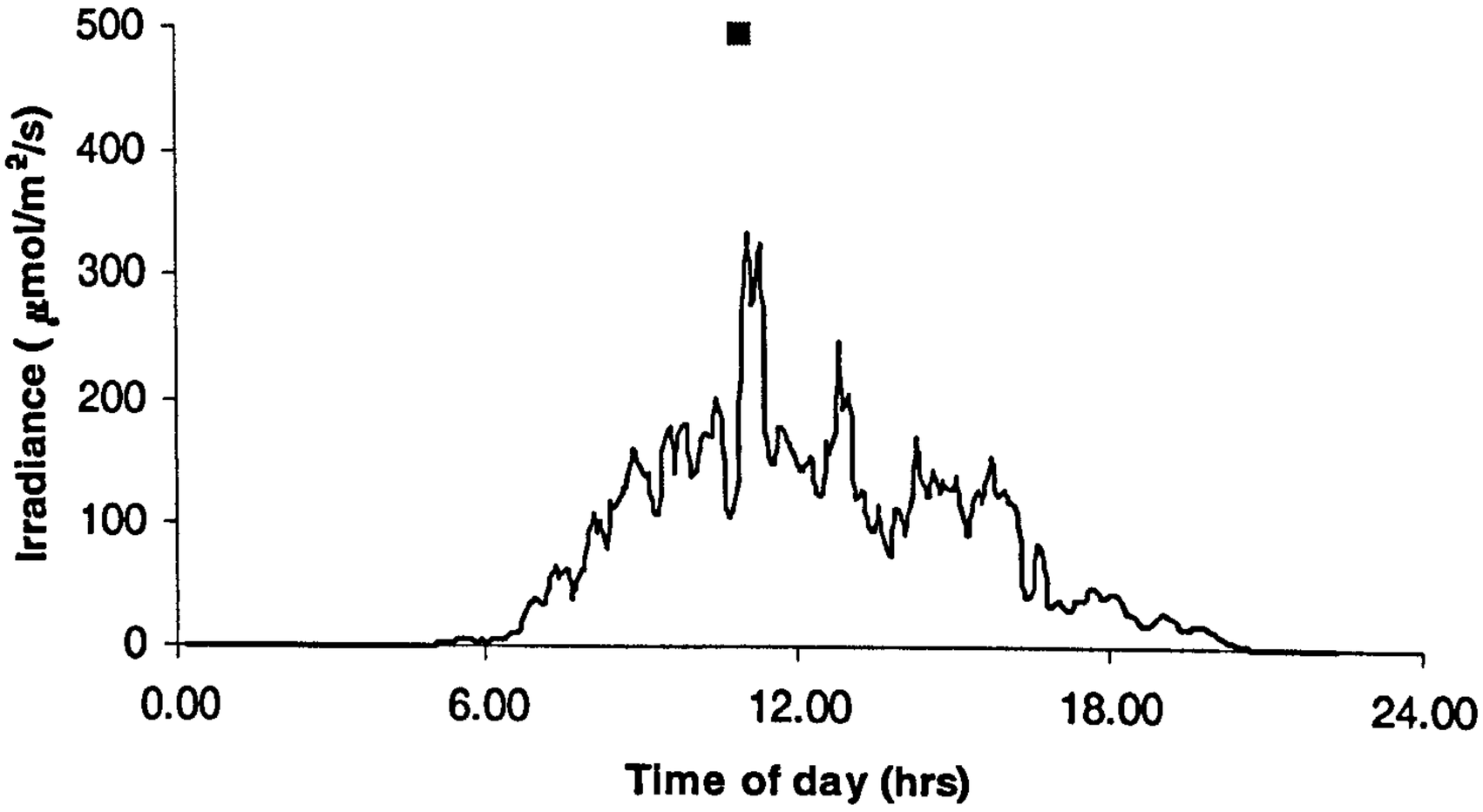


Figure 2.11. Mean downwelling irradiance (μmol m^{-2} s^{-1}) during the **summer** (7/98) over a daily cycle at 6 m water depth on a) a sunny day with some cloud b) a dull, rainy day in Lough Hyne M.N.R., Ireland. ■ represents the maximum irradiance recorded over the whole cycle; at 11.00 hrs on a sunny day a maximum irradiance of 497 μmol m^{-2} s^{-1} was recorded compared with 149 μmol m^{-2} s^{-1} at 13.35 hrs on a dull, rainy day.

a)


Irradiance (µmol/m²/s)
500
400
300
200
100
0
0.00
6.00
12.00
18.00
24.00
Time of day (hrs)

b)

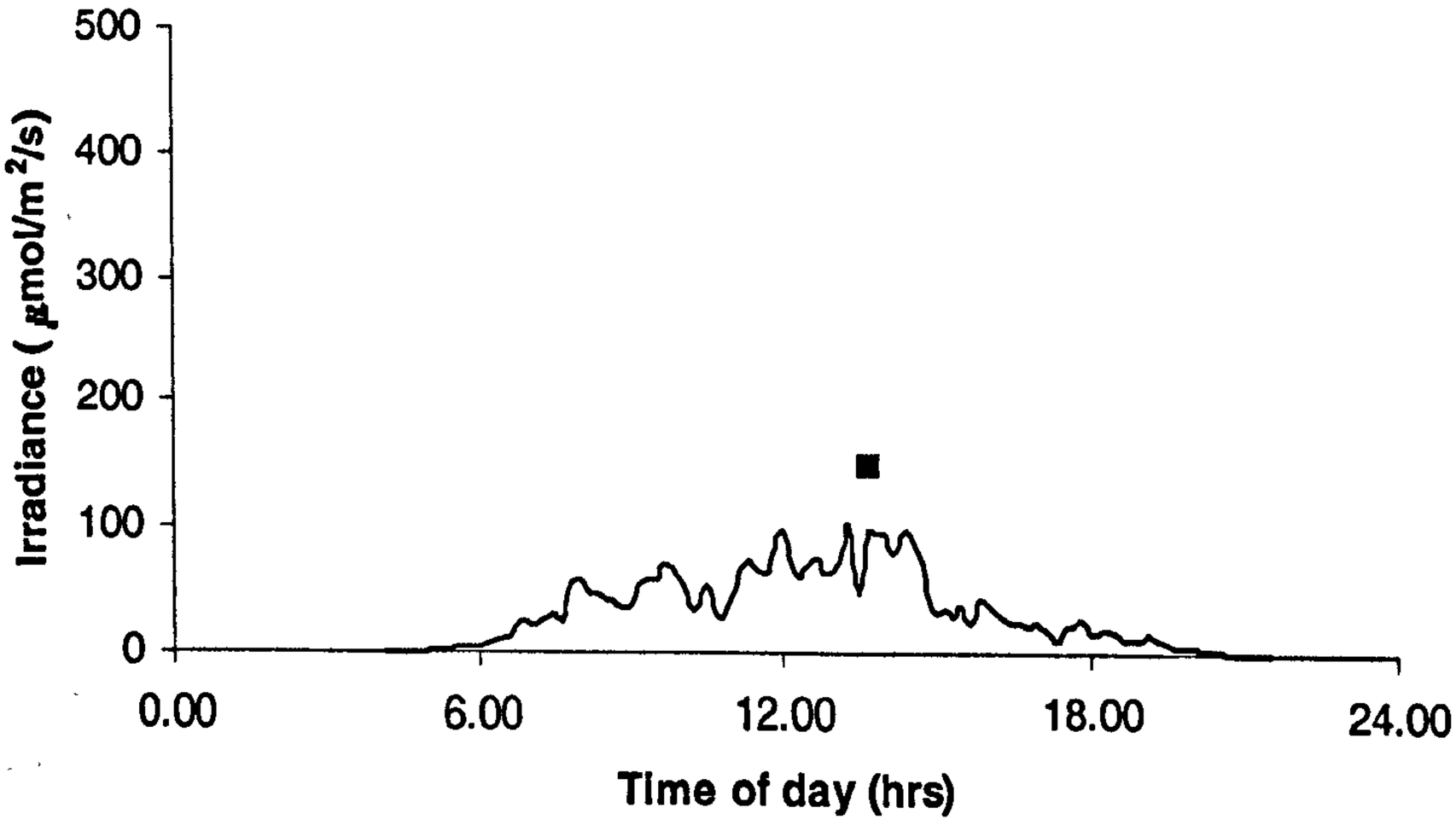

Irradiance (µmol/m²/s)
500
400
300
200
100
0
0.00
6.00
12.00
18.00
24.00
Time of day (hrs)

Figure 2.12. Mean downwelling irradiance (µmol m^{-2} s^{-1}) over a daily cycle at 6 m water depth on a dull, overcast day during **winter** (2/98) in Lough Hyne M.N.R., Ireland. ■ represents the maximum irradiance recorded over the whole cycle; a maximum irradiance of 70 µmol m^{-2} s^{-1} was recorded at 10.00 hrs.

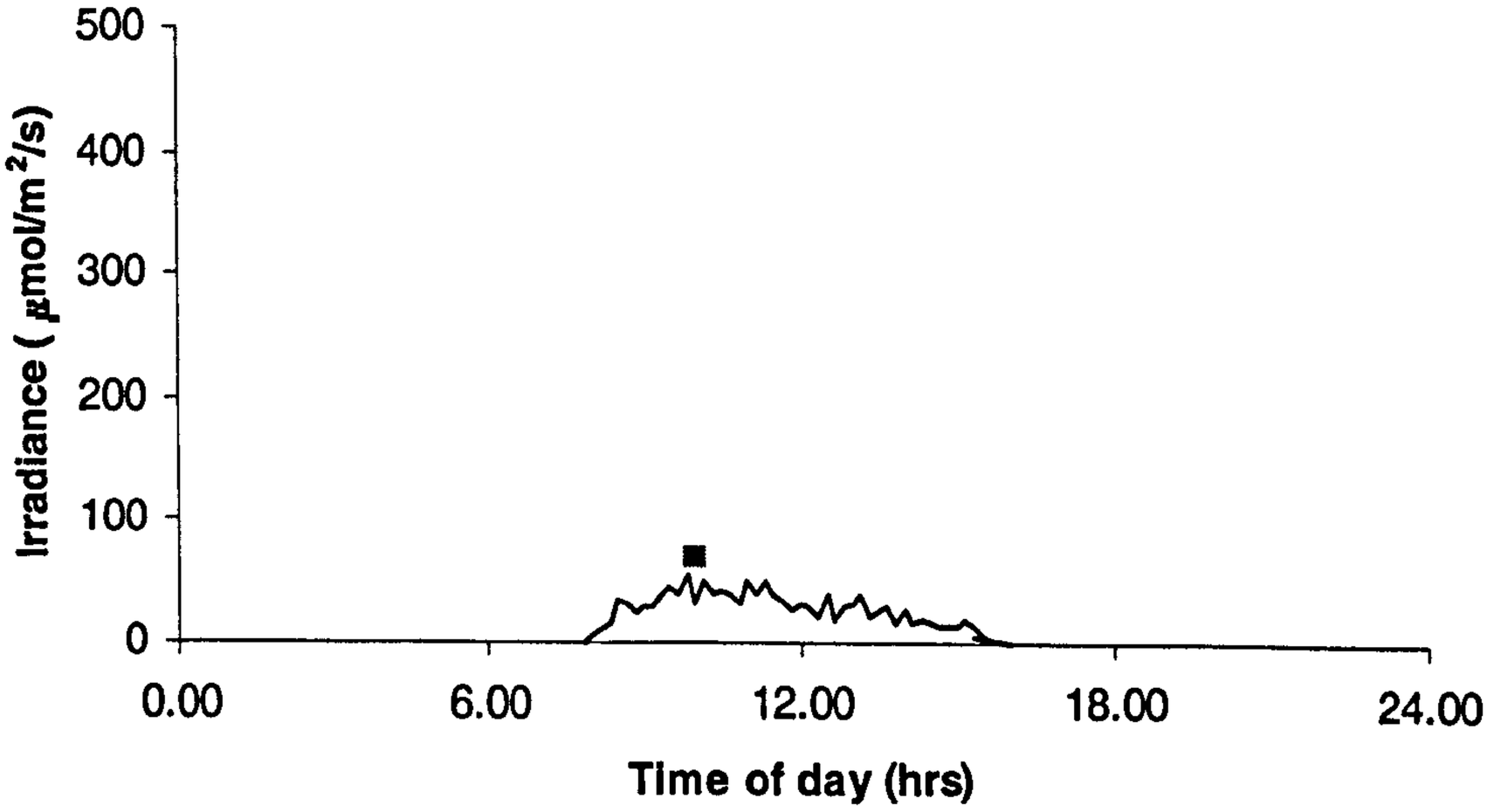
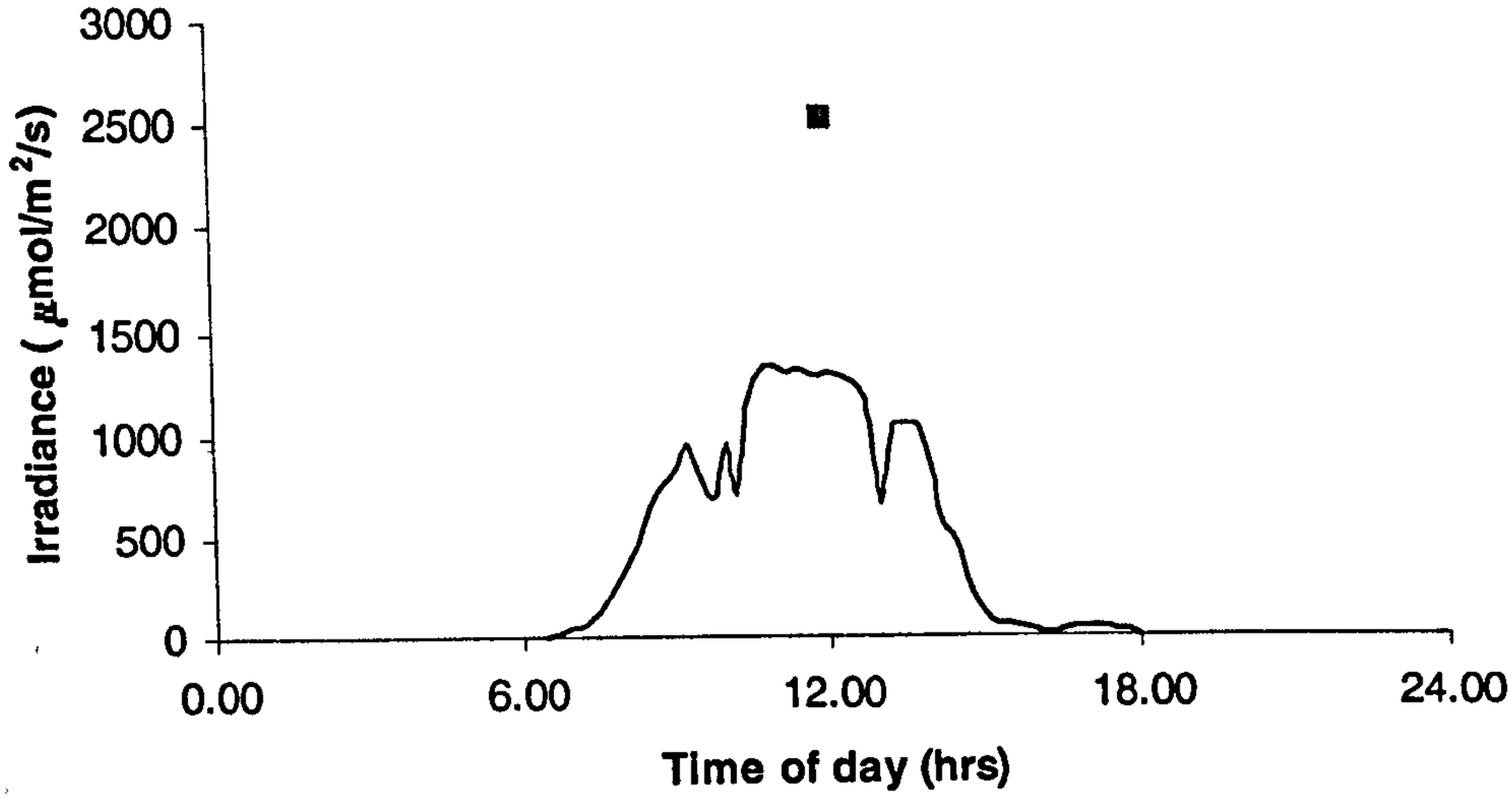

Irradiance (µmol/m²/s)
500
400
300
200
100
0
0.00
6.00
12.00
18.00
24.00
Time of day (hrs)

Figure 2.13. Mean downwelling irradiance (μmol m^{-2} s^{-1}) over a daily cycle at 0.5-1 m water depth on a) a bright, sunny day during April 1998 and b) a dull, overcast day during April 1997 in Trou D' Eau Douce lagoon, Mauritius, Indian Ocean. ■ represents the maximum irradiance recorded over the whole cycle; on a sunny day a maximum irradiance of 2522 μmol m^{-2} s^{-1} was recorded at 10.30 hrs, whilst on a dull, overcast day a maximum irradiance of 1985 μmol m^{-2} s^{-1} was recorded at 11.30 hrs.

a)


Irradiance (µmol/m²/s)
3000
2500
2000
1500
1000
500
0
0.00
6.00
12.00
18.00
24.00
Time of day (hrs)

b)

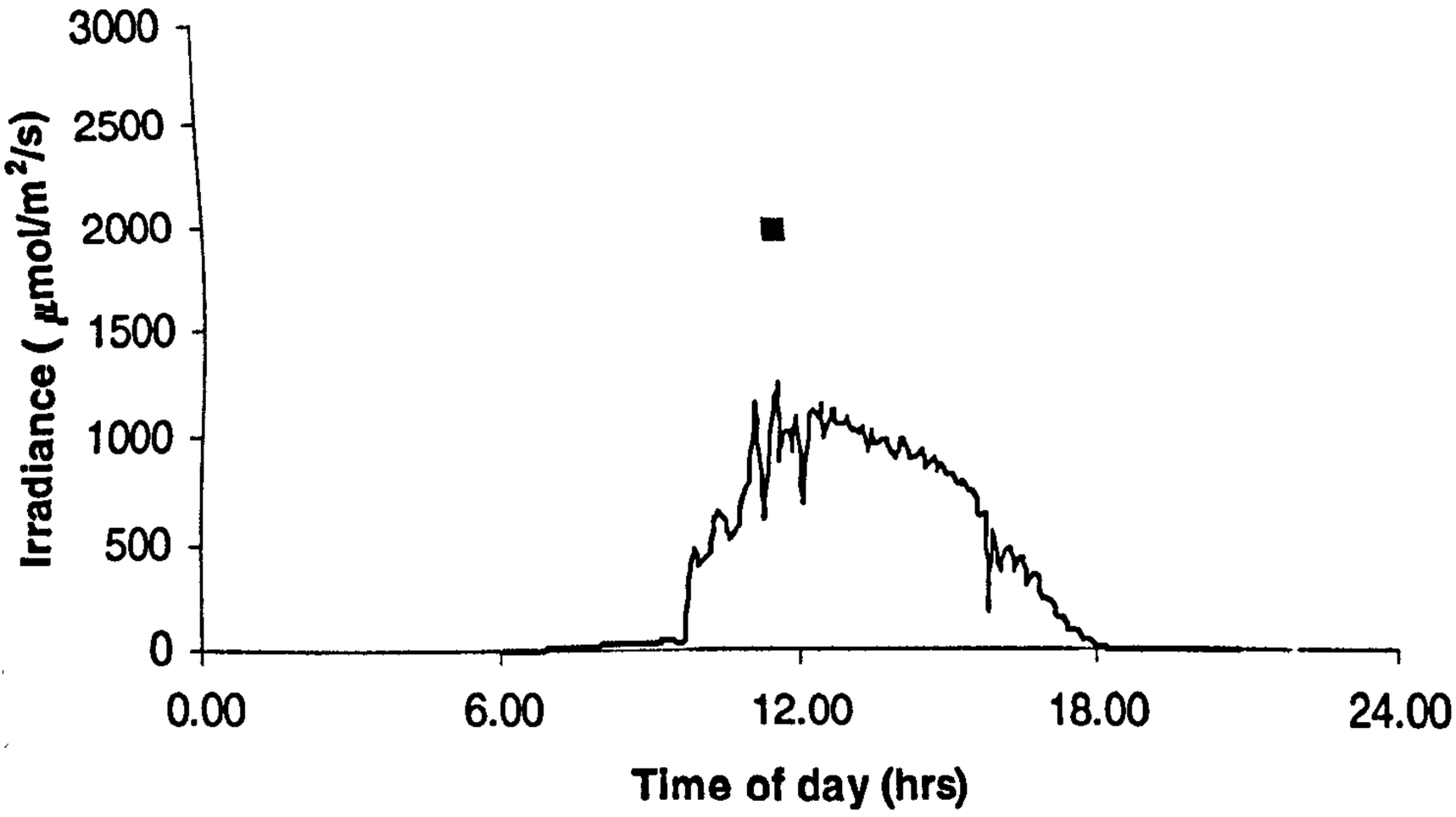

Irradiance (µmol/m²/s)
3000
2500
2000
1500
1000
500
0
0.00
6.00
12.00
18.00
24.00
Time of day (hrs)

Figure 2.14. Attenuation of downwelling irradiance (PAR, $\mu molm^{-2}\ s^{-1}$) measured beyond the reef front at Trou aux Biches, Mauritius (1/1/96). Measurements were only made to a depth of 30 m using an under water quantum sensor.

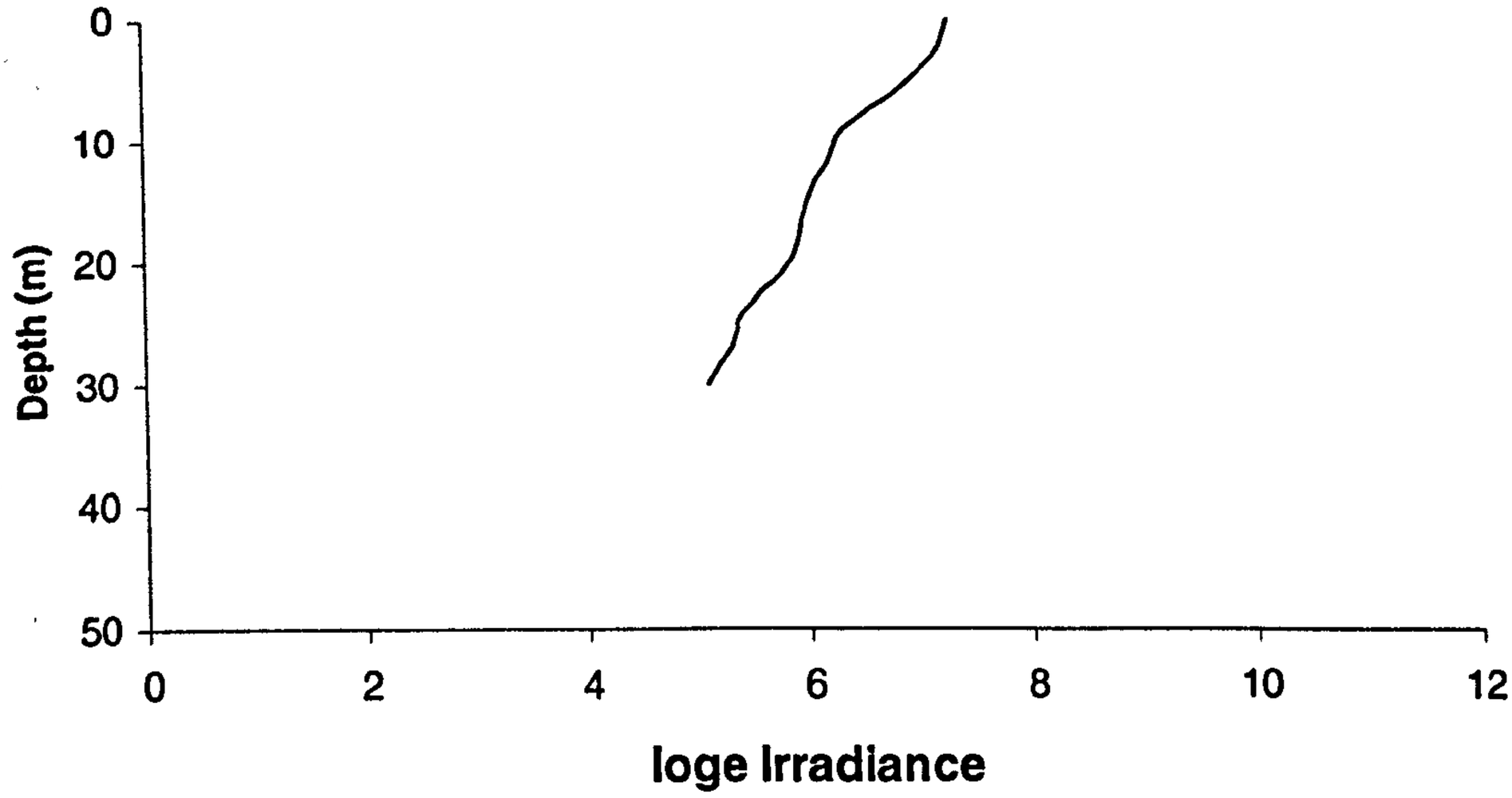
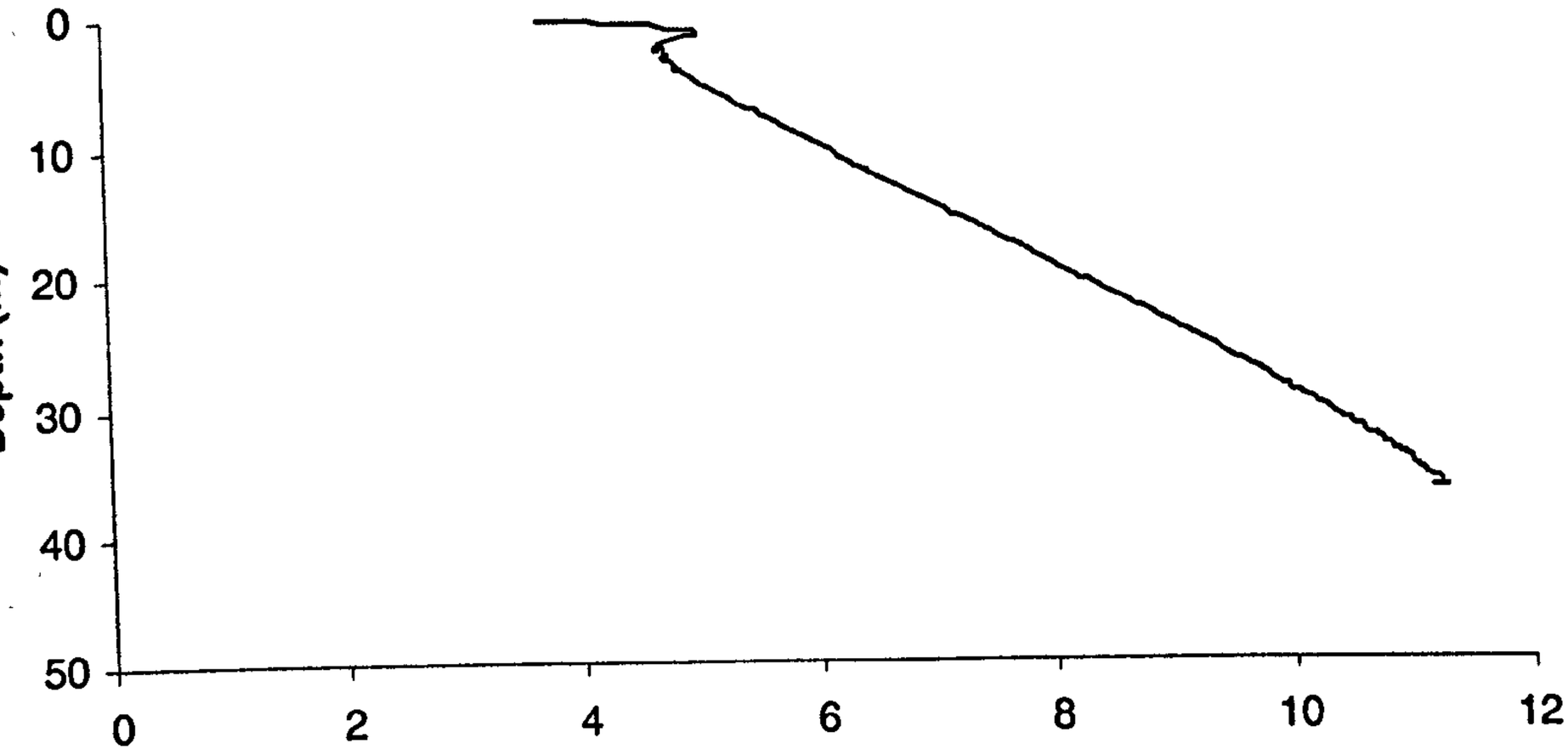

0
10
20
30
40
50
Depth (m)
0
2
4
6
8
10
12
loge Irradiance

Figure 2.15. Attenuation of downwelling irradiance (PAR, μmol m^{-2} s^{-1}) in Lough Hyne during a) winter(2/98) and b) summer (8/98).

a)


0
10
20
30
40
50
Depth (m)
0
2
4
6
8
10
12
loge Irradiance

b)

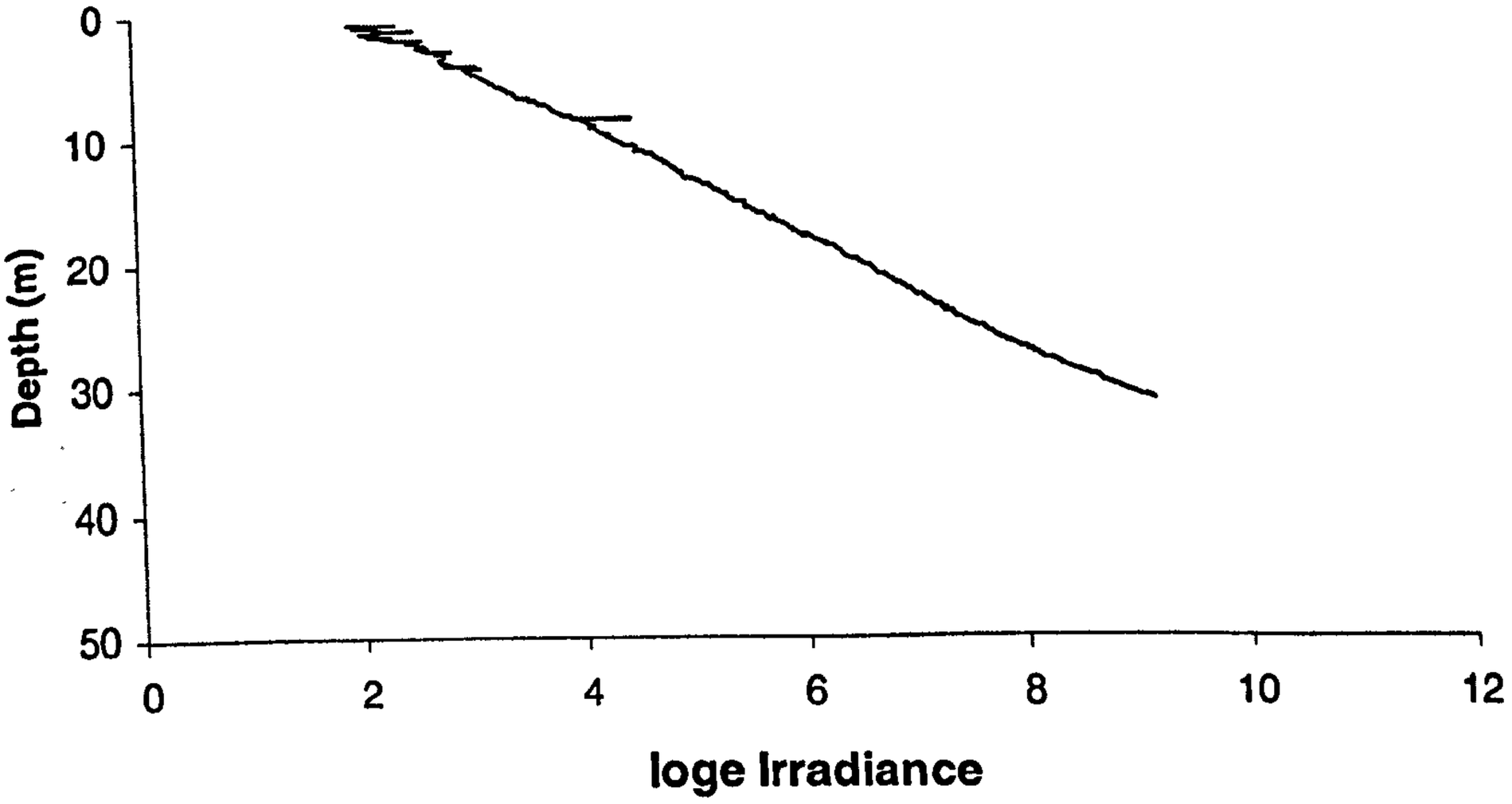
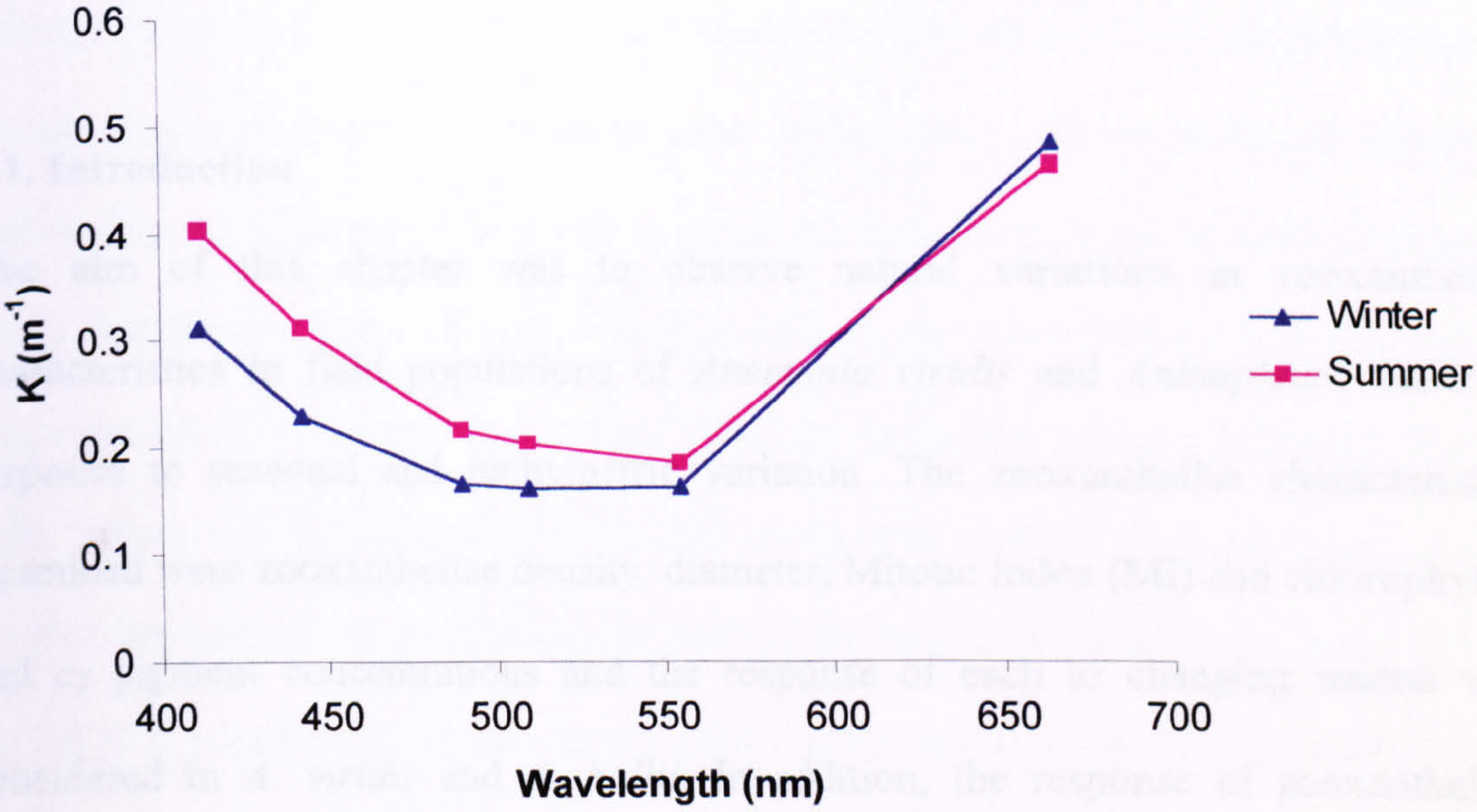

0
10
20
30
40
50
Depth (m)
0
2
4
6
8
10
12
loge Irradiance

Figure 2.16. Spectral variation of vertical attenuation coefficient (K, m^{-1}) for downwelling irradiance (PAR) in Lough Hyne during winter (2/98) and summer (8/98).


0.6
0.5
0.4
0.3
0.2
0.1
0
K (m-1)
400
450
500
550
600
650
700
Wavelength (nm)
Winter
Summer

CHAPTER 3

Natural variations in zooxanthellae characteristics in field populations of *Anemonia viridis* and *Anthopleura ballii*.

3.1. Introduction

The aim of this chapter was to observe natural variations in zooxanthellae characteristics in field populations of *Anemonia viridis* and *Anthopleura ballii* in response to seasonal and bathymetric variation. The zooxanthellae characteristics examined were zooxanthellae density, diameter, Mitotic Index (MI) and chlorophyll *a* and c_2 pigment concentrations and the response of each to changing season was considered in *A. viridis* and *A. ballii*. In addition, the response of zooxanthellae density, diameter and MI to water depth were considered in terms of variations in the prevailing light climate and the possibility of photoadaptation addressed.

Anemonia viridis or snakelocks anemone (Plate 3.2) grows to approximately 70 mm across the base with a tentacle span of up to 180 mm (Manuel, 1981). The tentacles are long and stout but flexible, are irregularly arranged and number up to 200. Usually the tentacles are extended but may be retracted into the column on occassion, perhaps for protection from predators (Turner, 1988). The anemones are either green, brown or grey in colour often with purple tips to the tentacles. Reproduction is oviparous, or more frequently, asexual by longitudinal fission (Shick, 1991). *A. viridis* is locally abundant around the south and west coasts of Britain and north to Scotland, being found from mid-shore to approximately 20 m. *A. viridis* is also abundant on all soth-west coasts of Europe and in the Mediterranean where it favours well-lit, low to mid-

energy sites. The anemones are usually attached to rocks or algae (particularly on leaves of the seas grass *Zostera* sp.) (Davy, 1994).

Anthopleura ballii (Plate 3.3) possesses a moderately adhesive base and a trumet shaped column when fully extended. The oral disc is wide and flat with stout tentacles arranged towards the outer edge. The tentacles number up to 96 and are usually curled at the tips. The maximum tentacle span is 120 mm. The animals are brown to yellow/brown in colour, often with a purple or pink flush to the column. The whole body is normally flecked with white markings and dark stripes (Manuel, 1981). Reproduction is via oviparity (Turner, 1988) or very rarely transverse fission (Turner, pers. comm.). *A. ballii* inhabits holes and crevices in rocks or is found buried in sand or mud attached to a hard substrate beneath, from mid-shore to 25 m (Manuel, 1981). A. ballii is a common species in the Mediterranean but is locally abundant around Britain. It is confined to the south and west, from the Isle of Wight to the Isle of Man in the north and south-west Ireland in the west (Manuel, 1981; Turner, 1988).

Photoadaptation describes changes in photosynthetic performance with changes in radiation during growth (Brown, 1997) and occurs on a time scale shorter than or comparable to a cell's generation. Several responses are observed and include changes in photosynthetic pigments, chloroplast structure and photosynthetic response which act to increase the light-harvesting ability of the zooxanthellae. Barnes & Chalker (1990) in a review of the biochemical and physiological adaptations of corals to varying irradiance, noted that photoadaptation to decreasing light generally results in a marked change in concentrations of photosynthetic pigments contained within the zooxanthellae. In symbiotic invertebrates such as anemones and corals, there are other means of homeostatic adjustment available, such as the alteration of the number and spatial arrangement of the zooxanthellae. Increases in the number of zooxanthellae

occur as a result of their cell division or a change in the expulsion rate. The frequency of dividing cells occurring in a population may be measured by determining the fraction of cells occurring as doublets (eg. Swift *et al.* 1976, Wilkerson *et al.* 1983, Muscatine *et al.* 1984, Stambler & Dubinsky 1987), and is termed the mitotic index (MI). *In situ* MI measurements have proved invaluable in understanding the flux of nutrients and energy through cnidarian-algal symbioses, where increases in the availability of energy and nutrients are associated with an increase in MI (Wilkerson *et al.* 1983, Muscatine *et al.* 1989). The regulation of the symbiont population by the cnidarian host is thought to be controlled by this relationship (Douglas & Smith, 1984).

Previous studies of photoadaptation in symbiotic anthozoans have been carried out on shade- or light-adapted tropical corals (Wethey & Porter 1976, Titlyanov *et al.* 1980, Falkowski & Dubinsky 1981, Muscatine *et al.* 1984, Porter *et al.* 1984), soft corals (Berner *et al.* 1987) or sea anemones (Muller-Parker, 1987) in the field and have shown that the symbiosis adapts to a wide range of natural photic environments.

In the field, several environmental variables exist which may influence the photoadaptive response and include season, water depth, spectral composition of light, ultraviolet radiation (UV), temperature and nutrient availability (Harland & Davies, 1994). Stimson (1997) comprehensively reviewed the literature on the influence of irradiance, seawater temperatures and nutrients upon photophysiological parameters in reef corals manipulated in controlled field and laboratory experiments. The majority of the work showed no changes in zooxanthellae density with altered irradiance. However, in a comparison with studies on colonies in their natural environment from different depths and different sun and shade habitats, he revealed evidence of an inverse relationship between irradiance and zooxanthellae density.

Seasonal fluctuations encompass seawater irradiance, rainfall, salinity, nutrients and temperature. There have been few quantitative studies of the effects of seasonality on anthozoan-algal symbioses (Dykens & Shick 1984, Muller-Parker 1987, Fagoonee *et al.* 1999, Brown *et al.* 1999) and little is known of the influence of seasonality on zooxanthellae densities and pigment content. In a study of the tropical anemone *Aiptasia puchella*, Muller-Parker (1987) observed that during the fall, both shade and sun anemones were larger but the densities of zooxanthellae were lower than those of summer anemones. It was also suggested that total chlorophyll content was elevated as a response to lower seasonal irradiance. In the only previous study of seasonal photoadaptation in a temperate anemone, Dykens & Shick (1984) found that seasonal changes in total chlorophyll (*a* and c_2) in *Anthopleura elegantissima* were not due to changes in zooxanthellae density but to the amount of pigment per alga; the higher values in winter were presumed to compensate for lower prevailing solar flux. In addition, the disproportional winter increase in chl c_2 may have been a further adaptation to reduced light availability, analogous to the situation in *Aiptasia pallida* transplanted from bright to dim habitats (Lesser & Shick, 1989). More recent studies of tropical scleractinian corals have revealed marked seasonal fluctuations in environmental parameters in shallow tropical waters which are reflected in both zooxanthellae density (Brown, 1997, Fagoonee *et al.* 1999, Brown *et al.*, 1999, Fitt *et al.* 2000) and chlorophyll content (Brown 1997, Fitt *et al.* 2000). By comparsion, Verde & McCloskey (1998) observed no seasonal difference in zooxanthellae density but higher chlorophyll per zooxanthella during January in Florida U.S.A. when daylength and irradiance were lower, in the mangrove jellyfish, *Cassiopea xamachana*. While seasonal fluctuations in light may have an important role, zooxanthellae density may also be altered by seasonal nutrient and temperature

variations. Fagoonee *et al.* (1999) observed that zooxanthellae density in field colonies of the tropical coral *Acropora formosa* was positively correlated with nitrate concentration. Similarly, after 2 weeks exposure to artificial ammonium (20μm) in the laboratory the density of zooxanthellae in the tropical coral *Stylophora pistillata* had nearly doubled (Muscatine *et al.*, 1989). In comparison, elevated water temperature can cause a decrease in the density of zooxanthellae in corals (Lasker *et al.* 1984, Hoegh-Guldberg & Smith 1989).

Zooxanthellae of Anthozoa with broad depth distributions are exposed to a wide range of light intensities of varying spectral composition. As light intensity decreases exponentially with depth underwater(Chapter 2, section 2.3.3.) zooxanthellae of increasingly deep water hosts receive rapidly declining amounts of light energy for photosynthesis. As the red wavelengths of light are most rapidly attenuated by sea water, in deeper water temperate anemones must also adapt to a spectrum containing an increasing proportion of energy in green wavelengths (Kirk, 1994; see chapter 2 section 2.3.3.). Anthozoa which span a wide depth range show physiological alterations analogous to the light/shade adaptations of higher plants (Wethey & Porter 1976, Falkowski & Dubinsky 1981, Berner *et al.* 1987). Studies on corals at different water depths (Wethey & Porter, 1976; Titlyanov *et al.* 1980; Dustan, 1982; Chalker *et al.* 1983; McCloskey & Muscatine, 1984) have shown that zooxanthellae in corals from low light intensity environments contain more chlorophyll than zooxanthellae in corals in high light environments. In addition, measurement of productivity has indicated that although shade-adapted corals are less productive at photosynthetically saturating irradiances than sun-adapted corals, they have higher photosynthetic efficiencies and are thus able to utilize low light more efficiently than sun-adapted corals.

Previous studies on the relationship between zooxanthellae density and depth have revealed no clear patterns. Among those studies carried out on tropical corals, some indicate zooxanthellae density decreases with depth (Drew, 1972, McCloskey & Muscatine, 1984), others show no difference (Porter *et al.* 1984, Falkowski & Dubinsky, 1981) or even a slight increase with depth (Zvalinskii *et al.*, 1978, Titlyanov *et al.*, 1980). The only previous study on a temperate anemone showed no response of zooxanthellae density to depth in *Anemonia viridis* (Bythell *et al.*, 1997).

The relationship between chlorophyll concentrations and depth is much clearer; as depth increases zooxanthellae may increase their pigment content to enhance light harvesting ability. Battey & Porter (1988) found that the amount of chlorophyll a per cell increased from 1.2 pg at 0.5 m depth to 2.62 pg at 50 m depth in zooxanthellae from *Montastrea annularis*. Similarly, *S. pistillata* colonies at 3 m contained 2.2 pg $cell^{-1}$ and 6.6 pg $cell^{-1}$ at 35 m (Falkowski & Dubinsky, 1981; Dubinsky *et al.*, 1984). However, Bythell *et al.* (1997) observed no difference in pigment concentrations in the temperate anemone *A.viridis* with depth.

The effect of ultraviolet radiation on zooxanthellae has also been considered in both tropical and temperate Anthozoa. Gleason & Wellington (1993), Kinzie (1993) and Brown *et al.* (1994) report decreases in the number of zooxanthellae present in tropical, shallow-water corals on exposure to elevated solar UV radiation (280-400 nm), and cite UV as the cause of coral bleaching. Similarly, exposure of the temperate anemone *Cereus pedunculatus* to artificial UV induced an intensity-dependent decrease in the number of zooxanthellae, and in some cases caused bleaching (Hannack *et al.* 1997). In addition, Lesser & Shick (1989) observed lower

concentrations of chlorophyll per zooxanthella from *Aiptasia pallida* after exposure to environmentally realistic levels of artificial UV.

The hypothesis investigated in this study states that zooxanthellae characteristics in the temperate anemones *A. viridis* and *A. ballii* are affected by season and depth in both intertidal and subtidal populations.

The objectives of this chapter were to investigate the effect of season and depth on anemone density and distribution and zooxanthellae characteristics in field populations of the temperate anemones *A. viridis* and *A. ballii*. The effects of seasonal and depth-related variation on anemone density and distribution were assessed through surveys at each study site. The zooxanthellae characteristics considered were zooxanthellae density, MI, diameter and pigment concentration (chlorophyll *a* and c_2). Firstly, variations in zooxanthellae characteristics in *A.viridis* were compared through the seasons in an intertidal rock pool habitat; unique to the intertidal site was the consideration of zooxanthellae pigment concentrations due to the site's close proximity to the laboratory. Similarly, zooxanthellae characteristics were compared during winter (2/98) and summer (8/98) in a shallow subtidal habitat. Both intertidal and subtidal habitats may be described as shallow ' sun-habitats' exposed to high levels of solar radiation. However, anemones in the intertidal habitat may be exposed to conditions which are physiologically more stressful, such as wave action and temperature and salinity fluctuations. Thus zooxanthellae from intertidal and subtidal environments were compared. Finally, variations in zooxanthellae characteristics in subtidal *Anthopleura ballii* from bathymetric extremes (6 m and 18 m water depths) of thier distribution and during winter and summer were considered.

3.2. Methods

3.2.1. Study sites

Field sampling was carried out at 3 sites in two locations.

Site 1 was a mid-tide level rockpool in Trearrddur Bay, Anglesey, North Wales, UK (53° 16' N, 4° 4' W; chapter 2, Figure 2.1). It is an exposed site with a typical zonation of rocky shore organisms and was chosen for the abundance of easily accessible intertidal *A. viridis* which could be sampled through the seasons.

Sites 2 and 3 were located in Lough Hyne Marine Nature Reserve, Co. Cork, Ireland (51° 29' N, 9° 18' W; Chapter 2, figure 2.2, and figures 3.1). The very sheltered nature of the lough provides ideal conditions for both littoral and sublittotal studies in all weather, and has led to a long history of marine biological study in Lough Hyne. The intertidal shore of Lough Hyne was subdivided by Renouf (1931) into sectors and each permanently marked with small numbered discs; N1-N12 along the north shore, S1-S17 along the south, E1-E20 east, W1-W38 west and I1-I21 for Castle Island (Figure 3.2). These also provide useful surface reference points during sublittoral studies and have been used during this study.

The two sites were chosen for their abundance and distribution of symbiotic Anthozoa and presence of suitable light habitats.

Site 1: Castle Island bay (Figure 3.1), Renoufian sector I13 (Figure 3.2) was sampled as a sun-habitat with relatively uniform depth, and abundant *Anemonia viridis* for a comparison of seasonal variation in zooxanthellae characteristics. 'Subtidal sun-habitat' is defined here as a shallow, well-lit habitat permanently covered by the tide.

Site 2: Glannafeen cliff (Figure 3.1) was sampled as a subtidal cliff habitat with a depth and therefore light gradient. *Anthopleura ballii* was distributed over a depth

range from LW mark to 20 m which provided an ideal opportunity to investigate depth-related, as well as seasonal variation in zooxanthellae characteristics.

3.2.2. Survey of anemone distribution, density and habitat

3.2.2.1. Mid-tide level rock pool, Trearddur Bay, Anglesey

Conditions in the rockpool were considered by measuring water temperature and salinity for the both the rockpool and the adjacent open sea. To assess the density of *Anemonia viridis* a rough grid was marked out over the rockpool using two tape measures, and 0.25 x 0.25 m quadrat samples taken in alternate squares along the grid. The oral diameter of 42 brown anemones was measured to determine size distribution of the population during winter and summer. Finally notes on the habitat were made.

3.2.2.2. Castle Island Bay, Lough Hyne M.N.R., Ireland (Renoufian sector I13)

To assess distribution and density of *Anemonia* quadrat samples were taken along a transect of the bay, 15 m from a rock with Renoufian marker 14/13 and perpendicular to the shore. Two 1 m^2 quadrats were taken every 5 fin strokes (approx. 6 m) for a total of 7 stations along the transect; quadrats were dropped randomly onto the sea bed. The oral diameter of 50 anemones was measured during winter to investigate size distribution of the anemones in the population. Notes were made on the habitat to describe substrate and dominant organisms present.

3.2.2.3. Glannafeen cliff, Lough Hyne M.N.R., Ireland

Depth-related distribution and density of *Anthopleura ballii* was investigated by counting the number of anemones in two 1 m^2 quadrats at 18, 15, 12, 9 and 6 m vertical depths on the cliff using SCUBA. Mean densities of anemones per square metre were obtained for each depth. Diving activities were carried out according to

the University Scientific Diving regulations. Habitat information was collected during a survey in August 1996.

3.2.3. The effects of season and depth on zooxanthellae characersitics

3.2.3.1. Field sampling

Twelve *Anemonia viridis* were sampled from a mid-tide level rock pool in Trearddur Bay every month over a 2-year period to investigate seasonal changes in zooxanthella characteristics. Months sampled were May 1997-January 1998 and February-April 1999. Four tentacles were excised from each anemone, placed in glass vials with seawater and transported back to the laboratory. Two were pooled and used to assess zooxanthellae density, MI and diameter. The remaining two were also pooled and used for pigment concentration analysis.

Twenty brown *Anemonia viridis* were collected from 1.5 m water depth in Castle Island Bay, Lough Hyne by snorkelling during summer (8/97) and winter (2/98) and transported to the field laboratory.

Twelve *Anthopleura ballii* of similar size were collected from Glannafeen cliff, Lough Hyne at 18 m and 6 m depth during winter, 2/98 and summer, 8/98 using SCUBA. Animals were placed in plastic bags and transported back to the field laboratory where two tentacles were removed from each animal and preserved for subsequent zooxanthellae density, MI and diameter analysis. During August 1997 and February 1998 a further two tentacles were removed from 3 animals and pooled for zooxanthella ultrastructure analysis in Chapter 5 (p. 82).

3.2.3.2. Zooxanthellae characteristics

Preliminary experiments were carried out to secure the following methods and are detailed in Appendix I. Laboratory methods were modified to be compatible with the limitations of field sampling.

At all three sites changes in zooxanthellae density, MI and diameter were investigated by sampling the anemone tissue and isolating the zooxanthellae. Anemones were sampled for subsequent zooxanthellae analysis by excising pairs of tentacles.

Two tentacles were excised from each anemone, blotted dry and weighed on a 4 decimal place balance; exposure of tentacles to the air was standardised during weighing to account for any weight loss due to moisture evaporation. Tentacles were preserved for subsequent analysis by fixing in 0.1 M phosphate buffered 3 % glutaraldehyde overnight. The tentacles were then rinsed thoroughly with distilled water, stored in 0.1 M phosphate buffer and refrigerated for subsequent isolation of zooxanthellae.

Zooxanthellae were isolated from the animal tissue by homogenising the tentacles in a hand-held tissue grinder with 10 ml filtered seawater (FSW) followed by repeated centrifugation at 1200 rpm for 10 minutes with FSW and a surfactant, 0.05 % sodium dodecyl sulphate (SDS); the final wash was with FSW.

The final algal pellet was resuspended in 4 ml FSW. The number of zooxanthellae present was determined using a modified Fusch's-Rosenthal haemocytometer and standardised to wet weight to give density. The MI was measured as an indicator of zooxanthellae population growth by counting the number of dividing cells present in each sample and expressing this as a percentage of the total number of cells present; a cell was considered dividing if it appeared as a doublet. Finally the cell diameters of

10 cells were measured from each sample using x 1000 magnification (oil immersion) and an optical micrometer.

The seasonal variation of zooxanthellae chlorophyll *a* and c_2 concentrations was also determined for intertidal *Anemonia viridis* from Trearddur Bay. Pigment extraction was carried out immediately. The two additional tentacles from each anemone were wet weighed and the zooxanthellae isolated as described in section 3.2.3.2. above.

The final algal pellet was resuspended in 5 ml of cold 90 % acetone and homogenised in a hand-held tissue grinder, in dim light to prevent photodestruction of pigments. All glasswear was held in ice during extraction. The solution was then placed in a foil covered vial, sonicated in an iced sonic water bath for 2 minutes and finally transferred to a freezer. After an 18 hour extraction period the sample was filtered through a 0.7 μm Whatman filter into a washed, foil-covered vial and stored temporarily on ice.

Samples were decanted into 4 ml silica cuvettes and the absorbance measured at 630nm, 663nm and 750nm using a Shimadzu UV- 1201 spectrophotometer; absorbance of an acetone blank was also measured at each wavelength.

Chlorophyll *a* and c_2 concentrations were calculated from the absorption values using the equations of Jeffrey and Humphrey (1975) and standardised to wet weight of anemone tissue.

Further tentacle samples were taken at each site for zooxanthella ultrastructure observations as described in chapter 5, p. 81-82.

3.3 Results

3.3.1. Anemone distribution, density and habitat

3.3.1.1. Mid-tide level rock pool, Trearddur Bay, Anglesey

Plate 3.1 shows the rock pool in Trearddur bay at low water spring tide. The dimensions were 3 m x 14 m with a total area of approximately 42 m^2 and a maximum depth of 22 cm. The tidal range in Trearddur Bay is approximately 9 m on a spring tide and 5 m on a neap tide. Anemone distribution was patchy and showed a density of 16 - 448 anemones m^{-2}.

During the summer (7/98) rock pool temperature was recorded with a thermometer as 18.5 °C compared with a sea temperature of 14.6 °C and in winter (2/99) 2 °C in the rock pool compared with 4 °C in the sea. Thus, water temperature was higher in the rock pool than the sea during summer and lower during the winter.

The size frequency distributions of anemones were compared for summer and winter populations and found to be significantly different (Kolmogorov-Smirnov; $z= 1.762$, $p< 0.005$). During summer the modal size class was 13-15 mm compared with 7-9 mm during winter (Figure 3.3).

The rock pool community was characterised by red algae such as *Lithothamnion* sp., *Corallina* sp. and *Ceramium* sp., to which were attached the brown alga *Leathesia difformis* and the green algae *Enteromorpha* sp. and *Ulva lactuca*. Conspicuous gastropods included *Gibbula cineraria*, *Gibbula umbilicalis* and *Nucella lapillus* found grazing on barnacles and *Patella* sp. grazing over the rock surface. *Anemonia viridis* were found in patches among the algae often beneath the fronds, sheltered from direct sunlight.

3.3.1.2. Castle Island Bay, Lough Hyne M.N.R., Ireland

The transect of Castle Island bay during **summer** (Figure 3.4 a) showed a distribution that as the distance from low water mark increased so did anemone density. The density reached a maximum of 75 brown and green anemones m^{-2} at a distance of 30 m from the shore and where the water was 110 cm deep, compared with 9 brown and green anemones m^{-2}, 18 m from the shore and covered by 70 cm of water.

By contrast, the transect of Castle Island bay during **winter** (Figure 3.4 b) showed two peaks in anemone density of 68 and 79 anemones m^{-2}. Both patches of anemones occurred on patches of the same underlying substrate, namely soft sediment with the brown filamentous alga *Stylophora* sp..

Although maximum anemone densities for both winter and summer populations showed trends in distribution, observations suggest patchiness of *Anemonia* distribution which does not correspond to water depth or substrate type. The dominant colour variety was golden brown; recorded much less frequently was a green anemone with crimson tips to the tentacles.

The size frequency of winter anemones showed an approximately normal size distribution (Figure 3.5a). The most frequent size class was 25-29 mm accounting for 28 % of the anemones measured, compared with only 2 % with an oral diameter between 10 and 14 mm. Summer anemones also showed a normal size distribution (Figure 3.5b) with 25-29 mm anemones being the most frequently recorded (26 %).

The size frequency distributions for summer and winter anemones were not significantly different (Kolmogorov-Smirnov; $z= 1.015$ $p> 0.05$). It may therefore be assumed that the population of *Anemonia viridis* in Castle Island Bay is made up of the same sized individuals during summer and winter.

The habitat was soft sediment with occasional small boulders, large gravel, abundant empty *Anomia ephippium* and *Venus verrucosa* shells (Plate 3.2) and patches where the sediment was covered with a layer of the filamentous alga *Stylophora* sp. more than 10 cm thick. *Anemonia viridis* was found attached to all of the hard substrata listed above. *Venus verrucosa* is found in sublittoral sediments at depths exceeding 20 m suggesting that the empty shells had been transported there by physical processes in the lough. However the bay is very sheltered and not subject to wave action or high currents.

3.3.1.3. Glannafeen cliff, Lough Hyne M.N.R., Ireland

A. ballii were distributed according to depth (Figure 3.6) for example, at 6 m there were 70 anemones m^{-2} compared with only 6 anemones m^{-2} at 18 m depth. Plate 3.3 (a-b) shows *A. ballii* on Glannafeen cliff at various depths during summer (8/97).

The topography of the cliff changed with water depth, rising vertically from thick sediment at 18 m for several metres; the angle eased towards the top of the cliff at 6 m, whereafter the substrate became horizontal and the habitat changed to algae-covered boulders. The community changed also with depth gradient showing a typical distribution of plants and animals in two zones, the infralittoral and circalittoral, dictated by light attenuation (see table 1, Appendix II). Species observed as dominant members of the cliff communities at 6 m and 18 m are listed in Table 3.1. Generally the cliff is characterised by sponges which are distributed over the whole cliff from 6 m to 18 m. Sponges are most dominant in the circalittoral zone which begins between 12 and 15 m on Glannafeen cliff. Above 15 m is the infralittoral zone which is dominated by a variety of encrusting, foliaceous and filamentous red algae as well as brown algae such as *Stilophora rhizoides* and *Asperococcus turneri*, and the small green alga *Ulva* sp. (Table 3.1).

3.3.2. The effects of season & depth on zooxanthellae characteristics

3.3.2.1. Seasonal variation in intertidal *Anemonia viridis*, Trearddur Bay

Zooxanthellae density

Figure 3.7 shows there are significant differences in mean monthly zooxanthellae densities through the year. The lowest mean density was recorded during June with a value of 0.7 (± 0.06) x 10^8 cells g^{-1} wet weight and the highest in January and December with values of 5.3 (± 0.20) and 2.7 (± 0.18) x 10^8 cells g^{-1} wet weight respectively. There appears to be three groupings of density according to month. The first includes June, July and August with densities less than or equal to 1.0 x 10^8 cells g^{-1} wet weight; the second February, March, April, May and September, October, November with densities in the range 1.5 - 1.8 x 10^8 cells g^{-1} wet weight. The final group comprises January and December with densities greater than 2.0 x 10^8 cells g^{-1} wet weight.

The three groups may be tentatively referred to as Summer, Autumn/Spring and Winter. However when each month was compared statistically significant differences were found which showed a slightly different pattern. One-way ANOVA and *post-hoc* Tukey tests were performed on reciprocal-transformed data. Table 3.2 shows the mean monthly zooxanthellae densities and groups months which are not significantly different using underlining. June and August densities were not found to be significantly different (0.68 ± 0.06 and 1.01 ± 0.05 x 10^8 cells g^{-1} wet weight respectively) but densities of zooxanthellae in both months were significantly different from November, December and January densities (1.76 ± 0.07, 2.73 ± 0.18 and 5.33 ± 0.20 x 10^8 cells g^{-1} wet weight respectively). Similarly, zooxanthellae density in July was significantly different from November, December and January densities but was also different from the density of zooxanthellae in June. Finally,

February, March, April, May, September and October zooxanthellae densities were significantly different from January, June and December densities but not from each other.

Comparing the statistical data with the graphs, actual differences in densities lie between June, July, August (= summer) and November, December, January (= winter) and February, March, April, May, September, October, November (= spring/autumn) and January, June, December. However, densities in two of the summer months, July and August are not significantly different from those recorded from spring and autumn months, February- May and September- November.

Mitotic Index

Figure 3.9 shows that the mean monthly MI was relatively consistent throughout the year except during May. Dividing cells increased from 0.88 (± 0.13) % during April to 3.07 (± 0.26) % during May which represents a 3.5-fold increase, and dropped to the lowest annual mean value of 0.29 (± 0.10) % during June. Table 3.3 shows the results of one-way ANOVA and *post-hoc* Tukey tests and confirms that only anemones collected during May harboured zooxanthellae with a MI significantly different from zooxanthellae in anemones collected during any other month.

Diameter

The smallest mean zooxanthellae diameter was recorded during November and was 8.5 (± 0.12) μm compared with the largest mean diameter of 10.0 (± 0.05) μm recorded during February (Figure 3.8). Table 3.4 shows the results of one-way ANOVA and *post-hoc* Tukey tests. The mean diameter of zooxanthellae sampled during November was significantly different from all months except July. Mean diameters during January, February, March, April, August and September were not significantly different.

Chlorophyll concentration

Figure 3.10a-c shows the seasonal variation in mean concentrations of chlorophyll *a*, c_2 and the ratio of chl $a:c_2$ (μg g^{-1} wet weight). Table 3.5 shows the results of a Kruskal-Wallis test on monthly samples of chlorophyll *a* and a significant difference was found among months ($H= 58.15$, $p> 0.05$). Median chlorophyll *a* concentrations for January, December and February were 1110, 810 and 441 μg g^{-1} wet weight respectively which differed significantly from all other months. The lowest and highest median chlorophyll *a* concentrations were 282 and 1110 μg g^{-1} wet weight and were recorded in August and January respectively.

One-way ANOVA followed by *post-hoc* Tukey tests were carried out on reciprocal transformed chlorophyll c_2 data and significant differences were found among months (ANOVA $F= 4.59$ $p< 0.001$). Table 3.6 shows mean monthly chlorophyll c_2 concentrations (μg g^{-1} wet weight) listed in ascending order of magnitude. July, May, August, April, March, February and October shared the same underlining and were therfore not significantly different from each other. December, September and January (309 ± 28.2, 440 ± 119 and 484 ± 83.6 μg g^{-1} wet weight respectively) were significantly different from July, May and August (178 ± 15.7, 193 ± 12.9 and 194 ± 23.1 μg g^{-1} wet weight respectively) but neither Dec, Sep, Jan nor July, May, Aug were significantly different from April, March, February and October.

The ratio of chlorophyll $a:c_2$ in μg g^{-1} wet weight was compared for each month using one-way ANOVA and *post-hoc* Tukey tests and significant differences were evident (ANOVA $F= 7.49$ $p> 0.05$; table 3.7). The highest mean ratio of 2.76 ± 0.16 was found in December which was significantly different from the mean ratios of February- October (range 1.96-1.14) but not January (2.49 ± 0.24). By comparison, the lowest mean ratio of 1.14 ± 0.23 was found in September and was significantly

different from May (1.96 ± 0.13), January (2.49 ± 0.24) and December (2.76 ± 0.16) only (Table 3.7).

Pigment concentration was standardized to zooxanthellae density to show concentration of pigments per cell. Figure 3.11a-c shows mean monthly chlorophyll *a*, chlorophyll c_2 and the ratio of chl a:c_2 in pg/zooxanthella. One-way ANOVA was performed on chlorophyll *a* data and square-transformed chlorophyll c_2 data and no significant differences were found between months for either pigment (Chl *a*: ANOVA, F= 1.98 p>0.05; Chl c_2: ANOVA, F= 1.66 p>0.05). By comparison, the results of one-way ANOVA and *post-hoc* Tukey tests on cube-transformed chl a:c_2 (pg cell^{-1}) ratio data revealed significant differences between months (ANOVA, F= 5.62 p< 0.05; table 3.8). The highest ratio was recorded during December (2.76 ± 0.19) and was significantly different from all months except January; the lowest ratio of 1.39 ± 0.16 was recorded during March and was significantly different from December alone (Table 3.8).

3.3.2.2. Seasonal variation in subtidal *Anemonia viridis*, Castle Island Bay

Zooxanthellae densities from summer (8/97) and winter (2/98) populations of *Anemonia viridis* differed significantly (T= 7.67, p< 0.001); winter zooxanthellae densities were significantly higher than summer densities (Table 3.9). Anemones collected during **winter** yielded a zooxanthellae density of **2.06** (± 0.11) x 10^8 cells g^{-1} wet weight (n=22), whereas anemones from the **summer** population bore **1.01** (± 0.09) x 10^8 cells g^{-1} wet weight (n=19).

The MI of zooxanthellae from the winter (0.68 ± 0.10 %) population of *A. viridis* was significantly higher than the MI of summer (0.39 ± 0.07 %) zooxanthellae (T= -2.32, p= 0.026) despite large variability in the samples (Table 3.9).

The diameter of zooxanthellae from summer (8/97) and winter (2/98) populations of *A. viridis* differed significantly ($T= -9.28$, $p< 0.001$). Zooxanthellae were significantly larger during winter (9.02 ± 0.05 μm) than summer (8.08 ± 0.10 μm; table 3.9).

3.3.2.3. Seasonal & bathymetric variation in *Anthopleura ballii*, Glannafeen cliff

All zooxanthellae density data were transformed by reciprocal square root to satisfy the assumptions for parametric statistics. Two-way ANOVA revealed a significant interaction between depth and season (ANOVA $F= 8.18$, $p< 0.05$); zooxanthellae density showed a different response to depth according to season (Table 3.10). Zooxanthellae density at 6 m depth on Glannafeen cliff during the summer was significantly lower ($0.45 \pm 0.04 \times 10^8$ cells g^{-1} wet weight) than the densities at 18 m during summer ($0.98 \pm 0.09 \times 10^8$ cells g^{-1} wet weight) and both 6 m and 18 m during winter (0.97 ± 0.10 and $0.82 \pm 0.10 \times 10^8$ cells g^{-1} wet weight; table 3.10).

Th low occurrence of dividing cells prevented statistical analysis of MI data. Instead the presence or absence of dividing cells was compared for summer and winter populations. The results of a Chi-square test of association showed that the number of samples with dividing cells was significantly different from the number of samples without dividing cells for summer and winter zooxanthellae at 6 m and 18 m ($X^2 = 10.35$ $p< 0.05$). Subsequent pairwise comparisons revealed a seasonal difference for 6 m zooxanthellae only ($X^2 = 7.048$ $p< 0.05$). Summer anemones at 6 m had a greater number of dividing zooxanthellae than winter anemones at 6 m. No depth related differences were found (Table 3.11).

Two-way ANOVA revealed that diameter is significantly affected by season (ANOVA $F= 4.55$ $p< 0.05$) but not depth (ANOVA $F= 0.00$ $p> 0.05$) and that there is no interaction between depth and season (ANOVA $F= 1.39$ $p> 0.05$).

Mean cell diameter of 6 m **summer** zooxanthellae was 11.3 ± 0.2 μm and was not significantly different from 18 m summer zooxanthellae which had a mean diameter of 11.1 ±0.2 μm (Table 3.10). The mean diameter of 6 m **winter** zooxanthellae was 11.5 ± 0.15 μm and 11.7 ± 0.18 μm for 18 m winter zooxanthellae and were not significantly different (Table 3.10). Zooxanthellae diameter showed a significant seasonal variation between summer and winter zooxanthellae at 18 m water depth but not at 6 m. Finally, table 1 Appendix VII provides a summary of all zooxanthellae characteristic data.

3.4. Discussion

Distribution and density of anemones

In the Trearddur Bay intertidal population of *Anemonia viridis*, anemones were significantly larger in summer than winter. Similarly, in studies of mapped individuals of *Actinia tenebrosa*, *Anthopleura elegantissima* and *A. xanthogrammica* on the shore which spanned several years, Ottaway (1980) and Sebens (1982 and 1983) found that major size increases occurred in spring and summer, and that decreases occured in late summer, autumn and winter in. In contrast, Muller-Parker (1987) found that the tropical anemone *Aiptasia pulchella* from subtidal sites were larger in the fall than the summer. In the present study there are two suggestions for the seasonal size difference observed. Firstly, the seasonal size variation in *A. viridis* from Trearddur Bay may be due to age differences in the population. If size is proportional to age, it may be inferred that a higher frequency of young individuals were present during the winter, which may have been due to emigration of older individuals. Schmidt (1972) and Chintiroglou & Koukouras (1992b) indicated that migration was important to the distribution of *A. viridis* and that larger individuals were found living in deeper water. In addition, Chintiroglou & Koukouras (1992a) report competitive behaviour of

younger individuals which out-compete older individuals, thus eliminating them from the population. Such intraspecific competition may begin after periods of rapid asexual and sexual reproduction prompted by abrupt temperature changes. Similar intraspecific competitive behaviour has been observed between individuals of *Anthopleura elegantissima* and *A. xanthogrammica* (Francis, 1973 and Sebens, 1984) which avoid competition for space and feeding resources either by reproducing asexually or migrating to other areas. Koehl (1977) observed that large *Metridium senile* were more abundant in deeper-water habitats, whilst small individuals were more abundant in shallow-water and attributed this to differences in drag forces experienced by different sized anemones. Larger individuals experience greater drag forces and thus are more likely to become dislodged by waves experienced during winter storms in shallow water than smaller individuals. Seasonal migration to deeper water avoids such physical stresses. The overall result is a population comprised of small, young individuals, comparable with the winter population of *A.viridis* in the present study. However, according to Shick (1991) sea anemones are classic examples of organisms showing indeterminate growth, in which body size is not fixed genetically and is variable according to environmental factors that affect the balance of energy intake and maintenance costs.

Thus, an alternative explanation for the observed size differences involves changes in the size of individual anemones in response to seasonality of temperature, irradiance, prey availability, sexual reproduction and asexual fission. During winter, growth rates are reduced by a combination of low temperatures, which slow metabolism, low solar irradiances which limit photosynthesis in the zooxanthellae and therefore limit the amount of carbon fixed in photosynthesis for translocation to the host, and low prey availability. Davy *et al.* (1996) demonstrated that at 1.5 m on sunny days in summer,

zooxanthellae in *A. viridis* would require 1.80 to 5.89 % of the carbon fixed in photosynthesis for respiration and growth, and translocate the remaining 94.11 to 98.20 % to the host. By comparison, at 9 m on cloudy days 37.82 to 87.84 % of the carbon fixed in photosynthesis would be required for zooxanthellae respiration and growth, leaving only 12.16 to 62.18 % for translocation. If an analogy is drawn between summer and 1.5 m on a sunny day, and winter and 9 m on a cloudy day, corresponding to high and low irradiance regimes, it is evident that more carbon is translocated to the anemone host in high solar irradiance situations (summer) than low irradiance situations (winter). However, in addition to harbouring symbiotic dinoflagellates, *A. viridis* is an opportunistic, omnivorous suspension feeder, predator (Plate 3.3a) and is able to take up glucose and amino acids from the water column (Chintiroglou & Koukouras, 1992b; Davy *et al.* 1997) and therefore may not be dependent upon symbionts for nutrition (Davy *et al.* 1997). Nevertheless, Davy *et al.* (1996 and 1997) concluded that whilst zooxanthellae may not be essential to their host's energy requirements they may still convey an energetically competitive advantage over non-symbiotic Anthozoa.

Ottaway (1979 and 1980) and Sebens (1981) suggested that some of the size increase in *Actinia tenbrosa*, and the symbiotic anemones *Anthopleura elegantissima* and *A. xanthogrammica* observed in spring and summer may have been due to the growth and maturation of gonads. They also suggested that the continuing decrease in size in winter appeared to relate to a decrease in prey, in both *A. elegantissima* and *A. xanthogrammica* and seasonal asexual fission in *A. elegantissima*, a process initiated by food deprivation (Sebens, 1980). In addition, size also declined in non-dividing specimens (Sebens, 1982) associated not only with spawning but also with somatic shrinkage (Shick, 1991). In the present study *Anemonia viridis* may have been

similarly affected by seasonal variation in environmental factors, resulting in a population comprised of significantly smaller individuals in the winter.

In Castle Island Bay the shallow, subtidal population of *A. viridis* showed a different spatial distribution according to season, which showed no correlation with depth (Figure 3.4). In addition, summer and winter populations of *A. viridis* showed no difference in size frequency distributions. Comparison with the Trearddur Bay population suggested that the more stable physical conditions experienced at the shallow subtidal site than a mid-tide level rock pool, produced a more stable population structure which was not effected by seasonal variations. It may be assumed that zooxanthellae photosynthesis in intertidal and shallow subtidal *A. viridis* was similarly affected by season, nevertheless such variation was not reflected in anemone size frequency distribution in subtidal *A. viridis*. Such an observation suggests that the factor (or combination of factors) causing seasonal variations in size frequency distributions, was only present or effective in the intertidal habitat. Further knowledge of seasonal variation in environmental and biological factors in these two habitats is required to enable interpretation.

The observed inverse relationship of *A. ballii* density with depth suggests a direct relationship with the exponential attenuation of irradiance with water depth (Chapter 2, section 2.3.3.2). Turner (1988) also observed that the depth distribution of *A. ballii* in Lough Hyne was determined by light availability, suggesting that these anemones actively seek out exposed, well-illuminated positions.

Zooxanthellae characteristics

Seasonal variation in zooxanthellae density, mitotic index (MI) and diameter were observed in the Trearddur Bay mid-tide level rock pool population of *A. viridis*. Winter (November, December, January) zooxanthellae densities were up to four times

higher than summer (June, July, August) zooxanthellae densities (Table 3.2). By comparison, the only month with a significantly different MI was May which demonstrated an increase on all other months of up to 3.5 months (Table 3.3). Zooxanthellae diameter was smallest in November, July and May and largest in February, March and August (Table 3.4). Explanation for the patterns observed was provided by considering zooxanthellae density, MI and diameter together. When a cell divides it becomes two smaller cells. The peak in cell division during May corresponded with a mean cell diameter which was smallest during May. Despite this peak zooxanthellae densities were lowest during summer, suggesting that population density was being regulated. The high rate of cell division in May produced new cells to replace old, degenerate cells, which may have been expelled to rid the zooxanthellae of waste products (Taylor, 1969) accumulated during the winter months. Thus, despite a high division rate and smaller size during May, no accompanying change in zooxanthellae density was observed. In subsequent months division was maintained at a background level. The maintenance of steady-state zooxanthellae density in the presence of on-going cell division may be through either pre-mitotic control of the zooxanthellae (occurring before division) or post-mitotic control (occurring after division) (Jones & Yellowlees, 1997). Pre-mitotic control mechanisms include the production of growth inhibiting factors and/or the limitation of zooxanthellae nutrient supply. Post-mitotic control mechanisms may involve the digestion of healthy or senescent zooxanthellae and/or the expulsion of excess or senescent zooxanthellae (Muscatine & Pool, 1979). Both mechanisms appear to be operating in the Trearddur population of *A. viridis*; regulation of zooxanthellae density occurs before and after division. During May, zooxanthellae numbers were regulated after division only, as a peak in zooxanthellae division rate (mitotic index)

did not result in an increase in zooxanthellae density. By contrast, during all other months zooxanthellae density appears to be regulated by inhibition of cell division, indicated by the low MI during all other months. As levels of solar radiation fall through the autumn months due to decreasing daylength, photosynthesis and therefore carbon fixation is reduced. In an attempt to enhance the light harvesting potential of the zooxanthellae during the autumn and winter, cell division may have continued at the background level, but with a decline in expulsion, producing an increase in zooxanthellae density. McCloskey *et al.* (1996) observed that reduced irradiance resulted in diminished expulsion rates for zooxanthellae from the temperate anemone *Anthopleura elegantissima.* If reduced autumn irradiances produced a similar diminution of zooxanthellae expulsion in *A. viridis*, a concomittant increase in zooxanthellae might be expected for winter months. By January density reached a maximum level and division ceased, possibly to prevent depletion of vital food reserves accumulated during summer or alternatively because zooxanthellae density had reached an optimum level for the host tissue. For example, Jones & Yelowlees (1997) suggested that space availability limits algal densities in colonies of *Acropora formosa*. During January, zooxanthellae may have reached the maximum possible level for the area available in *A. viridis*, such that space availablity limited further zooxanthellae division. Between January and February zooxanthellae density fell but size remained the same, possibly due to pre-mitotic control mechanisms and limited growth under winter conditions.

Although the division rate increased during February, March and April it was to a consistently low, background level and mean zooxanthellae size was consistent. Despite the smaller size and high division thereafter during May, no change in density was observed which suggests zooxanthellae numbers were being regulated by post-

mitotic control mechanisms such as digestion and/or expulsion of excess or senescent zooxanthellae. Hence, although high cell division was necessary to replenish a population which may have been laden with waste products from a long, dark winter, high zooxanthellae densities were not required by the anemone. Assuming light was a key seasonal variable, the high solar radiation levels experienced during May prompted post-mitotic regulation to maintain a small population of photosynthetically efficient zooxanthellae, where self-shading was minimised. It appears that a low density population of zooxanthellae was maintained throughout the summer and autumn by pre-mitotic control mechanisms, when low cell division was observed despite variations in daylength.

Fagoonee *et al.* (1999), Brown *et al.* (1999) and Fitt *et al.* (2000) observed strong seasonal cycles in zooxanthellae abundance in tropical scleractinian corals. Similar to the results of the present study, Fagoonee *et al.* (1999) showed that zooxanthellae densities in autumn and winter were three times the densities observed in spring and summer. Likewise zooxanthellae numbers showed a significant negative correlation with monthly irradiance (PAR) in four Indo-Pacific coral species (Brown *et al.* 2000). Increasing PAR in the dry season was paralleled by decreasing zooxanthellae numbers, whilst recovery of zooxanthellae numbers followed reductions in PAR at the end of the wet season. Similarly, Fitt *et al.* (2000) reported a significant increase in zooxanthellae densities during the winter in 5 species of reef-building corals over 4 consecutive years. By contrast, zooxanthellae densities in the tropical anemone *Aiptasia pulchella* were lower in the fall than the summer (Muller-Parker, 1987) whilst no seasonal variation was observed in zooxanthellae density of the temperate anemone *Anthopleura elegantissima* (Dykens & Shick, 1984).

Pigments

Seasonal variations in pigment concentrations were observed in zooxanthellae from Trearddur Bay *A.viridis*. Chlorophyll *a* concentrations per g of anemone tentacle wet weight were significantly higher during January and December than those of zooxanthellae from all other months. The pattern in chl c_2 concentrations per g of anemone wet weight was less obvious but nevertheless showed significantly higher concentrations in certain winter months (December and January) than summer months (May, July, August). Both chl *a* and c_2 concentrations per g anemone wet weight reflected seasonal variation in zooxanthellae density, as might be expected. By contrast, chl a:c_2 ratio showed no obvious winter/summer contrast, although December ratios were significantly higher than all other months except January, suggesting no seasonal variation in chl a:c_2 ratios.

No seasonal variation was observed in chl *a* or c_2 concentrations per zooxanthella, whilst the ratio of chl a:c_2 showed significant variation between months. The highest ratio per zooxanthella was recorded in December indicating that proportionately more chl *a* was present than in zooxanthellae sampled in March, when the lowest chl a:c_2 ratio was observed. Fitt *et al.* (2000) observed a similar increase in zooxanthellae chlorophyll *a* per unit area during winter in 5 species of reef coral from the Bahamas. However, in contrast to the present study a significantly lower concentration of chlorophyll per zooxanthella was also observed during winter.

Seasonal change in total chlorophyll (*a* plus c_2) was considered in the temperate anemone *A. elegantissima* and in contrast to the results of the present study, was not due to changes in the numbers of zooxanthellae but to the amount of pigment per alga (Dykens & Shick, 1984). The higher values in winter were presumed to compensate for the lower solar flux. In addition Dykens & Shick (1984) observed a

disproportional winter increase in chl c_2 (lower chl a:c_2 ratio) which they considered to be a further adaptation to reduced light availability. Similarly, the disproportional increase in chl a concentrations observed in the present study may be an adaptation to seasonally low irradiance in December.

The shallow subtidal population of *A. viridis* in Castle Island Bay showed a similar seasonal response to the intertidal population in Trearddur Bay. However, unlike the intertidal population, Castle Island Bay anemones were only sampled during one winter month (February) and one summer month (July), providing only part of the annual cycle of variation. Comparable to intertidal anemones, during certain winter months (2/98) zooxanthellae had a higher density, were larger but divided more frequently than in the summer (7/98). The low division rates in intertidal and subtidal zooxanthellae during August (summer) are comparable and reflect a relatively small, stable population of zooxanthellae. Similarly, the largest zooxanthellae diameter was recorded during February in both intertidal and subtidal anemones, suggesting that both populations were affected by seasonally varying conditions prevailing in intertidal and subtidal habitats.

Zooxanthellae characteristics in *A. ballii* from Glannafeen cliff showed both season and depth-related responses. Zooxanthellae density was lower at 6 m during summer than 18 m during summer, whilst neither presence of dividing cells nor zooxanthellae diameter was effected by depth on Glannafeen cliff in winter or summer. Winter zooxanthellae densities were not affected by depth. By comparison, zooxanthellae density was significantly lower at 6 m during summer than both 6 m and 18 m during winter. However, zooxanthellae density in 18 m summer anemones was not significantly different from winter densities, suggesting a depth/season interaction (Table 3.10). No season or depth response was observed in zooxanthellae diameter or

mitotic index on Glannafeen cliff. Hence, the size and number of dividing zooxanthellae were similar during February and July and at 18 m and 6 m water depth, indicating no season or depth response on Glannafeen. Alternatively, these observations may represent lack of variation only during the months and at the depths sampled. Intermittent months and depths may have revealed variations in zooxanthellae diameter and MI, similar to those observed over an annual cycle for *A. viridis* at Trearddur Bay.

Considering the influence of depth, the consistent zooxanthellae densities in winter at 6m and 18 m, and at 18 m in winter and summer suggest comparable irradiance regimes in these two situations and will be discussed further in chapter 6. Bythell *et al.* (1997) sampled two populations of *A. viridis* inhabiting environments characterised by different levels of solar radiation. The first population was intertidal, in mid-shore rock pools of 1-3 m depth at high tide; the second was found in the subtidal zone at 5-10 m water depth. Similar to depth responses observed in winter in the present study but in contrast to summer, no depth-related variation was observed in zooxanthellae density (i.e. $6.8 \pm 1.24 \times 10^8$ algae g^{-1} dry weight in intertidal anemones compared with $5.6 \pm 1.31 \times 10^8$ algae g^{-1} dry weight in subtidal anemones). However, the obsevations of Bythell *et al.* (1997) contrast sharply with many previous studies, which describe marked effects of irradiance or depth on symbiotic associations. Studies by Zvalinskii *et al.* (1978) and Titlyanov *et al.* (1980) on tropical corals showed increases in zooxanthellae density with depth, supporting the response observed in temperate *A. ballii* on Glannafeen cliff in summer. The higher zooxanthellae densities observed in 18 m anemones than 6 m anemones during the summer on Glannafeen cliff show analogies with the observations of zooxanthellae

during summer in *A. pulchella* in Hawaii where higher zooxanthellae densities were observed in shade anemones than sun anemones. Conversely, the lack of variation between 18 m and 6 m zooxanthellae from anemones on Glannafeen cliff contrasts with the observations of zooxanthellae in *A. pulchella* in autumn, which show higher zooxanthellae densities in shade anemones than sun anemones (Muller-Parker, 1987). The consistent winter zooxanthellae densities may be explained by consistencies in physical environmental conditions with depth. For example, there may have been no photosynthetic difference in prevailing light conditions between 6 m and 18 m water depths in winter, due to high turbidity and low solar irradiance.

It is tempting to draw parallels between shallow and deep water environments, and sun and shade habitats in terms of prevailing light conditions. However, the diminution of light with depth is accompanied by changes in spectral composition (chapter 2) which may effect zooxanthellae differently to variations in light intensity alone. Season and depth-related differences in prevailing physical environmental parameters were investigated in Lough Hyne in chapter 2. The effects of such factors on variations in zooxanthellae characteristics are discussed in chapter 6.

As an alternative to photoadaptation of the same species of zooxanthellae, some workers have suggested that different strains or species of zooxanthellae may occur in Anthozoa of the same species in different photic environments. Iglesias-Prieto & Trench (1994) demonstrated that the photoacclimatory responses of the zooxanthellae to irradiance varied between *Symbiodinium* taxa in laboratory culture. Similarly, Rowan & Knowlton (1995) found that the dominant genotype of *Symbiodinium* in the coral *Montastrea annularis* varied consistently with depth. In addition, Dustan (1982) observed depth-dependent photoadaptation of zooxanthellae in *Montastrea annularis*. However, in a study of algal genotype in *Anemonia viridis* from Cornwall, UK,

Bythell *et al.* (1997) concluded that there was no variation in genotype between natural populations living in different solar radiation environments. In light of these results photoadaptation is assumed in the present study. Further work on seasonal and depth variation in zooxanthellae of temperate symbiotic Anthozoa is required. Not considered in the present study was pigment variation with depth and seasonal pigment variation in a subtidal population of symbiotic anemones.

Conclusions

Anemones from the intertidal population of *A. viridis* were larger in summer than winter suggesting shrinkage, or emigration to deeper water, of large individuals in winter. The seasonal pattern in temperate intertidal and shallow subtidal *A. viridis*, and subtidal *A.ballii* showed an increase in zooxanthellae density in winter months. Both chl *a* and c_2 in intertidal *A. viridis* showed proportional increases in winter (mg g^{-1} of anemone wet weight) which reflected increases in zooxanthellae density. Thus, zooxanthellae may have been photoadapating to low winter irradiance by increasing the number of cells available for light harvesting. No seasonal differences in chlorophyll per zooxanthellae were observed, although proportionately more chl *a* was present in December.

In intertidal *A. viridis*, small zooxanthellae populations were regulated through the summer, when cell division was low. Zooxanthellae densities increased through the autumn to a maximum in winter. This may represent photoadaptation to seasonally low irradiances brought about by lack of post-mitotic control mechanisms. The peak in cell division in May produced small zooxanthellae which refreshed a senescent winter population.

Water depth had an effect on zooxanthellae density in subtidal *A. ballii* during summer but not winter, suggesting photoadaptation to differences in summer irradiances only. Zooxanthellae diameter and MI in *A. ballii* did not vary with water depth.

DEPTH	DOMINANT ORGANISMS
6 m	*Stilophora rhizoides* *Asperococcus turneri* *Ulva* sp. *Lithothamnion* sp. various foliaceous & filamentous red algae *Anthopleura ballii* *Anemonia viridis*
18 m	*Caryophyllia smithii* encrusting orange sponge *Raspailia* sp. *Ascidiella aspersa* *Isozoanthus sulcatus* *Anthopleura ballii*

Table 3.1. Dominant species at 6 m and 18 m on Glannafeen cliff; at 6 m the community was characterised by algae, whilst at 18 m fauna was dominant.

Table 3.2. Mean monthly zooxanthellae density (x 10^8 cells g^{-1} of wet weight) over an annual cycle in the Trearddur Bay intertidal population of *A. viridis*. Months sharing the same underlining indicates no significant difference was found using one-way ANOVA and the *post-hoc* Tukey test.

Table 3.3. Mean monthly MI (%) over an annual cycle in the Trearddur Bay intertidal population of *A. viridis*. Months sharing the same underlining indicates no significant difference was found using one-way ANOVA and the *post-hoc* Tukey test.

Month	June	Aug	July	April	Mar	Feb	May	Oct	Sep	Nov	Dec	Jan
Density (x 10^8 cells/g)	0.68	1.01	1.24	1.48	1.58	1.58	1.61	1.61	1.70	1.76	2.73	5.33
SE (+/-)	0.06	0.05	0.09	0.06	0.08	0.08	0.09	0.12	0.17	0.07	0.18	0.20

Month	June	Feb	Nov	Aug	Sep	Dec	April	July	Oct	Mar	May
MI (%)	0.29	0.53	0.61	0.65	0.74	0.77	0.88	0.93	1.01	1.02	3.07
SE (+/-)	0.10	0.12	0.20	0.19	0.10	0.20	0.13	0.10	0.25	0.13	0.26

Table 3.4. Mean monthly zooxanthellae diameter (μm) in the Trearddur Bay intertidal population of *A. viridis*. Months sharing the same underlining indicates no significant difference was found using one-way ANOVA and the *post-hoc* Tukey test.

Month	Nov	Jul	May	Dec	Oct	June	Sep	April	Jan	Mar	Aug	Feb
Diameter (μm)	8.50	8.81	9.07	9.16	9.23	9.38	9.71	9.78	9.87	9.91	9.94	10.00
SE (+/-)	0.12	0.12	0.05	0.10	0.16	0.15	0.06	0.09	0.07	0.09	0.10	0.05

MONTH	n=	MEDIAN	Z
January	10	1110	4.66
December	11	810	4.60
February	10	441	0.99
April	12	381	-0.01
October	11	326	-0.61
May	12	372	-0.86
September	10	333	-0.98
August	11	282	-2.38
March	12	311	-2.44
July	11	289	-2.63

Table 3.5. Medians and z values for monthly chlorophyll *a* concentrations ($\mu g\ g^{-1}$ wet weight) in intertidal *Anemonia viridis*. Mean ranks are shown for each month (z) and listed from greatest to least different from mean rank for all observations.

Table 3.6. Mean monthly chlorophyll c_2 concentrations ($\mu g\ g^{-1}$ of wet weight) in zooxanthellae from the Trearddur Bay intertidal population of *A. viridis*. Months sharing the same underlining indicates no significant difference was found using one-way ANOVA and the *post-hoc* Tukey test.

Table 3.7. Mean monthly chl a:c_2 ratio ($\mu g\ g^{-1}$ of wet weight) in zooxanthellae from the Trearddur Bay intertidal population of *A. viridis*. Months sharing the same underlining indicates no significant difference was found using one-way ANOVA and the *post-hoc* Tukey test.

Month	July	May	Aug	April	Mar	Feb	Oct	Dec	Sep	Jan
Chl c_2 (µg/g)	178	193	194	215	236	261	270	309	440	484
SE (+/-)	15.7	12.9	23.1	16.1	24.4	35.3	41.2	28.2	119	83.6

Month	Sep	Mar	Oct	July	Aug	April	Feb	May	Jan	Dec
Ratio (Chl a:c_2)	1.14	1.50	1.59	1.79	1.82	1.88	1.90	1.96	2.49	2.76
SE(+/-)	0.23	0.11	0.15	0.18	0.20	0.07	0.20	0.13	0.24	0.16

Table 3.8. Mean monthly chlorophyll $a{:}c_2$ ratio (pg/cell) in zooxanthellae from the Trearddur Bay intertidal population of *A. viridis*. Months sharing the same underlining indicates no significant difference was found using one-way ANOVA and the *post-hoc* Tukey test.

Month	Mar	Oct	Aug	Jul	April	Feb	May	Jan	Dec
Ratio (Chl *a*:c_2)	1.39	1.63	1.82	1.86	1.89	1.90	1.96	2.01	2.76
SE (+/-)	0.16	0.17	0.20	0.18	0.06	0.20	0.13	0.09	0.19

Table 3.9. Zooxanthellae characteristics for winter (2/98) and summer (8/97) subtidal, sun-habitat *Anemonia viridis* from Castle Island Bay, Lough Hyne. Values shown are means ± 1 standard error.

Table 3.10. Zooxanthellae characteristics for *Anthopleura ballii* from 6 m and 18 m on Glannafeen cliff during winter (2/98) and summer (8/98). Values shown are means ± 1 standard error.

Zooxanthellae characteristics	SUMMER mean	n=	WINTER mean	n=
Density (x 10^8 algae g^{-1} wet weight)	**1.01** ± 0.09	19	**2.06** ± 0.11	22
MI (%)	**0.39** ± 0.07	18	**0.68** ± 0.10	22
Diameter (μm)	**8.08** ± 0.10	19	**9.02** ± 0.05	22

Zooxanthellae characteristics	SUMMER 6 m mean	n=	SUMMER 18 m mean	n=	WINTER 6 m mean	n=	WINTER 18 m mean	n=
Density (x 10^8 algae g^{-1} wet weight)	**0.45** ± 0.04	12	**0.98** ± 0.09	12	**0.97** ± 0.10	12	**0.82** ± 0.10	12
Diameter (μm)	**11.3** ± 0.20	12	**11.1** ± 0.21	12	**11.5** ± 0.15	12	**11.7** ± 0.18	12

	X^2	S/NS
Summer 6 m vs Summer 18 m	0.013	NS
Winter 6 m vs Winter 18 m	0.361	NS
Summer 6 m vs Winter 6 m	7.048	S
Summer 18 m vs Winter 18 m	3.308	NS

Table 3.11. Statistical comparison of anemones with dividing zooxanthellae and without dividing zooxanthellae during summer and winter at 6 m and 18 m water depths on Glannafeen cliff. S = significant NS= not significant difference, p= 0.05).

Figure 3.1. Location of Castle Island Bay and Glannafeen subtidal cliff in Lough Hyne M.N.R., Co. Cork, Ireland.

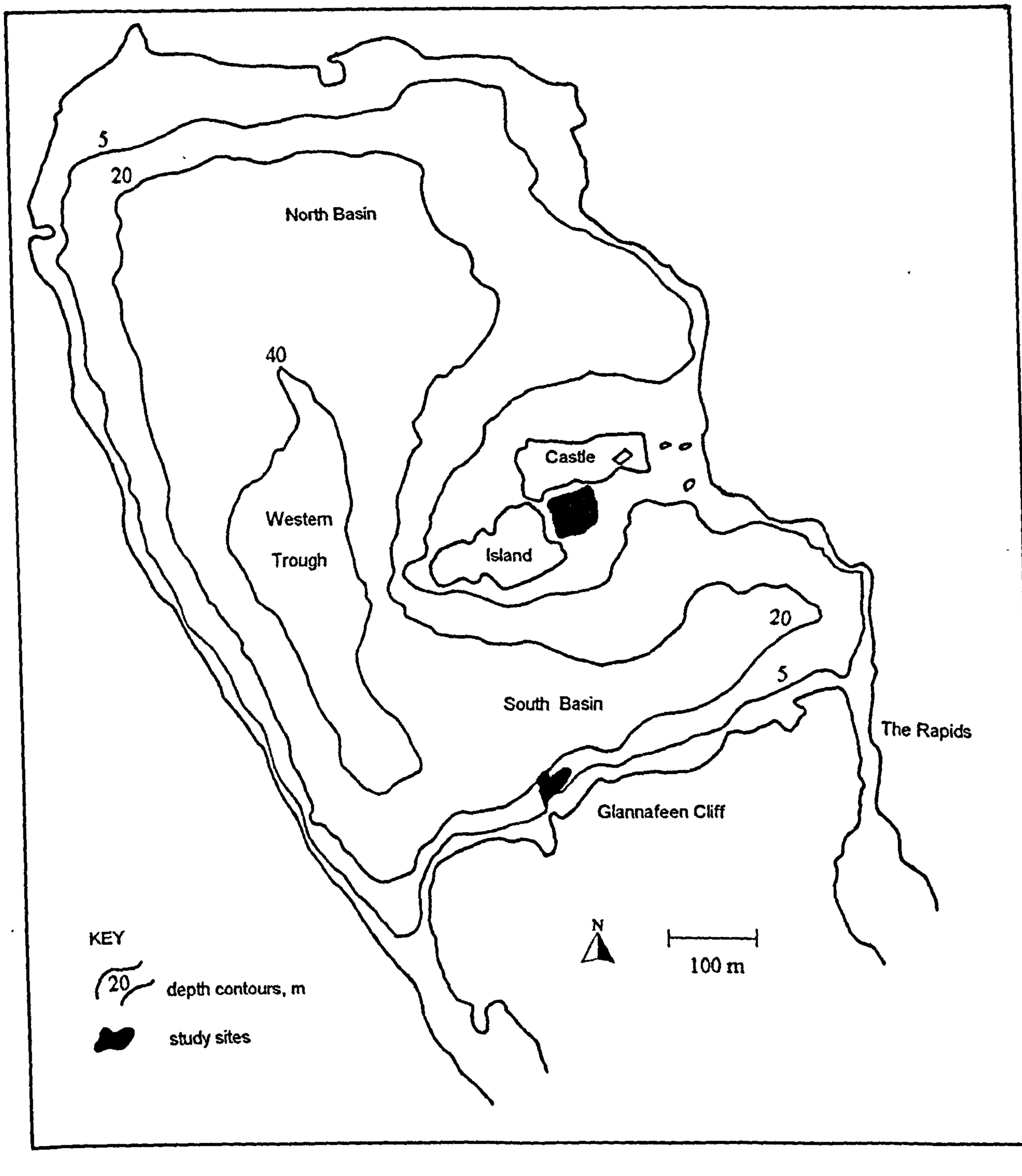

5
20
North Basin
40
Western
Trough
Castle
Island
20
5
South Basin
The Rapids
Glannafeen Cliff
N
100 m
KEY
20
depth contours, m
study sites

Figure 3.2. Location of Renoufian sectors in Lough Hyne, Co. Cork, Ireland. These subdivisions of the intertidal shore of Lough Hyne are permanently marked with numbered discs and provide useful reference points in ecological studies.

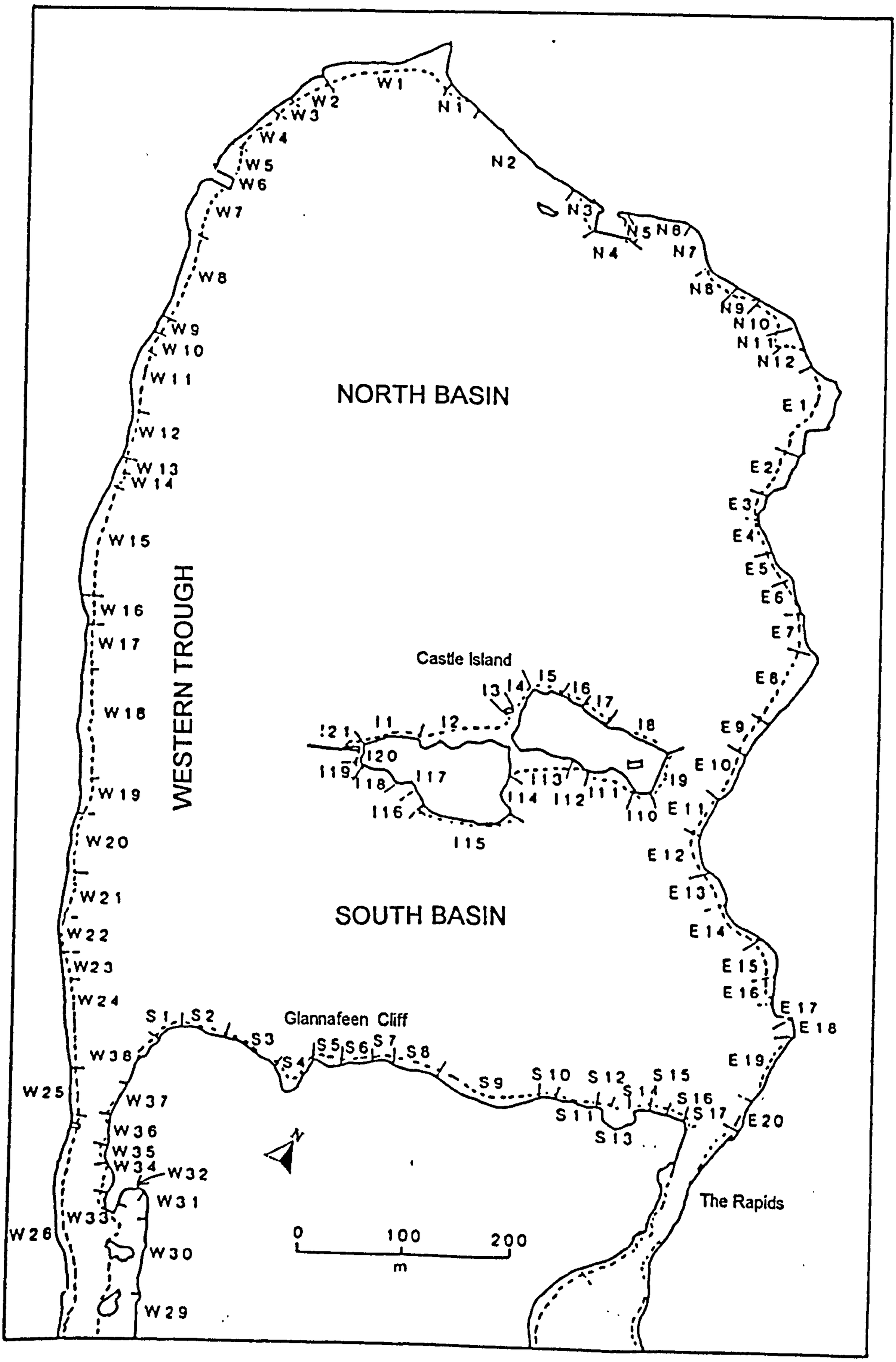
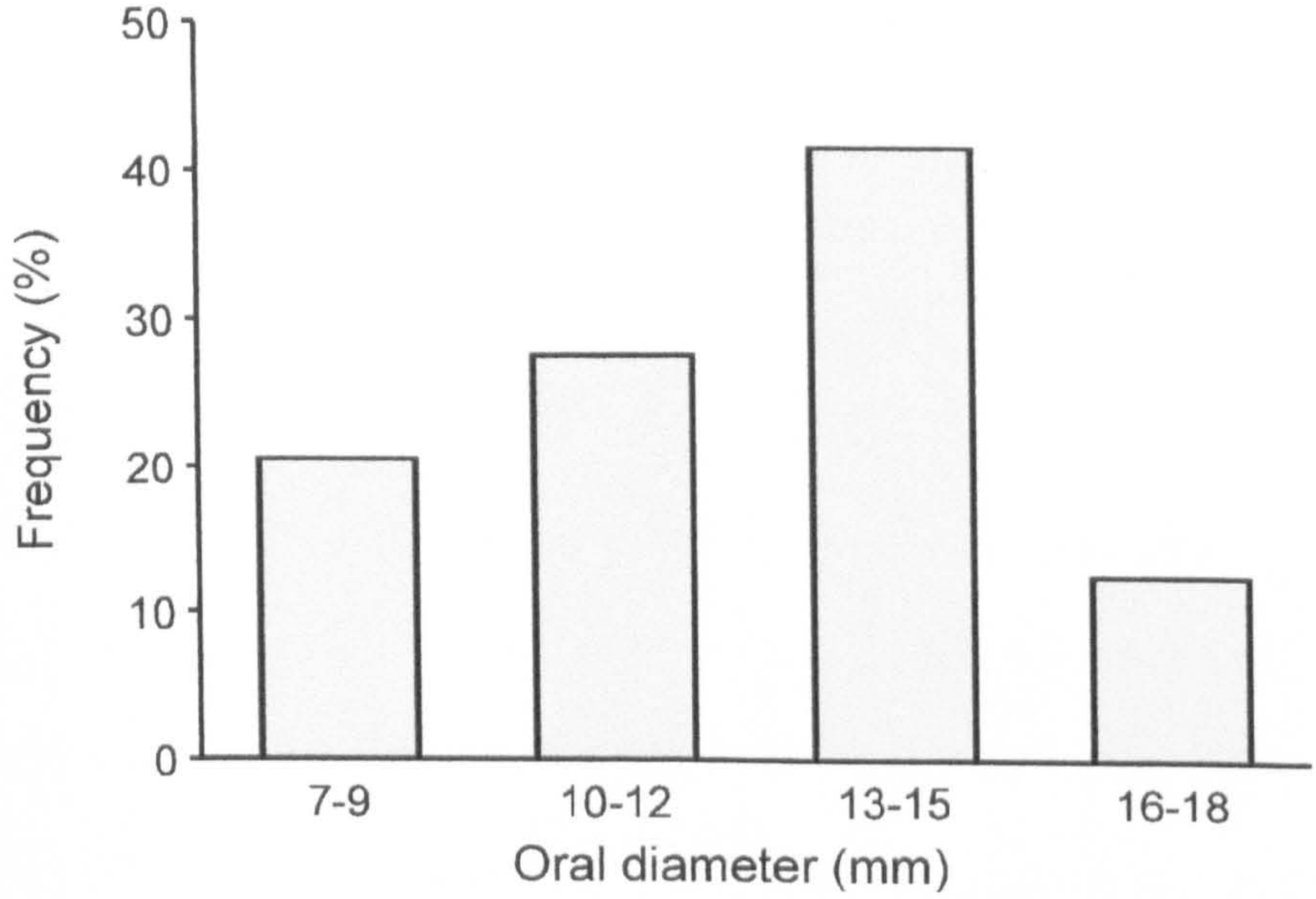

W1
W2
W3
W4
W5
W6
W7
W8
W9
W10
W11
W12
W13
W14
W15
W16
W17
W18
W19
W20
W21
W22
W23
W24
W25
W26
W27
W28
W29
W30
W31
W32
W33
W34
W35
W36
W37
W38
WESTERN TROUGH
N1
N2
N3
N4
N5
N6
N7
N8
N9
N10
N11
N12
NORTH BASIN
E1
E2
E3
E4
E5
E6
E7
E8
E9
E10
E11
E12
E13
E14
E15
E16
E17
E18
E19
E20
Castle Island
11
12
13
14
15
16
17
18
19
110
111
112
113
114
115
116
117
118
119
120
121
SOUTH BASIN
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12
S13
S14
S15
S16
S17
Glannafeen Cliff
The Rapids
N
0
100
200
m

Figure 3.3 Size distribution of brown *Anemonia viridis* from the Trearddur Bay population during a) **summer** (7/98) and b) **winter** (2/99). The oral diameters of 40 animals were measured; during summer the modal size class was 13-15 mm compared with 7-9 mm during winter.

a)


Frequency (%)
50
40
30
20
10
0
7-9
10-12
13-15
16-18
Oral diameter (mm)

b)

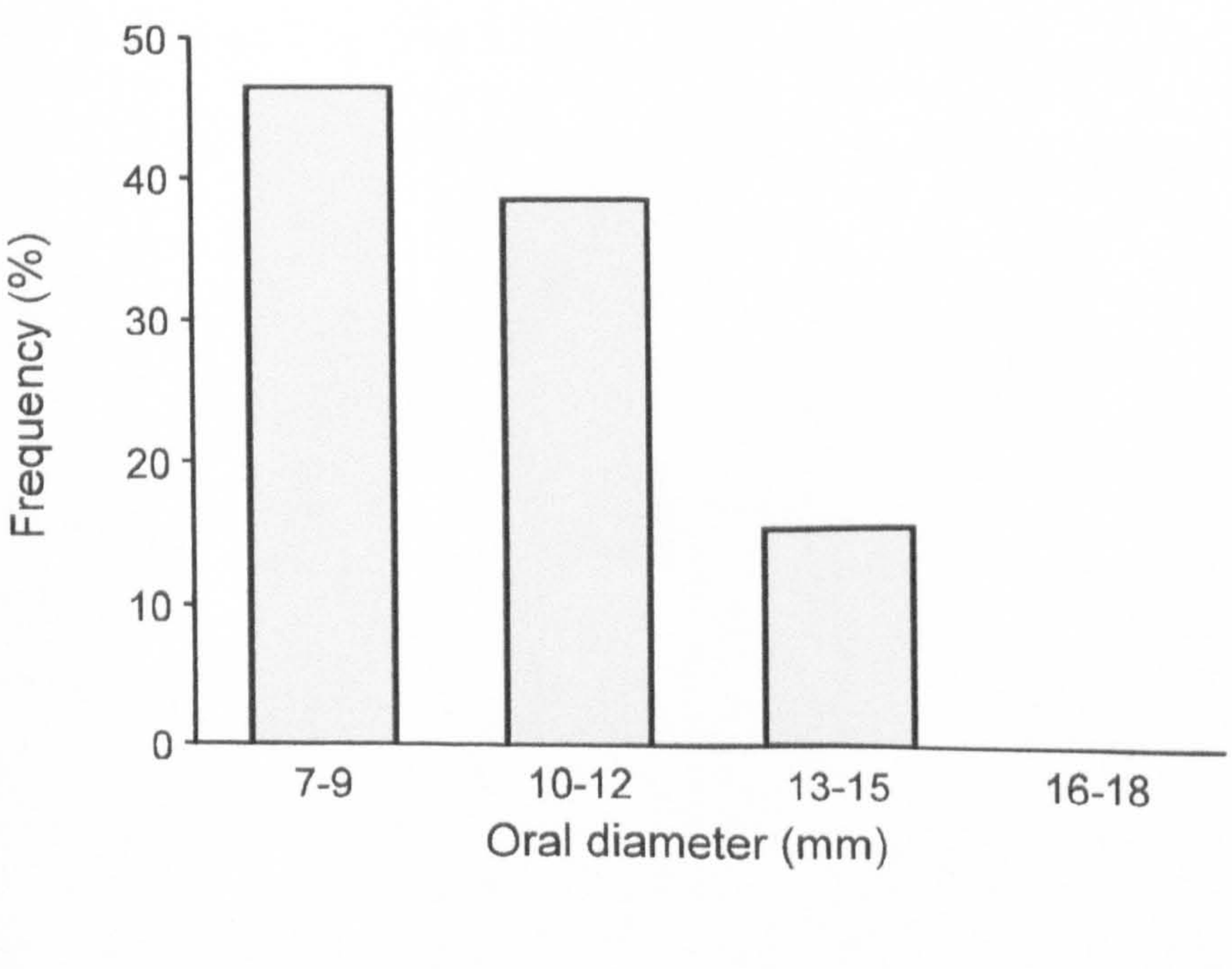
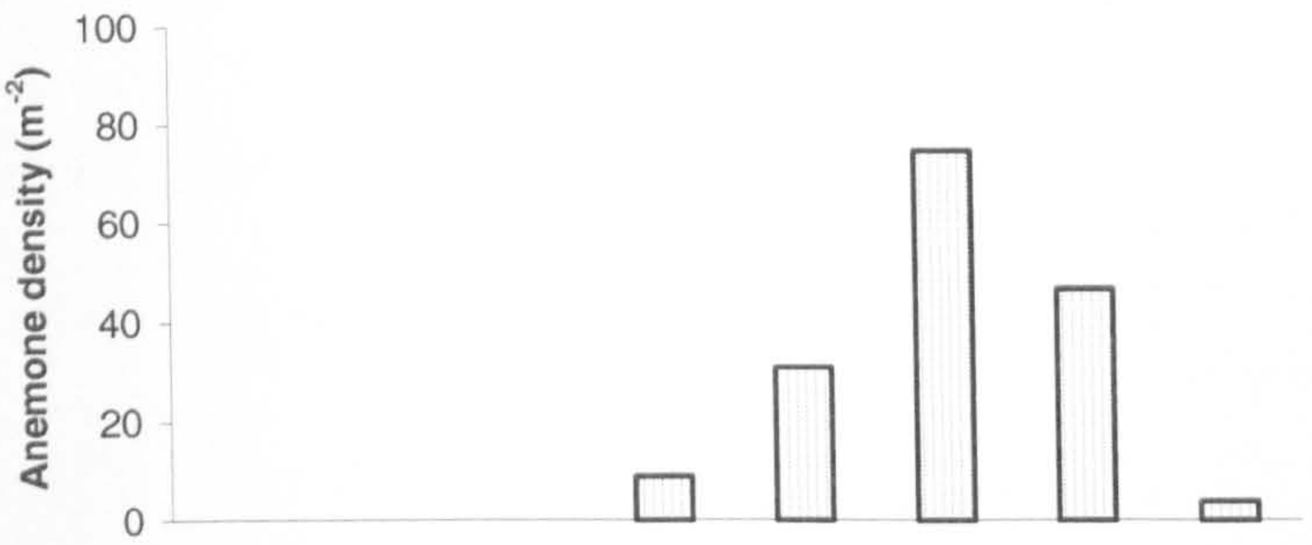
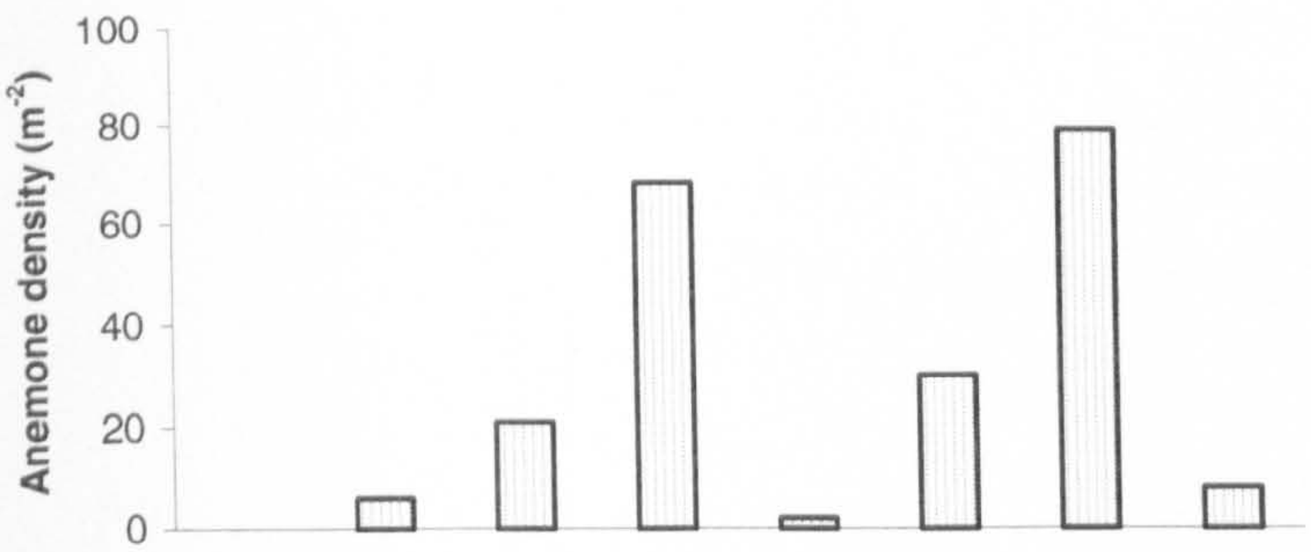

Frequency (%)
50
40
30
20
10
0
7-9
10-12
13-15
16-18
Oral diameter (mm)

Figure 3.4. Density and distribution of *Anemonia viridis* along a transect of Castle Island bay, Renoufian sector I13 (c) during a) summer (8/98) and b) winter (2/98). Two 1 m^2 quadrat samples were taken approximately every 6 m along the transect starting at LW mark (distance 0 m) and ending at 42 m perpendicular distance from the shore. Density was recorded as the number of anemones per square metre (m^{-2}) and a mean value of the two samples plotted against distance along the shore. In b) for example, 20 anemones m^{-2} occurred at 12 m perpendicular distance from the shore and at a water depth of 0.4 m.

a)


Anemone density (m-2)
100
80
60
40
20
0

b)


Anemone density (m-2)
100
80
60
40
20
0

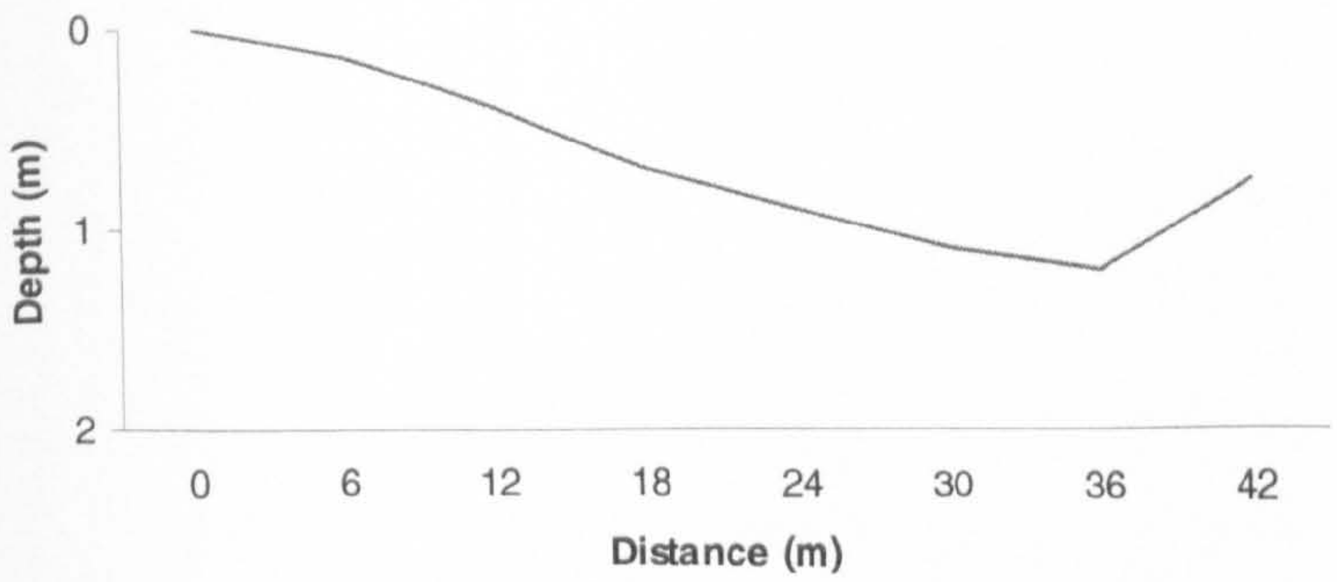
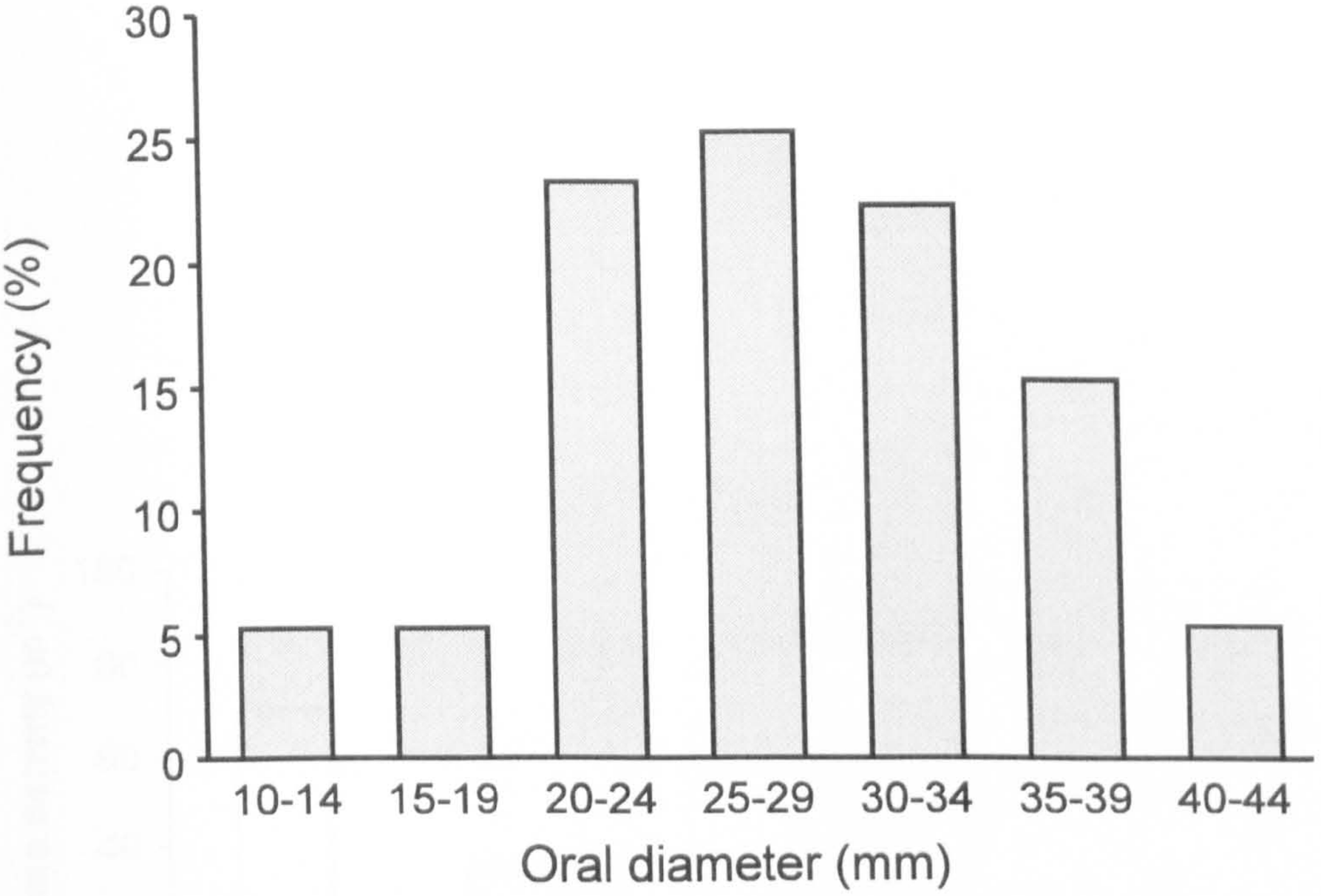
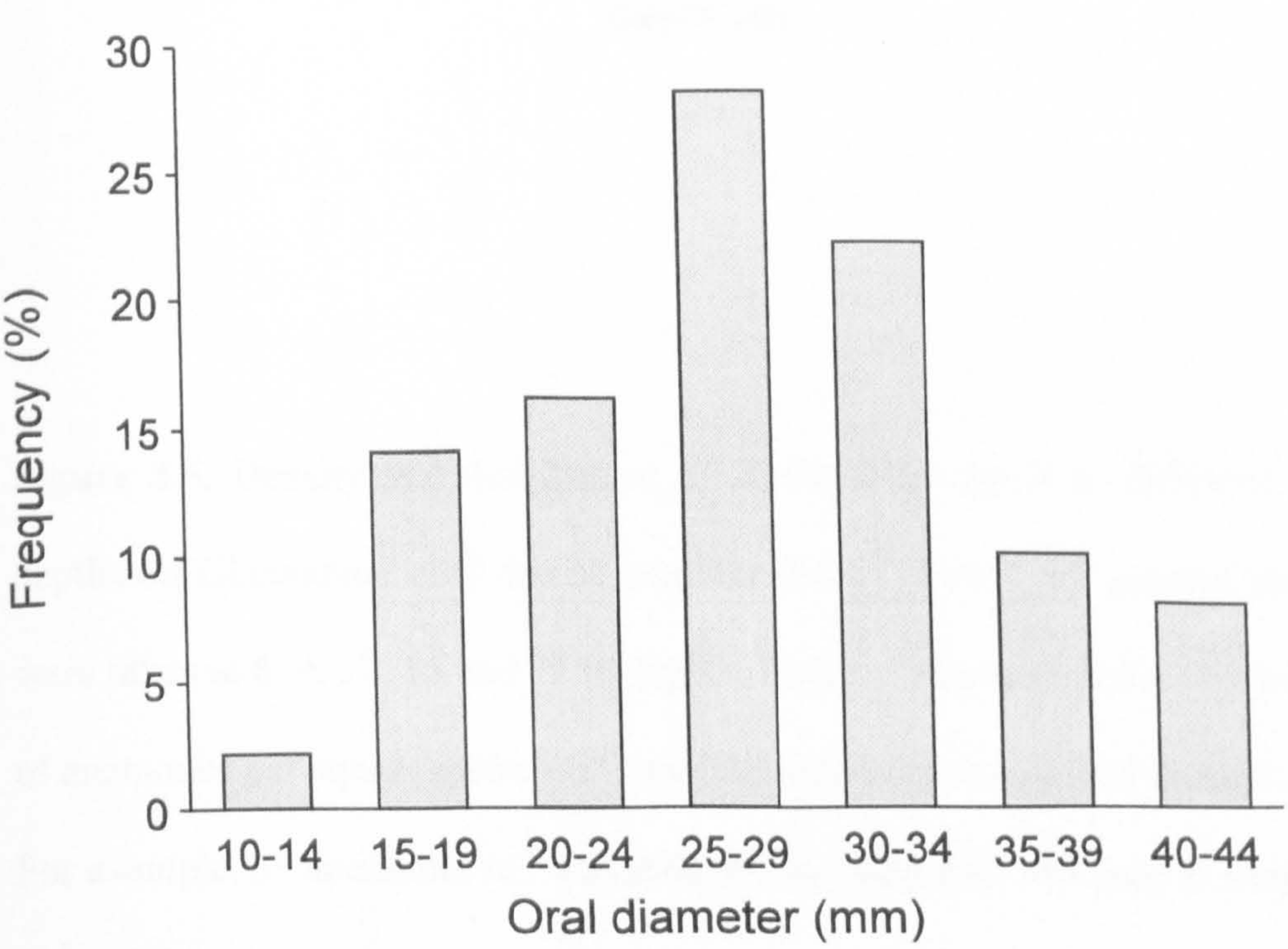

Depth (m)
0
1
2
0
6
12
18
24
30
36
42
Distance (m)

Figure 3.5. Size frequency distribution of brown *Anemonia viridis* from Castle Island Bay, Lough Hyne during a) summer and b) winter. The oral diameters of 50 animals were measured; during both summer and winter the modal size class was 25-29 mm.

a)


Frequency (%)
30
25
20
15
10
5
0
10-14
15-19
20-24
25-29
30-34
35-39
40-44
Oral diameter (mm)

b)


Frequency (%)
30
25
20
15
10
5
0
10-14
15-19
20-24
25-29
30-34
35-39
40-44
Oral diameter (mm)

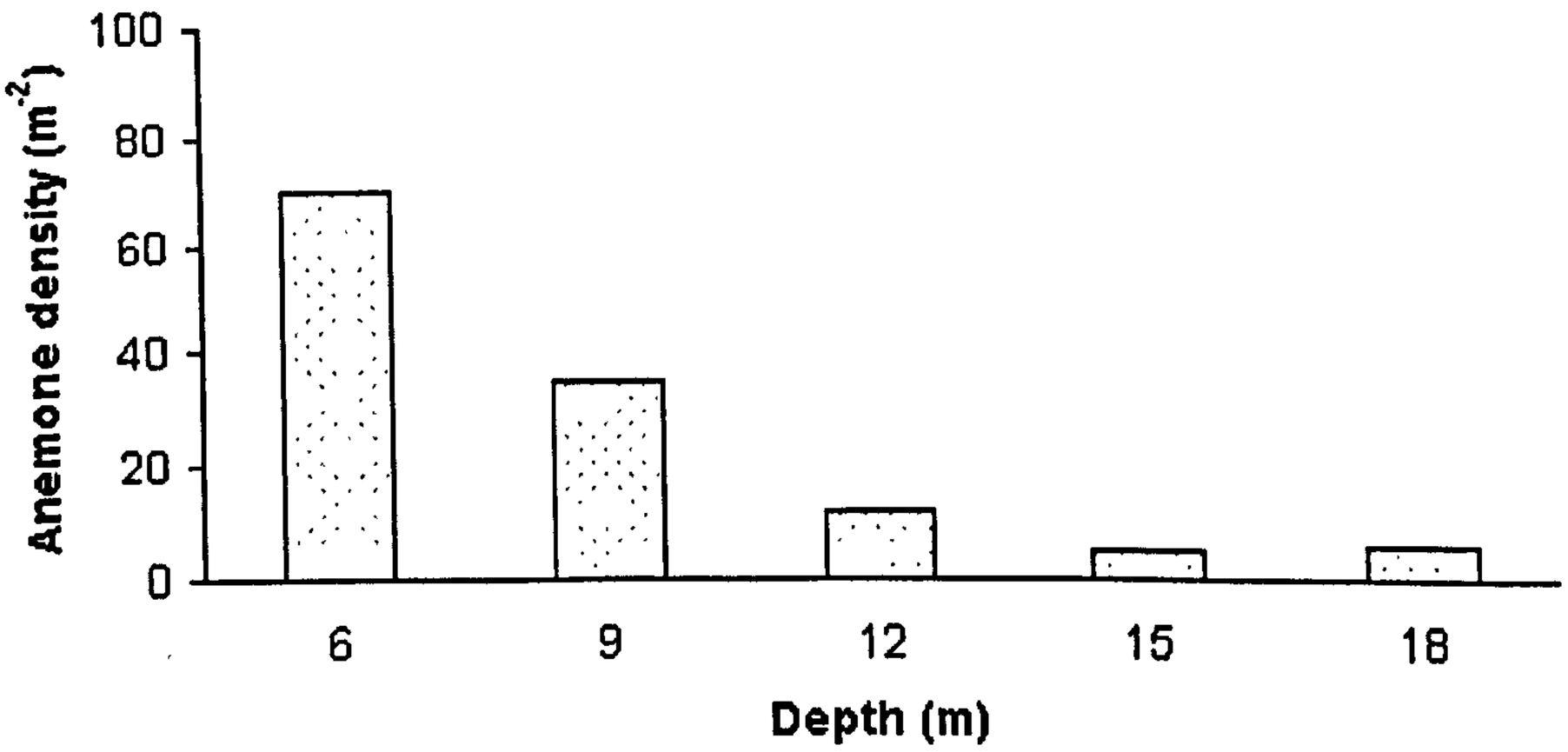
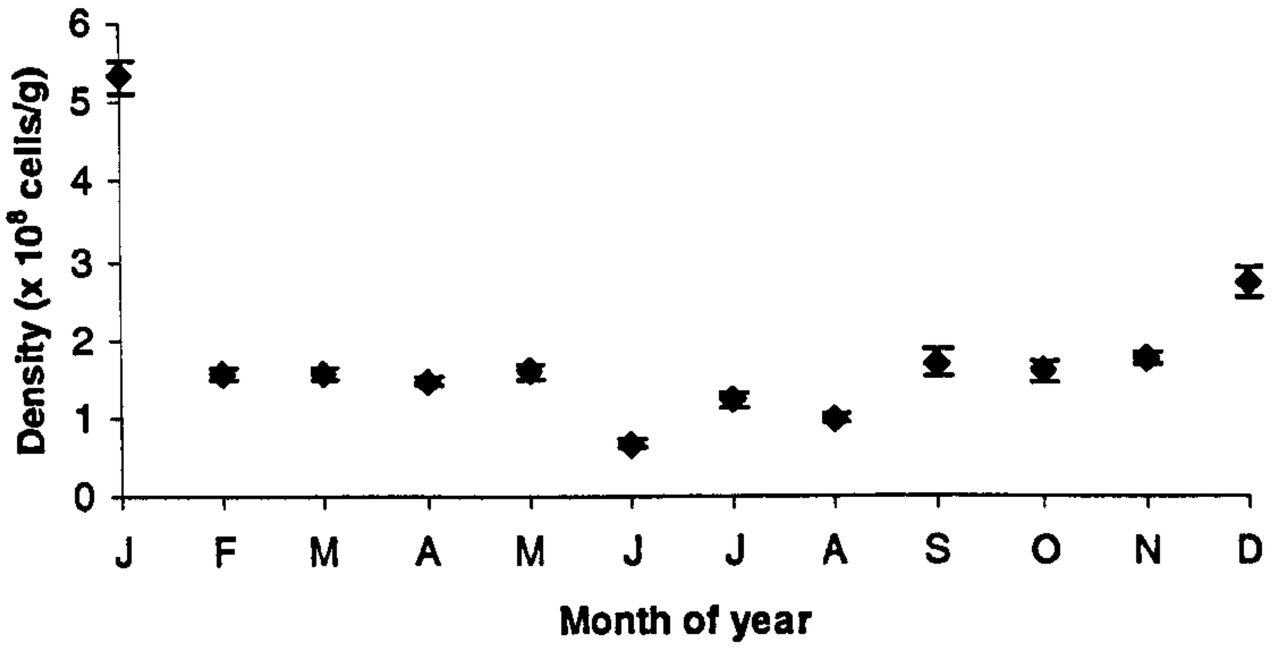


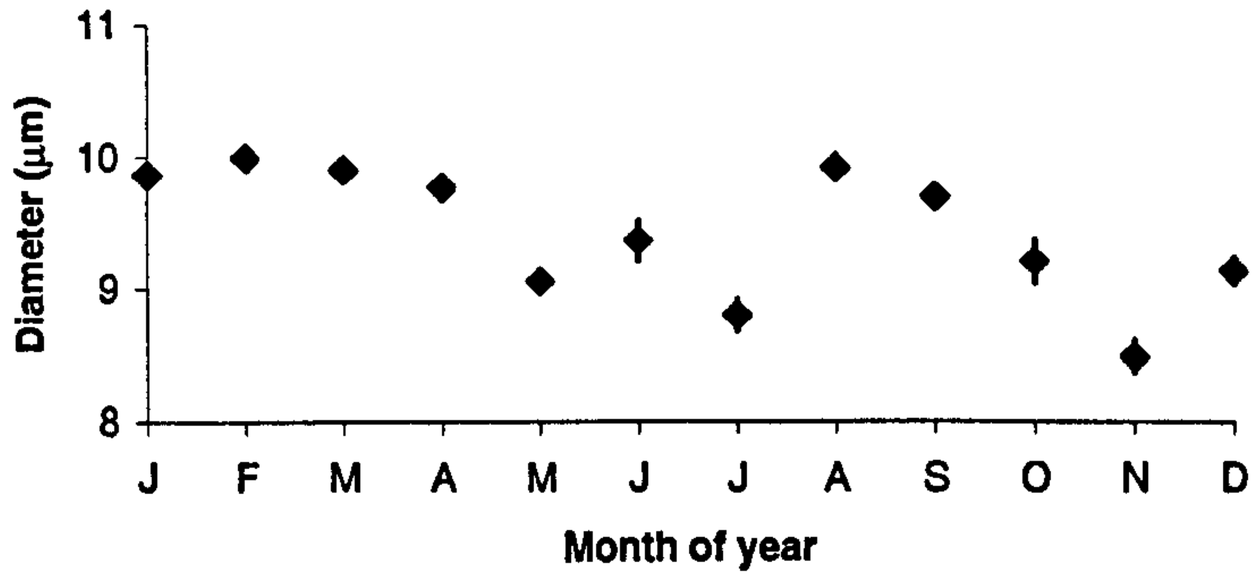
Figure 3.6. Density and distribution of *Anthopleura ballii* at different water depths on Glannafeen cliff during summer (8/98). Two 1 m^2 quadrat samples were taken at 6, 9, 12, 15 and 18 m depths. Density was recorded as the number of anemones per square metre (m^{-2}) and the mean density plotted at each depth. For example, 35 anemones m^{-2} occurred at 9 m compared with only 6 anemones m^{-2} at 18 m.

Figure 3.7. Mean monthly zooxanthellae density in *Anemonia viridis* tentacles from an intertidal rock pool, Trearddur Bay, Anglesey (May 1997 to January 1998 and February to April 1999). Units are x 10^8 cells g^{-1} wet weight and error bars are ± 1 standard error.

Figure 3.8. Mean monthly zooxanthellae diameter in *Anemonia viridis* tentacles from an intertidal rock pool, Trearddur Bay, Anglesey (May 1997 to January 1998 and February to April 1999). Diameter was measured in units μm and error bars are ± 1 standard error.

Figure 3.9. Mean monthly mitotic index of zooxanthellae in *Anemonia viridis* tentacles from an intertidal rock pool, Trearddur Bay, Anglesey (May 1997 to January 1998 and February to April 1999). MI was measured as percentage and error bars show ± 1 standard error.


Density (x 10⁸ cells/g)
6
5
4
3
2
1
0
J F M A M J J A S O N D
Month of year


Diameter (µm)
11
10
9
8
J F M A M J J A S O N D
Month of year

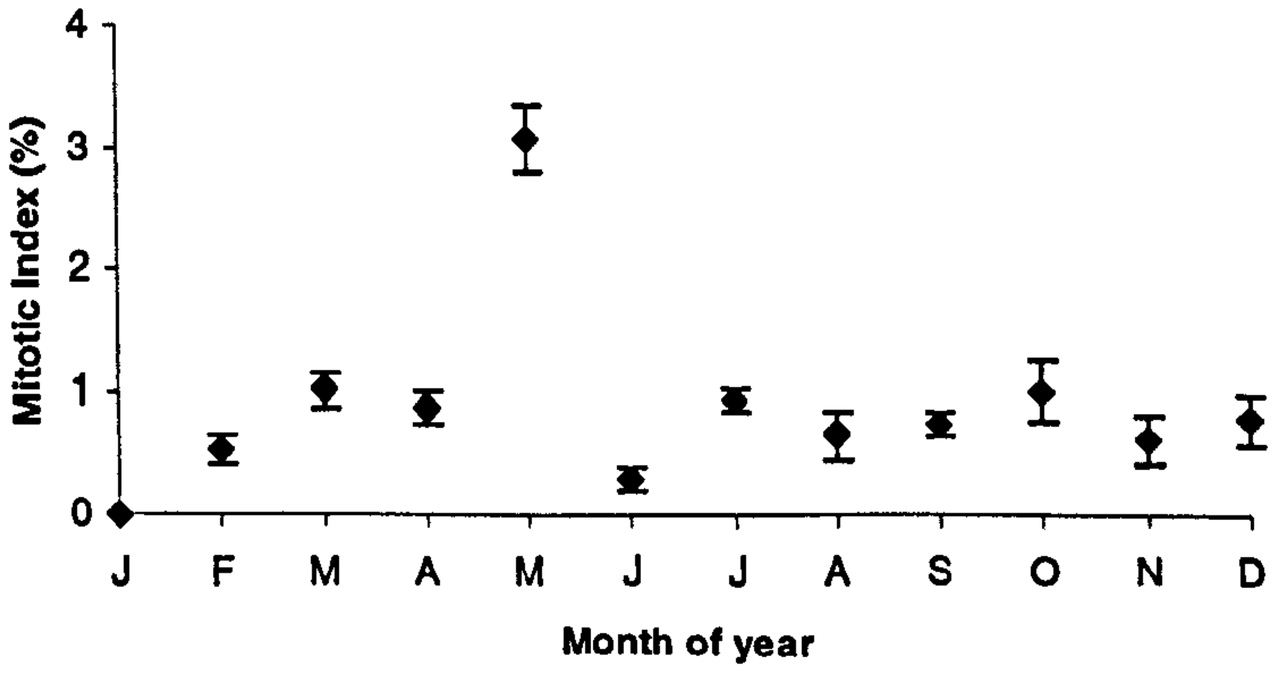
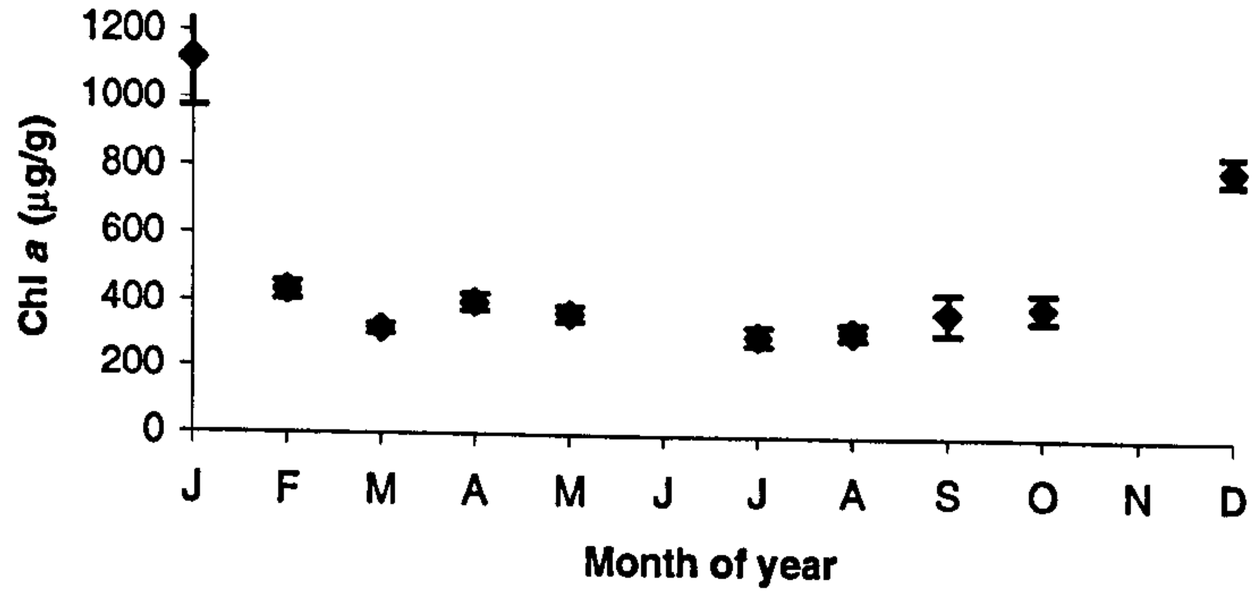
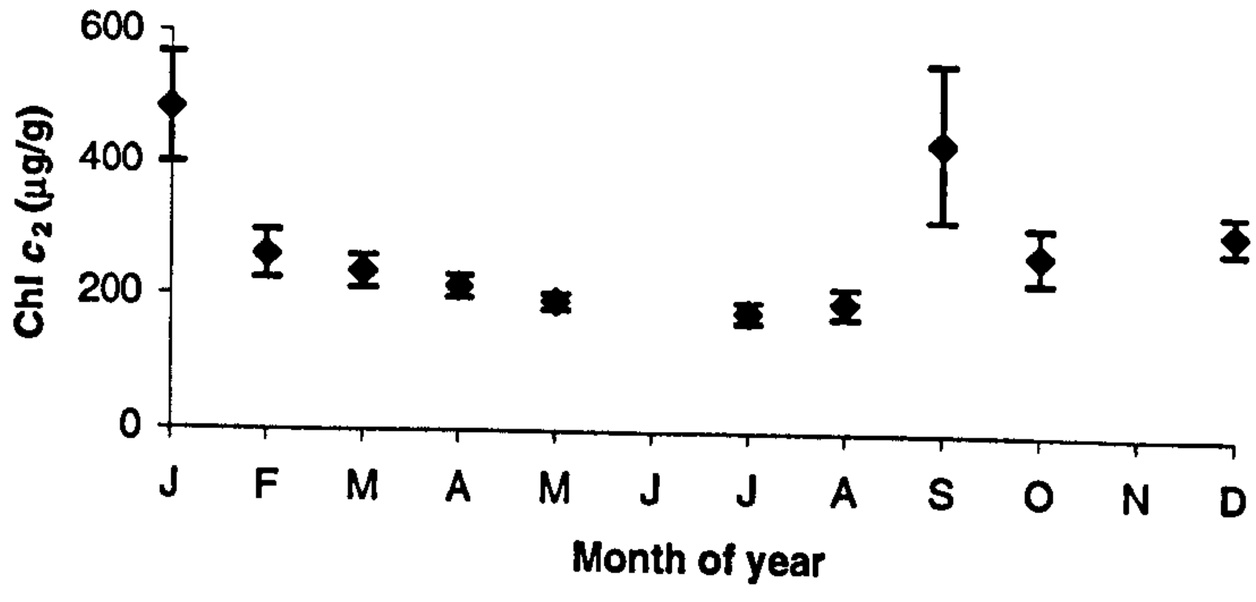

Mitotic Index (%)
4
3
2
1
0
J F M A M J J A S O N D
Month of year

Figure 3.10. Mean monthly chlorophyll concentrations in *Anemonia viridis* tentacles from an intertidal rock pool, Trearddur Bay, Anglesey (sampled May 1997 to January 1998 and February to April 1999); a) chlorophyll *a* (chl *a*); b) chlorophyll c_2 (chl c_2); c) ratio of chlorophyll a:c_2 (chl a: c_2). Units are µg/g wet weight of anemone tissue and error bars are ± 1 standard error.

a)


1200
1000
800
600
400
200
0
Chl a (µg/g)
J F M A M J J A S O N D
Month of year

b)


600
400
200
0
Chl c2 (µg/g)
J F M A M J J A S O N D
Month of year

c)

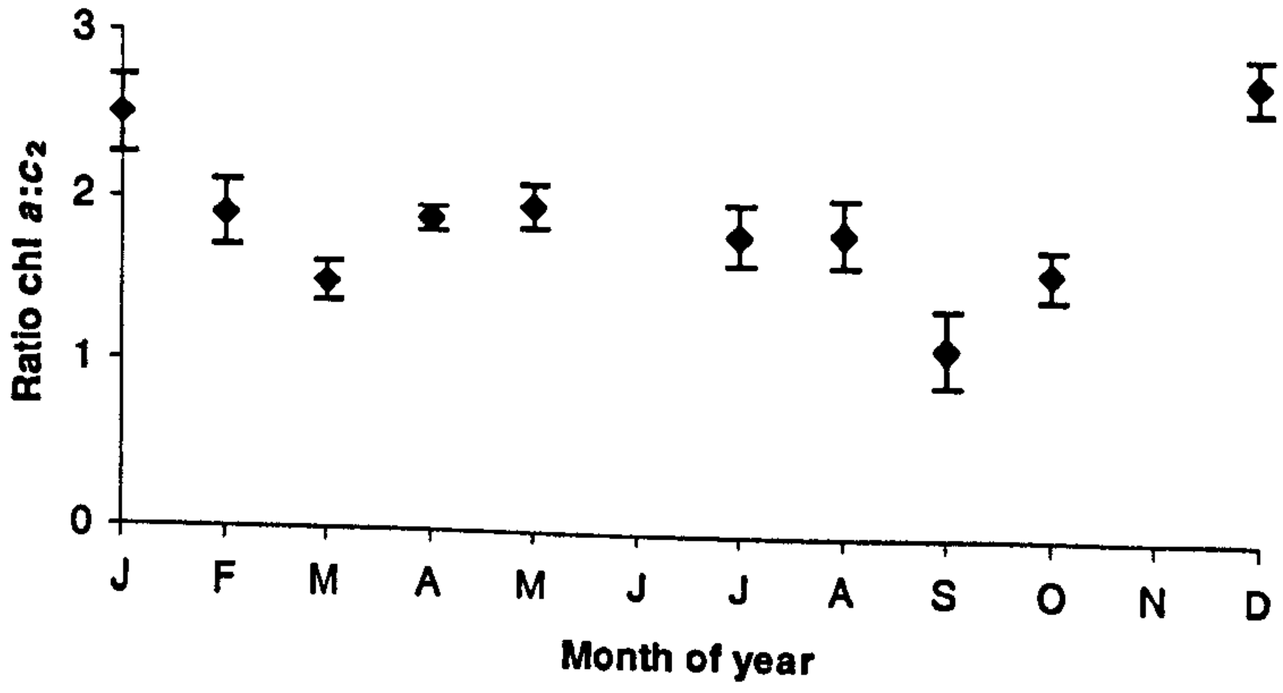
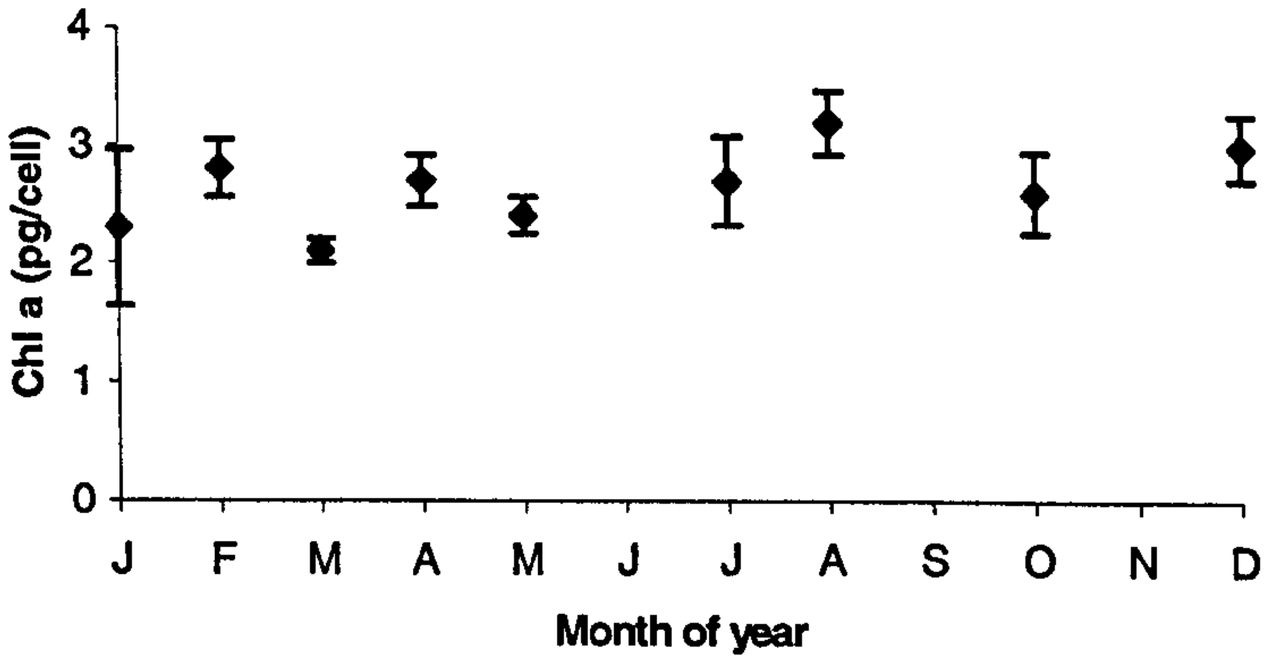
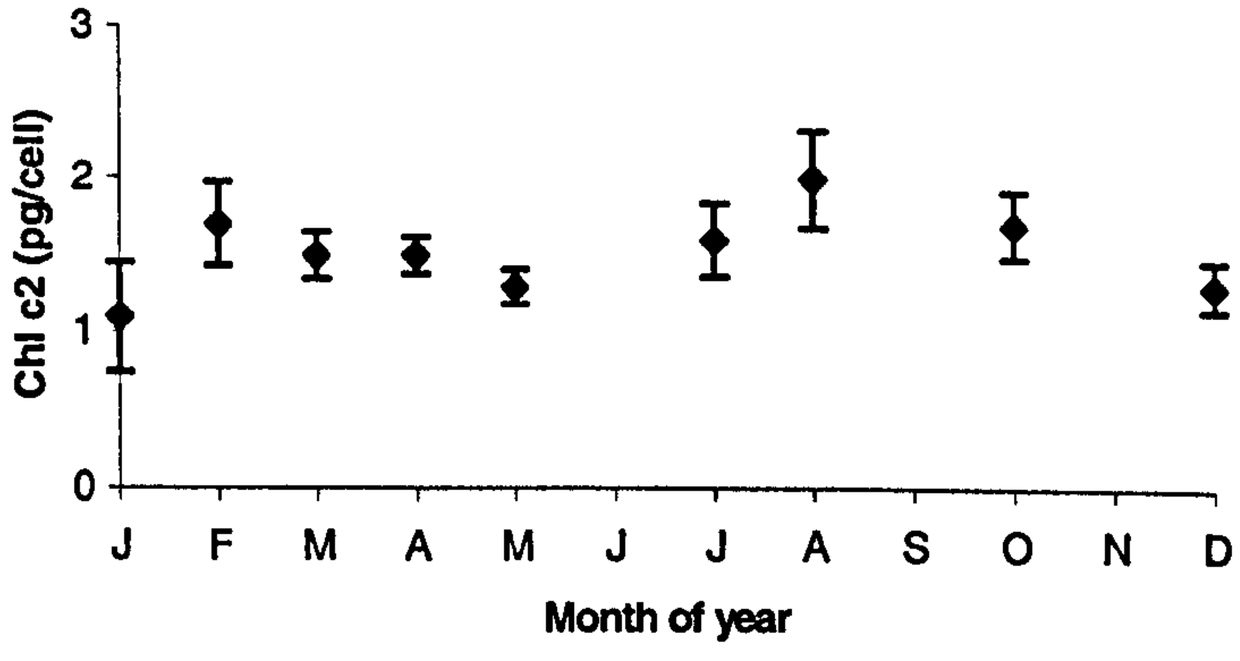

3
2
1
0
Ratio chl a:c2
J F M A M J J A S O N D
Month of year

Figure 3.11. Mean monthly chlorophyll concentrations in zooxanthellae from *Anemonia viridis* tentacles in an intertidal rock pool, Trearddur Bay, Anglesey (samples taken May 1997 to January 1998 and February to April 1999; see text, p. 36); a) chlorophyll a (Chl *a*); b) chlorophyll c_2 (Chl c_2); c) ratio of chlorophyll a:c_2 (Chl a:c_2). Units are pg/zooxanthella and error bars are ± 1 standard error.

a)


Chl a (pg/cell)
4
3
2
1
0
J F M A M J J A S O N D
Month of year

b)


Chl c2 (pg/cell)
3
2
1
0
J F M A M J J A S O N D
Month of year

c)

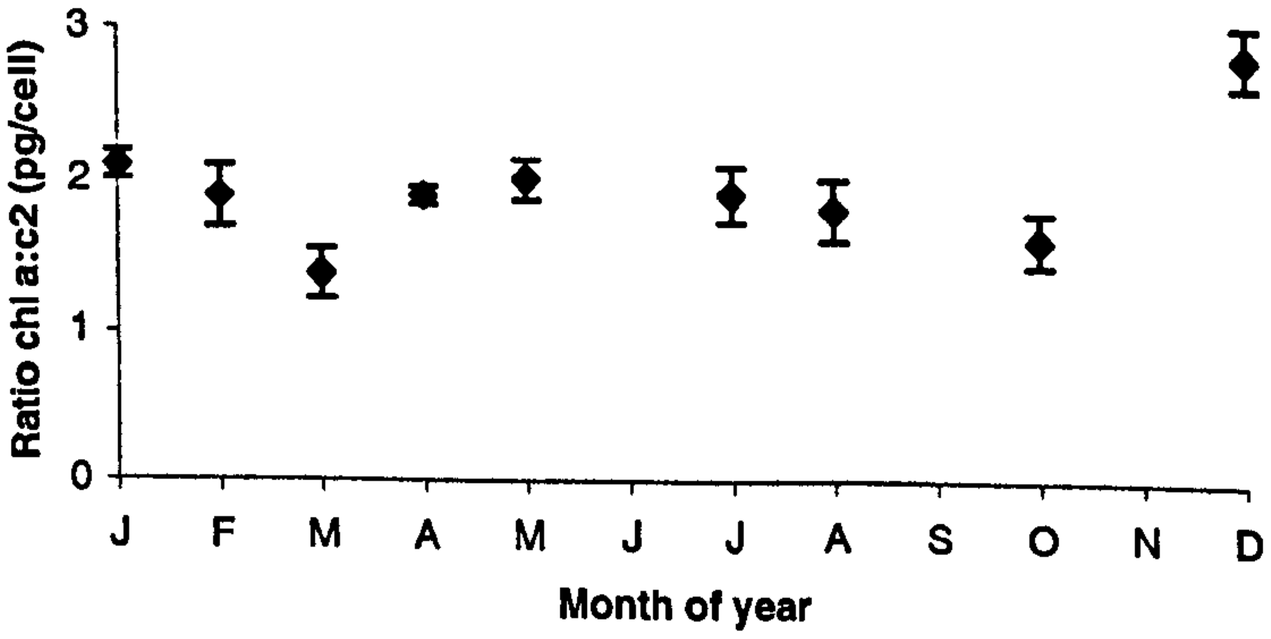

Ratio chl a:c2 (pg/cell)
3
2
1
0
J F M A M J J A S O N D
Month of year

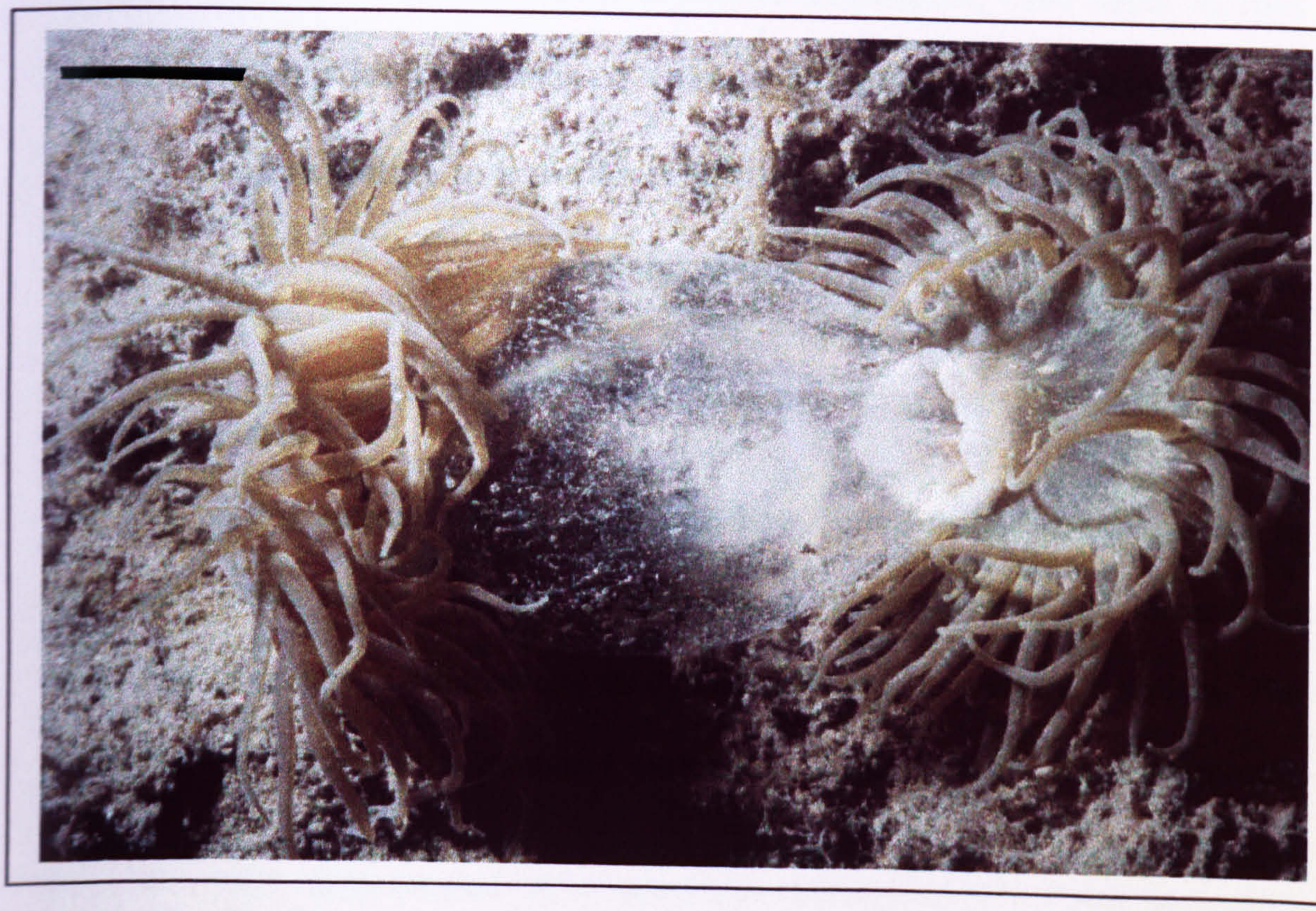
Plate 3.1. Mid-tide level rock pool, Trearddur Bay, Anglesey at low water.

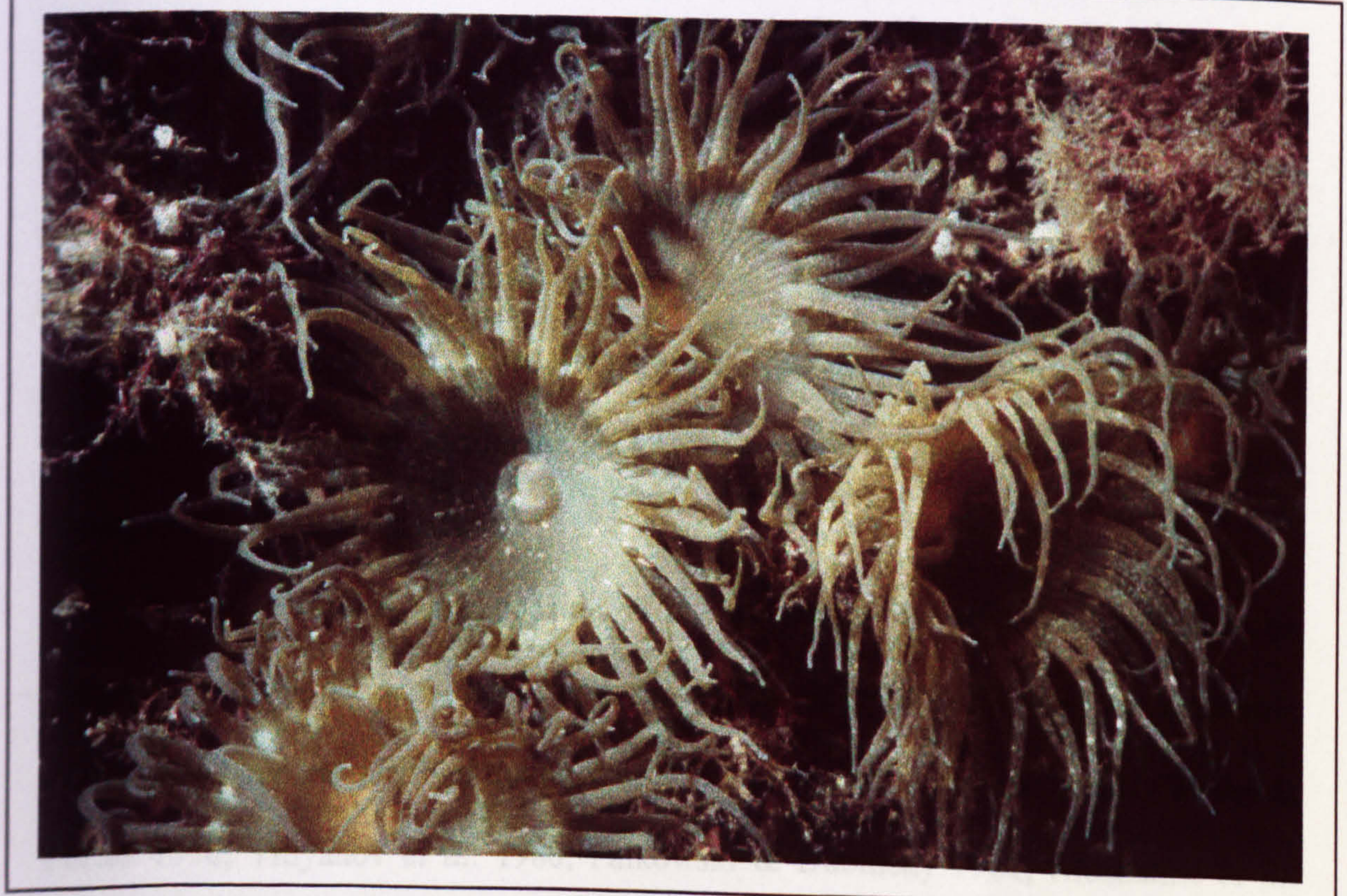
Plate 3.2. *Anemonia viridis* among soft sediment and *Anomia ephippium* shells, Castle Island Bay, Lough Hyne. Scale bar = 10cm.

Plate 3.3a. *Anthopleura ballii* on Glannafeen cliff, Lough Hyne feeding on a jellyfish.

Scale bar = 2 cm.

Plate 3.3b. *Anthopleura ballii* at 6m among macroalgae. Scale bar = 2 cm.





CHAPTER 4

Photoadaptive responses of *Anemonia viridis* and associated zooxanthellae to irradiance of altered spectral quality and intensity

4.1. Introduction

The aim of this chapter was to investigate how zooxanthellae characteristics in *Anemonia viridis* respond to light of altered spectral composition and intensity in controlled laboratory conditions and to monitor changes in anemone biomass. The zooxanthellae characteristics examined were zooxanthellae density, diameter, mitotic index (MI) and chlorophyll *a* and c_2 pigment concentrations, and the response of each to changing irradiance was considered.

Photoadaptation describes changes in photosynthetic performance with changes in radiation during growth (Brown, 1997). Several mechanisms are utilised and include changes in photosynthetic pigments, as well as changes in chloroplast structure and photosynthetic response which increase the light-harvesting abilitiy of the zooxanthellae. In symbiotic invertebrates such as anemones, there are other means of homeostatic adjustment available, such as alteration of the number and spatial arrangement of the zooxanthellae.

Few laboratory studies have considered the photoadaptive affect of irradiance on temperate symbiotic Anthozoa. Previous studies of photoadaptation in symbiotic anthozoans have been carried out on shade- or light-adapted tropical corals (Wethy & Porter 1976, Titlyanov *et al.* 1980, Falkowski & Dubinsky 1981, Muscatine *et al.* 1984, Porter *et al.* 1984), soft corals (Berner *et al.* 1987) or sea anemones (Muller-

Parker, 1987) in the field. However, in field situations the photoadaptive response may also be influenced by other variables such as temperature, water depth, nutrient availability and the spectral composition of light (Harland & Davies, 1994). As described in chapter 2 light is attenuated with increasing water depth. Anthozoa which span a wide depth range show physiological alterations analogous to the light/shade adaptations of higher plants (Wethy & Porter 1976, Falkowski & Dubinsky 1981, Berner *et al.* 1987). Since attenuation of light with depth is accompanied by marked alterations in spectral composition (Dring, 1982) symbiotic Anthozoa are also exposed to light of altered spectral quality at different depths. Studies on planktonic algae have demonstrated many responses to spectral changes and include changes in pigment composition (Brody & Emerson 1959, Jones & Meyer 1965), metabolism (Bird *et al.* 1981) and ultrastructure (Lichtenthaler *et al.* 1980). Light quality effects have also been demonstrated in macrophytes (Dring, 1981) which are more comparable with sessile symbiotic Anthozoa than planktonic microalgae. Saffo (1987) reviewed the role of light-harvesting pigments in depth zonation of seaweeds and concluded that at least 3 mechanisms are exploited for photosynthesis in dim, spectrally limited deep water:

1/produce more accessory pigment relative to chlorophyll *a*

2/ produce more pigments generally

3/ possess structural features that maximize the efficiency of light absorption; in the case of macrophytes this may include thick fronds or special arrangement of chloroplasts.

To illustrate, Ramus *et al.* (1976) demonstrated that the green seaweed *Ulva* can acclimate to life in deeper water by making more of the photosynthetic pigments that absorb available light there.

In order to overcome the existence of several variables in the field situation experiments have been carried out under controlled conditions in the laboratory with tropical reef corals, *Pocillopora damicornis* and *Montipora verrucosa* (Kinzie *et al.*, 1984) *Acropora formosa* and *Acropora cuneata* (Thinh, 1991) the tropical sea anemone, *Aiptasia pulchella* (Muller-Parker, 1985) and the temperate sea anemone, *Anemonia viridis* (Harland & Davies, 1994). Kinzie *et al.* (1984) investigated the effects of spectral quality on zooxanthellae density and pigment concentration whilst Muller-Parker (1985), Thinh (1991) and Harland & Davies (1994) all considered the influence of intensity of irradiance on zooxanthellae density and pigments.

Similarly, Chang *et al.* (1983) considered photoadaptation of different zooxanthellae strains isolated from 3 tropical hosts, *Tridacna maxima*, *Aiptasia pulchella* and *Montipora verrucosa* and maintained in a range of irradiances (22-248 μE m^{-2} s^{-1} = μmol m^{-2} s^{-1}).

Other laboratory studies have examined the regulation of zooxanthellae density alone in different irradiance intensities, for example, Steele (1976) considered the role of irradiance in the regulation of zooxanthellae from the tropical anemone *Aiptasia tagetes.* Similarly, Saunders & Muller-Parker (1997) considered relative densities of zooxanthellae and zoochlorellae different intensity irradiance maintained *Anthopleura elegantissima,* whereas McCloskey *et al.* (1996) examined symbiont expulsion from the same temperate anemone during exposure to different irradiance intensities. No other zooxanthellae characteristics, such as size or pigment concentration were considered in these studies. Furthermore, no previous laboratory study has considered the effects of different light spectra on temperate symbiotic Anthozoa; the only study to consider such effects examined tropical reef corals (Kinzie *et al.* 1984). Finally, Tsuchida & Potts (1994) monitored the growth of the temperate anemone

Anthopleura elegantissima under different irradiance and feeding regimes and found that both had a positive effect on weight change in anemones.

The hypothesis investigated in this study states that zooxanthellae characteristics are affected by changes in artificial irradiance in respect of intensity or spectral composition and are a means of photoadaptation.

The objectives of this study were firstly to monitor the effects of irradiance of altered spectral quality and intensity on the buoyant weight of well-fed, laboratory maintained anemones. Secondly the effects of different intensity irradiance and of spectral composition on zooxanthellae characteristics were determined using mesh to reduce intensity and red and green coloured filters to alter spectral quality of artificial irradiance. Zooxanthellae density, MI, diameter and chlorophyll *a* and c_2 concentrations were considered collectively in each treatment as mechanisms of photoadaptation. Finally, the intensity and spectral composition of artificial irradiance were measured using a spectroradiometer to determine the nature of irradiance reaching the anemones in each treatment.

4.2. Methods

4.2.1. Collection and maintenance of anemones

Thirty six *Anemonia viridis* of all sizes were collected from mid-tide level rock pools at Shell Island, Gwynedd, Wales (52° 47' N, 004° 06' W; figure 4.1) on 17th March 1999. Each anemone was placed in a numbered glass honey jar and put into experimental aquaria provided with aerated, running sea water at 6 °C and 54 μmol m^{-2} s^{-1} in air on a 12 hour light:dark cycle to acclimate for 2 weeks. The light and temperature regimes were similar to those experienced at Shell Island underwater on a

dull winters day and represented a natural photoperiod for spring. The aquaria comprised 2 large tanks covered over with light-proof black polythene with 2 square windows 25 x 25 cm cut out. Beneath each window were placed 9 anemones in their numbered jars. Both aquaria were surrounded by light-proof black polythene curtains to exclude any ambient light. Reflective aluminium foil was used to cover the ceiling to ensure maximum light reached the aquaria.

Anemones were fed weekly with similar sized pieces of chopped mussel (*Mytilus edulis*) flesh. Care was taken to ensure each anemone ingested thier ration.

4.2.2. Measuring intensity and spectral composition of artificial light

Irradiance was provided by three 40 W daylight fluorescent strip lights and was measured both in air and under water, initially with a LiCor underwater quantum sensor (400-700 nm) and subsequently with a PRR600 spectroradiometer. The PRR600 sensor was submerged in a dustbin filled with seawater and placed beneath the light source. Each colour filter was placed over the sensor at a height of approximately 30 cm from the sensor, and sealed around the edges with light-proof polythene to exclude ambient light. Irradiance was recorded by underwater and air sensors simultaneously at 6 wavelengths 412, 443, 490, 510, 555 and 665 nm; total downwelling irradiance was also measured.

4.2.3. Buoyant weighing

Anemones were buoyant weighed at the beginning and end of the experiment to monitor growth as a measure of health during exposure to each light treatment.

The buoyant weight of an anemone is the weight of the anemone tissue minus the weight of the volume of water it displaces when weighed in seawater. Prior to

feeding, five days after collection and each week thereafter, each anemone was buoyant weighed using a torsion balance (range 0-1000 mg in 2 mg intervals) and the method of Turner (1988). Anemones were hooked through the pedal disc and weighed while submerged in SW. Several precautions were taken while weighing to ensure accurate readings:

1/ anemones allowed a minimum of 3 days captivity to digest any ingested material

2/ anemones were cleaned of any sediment or debris

3/ each anemone was squeezed while submerged to expel air bubbles within the coelenteron

4/ seawater was of constant density

5/ to ensure no changes in tissue density had occurred between weighings a check was made by comparing weight differences of a few anemones in SW and distilled water.

4.2.4. The effect of light of altered spectral composition and intensity

Experimental light treatments were begun 2 weeks after anemone collection. Four treatments were provided via windows in the aquaria covering by using fine plastic mesh ($2mm^2$) to reduce the intensity and colour filters to alter the spectral composition of downwelling irradiance:

Tank 1/ three layers of mesh; underwater irradiance 4 $\mu mol\ m^{-2}\ s^{-1}$

Tank 2/ no mesh; underwater irradiance 20 $\mu mol\ m^{-2}\ s^{-1}$

Tank 3/ red coloured filter and 1 layer of mesh; irradiance 3 $\mu mol\ m^{-2}\ s^{-1}$

Tank 4/ green coloured filter; irradiance 3 $\mu mol\ m^{-2}\ s^{-1}$.

To ensure comparable intensities in tanks 3 and 4 an additional layer of mesh was required in tank 3. Red and green colour filters were selected from knowledge of spectral attenuation of irradiance with depth in temperate waters (see chapter 2, p.8

and figure 2.16). The red filter was used to represent shallow water depths where red wavebands occur and the green filter was used to represent deeper water where irradiance is rich in green wavebands.

Anemones were sampled after 5 weeks for zooxanthellae density, MI, diameter chlorophyll *a* and c_2 and ultrastructure as described in Chapter 3 section 3.2.3.2.

4.3. Results

4.3.1. Spectral composition of artificial light

The mean irradiance recorded for each of 6 wavelengths tested was used to calculate percentage contribution to the total spectrum. Figures 4.2a-c show how the spectral composition is altered by red and green colour filters in tanks 3 and 4. With no filters, in air (Figure 4.2a) the 'daylight' artificial lights used have a relatively broad spectrum from 400 to 700 nm, the most penetrative light being yellow/green between 525 nm and 625 nm). The most penetrative wavelength tested was 555 nm which comprised 42 % of the total spectrum. By comparison, the red filter absorbed short wavelengths below 550 nm such that 665 nm irradiance accounted for nearly 90 % of the total spectrum measured and no blue or green light was able to penetrate; only red light remained (Figure 4.2 b).

The green filter had the opposite effect to the red filter absorbing all irradiance of 665 nm; mostly green and green/yellow light penetrated such that 555 nm irradiance accounted for 60 % of the total spectrum (Figure 4.2c).

4.3.2. Buoyant weight

Anemones showed a range of sizes, 38-264 mg buoyant weight. Figure 4.3 shows the mean buoyant weights of *A. viridis* before (a) and after (b) exposure to different light

treatments. Anemones in tank 4 (green filter) were significantly smaller than anemones in other tanks before exposure to experimental irradiances (Kruskal-Wallis, H= 9.89 $p< 0.05$; Figure 4.3a). But, at the end of the 5 week experimental period buoyant weights were not significantly different between treatments (Kruskal-Wallis, H= 5.31 $p > 0.05$; Figure 4.3b).

Anemones exposed to irradiance spectrally altered by red and green filters had significantly larger buoyant weights at the end of the experiment (red light, T= -2.50 $p< 0.05$; green light T= 3.47 $p< 0.05$). Red light anemones showed an increase in buoyant weight of 68 ± 48 % compared with 98 ± 31 % in green light anemones (Figure 4.4). Buoyant weight data from anemones exposed to green light required reciprocal transformation to satisfy the assumptions for statistical analysis.

By comparison, in 4 µmol m^{-2} s^{-1} (tank 1) and 20 µmol m^{-2} s^{-1} (tank 2) full spectrum irradiance mean buoyant weights increased by 50 ± 14 % and 46 ± 20 % respectively (Figure 4.4), but these differences were not statistically significant either (Tank 1, T= -1.90 $p > 0.05$; Tank 2, Kruskal-Wallis W= 68.0 $p >0.05$). Thus, the final mean weights of different experimental groups were similar; the least increase in weight was observed in the group with the largest original weight.

4.3.3. The effect of light of altered intensity and spectral composition

4.3.3.1. Intensity experiment

After 5 weeks anemones in tank 1 (irradiance of 4 µmol m^{-2} s^{-1}) bore 0.69 ± 0.06 x 10^6 zooxanthellae g^{-1} wet weight which did not differ significantly from the density of 0.82 ± 0.07 x 10^6 zooxanthellae g^{-1} wet weight of anemone in tank 2 (irradiance 20 µmol m^{-2} s^{-1};)T = -1.48 $p>0.05$; Figure 4.5).

Anemones in medium intensity irradiance bore zooxanthellae with a significantly lower mitotic index (MI) than those in low intensity irradiance (T= 2.28 p< 0.05); zooxanthellae had a MI of 0.55 ± 0.08 % in low light compared with only 0.33 ± 0.05 % in medium light anemones (Figure 4.6).

The diameter of zooxanthellae in anemones from low irradiance was 10.6 ± 0.08 μm compared with 11.0 ± 0.11 μm in medium irradiance; zooxanthellae were significantly smaller in low intensity than in medium intensity anemones (T= -2.93 p< 0.05; figure 4.7).

Zooxanthellae from anemones in low irradiance had a chlorophyll *a* concentration of 263.1 ± 24 μg g^{-1} wet weight which differed significantly from a concentration of 423.7 ± 33 μg g^{-1} wet weight in medium intensity irradiance (T= -3.96 p< 0.05). Similarly, chlorophyll c_2 concentration was significantly higher in medium light maintained zooxanthellae (191.1 ± 14 μg g^{-1} wet weight) than low light maintained zooxanthellae (124.6 ± 8 μg g^{-1} wet weight; T= -3.98 p < 0.05). By contrast anemones in low level light had a ratio of chlorophyll a:c_2 of 2.09 ± 0.07 which was not significantly different from medium level light chlorophyll a:c_2 of 2.22 ± 0.05 (T= -1.46 p>0.05; figure 4.8a-c).

Pigment concentration was standardized to zooxanthellae density to show the concentration of pigments per cell. Zooxanthellae in anemones from low light had a chlorophyll *a* concentration of 4.0 ± 0.52 pg $cell^{-1}$ which was not significantly different from a concentration of 5.4 ± 0.67 pg $cell^{-1}$ in medium light zooxanthellae (T= -1.65 p>0.05; figure 4.9 a).

Similarly, neither the chlorophyll c_2 nor the ratio of chlorophyll *a*: c_2 per cell were significantly different in low and medium intensity light (T= -1.48 p>0.05 and T= -1.43 p>0.05 respectively; figure 4.9 b and c).

4.3.3.2 Spectra experiment

In full spectra irradiance (tank 1) anemones bore **0.69 ± 0.06 x 10^8** zooxanthellae g^{-1} wet weight compared with **0.57 ± 0.03** and **0.44 ± 0.04 x 10^8** zooxanthellae g^{-1} wet weight of anemone in green and red light (tanks 3 & 4) respectively. One-way ANOVA followed by *post-hoc* Tukey test showed there were significantly more zooxanthellae in anemones from the full spectrum treatment than the red light treatment (ANOVA F= 7.15 p< 0.05). However, full spectrum and red anemone zooxanthellae densities were not significantly different from green treatment zooxanthellae density (Figure 4.10).

Dividing cells were absent from some of the zooxanthellae samples preventing a comparison of mitotic indices between treatments. As an alternative, the actual number of samples with and without dividing cells in the three treatments were compared and found to be significantly different (X^2 = 8.89 p< 0.05). Pairwise comparisons revealed that full spectrum light anemones had significantly more samples with dividing zooxanthellae than anemones in both red (X^2 = 5.14 p< 0.05) and green (X^2 = 9.00 p< 0.05) light. However, the number of dividing zooxanthellae in red and green light were not significantly different (X^2 = 0.90 p> 0.05; figure 4.11). Mean zooxanthellae diameter in full spectrum light was **10.8 ± 0.10** µm compared with **10.6 ± 0.08** µm in green light and **11.0 ± 0.11** µm in red light. Zooxanthellae from anemones in red light were significantly larger than those in green light

(ANOVA, F= 4.14 p< 0.05; figure 4.12). However, neither red nor green light zooxanthellae were significantly different from full spectrum zooxanthellae.

Zooxanthellae in full spectrum anemones had a chlorophyll *a* concentration of 263.1 ± 24 μg g^{-1} wet weight of anemone which did not differ significantly from a concentration of 212 ± 25 or 250 ± 17 μg g^{-1} wet weight of anemone in green and red light treatments respectively; similarly chlorophyll *a* concentrations from anemones in green and red light did not differ significantly (ANOVA, F= 1.46 p> 0.05; figure 4.13).

Chlorophyll c_2 concentrations in zooxanthellae from full spectrum, green and red irradiance treatments were 125 ± 9, 100 ± 11 and 109 ± 7 μg g^{-1} wet weight of anemone respectively and no significant differences were found (ANOVA, F= 1.91 p> 0.05; figure 4.13b):

By contrast, significant differences were found between treatments in the ratio of chlorophyll $a:c_2$ (ANOVA, F= 5.14 p<0.05). The mean ratios of 2.09 ± 0.07 and 2.11 ± 0.05 (figure 4.13c) for full spectrum and green light treatments respectively, were not significantly different. However, both were significantly lower than the ratio for red light zooxanthellae which had a ratio of 2.31 ± 0.03.

Cellular pigment concentrations showed a different pattern (Figure 4.14 a-c). The mean chlorophyll *a* concentrations in full spectrum and green light were 4.0 ± 0.52 and 3.7 ± 0.31 pg $cell^{-1}$ respectively which were significantly lower than for red light zooxanthellae which had a mean concentration of 6.0 ± 0.63 pg $cell^{-1}$ (ANOVA, F= 5.99 p<0.05). Zooxanthellae chlorophyll c_2 concentrations were significantly higher in red light (2.6 ± 0.28 pg) than green light (1.7 ± 0.14 pg; ANOVA, F= 4.26 p< 0.05).

However, no significant differences were found in chlorophyll c_2 concentrations between red and full spectrum light and green and full spectrum light (Figure 4.14 b). By comparison, the ratio of chlorophyll a:c_2 per zooxanthella was significantly higher in red light (2.3 ± 0.03 pg) than full spectrum light (2.1 ± 0.07 pg; ANOVA, F= 5.03 $p< 0.05$). However, chl a: c_2 ratios in green & full spectrum light, and red & green light were not significantly different.

4.4. Discussion

Anemonia viridis and associated zooxanthellae respond to light of altered spectral composition and intensity in the laboratory. Buoyant weight increased after 5 weeks exposure to light of altered spectral quality and intensity. However, buoyant weights between treatments were not different at the end of the experiment (p.68) suggesting either that smaller anemones were growing more than larger anemones in different treatments, or that red and green light enhanced growth. Similarly, Steele (1976) buoyant weighed the tropical anemone *Aiptasia tagetes* before and after a 14 day experiment with different light treatments. The final mean weights of different experimental groups were similar, such that the least increase in weight occurred in the group with the largest original weight.

In the present study, the percentage increase in mean weights was not significantly different between treatments in intensity or spectra experiments. By contrast, Steele (1976) found that the increase in mean buoyant weight of *Aiptasia* was least at the lowest light intensity and became greater as the light intensity increased. Similarly, Tsuchida & Potts (1994) found that growth of *A. elegantissima* was positively affected by exposure to high intensity light (130 $\mu E.\ m^{-2}\ s^{-1}$) for 3 months. By contrast, Davy *et al.* (1997) reported that the average weight of both *A. ballii* and *A.*

viridis changed by no more than ± 5 % in most treatments over 1 month in the field; weight changes were not significant under various light and feeding regimes and were small compared to laboratory investigations run over a comparable period of time. For example, over 30 days *A. elegantissima* and *A. viridis* changed weight (buoyant or wet) by -35 % to 95 % depending on light and feeding regimes (Muscatine 1961, Taylor 1969b, Sebens 1980, Tsuchida & Potts, 1994). Similar to the present study, Muscatine (1961), Sebens (1980) and Tsuchida and Potts (1994) used a range of anemone sizes, whereas Davy *et al.* (1997) used anemones of similar size.

Zooxanthellae density was not affected by the light intensities used in the experiment; the number of zooxanthellae per unit of anemone tentacle biomass did not change in response to light intensity (Figure 4.5). Similar results have been observed in comparisons between high and low irradiance adapted corals (Svoboda & Porrmann, 1980) and for *Anemonia viridis* in high and low irradiance treatments in the laboratory (300 and 10 $\mu E.m^{-2}\ s^{-1}$; Harland & Davies, 1994) and in the field in intertidal (300-1700 $\mu mol.m^{-2}\ s^{-1}$) and subtidal (30-90 $\mu mol.m^{-2}\ s^{-1}$) habitats (Bythell *et al.* 1997). But, Steele (1976) found that zooxanthellae density decreased in low intensity maintained *Aiptasia tagetes*, whilst Saunders & Muller-Parker (1997) and Porter *et al.* (1984) found density increased in low irradiance maintained (10 $\mu mol.m^{-2}\ s^{-1}$) *A. elegantissima* and Red Sea shade-adapted colonies of *Stylophora pistillata.*

The higher MI in 4 $\mu mol.m^{-2}\ s^{-1}$ compared with 20 $\mu mol.m^{-2}\ s^{-1}$ was accompanied by a decrease in zooxanthellae size which might be expected where cells are dividing (Figures 4.6, 4.7). The stability of zooxanthellae density in response to changes in irradiance intensity however, indicates the presence of some regulatory mechanism of their numbers. In comparison, Saunders & Muller-Parker (1997) found that the MI of zooxanthellae in *A. elegantissima* remained low (again under 1 %) and variable over

time in high and low irradiance treatments.

Changes in zooxanthellae density might also result from a change in anemone biomass since algal density was measured as the number of zooxanthellae per gram of anemone wet weight. However, although all anemones gained weight during both experiments the increase between treatments was extremely variable and not statistically significant. Despite statistical evidence, any changes observed in zooxanthellae were interpreted with caution.

Full spectrum irradiance (Tank 1) produced higher density and increased division of cells compared with red and green irradiance, but no decrease in their size was observed. No change in zooxanthellae density was observed between anemones maintained in green waveband irradiance and red waveband irradiance (Figure 4.10). Zooxanthellae were larger in red irradiance than green irradiance (Figure 4.12). Kinzie *et al.* (1984) found no change in zooxanthellae density of *Pocillopora damicornis* maintained in blue, white, green and red light treatments, whilst *Montipora verrucosa* colonies grown in blue light showed higher zooxanthellae densities than colonies grown in white, green or red light. *M. verrucosa* was described as a typically low light species in Hawaii (Kinzie *et al.*, 1984) and as *Anemonia viridis* shows lower zooxanthellae density in red light. Such a response may minimise self-shading of zooxanthellae and thus maximize the surface area for efficient light capture.

Both chlorophyll *a* and c_2 concentrations per g of anemone wet weight were higher in tank 2 (20 $\mu mol.m^{-2}\ s^{-1}$) than tank 1 (4 $\mu mol.m^{-2}\ s^{-1}$; figure 4.8 a,b). However, no attendant increase in density, ratio chl *a:* c_2 or concentrations per cell were observed (Figures 4.8 c, 4.9) providing no explanation as to the mechanism for the observed increase. In contrast, a similar experiment with *Anemonia virdis* by Harland & Davies

(1994) showed that in artificial irradiance of 10 μmol.m^{-2} s^{-1} zooxanthellae possessed higher concentrations of chlorophyll *a* per zooxanthella and per gram of anemone tissue than those from anemones maintained in 300 μmol.m^{-2} s^{-1}. Furthermore, they observed no change in chlorophyll c_2 concentrations in 10 μmol.m^{-2} s^{-1}, giving higher ratios of chl a:c_2. Similar results were observed between light and shade adapted colonies of the soft coral, *Litophyton arboreum* and *Stylophora pistillata* where more chl *a* per cell was present in shade-adapted colonies (Berner *et al.* 1987, Porter *et al.* 1984). In all of these studies chlorophyll concentrations per zooxanthella were increased as a photoadaptive response to low levels of irradiance.

Chlorophyll *a* and c_2 concentrations per g showed no spectral response; the ratio of chl a:c_2 however was higher in red irradiance than full spectrum or green irradiance (Figure 4.13). By contrast, for the corals *Pocillopora damicornis* and *Montipora verrucosa* Kinzie *et al.* (1984) found that chl *a* per unit area was significantly different between white, blue, green and red light treatments, whilst chl c_2 showed no difference.

In the present study (Figure 4.14), chl *a* per zooxanthella was higher in red irradiance than full spectrum irradiance or green irradiance, whilst chl c_2 was higher in red irradiance than green but not full spectrum irradiance. The opposite pattern was reported for chl *a* concentrations per cell in *P. damicornis*, whilst no significant difference was found between treatments in zooxanthellae from *M. verrucosa* (Kinzie *et al.* 1984). The higher ratio of chl a: c_2 per zooxanthella in red irradiance than full spectrum light (Figure 4.14 c) indicates proportionately more chl *a* was present than chl c_2 in red irradiance.

With reference to the conclusions of Saffo (1987) of the role of photosynthetic pigments in depth zonation of seaweeds (see p. 62), the zooxanthellae in *Anemonia*

viridis in red irradiance in the laboratory, appear to be producing more pigments generally per cell and proportionately more chl *a* than chl c_2. Observation of the absorption spectrum for zooxanthellae from *Anemonia viridis* (Figure 4.15, adapted from Taylor, 1967) and the transmission spectra for each irradiance treatment (Figure 4.2 a-c) provide an explanation for the pigment variations. The absorption peak for chl *a* between 650 and 680 nm (red waveband) corresponds to a peak in the transmission spectrum for the red filter (Figure 4.2b). Chlorophyll *a* has a greater capacity to absorb red waveband irradiance than chl c_2, which may explain why proportionately more chl *a* than chl c_2 was observed in zooxanthellae maintained in red irradiance. Similarly the higher concentration of chl c_2 per zooxanthellae in red irradiance compared to green irradiance (Figure 4.14) may be explained by the greater absorbance of chl c_2 (Figure 4.15) for wavebands present in red filter irradiance (440-460 nm and 628 nm; Figure 4.15 and 4.2b). Ramus *et al.* (1976) demonstrated a comparable response in the green seaweed *Ulva* which acclimated to low intensity, spectrally limited irradiance of deeper water, by producing more of the photosynthetic pigments able to absorb the light available. Thus in the present study zooxanthellae in red irradiance appeared to be photoadapting by increasing chl *a* and chl c_2 concentrations.

Conclusions

Neither intensity or spectral composition of artificial irradiance affected buoyant weight of *Anemonia viridis*; the original weight of the anemones affected weight increase during the experiment (Figures 4.3, 4.4). Zooxanthellae density was not affected by the low intensity, artificial irradiance used in the experiment, despite a higher division rate and accompanying decrease in diameter. Both chl *a* and c_2 concentrations per g of anemone wet weight were 1.5 times higher in 20 μmol.

m^{-2} s^{-1} intensity irradiance than in 4 μmol.m^{-2} s^{-1}, however no explanation as to the mechanism involved is evident.

Red irradiance produced a lower density of zooxanthellae (Figure 4.10) with a larger diameter (Figure 4.12) and a division rate (Figure 4.11) equivalent to zooxanthellae exposed to green and full spectrum irradiance. The ratio of chl *a*:c_2 per g of anemone wet weight and chl *a* concentration per cell were higher in zooxanthellae exposed to red light (2.31 ± 0.03 and 6.0 ± 0.63 pg cell $^{-1}$) than green (2.11 ± 0.05 and 3.7 ± 0.31 pg cell $^{-1}$) or full spectrum (2.09 ± 0.07 and 4.0 ± 0.52 pg cell $^{-1}$) irradiance. Similarly, chl c_2 per cell and the ratio of chl *a*:c_2 per cell were higher in zooxanthellae exposed to red irradiance (2.6 ± 0.28 pg and 2.3 ± 0.03) than green irradiance (1.7 ± 0.14 pg and 2.1 ± 0.07). Zooxanthellae exposed to red irradiance were photoadapting by inceasing the proportion of pigment best suited to absorbing red waveband irradiance. Zooxanthella density was also regulated perhaps to minimise self-shading and thus maximise the surface area for efficient light capture.

Figure 4.1. Location of Shell Island (52° 47' N, 004° 06' W), North Wales.

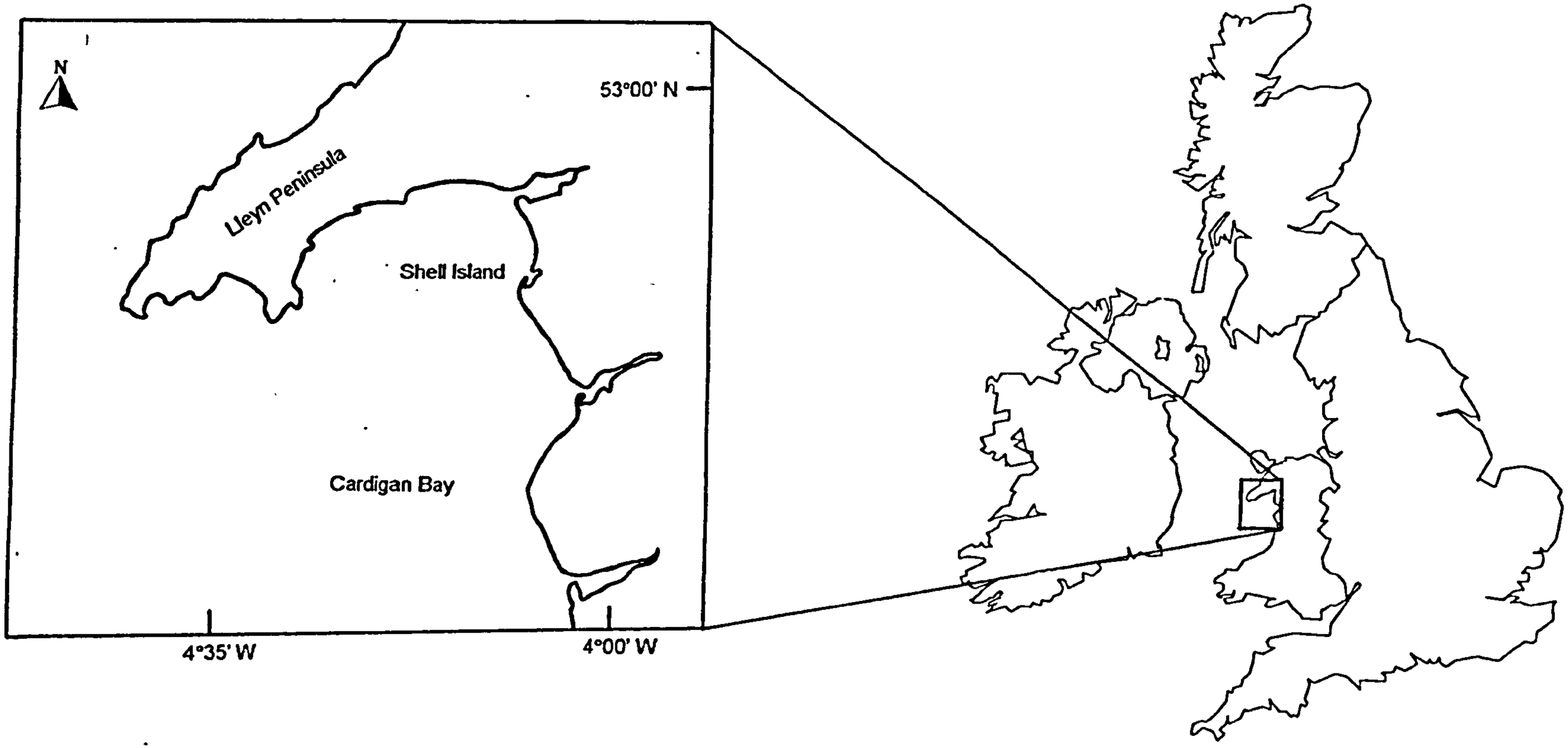
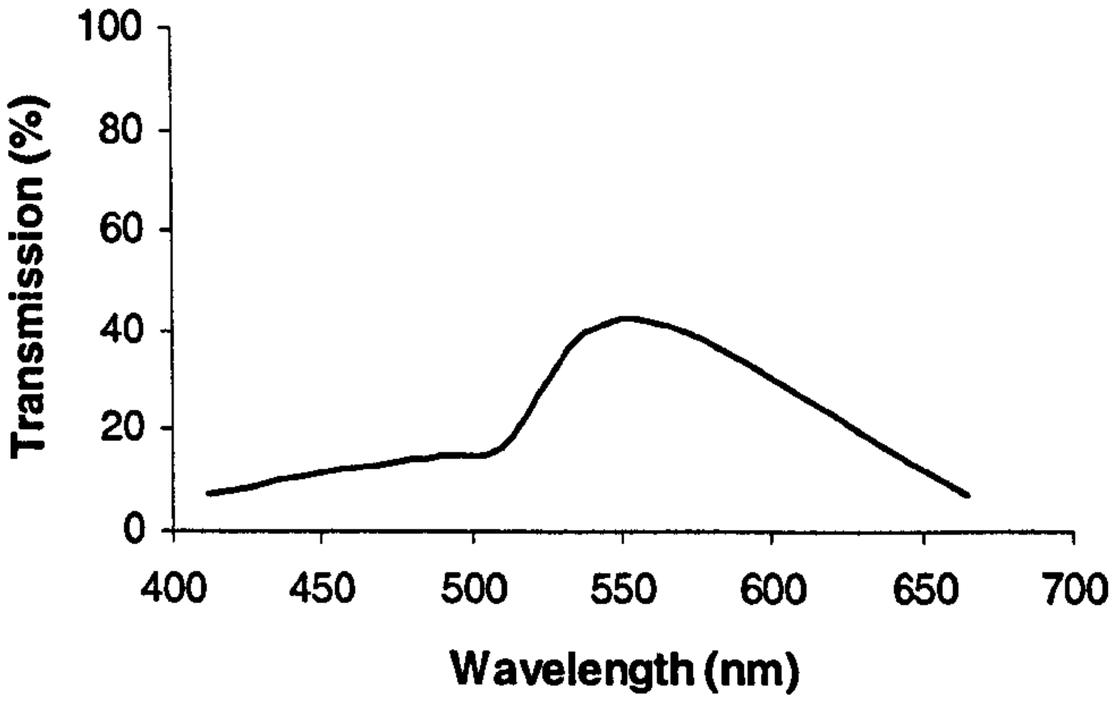
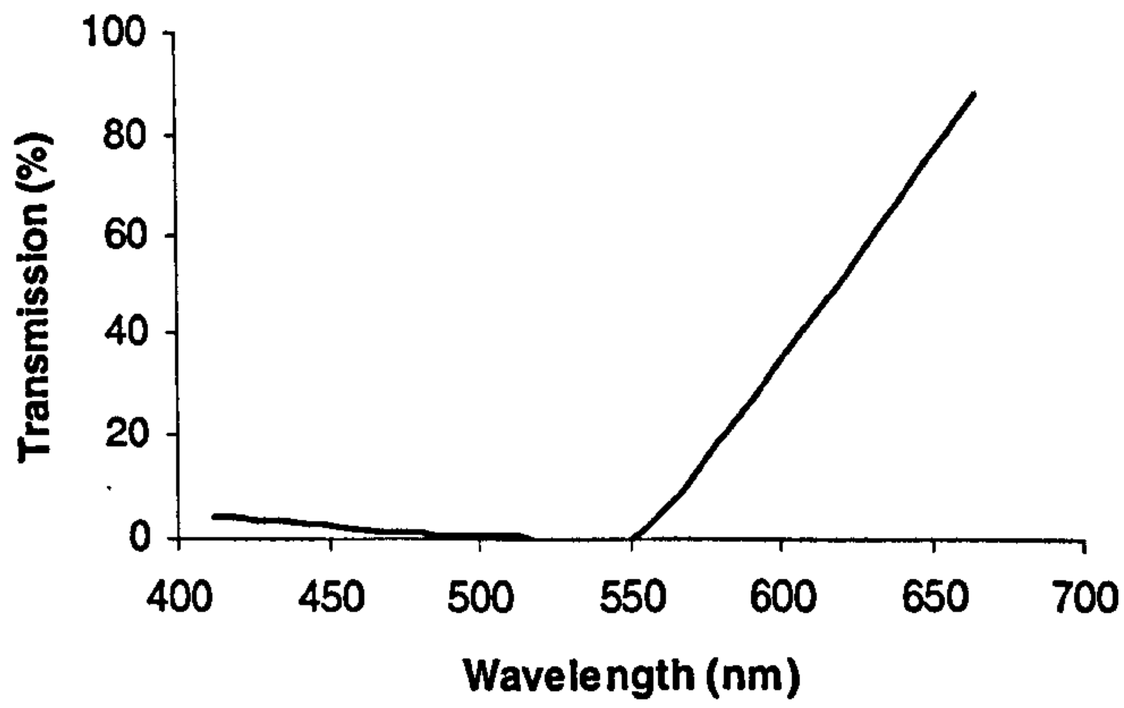

N
53°00' N
Lleyn Peninsula
Shell Island
Cardigan Bay
4°35' W
4°00' W

Figure 4.2. Transmission spectra for 'daylight' artificial light a) in air, no filter; b) underwater, red filter; c) underwater green filter. Irradiance was measured at 6 wavelengths and the mean value used to calculate percentage contribution of each wavelength to the total spectrum.

a)


100
80
60
40
20
0
Transmission (%)
400
450
500
550
600
650
700
Wavelength (nm)

b)


100
80
60
40
20
0
Transmission (%)
400
450
500
550
600
650
700
Wavelength (nm)

c)

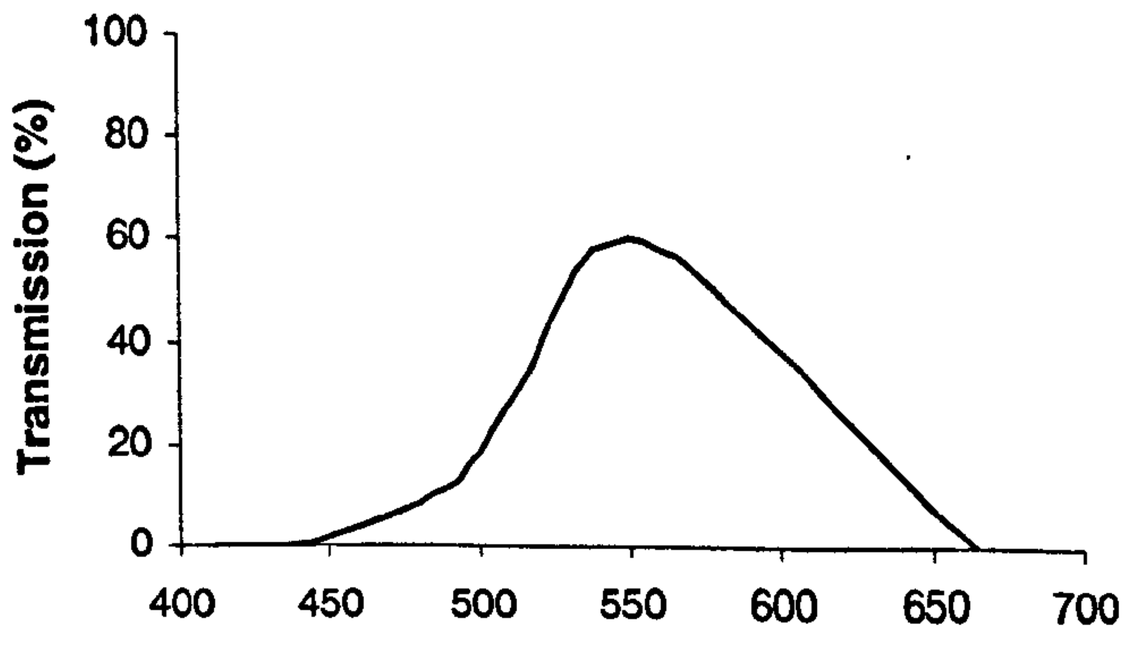
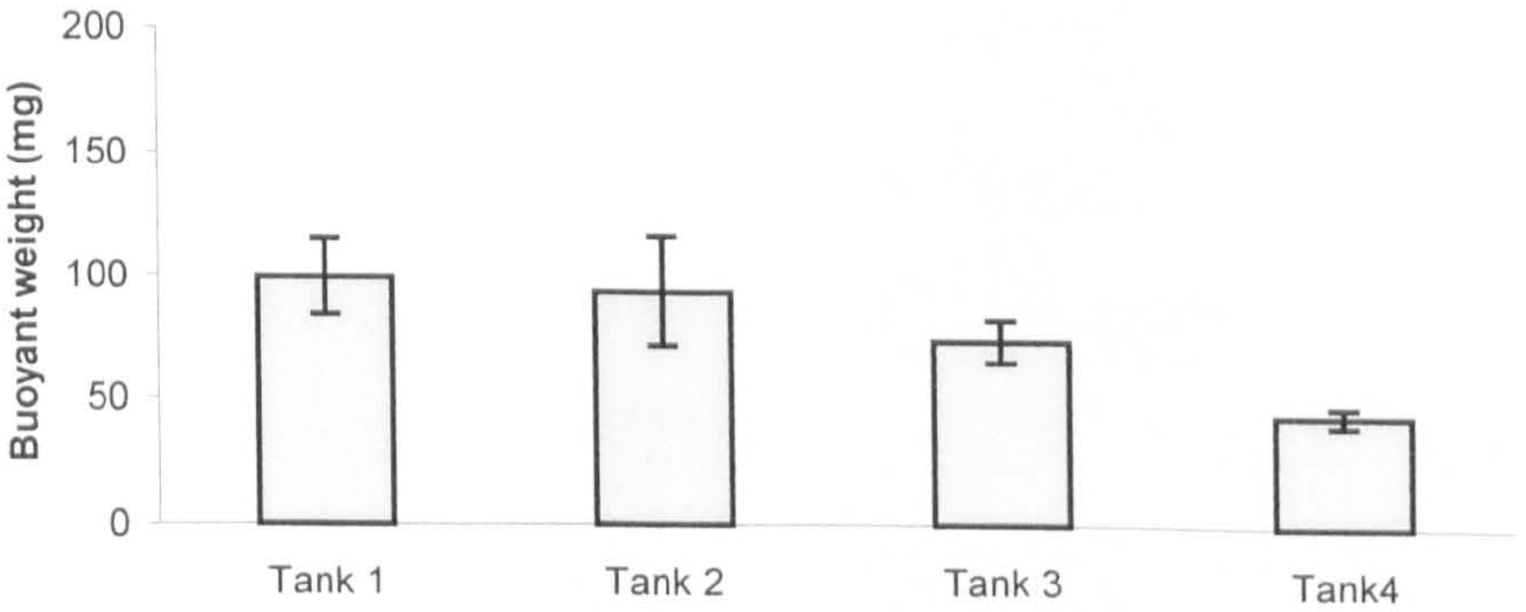

100
80
60
40
20
0
Transmission (%)
400
450
500
550
600
650
700
Wavelength (nm)

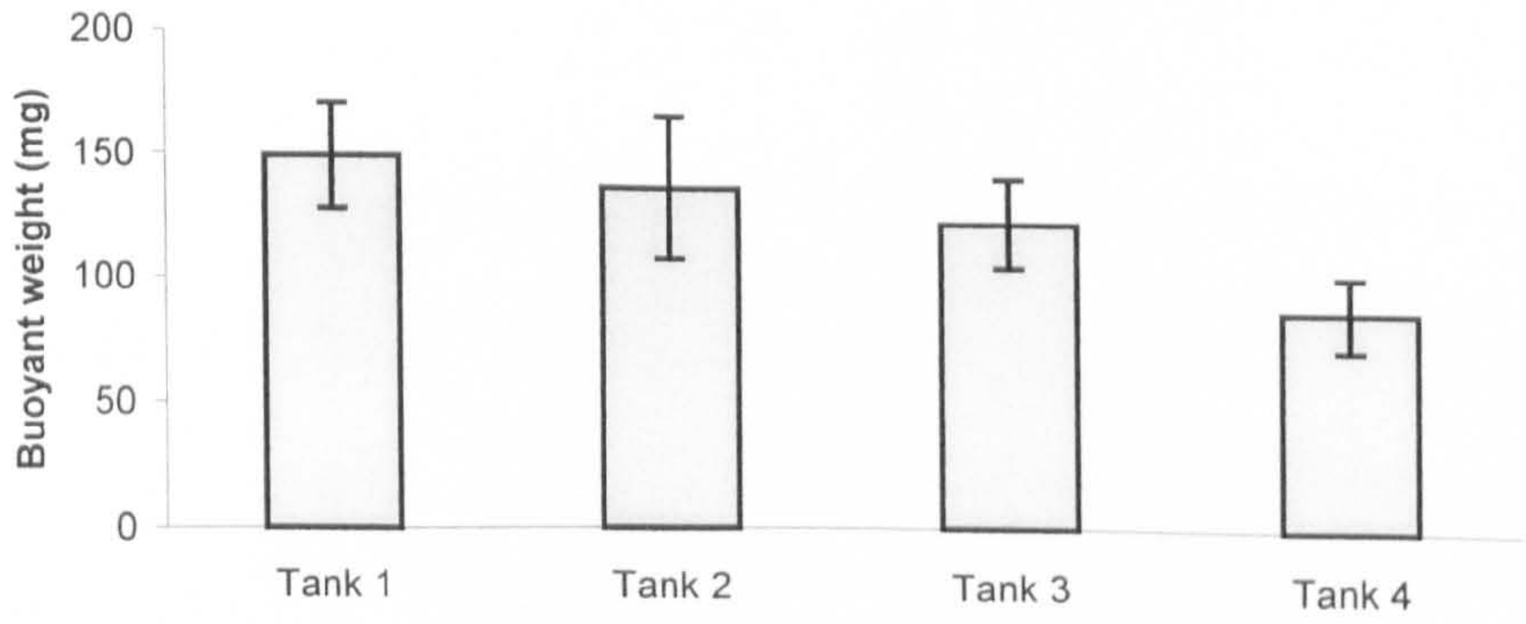
Figure 4.3. Buoyant weights (mg) of *Anemonia viridis* a) after 2 weeks acclimation in 54 μmol m^{-2} s^{-1} but before exposure to experimental light treatments; b) after 5 weeks exposure to experimental light treatments and weekly feeding with chopped mussel flesh. Tank 1= 4 μmol m^{-2} s^{-1} irradiance; Tank 2= 20 μmol m^{-2} s^{-1} irradiance; Tank 3= 3 μmol m^{-2} s^{-1} irradiance with red-coloured filter; Tank 4= 3 μmol m^{-2} s^{-1} irradiance with green coloured filter. Means ± 1 standard error are shown (n= 9).

Figure 4.4. Percentage increase in mean buoyant weight of *Anemonia viridis* after 5 weeks exposure to experimental light treatments. Tank 1= 4 μmol m^{-2} s^{-1} irradiance; Tank 2= 20 μmol m^{-2} s^{-1} irradiance; Tank 3= 3 μmol m^{-2} s^{-1} irradiance with red-coloured filter; Tank 4= 3 μmol m^{-2} s^{-1} irradiance with green coloured filter.

a)


Buoyant weight (mg)
200
150
100
50
0
Tank 1
Tank 2
Tank 3
Tank4

b)


Buoyant weight (mg)
200
150
100
50
0
Tank 1
Tank 2
Tank 3
Tank 4

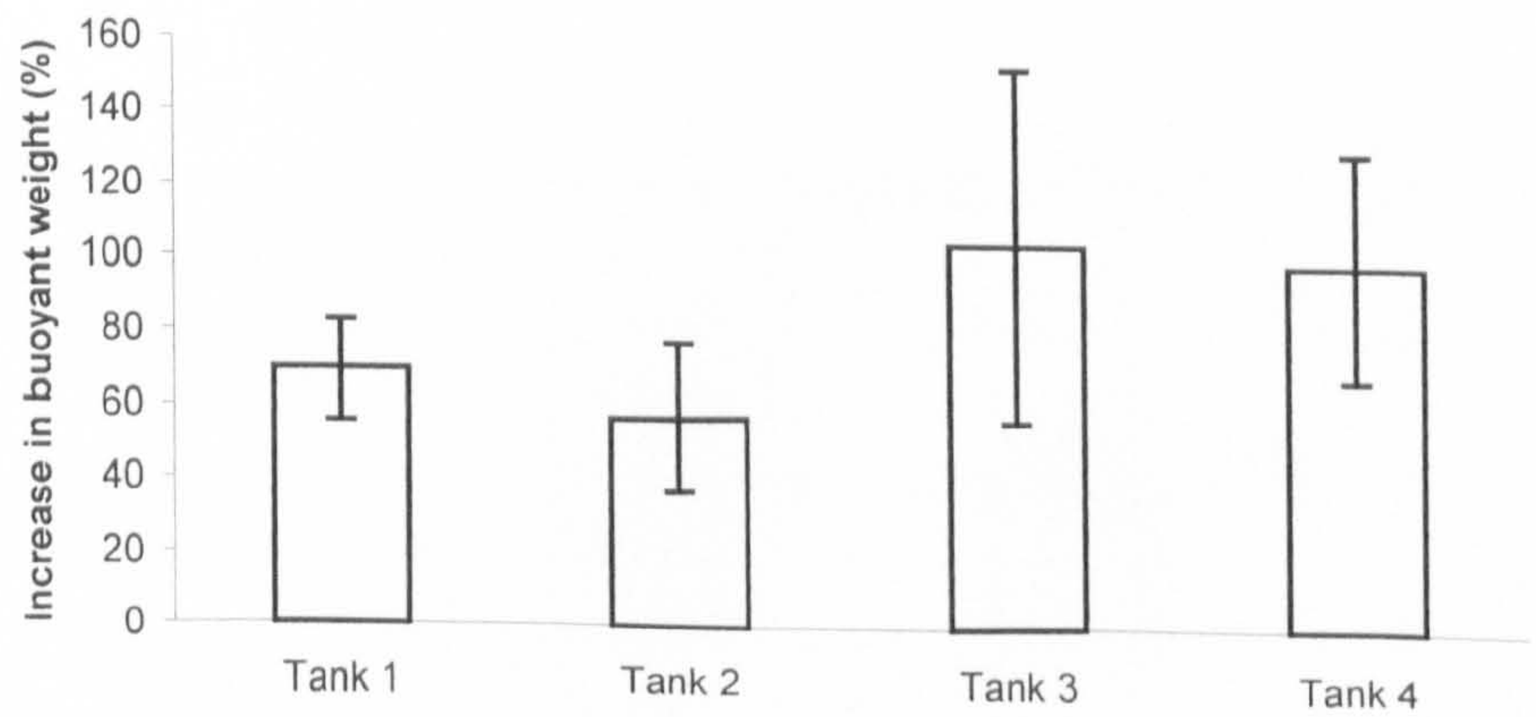
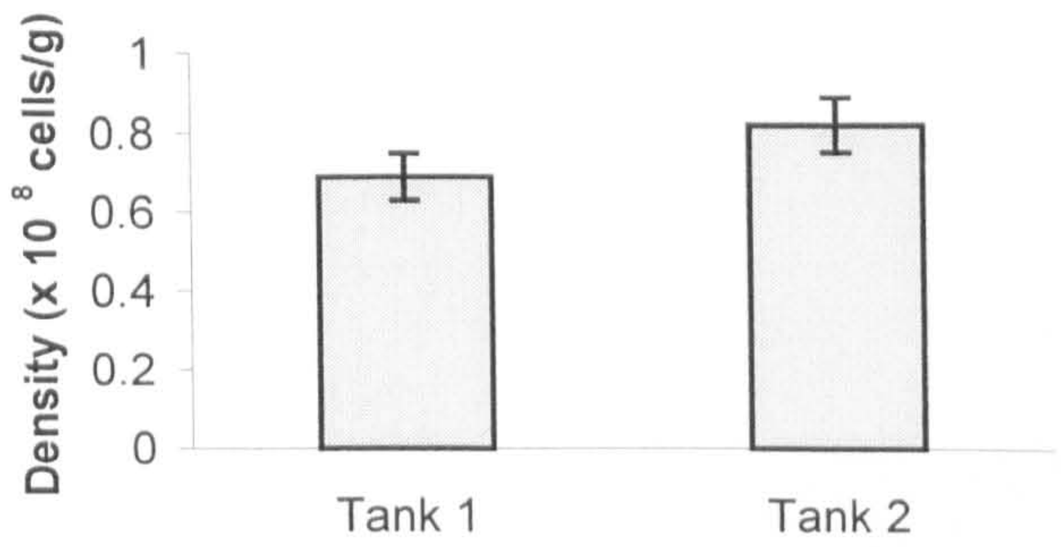
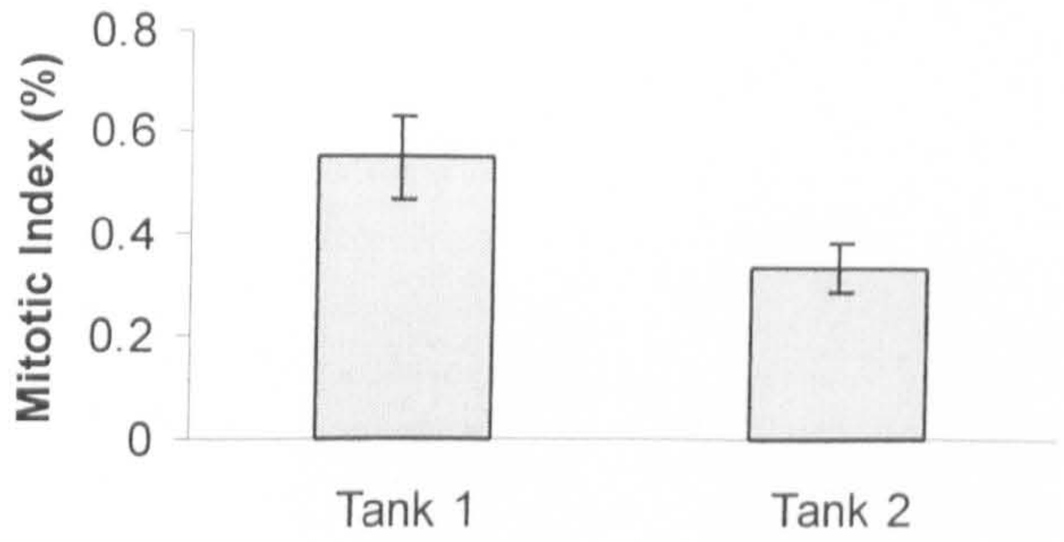

Increase in buoyant weight (%)
160
140
120
100
80
60
40
20
0
Tank 1
Tank 2
Tank 3
Tank 4

Figure 4.5. Mean zooxanthellae density in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} (Tank 1) and 20 μmol m^{-2} s^{-1} (Tank 2) artificial irradiance. Units are x 10^8 cells g^{-1} of anemone wet weight and error bars show ± 1 standard error.

Figure 4.6. Mean Mitotic Index (%)in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} (Tank 1) and 20 μmol m^{-2} s^{-1} (Tank 2) artificial irradiance. Error bars show ± 1 standard error.

Figure 4.7. Mean zooxanthellae diameter (μm) in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} (Tank 1) and 20 μmol m^{-2} s^{-1} (Tank 2) artificial irradiance. Error bars show ± 1 standard error.


Density (x 10 8 cells/g)
1
0.8
0.6
0.4
0.2
0
Tank 1
Tank 2


Mitotic Index (%)
0.8
0.6
0.4
0.2
0
Tank 1
Tank 2

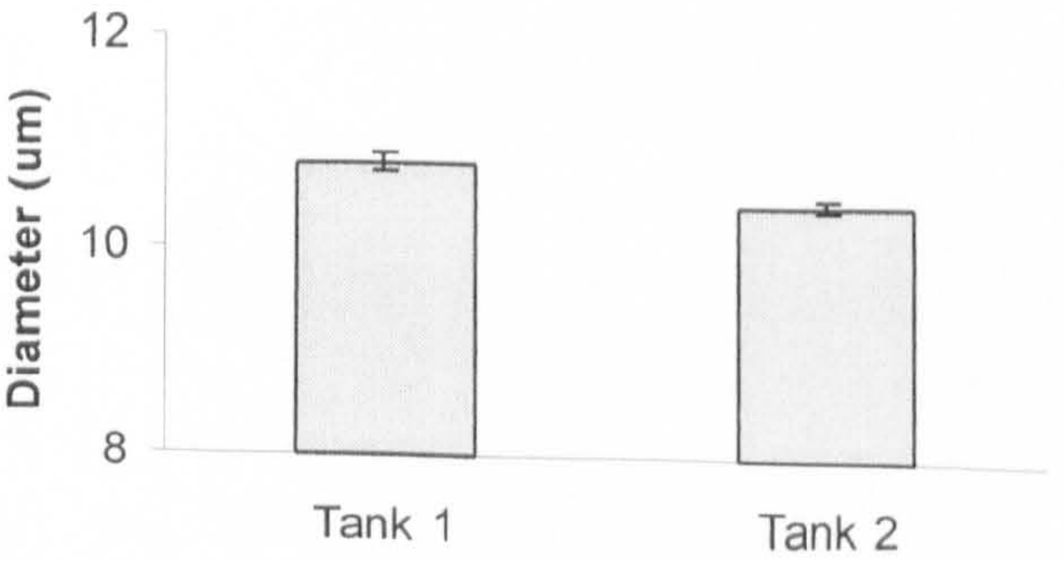
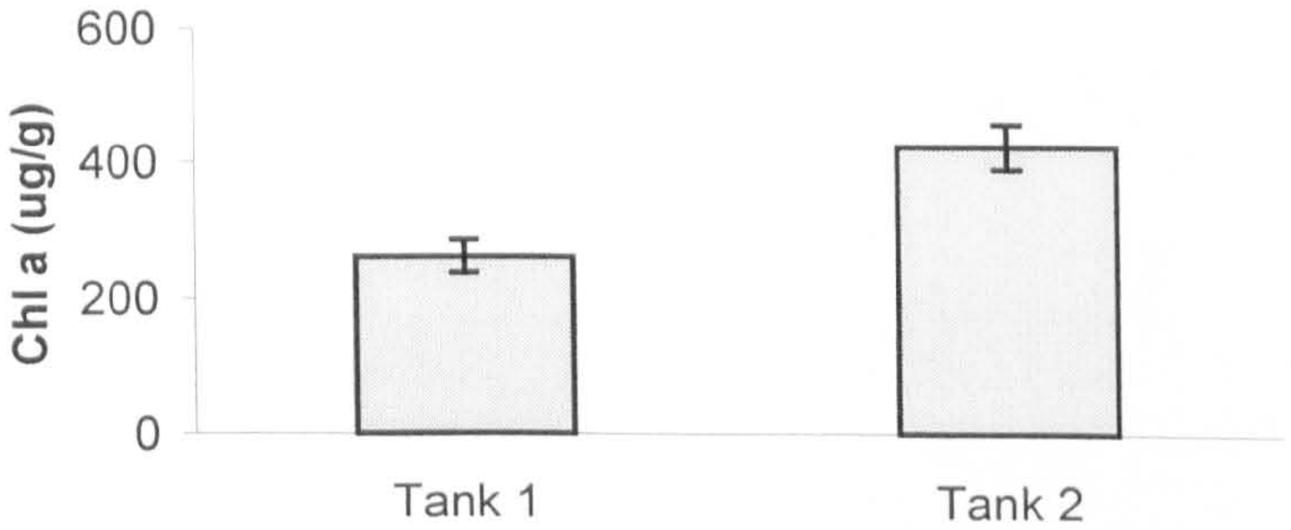
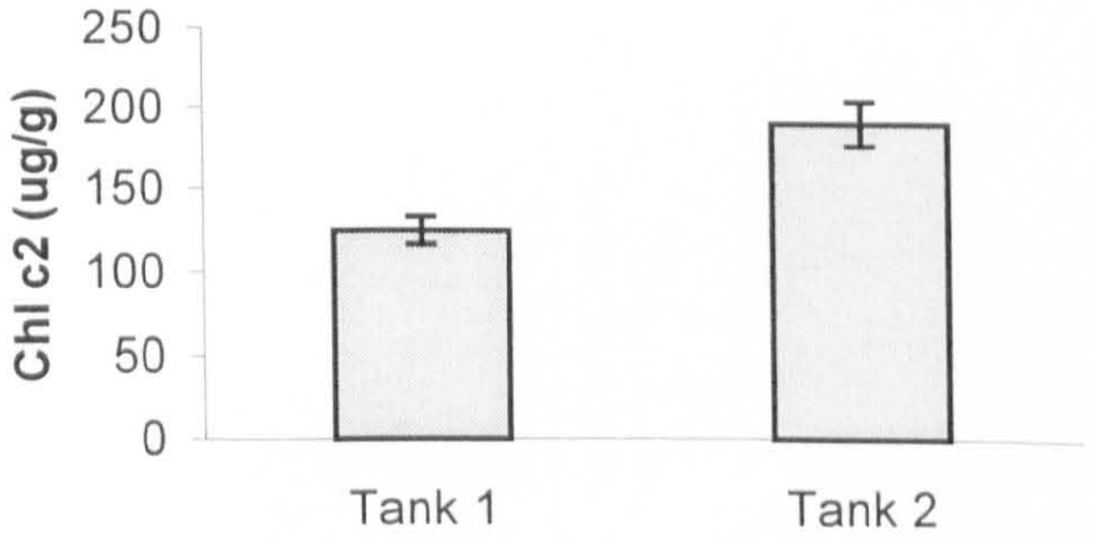

Diameter (um)
12
10
8
Tank 1
Tank 2

Figure 4.8. Mean zooxanthellae chlorophyll concentrations in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} (Tank 1) and 20 μmol m^{-2} s^{-1} (Tank 2) artificial irradiance; a) chlorophyll *a* (Chl *a*); b) chlorphyll c_2 (Chl c_2); c) ratio of chl *a*:c_2. Units are μg g^{-1} of anemone wet weight and error bars show ± 1 standard error.


600
400
200
0
Chl a (ug/g)
Tank 1
Tank 2


250
200
150
100
50
0
Chl c2 (ug/g)
Tank 1
Tank 2

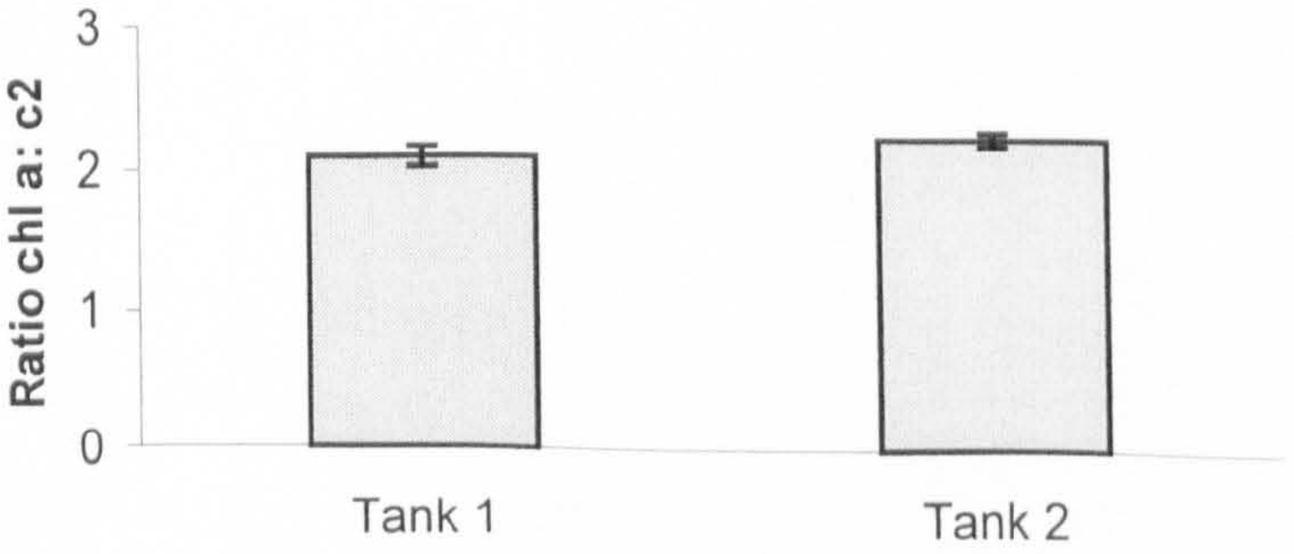
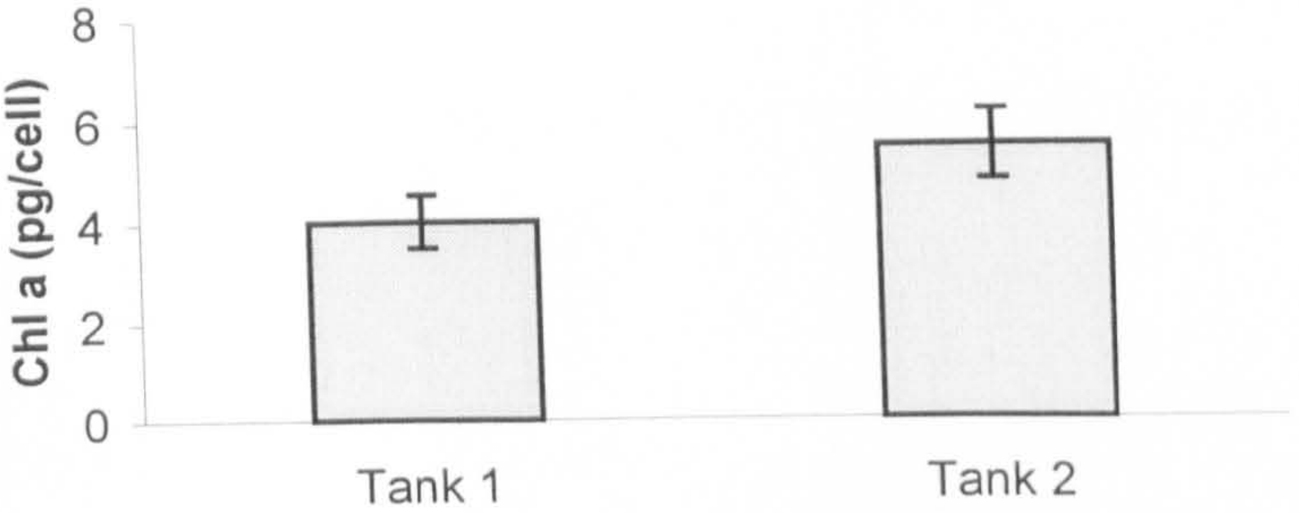
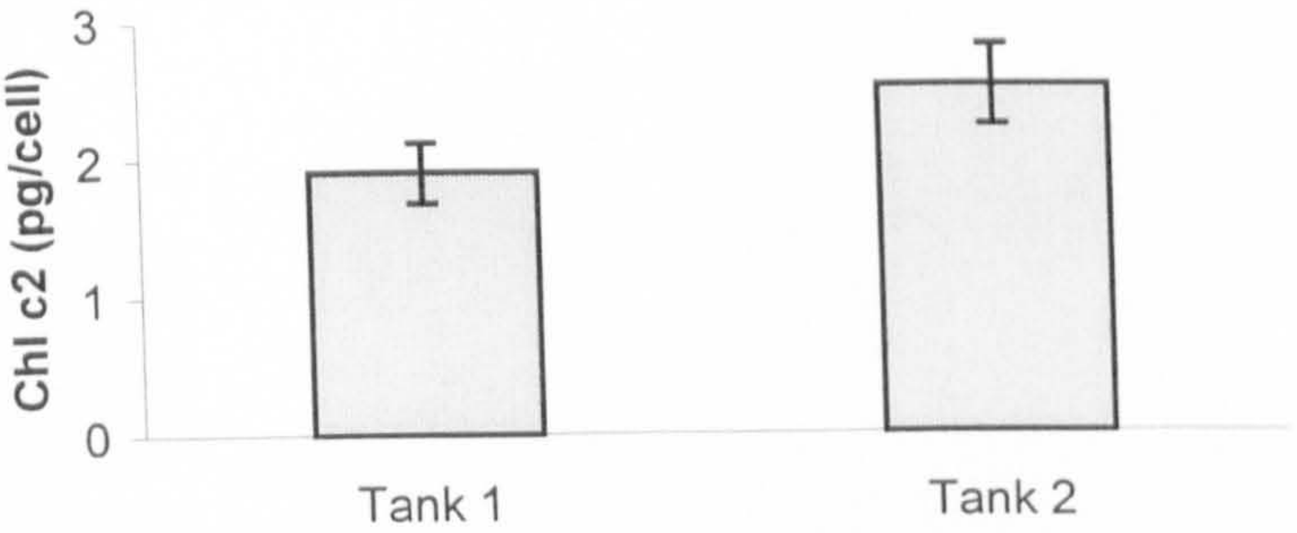

3
2
1
0
Ratio chl a: c2
Tank 1
Tank 2

Figure 4.9. Mean zooxanthellae chlorophyll concentrations in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} (Tank 1) and 20 μmol m^{-2} s^{-1} (Tank 2) artificial irradiance; a) chlorophyll *a*; b) chlorophyll c_2; c) ratio of chl a:c_2. Units are pg $cell^{-1}$ and error bars show ± 1 standard error.


Chl a (pg/cell)
8
6
4
2
0
Tank 1
Tank 2


Chl c2 (pg/cell)
3
2
1
0
Tank 1
Tank 2

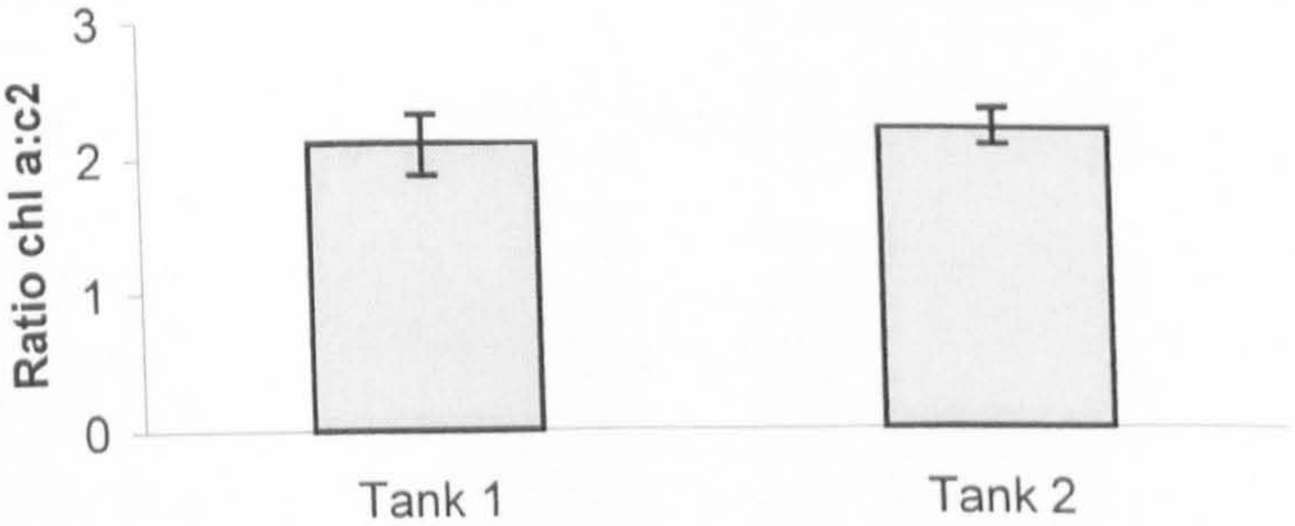
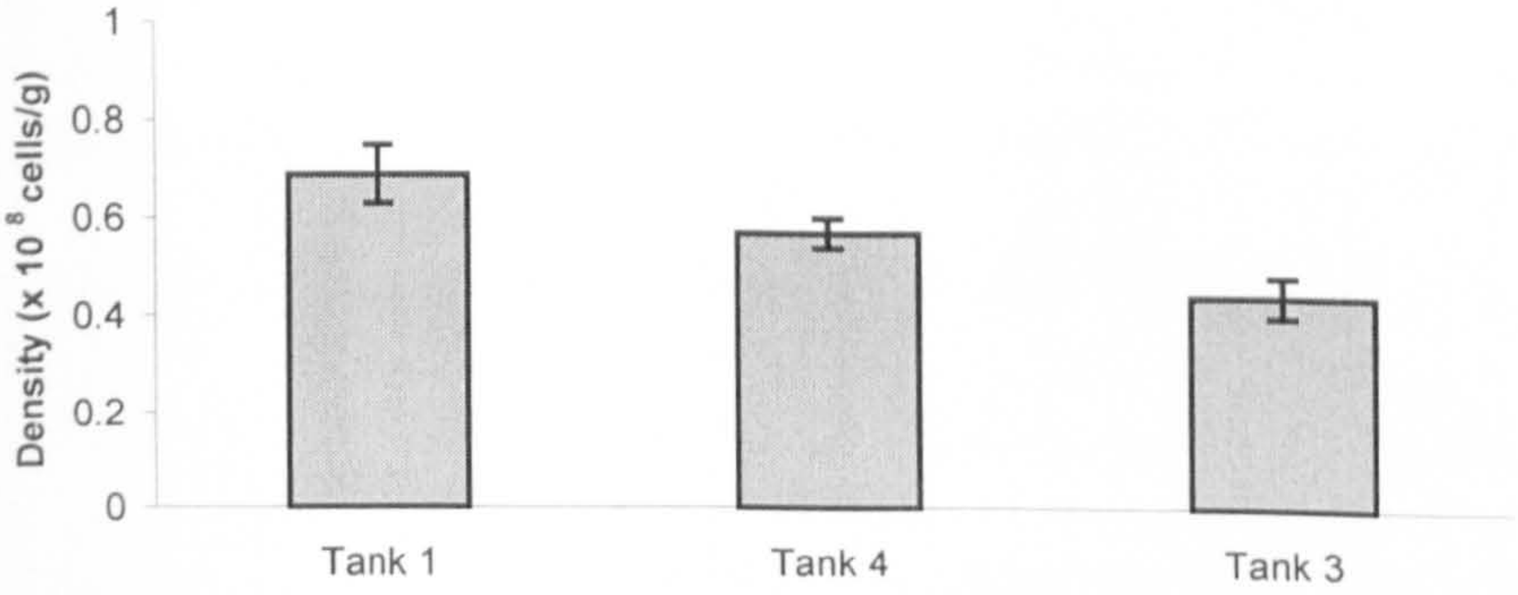
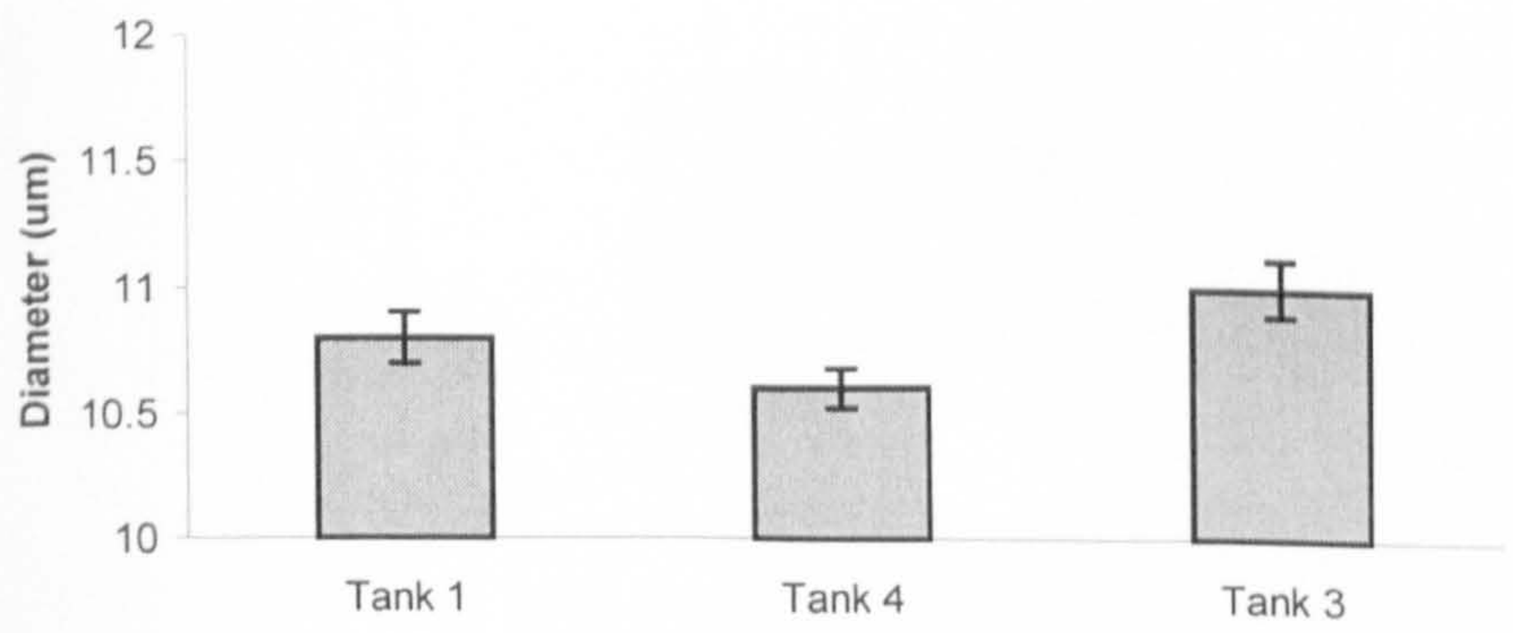

Ratio chl a:c2
3
2
1
0
Tank 1
Tank 2

Figure 4.10. Mean zooxanthellae density in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} full spectrum irradiance (Tank 1), 3 μmol m^{-2} s^{-1} with a red filter (Tank 3) and 3 μmol m^{-2} s^{-1} with a green filter (Tank 4). Units are x 10^8 cells g^{-1} of anemone wet weight and error bars show ± 1 standard error.

Figure 4.11. Mean zooxanthellae diameter (μm) in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} full spectrum irradiance (Tank 1), 3 μmol m^{-2} s^{-1} with a red filter (Tank 3) and 3 μmol m^{-2} s^{-1} with a green filter (Tank 4). Error bars show ± 1 standard error.

Figure 4.12. The number of dividing zooxanthellae (observed as doublets) in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} full spectrum irradiance (Tank 1), 3 μmol m^{-2} s^{-1} irradiance with a red filter (Tank 3) and 3 μmol m^{-2} s^{-1} irradiance with a green filter (Tank 4). Error bars show ± 1 standard error.


Density (x 10 8 cells/g)
1
0.8
0.6
0.4
0.2
0
Tank 1
Tank 4
Tank 3


Diameter (um)
12
11.5
11
10.5
10
Tank 1
Tank 4
Tank 3

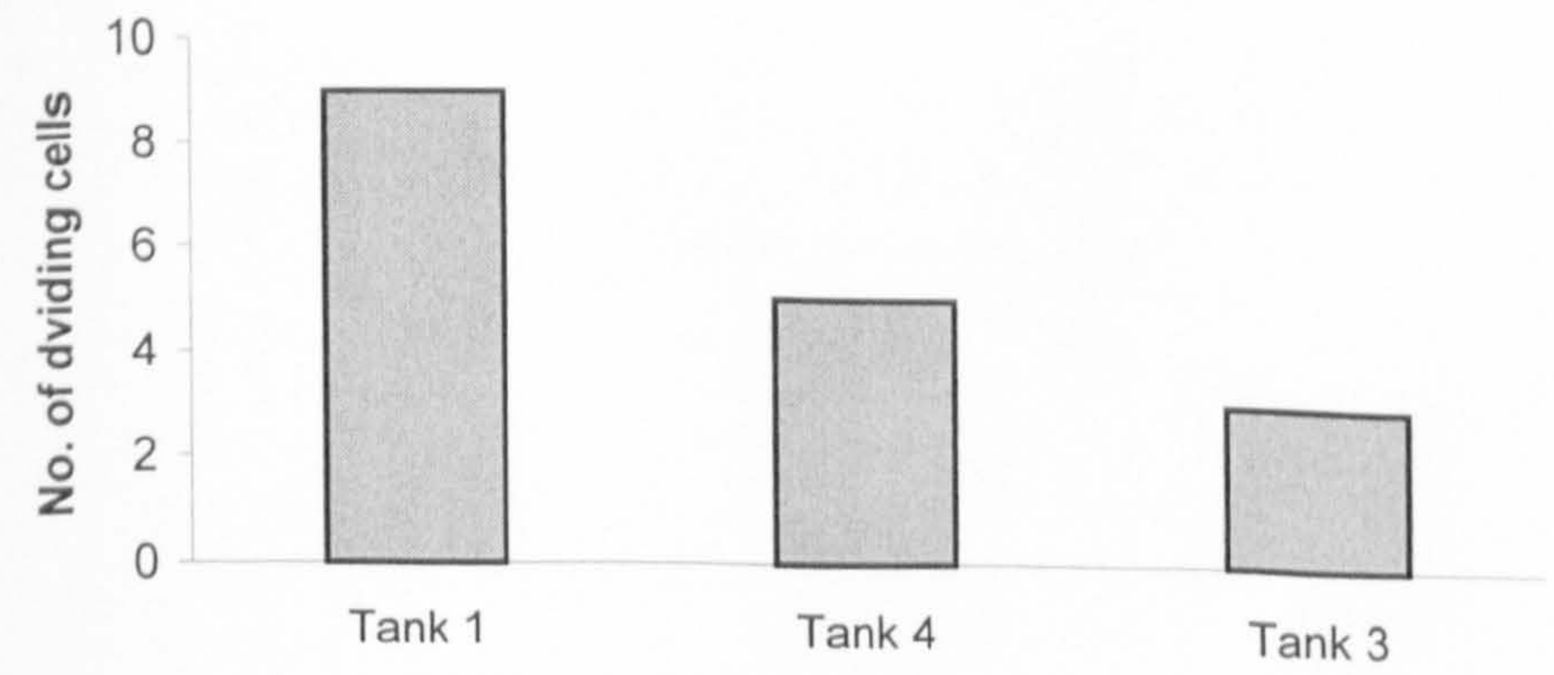
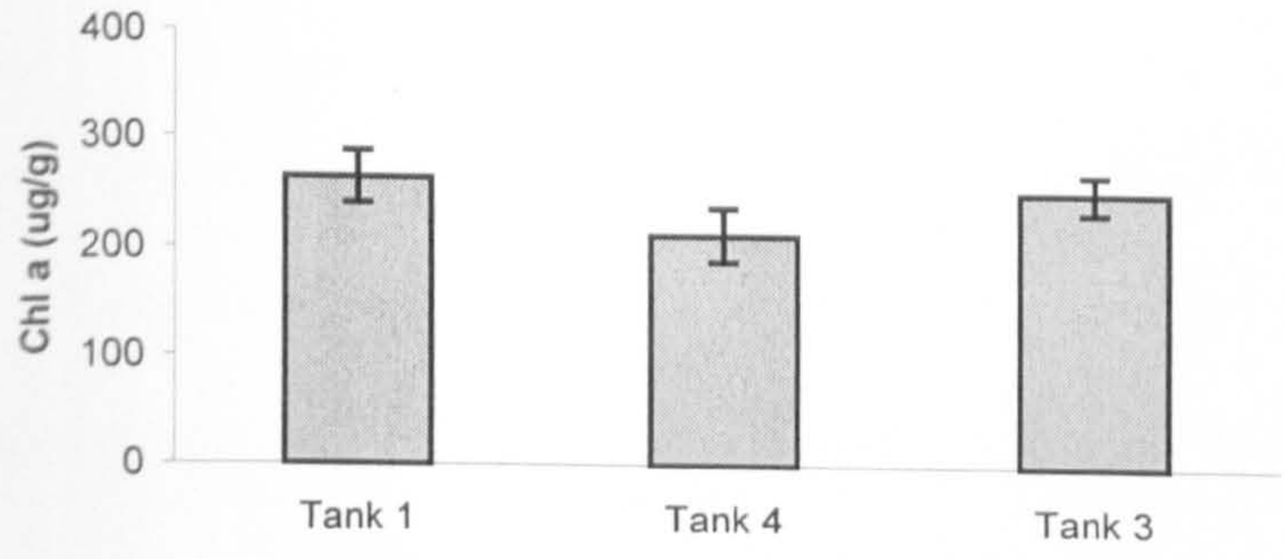
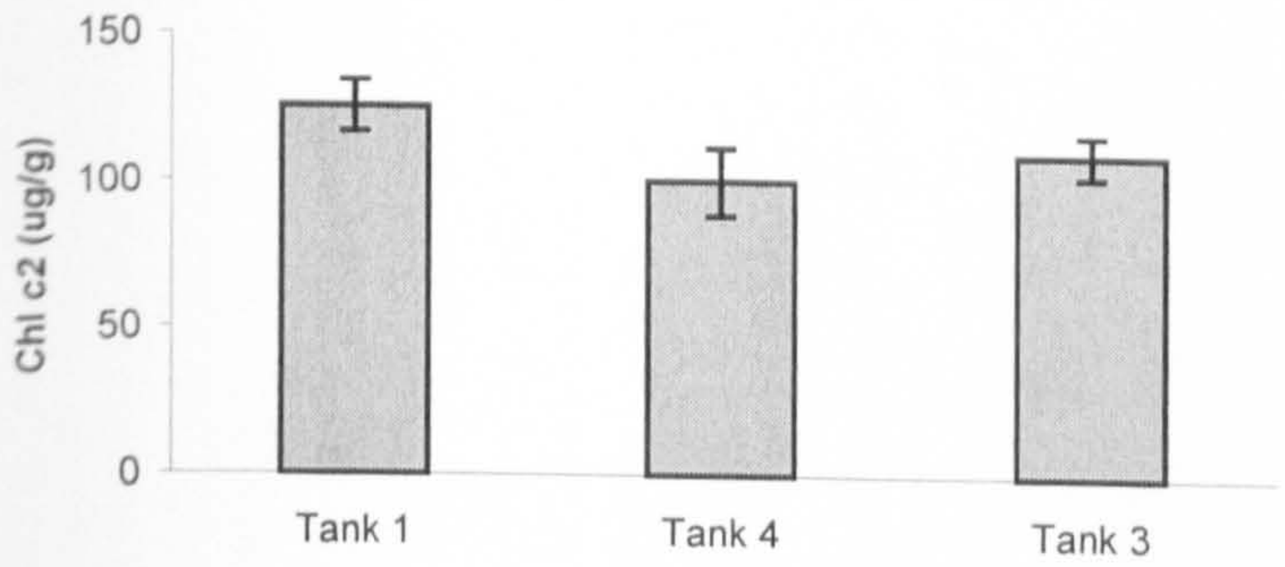

No. of dviding cells
10
8
6
4
2
0
Tank 1
Tank 4
Tank 3

Figure 4.13. Mean zooxanthellae chlorophyll concentrations in in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} full spectrum irradiance (Tank 1), 3 μmol m^{-2} s^{-1} with a red filter (Tank 3) and 3 μmol m^{-2} s^{-1} with a green filter (Tank 4); a) chlorophyll *a*; b) chlorophyll c_2; c) ratio of chl a:c_2. Units are μg g^{-1} of anemone wet weight and error bars show ± 1 standard error.


Chl a (ug/g)
400
300
200
100
0
Tank 1
Tank 4
Tank 3


Chl c2 (ug/g)
150
100
50
0
Tank 1
Tank 4
Tank 3

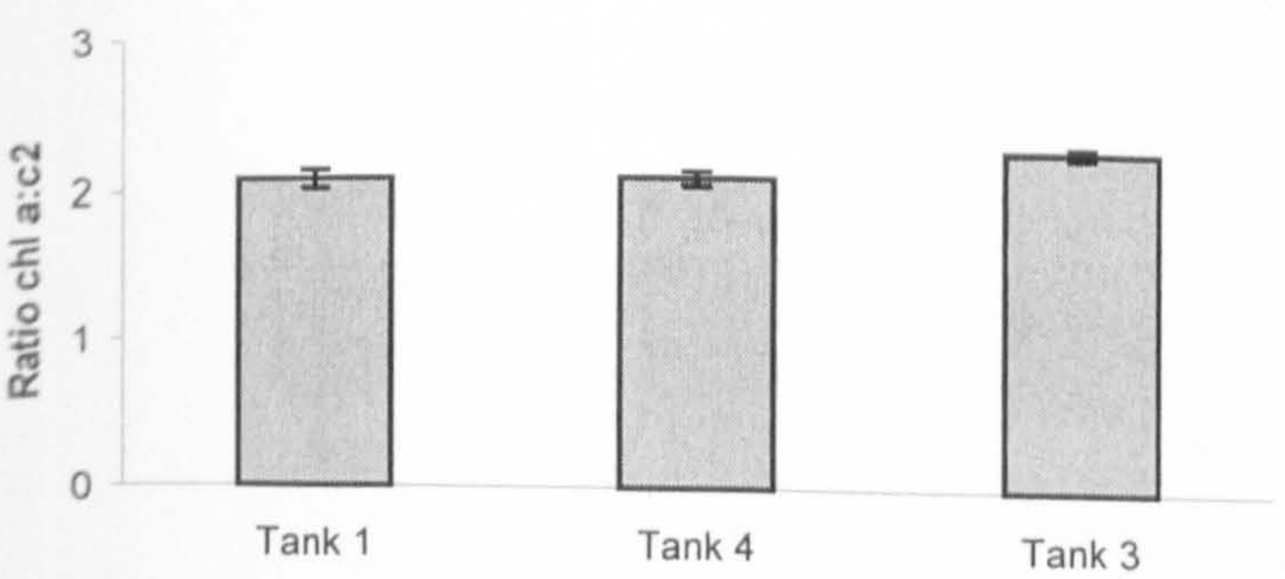
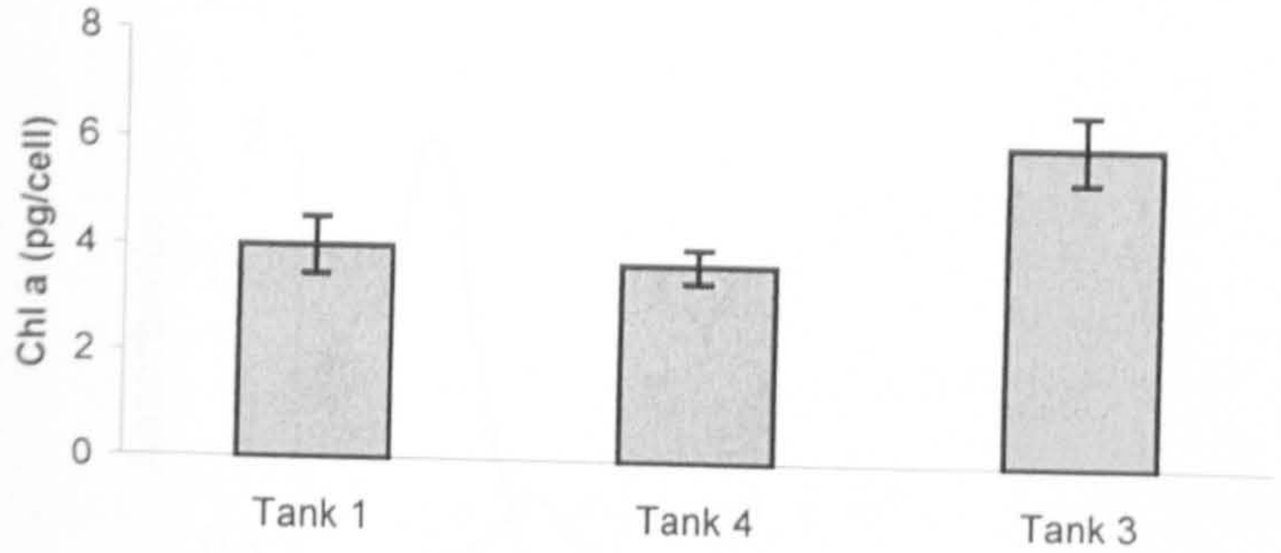
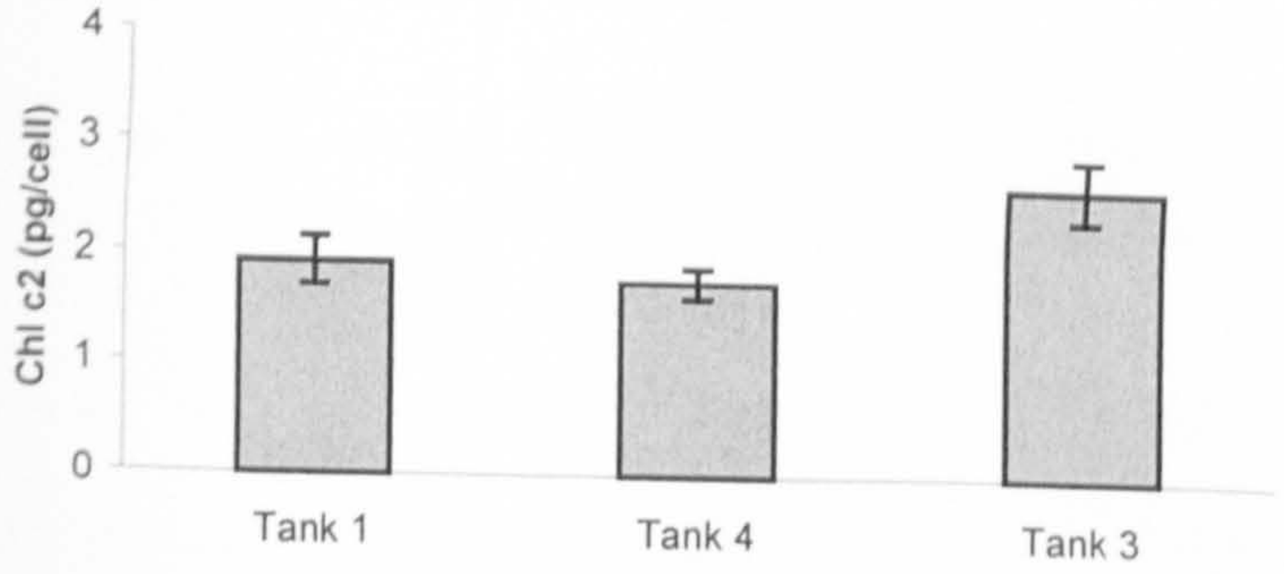
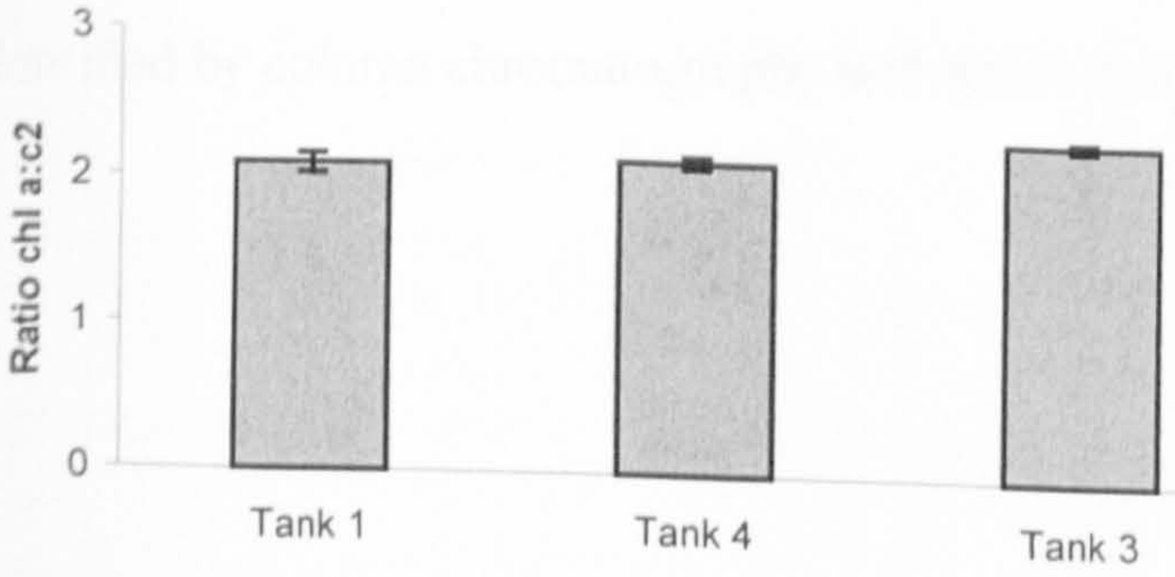

Ratio chl a:c2
3
2
1
0
Tank 1
Tank 4
Tank 3

Figure 4.14. Mean zooxanthellae chlorophyll concentrations in tentacles from *Anemonia viridis* maintained for 5 weeks in 4 μmol m^{-2} s^{-1} full spectrum irradiance (Tank 1), 3 μmol m^{-2} s^{-1} with a red filter (Tank 3) and 3 μmol m^{-2} s^{-1} with a green filter (Tank 4); a) chlorophyll *a*; b) chlorophyll c_2; c) ratio of chl *a*:c_2. Units are pg $cell^{-1}$ and error bars show ± 1 standard error.


Chl a (pg/cell)
8
6
4
2
0
Tank 1
Tank 4
Tank 3


Chl c2 (pg/cell)
4
3
2
1
0
Tank 1
Tank 4
Tank 3


Ratio chl a:c2
3
2
1
0
Tank 1
Tank 4
Tank 3

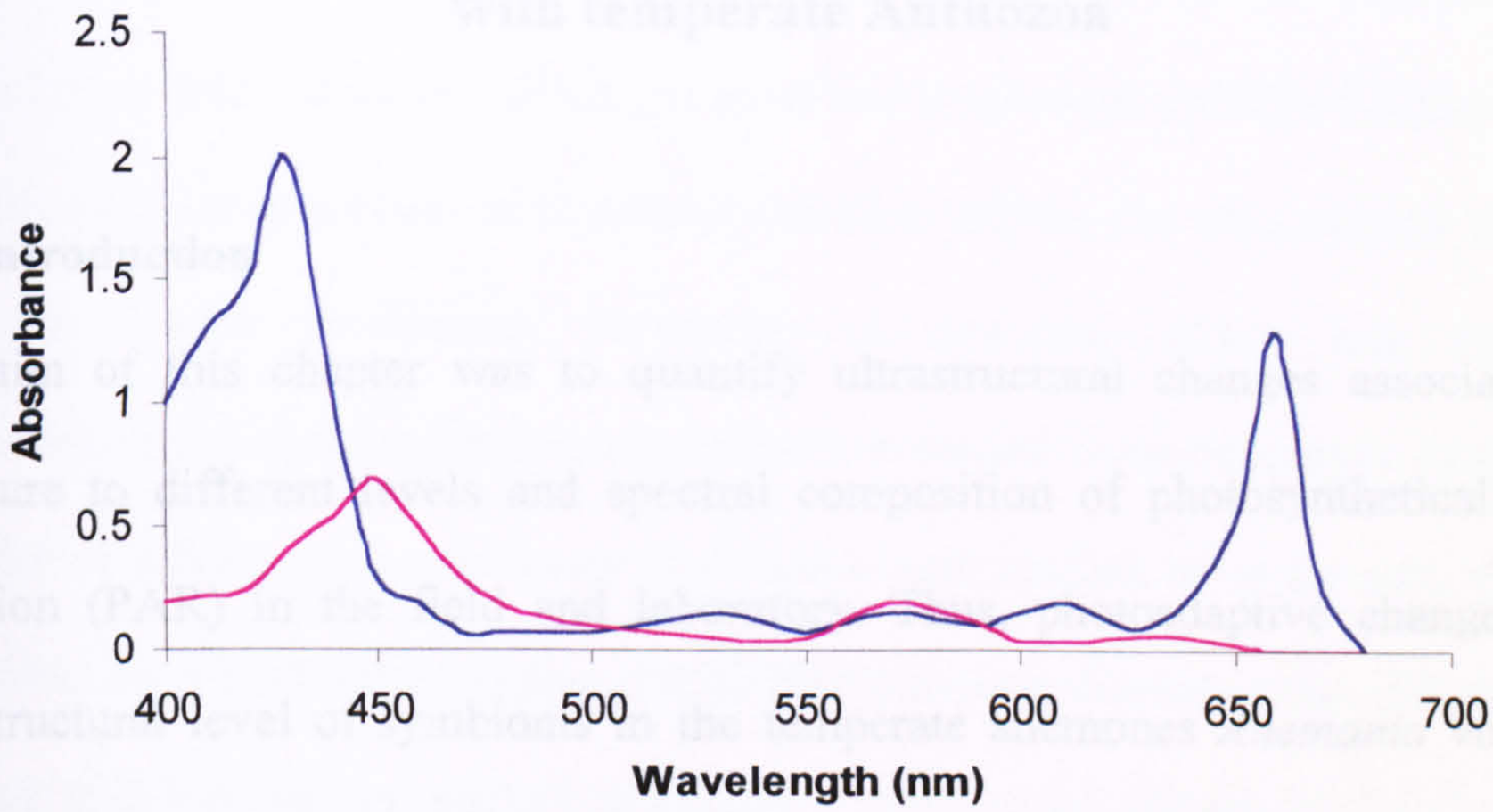


Figure 4.15. Absorption spectra for a) chlorophyll *a* (——) and *c* (——) extracted from zooxanthellae of *Anemonia sulcata* (adapted from Taylor, 1967). Pigments were identified by column chromatography and spectrophotometric analysis.

CHAPTER 5

Photoadaptive ultrastructure changes in zooxanthellae symbiotic with temperate Anthozoa

5.1. Introduction

The aim of this chapter was to quantify ultrastructural changes associated with exposure to different levels and spectral composition of photosynthetically active radiation (PAR) in the field and laboratory. Thus, photoadaptive changes at the ultrastructural level of symbionts in the temperate anemones *Anemonia viridis* and *Anthopleura ballii* have been quantified.

Zooxanthella ultrastructure may alter in response to changes in radiation during growth to enhance the capture of light energy for photosynthesis. Such responses are termed photoadaptation. Previous studies on zooxanthellae in tropical hosts have shown that irradiance may affect chloroplast morphology and lipid and starch content of zooxanthellae (Dubinsky *et al.* 1984, Lesser and Shick 1990, Muller-Parker *et al.* 1996). Photoadaptation may be accompanied by changes in cell volume, the number and density of thylakoid membranes (Berner *et al.* 1989), the size of pyrenoids and other storage bodies within plastids (Sukenik *et al.* 1987) and sometimes in the number of plastids. Taylor (1968, 1969) and Doyle & Doyle (1940) also showed that the number of vacuolar crystals increased in zooxanthellae from anemones maintained in the dark.

Sun and shade habitats in the field and the level of artificial irradiance in the laboratory may affect zooxanthellae ultrastructure. For example, the descriptive observations of Dubinsky *et al.* (1984), Berner *et al.* (1987) and Muller-Parker (1987)

showed that thylakoids in chloroplasts from shade-adapted colonies of *Stylophora pistillata* (hermatypic coral), *Litophyton arboreum* (soft coral) and *Aiptasia pulchella* (sea anemone) were more densely packed than those in light-adapted colonies. Similarly, Muller-Parker *et al.* (1997) and Lesser & Shick (1990) found that zooxanthellae from *Aiptasia pallida* grown at low irradiances exhibited higher volume fractions of chloroplast and of thylakoid lamellae within the chloroplasts than cells grown at higher irradiances. However, no previous study has considered photoadaptive changes in ultrastructure of zooxanthellae from temperate Anthozoa in the field or laboratory environment. Ultrastructural studies of temperate Anthozoa have either been qualitative (Taylor, 1968, 1969) or have examined the effects of light and dark and feeding and starvation (Taylor, 1969) or of ultraviolet (UV) radiation (Hannack *et al.*, 1997, Lesser & Shick, 1990)) on zooxanthellae.

Taylor (1969) examined changes in the ultrastructural morphology of *Anemonia sulcata* (= *viridis*) and its symbiotic zooxanthellae in the presence and absence of both light and food. However, photoadaptation was not considered and the differences observed were not quantified. Hannack *et al.* (1997) considered the ultrastructure of zooxanthellae from the temperate anemone *Cereus pedunculatus* maintained in UV light. Quantitative stereological analysis of electron micrographs was carried out, but not in relation to photoadaptation.

Quantitative studies of photoadaptive changes in zooxanthella ultrastructure have been carried out using tropical Anthozoa in both laboratory and field situations. Muller-Parker *et al.* (1996) and Lesser & Shick (1990) quantified the effects of different irradiance levels in the laboratory on zooxanthellae ultrastructure, in the tropical sea anemone, *Aiptasia pallida.* Whilst Muller-Parker (1987) previously considered the effects of natural variations in irradiance in both high and low

irradiance environments on the tropical sea anemone *Aiptasia pulchella*, and photoadaptive ultrastructure changes were considered only qualitatively. No study of temperate or tropical symbiotic Anthozoa has addressed the effects of seasonality or depth in the field, nor the effect of altering spectral composition in the laboratory, on zooxanthellae ultrastructure.

The hypothesis investigated in this study states that irradiance significantly affects the ultrastucture of zooxanthellae in temperate Anthozoa under both field and laboratory conditions. The objectives of this chapter were to carry out quantitative field and laboratory investigations of zooxanthella ultrastructure in *Anemonia viridis* and *Anthopleura ballii* using stereological analysis of electron micrographs. Consideration was given to chloroplast morphology, starch and lipid stores, mitochondria, the nucleus, pyrenoid, accumulation bodies and vacuoles with crystals when anemones were exposed to different light regimes. Firstly zooxanthella ultrastructure in *A. viridis* was compared during winter and summer, from anemones collected from a shallow sublittoral habitat and an intertidal rock pool habitat. Both habitats may be described as shallow 'sun-habitats' experiencing high intensity irradiance. However, anemones in the intertidal habitat may be exposed to conditions which are physiologically more stressful, such as wave action and temperature and salinity fluctuations. Thus zooxanthellae from intertidal and subtidal environments were compared.

Secondly, a comparison of zooxanthella ultrastructure in sublittoral *Anthopleura ballii* from bathymetric extremes of thier distribution and during different seasons was made. Thus the effects of light attenuation with depth were considered during winter and summer. Finally, the effects of different levels and spectral composition of artificial irradiance (PAR) on the ultrastructure of zooxanthellae in *A. viridis* was

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considered in controlled laboratory conditions. Low irradiance and winter temperatures were used to simulate the temperate climate experienced at depth in the field (see 2.3.3.1, 2.3.3.2).

5.2. Methods

5.2.1. The effect of season and habitat on zooxanthellae ultrastructure

Field sampling was carried out at 3 sites in two locations (described in chapter 3 section 3.2.1 and 3.2.3.1) which comprised two shallow water sun-habitats, one intertidal the other subtidal and a subtidal cliff habitat. The intertidal sun-habitat was a mid-tide level rock pool in Trearddur Bay, Anglesey (53° 16' N, 4° 4' W; chapter 2, figure 2.1; plate 3.1). The subtidal sun-habitat was Castle Island Bay, Lough Hyne M.N.R., Ireland (51° 29' S, 9° 18' W; Figures 2.2 and 3.1; plate 3.2). The third site was Glannafeen cliff, Lough Hyne M.N.R., Ireland (51° 29' S, 9° 18' W; figures 2.2 and 3.1; plate 3.3).

<u>Site 1:</u> Mid-tide level rock pool, Trearddur Bay, Anglesey.

Two tentacles were excised from three *Anemonia viridis* during selected winter and summer months (1/98 and 8/98), placed in glass vials with sea water and transported back to the laboratory, as mentioned in chapter 3, 3.2.3.1. Each tentacle was cut into 0.5 mm round segments with a razor blade and then cut in half and fixed in 0.1 M phosphate buffered (pH 7.3) 3 % glutaraldehyde at room temperature for 2 hours. The sample was then washed with distilled water for 30 seconds and stored in 0.1 M phosphate buffer (pH 7.3) until subsequent laboratory preparation was carried out.

<u>Site 2:</u> Castle Island Bay, Lough Hyne M.N.R., Ireland.

Anemonia viridis were collected from 1.5 m by snorkelling during summer (8/97) and winter (2/98) and transported back to the field laboratory. Two tentacles were excised

from 3 animals (as mentioned in chapter 3, 3.2.3.1.) and preserved as described for site 1.

<u>Site 3:</u> Glannafeen cliff, Lough Hyne M.N.R., Ireland.

Anthopleura ballii were collected from the cliff at 18 m and 6 m depth during winter (2/98) and summer (8/98) using SCUBA and transported back to the field laboratory. Two tentacles were excised from 3 anemones (as mentioned in chapter 3, 3.2.3.1) and preserved as described for site 1.

5.2.2. The effect of light intensity and spectral composition on zooxanthellae ultrastructure

Anemonia viridis were collected on 17 th March 1999 and maintained as described in chapter 4 section 4.2. The animals used for experiments in Chapter 4 were used in the present chapter. Two experiments were carried out simultaneously. Experiment 1 exposed the anemones to two different levels of irradiance; experiment 2 exposed the anemones to irradiance of altered spectral composition. The treatments (as used in section 4.2) were as follows:

Tank 1/ low light intensity; 4 μmol m^{-2} s^{-1} underwater irradiance

Tank 2/ medium intensity; 20 μmol m^{-2} s^{-1} underwater irradiance

Tank 3/ red coloured filter and 1 layer of mesh; 3 μmol m^{-2} s^{-1} underwater irradiance

Tank 4/ green coloured filter; 3 μmol m^{-2} s^{-1} underwater irradiance

After 4 weeks two tentacles were excised from 3 animals in each of the 4 treatments. Tentacle samples from animals in the same treatments were pooled then fixed and preserved as described in section 5.2.1. above.

5.2.3. Transmission electron microscopy

Zooxanthellae ultrastructure was revealed by observing microsections of anemone tentacle with a transmission electron microscope (TEM). Preparation of the tissue involved several stages and followed the methods of J.D. Russel (Pers. comms.) and Bozzola & Russel (1992).

After fixation (described in section 5.2.1. above) tentacle segments were post-fixed with 1 % osmium tetroxide (OsO_4) in 0.1 M phosphate buffer (pH7.3) for 1 hour at room temperature. Each was then dehydrated through an ethanol series e.g. 10 minutes in 50 % ethanol, 10 minutes in 70 %, 10 minutes in 95 % and 15 minutes in 100 % ethanol.

For thin sectioning of the tentacle segments each was embedded in an epoxy liquid embedding medium (Spurrs' resin). The samples were infiltrated overnight at room temperature with the resin to allow penetration into all the cells. Each piece of tentacle was then transferred to a dry BEEM capsule, the capsule filled with fresh Spurrs' resin and the resin finally polymerized in an oven at 70 °C for 72 hours.

Microsections were cut using an ultramicrotome, picked up on 200-mesh copper grids and stained with 2 % aqueous uranyl acetate and Reynolds lead citrate. Sections were examined with a Corinth electron microscope operated at 60 kV. Micrographs were taken of randomly selected zooxanthellae in which the nucleus was clearly visible. To obtain true images from the negatives taken using the TEM each micrograph was placed on a light box with a digital camera positioned above. The digital camera was able to convert the negative images of zooxanthellae to positive images on a monitor. A permanent record was obtained by videoing each zooxanthella for a few seconds. Stereology was used to quantitatively analyse zooxanthellae components.

5.2.4. Stereology

Background

Stereology is a branch of applied mathematics used for the three-dimensional analysis of organs and materials from two-dimensional measurements. It allows 3-D interpretation of flat images and is practised by measuring and counting profiles in sections by superimposing grids of lines or squares and circles over an image (Elias & Hyde, 1980). The grid used in this investigation was made up of an array of short lines described by Wiebel *et al.* (1966) as a multipurpose test system with 21 lines of length *z*, which are arranged on 7 equidistant and parallel rows, whereby distance between end-points of lines is *z* in every direction. These 42 end-points can thus be used as test points for point-counting volumetry which involves the application of simple formulae.

The Delesse principle states that the volume fraction, *Vvi* of a component, *i* in the tissue can be estimated by measuring the area fraction *AAi* of a random section occupied by a transection of *i*:

$$Vvi = Aai$$

It also proposes that fraction *Ppi* of points lying on transections of *i* would thus be an estimate of *Vvi*:

$$Vvi = Ppi$$

The measurements required consist merely of counting points, *Pi* coinciding with particular organelles or structures on the section and expressing this as a fraction of the total number of test points, *PT* :

$$\text{Volume fractions, } Vvi = \frac{Pi}{PT} = Ppi$$

Stereological analysis

The arrangement of organelles and storage products is assumed to be isotropic, whereby all organelles are evenly distributed, as assumed by other investigators conducting stereological analyses of zooxanthellae (Trench & Blank 1987, Lesser & Shick 1990, Berner & Izhaki 1994, Muller-Parker *et al.* 1996). Only cells with the nucleus clearly visible were used for analysis. Organelles and storage products were identified by comparison with previously published transmission micrographs (Van Thinh *et al.* 1986, Davy 1994, Muller-Parker *et al.* 1996 Hannack *et al.* 1997). The main ultrastructural features of the zooxanthella *Symbiodinium* are shown in Figure 5.1. Each zooxanthella was analysed stereologically by superimposing a grid with an array of short lines on the negative and using the point-count method to determine volume fractions of chloroplasts, nucleus, starch, mitochondria, lipid, vacuoles, and accumulation bodies as percentage of cell volume and thylakoid membranes as a percentage of chloroplast volume. Three grid sizes were used with line (z) spacing of 0.3, 0.5 and 0.8 cm according to magnification of the original micrograph.

5.2.5. Statistics

All percentage data were transformed by the angular ("arcsin") transformation. The parametric One-way ANOVA analysis followed by Tukey *post-hoc* ANOVA and Two-sample T-tests and the non-parametric Mann-Whitney and Kruskal-Wallis tests were performed on the data.

5.3. Results

5.3.1. Field populations

Seasonal variations in light affected zooxanthella ultrastructure in intertidal *Anemonia viridis* collected from Trearddur Bay. Table 5.1 shows the volume fractions of zooxanthellae from intertidal *Anemonia viridis* collected during winter (1/98) and summer (8/98). The volume fraction of chloroplasts in winter zooxanthellae was 32.1 (± 1.5) % which was significantly higher than summer anemones with a volume fraction of 21.8 (± 2.1) % ($T= 4.07$ $p< 0.05$; Figure 5.2a). By contrast, summer and winter volume fractions were not significantly different for the nucleus ($T= 1.34$ $p >0.05$), starch ($T= 1.12$ $p >0.05$; Figure 5.2b), nor mitochondria ($W= 84.0$ $p >0.05$). Zooxanthellae in summer anemones contained 19.8 (± 3.4) % lipid, whilst no lipid was found in zooxanthellae from winter anemones (Plate 5.1a & b). Vacuoles were absent from zooxanthellae in both summer and winter anemones (Plate 5.1).

In the shallow, subtidal 'sun-habitat' of Castle Island Bay, Lough Hyne only zooxanthellae samples from winter anemones were suitably preserved for stereological analysis (Table 5.2). However, chloroplast volume (34.9 ± 2.0 %) was comparable to that measured in intertidal *Anemonia viridis* during winter (32.1 ± 1.5 %) as was starch volume (12.9 ± 2.2 % compared with 11.1 ± 2.0 %). Lipid was

present in small amounts in winter subtidal zooxanthellae (2.4 ± 1.8 %) from Castle Island (Plate 5.2) but absent from zooxanthellae in winter intertidal Trearddur Bay anemones, as were vacuoles containing crystals of waste products.

From Glannafeen subtidal cliff, Lough Hyne only zooxanthellae samples from anemones at 6 m and 18 m during winter and 18 m during summer were suitably preserved for stereological analysis (Table 5.3). Among anemones collected from Glannafeen cliff during winter and summer, zooxanthellae from 6 m and 18 m water depths showed ultrastructural differences. Relative chloroplast volumes were significantly larger in zooxanthellae from anemones collected at 18 m in summer (41.4 ± 3.2 %) than 6 m in winter (22.0 ± 3.2 %; ANOVA, $F= 5.97$ $p < 0.05$; Figure 5.3a). Thus, when both depth and season were altered (e.g; summer 18 m *vs.* winter 6 m) chloroplast volume was significantly affected. Conversely, starch volume was significantly larger in zooxanthellae from anemones collected at 6m during the winter (14.3 ± 4.2 %) than from 18 m during both winter (4.7 ± 1.6 %) and summer (4.7 ± 1.1 %; ANOVA, $F= 6.04$ $p< 0.05$; Figure 5.3b). Similarly, lipid was present in zooxanthellae from 6 m anemones during winter (18.0 ± 10.4 %) and absent from 18 m zooxanthellae during both winter and summer (Plate 5.3a). Large vacuoles, with a mean relative volume of 19.5 ± 2.0 % were present in zooxanthellae from 18 m anemones collected during the summer (Plate 5.3c) but completely absent from zooxanthellae in winter anemones from both 6 m and 18 m (Plate 5.3a & b). The relative volumes of the nucleus and mitochondria were not significantly different (Kruskal-Wallis, $H= 4.90$, $p> 0.05$; ANOVA, $F= 1.04$, $p> 0.05$; Kruskal-Wallis, $H= 2.93$, $p> 0.05$). Comparison of relative thylakoid volumes in chloroplasts was not

possible in zooxanthellae from field anemones due to poor preservation and sectioning of samples.

5.3.2. Laboratory experiments

5.3.2.1. Intensity experiment

Table 5.4 shows volume fractions of zooxanthellae exposed to irradiance of 4 μmol m^{-2} s^{-1} and 20 μmol m^{-2} s^{-1}. No significant effect on zooxanthellae ultrastructure was observed between the two treatments; chloroplasts T= 1.94, p> 0.05; nucleus T= 1.30, p> 0.05 (Figure 5.4a); starch T= -0.60, p> 0.05, Figure 5.4b; mitochondria T= 0.39, p> 0.05; vacuoles T= -1.09, p>0.05; (Plate 5.4a,b). By contrast, the relative volume of thylakoids in a chloroplast in 20 μmol m^{-2} s^{-1} (37 ± 0.01 %) was significantly larger than of thylakoids in 4 μmol m^{-2} s^{-1} (30 ± 0.02 %; T= -2.78, p< 0.05, Figure 5.5a; plate 5.4; table 5.6).

5.3.2.2. Spectra experiment

Altering the spectral composition of artificial irradiance by using red and green coloured filters had a significant effect on the relative volume of chloroplasts only (Table 5.5). Zooxanthellae from anemones in full spectrum light had a significantly larger chloroplast volume (42.5 ± 1.5 %) than zooxanthellae in both green (33.0 ± 2.1 %) and red (34.5 ± 1.9 %) light maintained anemones (ANOVA, F= 9.06, p <0.001; Figure 5.6a; plate 5.5a,b). The relative volumes of vacuoles and mitochondria were not significantly different among anemones (ANOVA, F= 1.21, p> 0.05; Kruskal-Wallis, H= 1.31, p> 0.05). Mean starch volume in green and red light treatments was double that measured in full spectrum light zooxanthellae (5.9 ± 1.1 % and 5.4 ± 0.7 % compared with 2.4 ± 0.4 %; Figure 5.6b) which represented a significant difference (Kruskal-Wallis, H= 11.06, p< 0.05). Accumulation bodies were observed in

zooxanthellae from green (Plate 5.5b) and red light anemones but not in full light spectrum zooxanthellae (Table 5.5; Figure 5.6c). The relative volume of thylakoids was not affected by the spectral composition of artificial light (ANOVA, F= 1.31, p> 0.05; Table 5.6 and Figure 5.5b).

5.4. Discussion

The ultrastructure of zooxanthellae in the temperate sea anemones *Anemonia viridis* and *Anthopleura ballii* is influenced by irradiance. Figure 5.7 summarizes the effects of shade and sun on zooxanthellae ultrastructure. Intertidal zooxanthellae have higher chloroplast volumes in winter compared with summer and lipid is accumulated during the summer and absent during winter. Although shallow subtidal sun-habitat zooxanthellae were not sampled during summer, subtidal winter samples were compared with intertidal winter zooxanthellae. Similar starch and chloroplast volume fractions were obtained. Small amounts of lipid and vacuoles were also present in zooxanthellae from subtidal anemones but absent from intertidal zooxanthellae.

Previous quantitative studies of zooxanthella ultrastructure have examined the effects of artificial irradiances on laboratory maintained anthozoans (Hannack *et al.* 1997, Lesser & Shick 1990, Muller-Parker *et al.* 1996). Although no previous season or depth studies of zooxanthellae have been carried out in the field in either tropical or temperate habitats, the results of this study show consistencies with studies that have examined ultrastructural changes in response to irradiance intensity. Muller-Parker (1987) examined the tropical anemone, *Aiptasia pulchella* from a shaded mangrove lagoon in 1.5 m of turbid water (maximum summer irradiance of 100 $\mu E.m^{-2} s^{-1}$) and a sun-lit reef flat in 1 m of water (maximum summer irradiance of 1400 $\mu E.m^{-2} s^{-1}$) during the summer in Hawaii. The zooxanthellae in shade anemones contained more

chloroplast thylakoids than sun anemones. Similarly, Berner *et al.* (1987) found that zooxanthellae exposed to different light intensities within the same colony of the tropical Alcyonacean *Litophyton arboreum* displayed differences in ultrastructure; greater chloroplast volume and more thylakoid per chloroplast were observed in the most shaded parts of the coral compared with the upper, well-lit parts.

The higher chloroplast volume in temperate, intertidal *A. viridis* during winter (winter= 32 % and summer= 22 %; table 5.1) is comparable with higher volumes observed in tropical *A. pallida* well-fed and maintained at 5 and 50 $\mu E.m^{-2}$ s^{-1} (low light= 30 % and high light = 18 %; Muller-Parker *et al.*, 1996). The zooxanthellae of *Anemonia viridis* appear to photoadapt to low winter irradiances by increasing chloroplast volume, similar to shade adaptations observed in tropical Anthozoa maintained at low irradiances, which enhance light-harvesting capabilities.

No seasonal effect on chloroplast volume was observed in zooxanthellae at 18 m on Glannafeen and no depth affect was observed during winter. However, when both depth and season were altered (e.g. summer 18 m *vs.* winter 6 m; table 5.3) chloroplast volume was significantly affected; chloroplast volume was higher at 18 m during summer than at 6 m during winter. This suggests an interactive affect of depth and season on chloroplast volume fractions. The presence of lipid and higher starch volume fractions during winter at 6 m, and absence of lipid and smaller starch volume fractions at 18 m during both winter and summer indicate the presence of more favourable conditions for carbohydrate and lipid accumulation at 6 m during winter. Variations in the irradiance with water depth were considered in chapter 2 and will be discussed in terms of variations in zooxanthellae ultrastructure in chapter 6.

The higher lipid and starch volumes in zooxanthellae from summer intertidal anemones compared with winter intertidal anemones (Table 5.1) are comparable with

findings for the laboratory maintained, tropical *Aiptasia pallida*. Muller-Parker *et al.* (1996) found that zooxanthellae in high light (50 $\mu E.m^{-2}\ s^{-1}$) well-fed *A. pallida* contained more lipid and starch volume than zooxanthellae in low light (5 $\mu E.m^{-2}\ s^{-1}$) anemones maintained at 25 °C on a 12h: 12h light:dark cycle. Similarly, in a laboratory study of fed *Anemonia viridis*, Harland *et al.* (1992) observed an increase in zooxanthellae storage lipid after 60 days which was proportional to irradiance levels (10, 100 and 300 $\mu E.m^{-2}\ s^{-1}$).

The presence of lipid suggests higher light intensity however, anemones from the subtidal site were permanently submerged in water of 1 m depth or more. Anemones from the intertidal site would have experienced irradiance levels higher than the subtidal site due to shallower water but similar prevailing irradiance levels to the subtidal site (Chapter 2, section 2.3.3). Lipid may have been present as a result of greater reserves accumulated during the summer under the more stable conditions experienced in the subtidal habitat. The presence of vacuoles with crystals is consistent with the results of Muller-Parker *et al.* (1996) for *A. pallida* maintained in 5 $\mu mol.m^{-2}\ s^{-1}$. The crystals were originally identified by Doyle & Doyle (1940) as calcium oxalate and they noted that the number of crystals increased when the zooxanthellae were placed in the dark (Taylor, 1968). Thus, their low volume fraction may reflect the relatively high light intensity experienced in this shallow subtidal habitat.

At 18 m on Glannafeen cliff no photoadaptive differences were evident between zooxanthellae chloroplast volumes from *Anthopleura ballii* collected during winter and summer. This may reflect a negligible difference in light intensities between summer and winter at 18 m in terms of photosynthesis and the compensation irradiance (discussed in chapter 6). The greater chloroplast volume at 18 m during

summer than 6 m during winter would suggest that zooxanthellae are photoadapting to lower irradiances prevailing at 18 m during both winter and summer. Evidence presented by Muller-Parker *et al.* (1996) for laboratory maintained *A. pallida* supports these results. Furthermore, the presence of lipid and higher starch volume fractions at 6 m during winter, where the irradiance levels are higher than at 18 m (Figure 2.15), are consistent with the results of Muller-Parker *et al.* (1996) (see p. 92).

Similarly, Sukenik *et al.* (1989) found that *Nannochloropsis* sp. maintained under high irradiances contained twice as much lipid as cells maintained under low irradiance.

Feeding and nutrient supply may also affect zooxanthellae ultrastructure. Muller-Parker *et al.* (1996) showed that zooxanthellae in low irradiance, starved anemones had 10 times as much lipid (17.4 %) as those in low irradiance well-fed anemones (1.8 %). Similarly, Berner & Izhaki (1994) found that laboratory maintained *Pocillopora damicornis* enriched with nitrogen had lower lipid and starch stores than in nutrient stripped seawater. Ambariyanto and Hoegh-Guldberg (1996) also found that nitrogen addition produced decreases in the size of starch bodies in the zooxanthellae of the giant clam, *Tridacna maxima*. The reduction of lipid under enriched nutrient conditions and starch stores is thought to occur as a result of mobilisation of organic carbon stores in response to stimulated amino acid synthesis and is a possible explanation for variations observed in the present study.

The presence of lipid in intertidal zooxanthellae during summer and at 6 m during winter suggests that, either zooxanthellae are accumulating storage products, or that the pattern of translocation of carbon to the host may change according to seasonal conditions. For example, during the summer months when irradiance conditions are most favourable for photosynthesis and the supply of resources exceeds the

requirements for normal metabolism and growth, zooxanthellae may be able to accumulate lipid and starch. However, other studies show that it remains unknown whether differences in lipid and starch reflect the productivity of zooxanthellae and translocation of photosynthate to the animal host (Muller-Parker *et al.*, 1996). Whether the observed differences are responses to irradiance or food/nutrients or some other seasonally variable factor is discussed in chapter 6.

Chloroplast volume and thylakoid surface density per chloroplast may also be affected by nutrition level. Berner and Izhaki (1994) found thylakoid surface density of chloroplasts in zooxanthellae from *Pocillopora damicornis* was higher in nitrogen enriched water. Conversely, Muller-Parker *et al.* (1996) showed feeding affected chloroplast volume in both high and low irradiance but not thylakoid surface density. Thus, the differences observed here in the ultrastructure of zooxanthellae from 1.5 m in the subtidal habitat and > 1 m in the intertidal habitat and 6 m and 18 m water depths may be photoadaptation, but may also be the product of nutritional differences between the hosts.

Other factors that may vary with water depth and season in the field besides light intensity and nutrients/food include spectral composition of light, water temperature, pressure and plankton. Light intensity and spectral composition were considered in the laboratory in an attempt to control all other variables encountered under field conditions. In laboratory maintained *A. viridis* zooxanthellae had higher thylakoid volumes in medium intensity light than low intensity light. This contradicts the observations of Muller-Parker *et al.* (1996) which showed higher thylakoid volumes in chloroplasts from zooxanthellae in *Aiptasia pallida* in low intensity irradiance. In the present study, zooxanthellae exposed to spectrally altered irradiance showed higher chloroplast volume in full spectrum low intensity irradiance, than in either red

or green irradiance, but no effect on thylakoid volume was observed. In addition the presence of accumulation bodies in zooxanthellae exposed to spectrally altered irradiance indicate more waste products from metabolic decomposition were being formed, and suggest zooxanthellae were in poor health. If the influence of depth and season affect observed for zooxanthellae from *A. ballii* in Lough Hyne was due to differences in spectral composition, comparable results might be expected for anemones in red light and 6 m, where there was still a relatively high proportion of red light, and green light and 18 m where light attenuation produced a spectrum of light with a high proportion of green light (see chapter 2, section 2.3.3). However, no comparable results were obtained for field and laboratory anemones.

Mitochondria and nuclei showed no significant variations among field or laboratory sampled anemones. Comparable with these findings, Berner & Izhaki (1994) found no effect of nitrogen levels on nuclei, mitochondria or vacuoles on zooxanthellae of *Pocillopora damicornis*. Similarly, Muller-Parker *et al.* (1996) also found that relative volumes of pyrenoids and nuclei in zooxanthellae from *Aiptasia pallida* were conserved under low and high irradiances, and different feeding histories of the host, and concluded that lipid and starch were the cell components most responsive to irradiance and nutrient supply. The results presented here for field populations suggest the same may be true for zooxanthellae symbiotic with temperate hosts. Finally, vacuoles with crystals were conspicuous in zooxanthellae from all laboratory maintained *A. viridis* and from 18 m summer *A. ballii* which relates directly to low light intensity (see Doyle & Doyle, 1940).

Conclusions

The zooxanthellae of *Anemonia viridis* appear to photoadapt to low winter irradiances by increasing the chloroplast volume, however this may also be a response to seasonal fluctuations in nutrient levels or food availability and thus requires confirmation through controlled laboratory experiments. Similarly, starch and lipid stores in zooxanthellae from both *A. viridis* and *A. ballii* were responsive to seasonal fluctuations, whilst in *A. viridis* these stores were not responsive to low light intensities and spectral composition in the laboratory. Future work would involve feeding and light regimes similar to the investigations of Muller-Parker *et al.* (1996) but with levels of artificial irradiance high enough to be comparable with those experienced in the field during summer. No ultrastructural photoadaptation appeared to occur between different depths in winter, whilst no summer comparison was available. Thus, more extensive investigation of depth effects in the field are required. Photoadaptive ultrastructure changes in temperate symbiotic Anthozoa in general requires investigation.

Table 5.1. Volume fractions (%) of organelles and storage products in zooxanthellae from intertidal *Anemonia viridis* collected during winter (2/98) and summer (8/98) from Trearddur Bay, Anglesey. Mean percentages are shown ± 1 standard error. . n = number of zooxanthella sections analysed.

Table 5.2. Volume fractions (%) of organelles and storage products in zooxanthellae from subtidal *Anemonia viridis* collected during winter (2/98) from Castle Island Bay, Lough Hyne. Mean percentages are shown ± 1 standard error.

	Chloroplast	Nucleus	Starch	Mitochondria	Lipid
WINTER n= 11	**32.1** ± 1.5	**20.1** ± 2.0	**11.1** ± 2.0	**3.1** ± 0.5	**0**
SUMMER n=6	**21.8** ± 2.1	**16.7** ± 1.7	**8.0** ± 2.0	**2.2** ± 0.4	**19.8** ± 3.4

	Chloroplast	Nucleus	Starch	Mitochondria	Lipid	Vacuole
WINTER n= 10	**34.9** ± 2.0	**16.2** ± 1.6	**12.9** ± 2.2	**5.2** ± 0.5	**2.4** ± 1.8	**0.9** ± 0.5

Table 5.3. Volume fractions (%) of organelles and storage products in zooxanthellae from subtidal *Anthopleura ballii* collected during summer (8/98) and winter (2/98) from 6 m and 18 m on Glannafeen cliff, Lough Hyne. Mean percentages are shown ± 1 standard error. . n = number of zooxanthella sections analysed.

	Chloroplast	Nucleus	Starch	Mitochondria	Lipid	Vacuole
WINTER 6 m n= 4	**22.0** ± 3.2	**10.0** ± 0.4	**14.3** ± 4.2	**2.0** ± 0.7	**18.0** ± 10.4	**0**
WINTER 18 m n= 9	**34.2** ± 2.5	**12.7** ± 1.2	**4.7** ± 1.6	**3.1** ± 0.7	**0**	**0**
SUMMER 18 m n= 13	**41.4** ± 3.2	**15.3** ± 1.9	**4.7** ± 1.1	**3.5** ± 0.4	**0**	**19.5** ± 2.0

Table 5.4. Volume fractions (%) of organelles and storage products in zooxanthellae from *Anemonia viridis* maintained 4 μmol m^{-2} s^{-1} and 20 μmol m^{-2} s^{-1}. Mean percentages are shown ± 1 standard error. n = number of zooxanthella sections analysed.

Table 5.5. Volume fractions (%) of organelles and storage products in zooxanthellae from *Anemonia viridis* maintained in irradiance of altered spectral composition. Tank 1 (4 μmol m^{-2} s^{-1}), tank 4 (green colour filter; 3 μmol m^{-2} s^{-1}) and tank 3 (red colour filter; 3 μmol m^{-2} s^{-1}). Mean percentages are shown ± 1 standard error. n = number of zooxanthella sections analysed.

	Chloroplast	Nucleus	Starch	Mitochondria	Vacuole
4 μmol m^{-2} s^{-1}	**42.5**	**16.1**	**2.4**	**4.9**	**15.1**
n= 28	± 1.5	± 1.2	± 0.4	± 0.4	± 1.6
20 μmol m^{-2} s^{-1}	**38.8**	**14.3**	**2.9**	**4.7**	**17.6**
n= 21	± 1.2	± 0.8	± 0.6	± 0.4	± 1.7

	Chloroplast	Nucleus	Starch	Mitochondria	Vacuole	Accumulation body
4 μmol m^{-2} s^{-1}	**42.5**	**16.1**	**2.4**	**4.9**	**15.1**	**0**
n= 28	± 1.5	± 1.2	± 0.4	± 0.4	± 1.6	
GREEN	**33.0**	**13.9**	**5.9**	**4.3**	**18.9**	**1.9**
n= 17	± 2.1	± 0.7	± 1.1	± 0.5	± 3.0	± 1.1
RED	**34.5**	**14.9**	**5.4**	**4.8**	**14.8**	**0.4**
n= 20	± 1.9	± 0.7	± 0.7	± 0.5	± 2.3	± 0.4

	Light treatment			
	4 μmol m^{-2} s^{-1}	20 μmol m^{-2} s^{-1}	Green	Red
Thyl. (%)	**30.0**	**37.0**	**36.5**	**37.5**
S.E.	± 2.3	± 1.3	± 5.1	± 2.8

Table 5.6. Volume fractions (%) of thylakoid membranes (Thyl.) in chloroplasts of zooxanthellae from *Anemonia viridis* exposed to irradiance of different intensity and spectral composition. Treatments were 4 μmol m^{-2} s^{-1} (tank 1); 20 μmol m^{-2} s^{-1} (tank 2) green= green colour filter (tank 4); red= red colour filter (tank 3). Mean percentages are shown (n= 6 zooxanthella sections) ± 1 standard deviation.

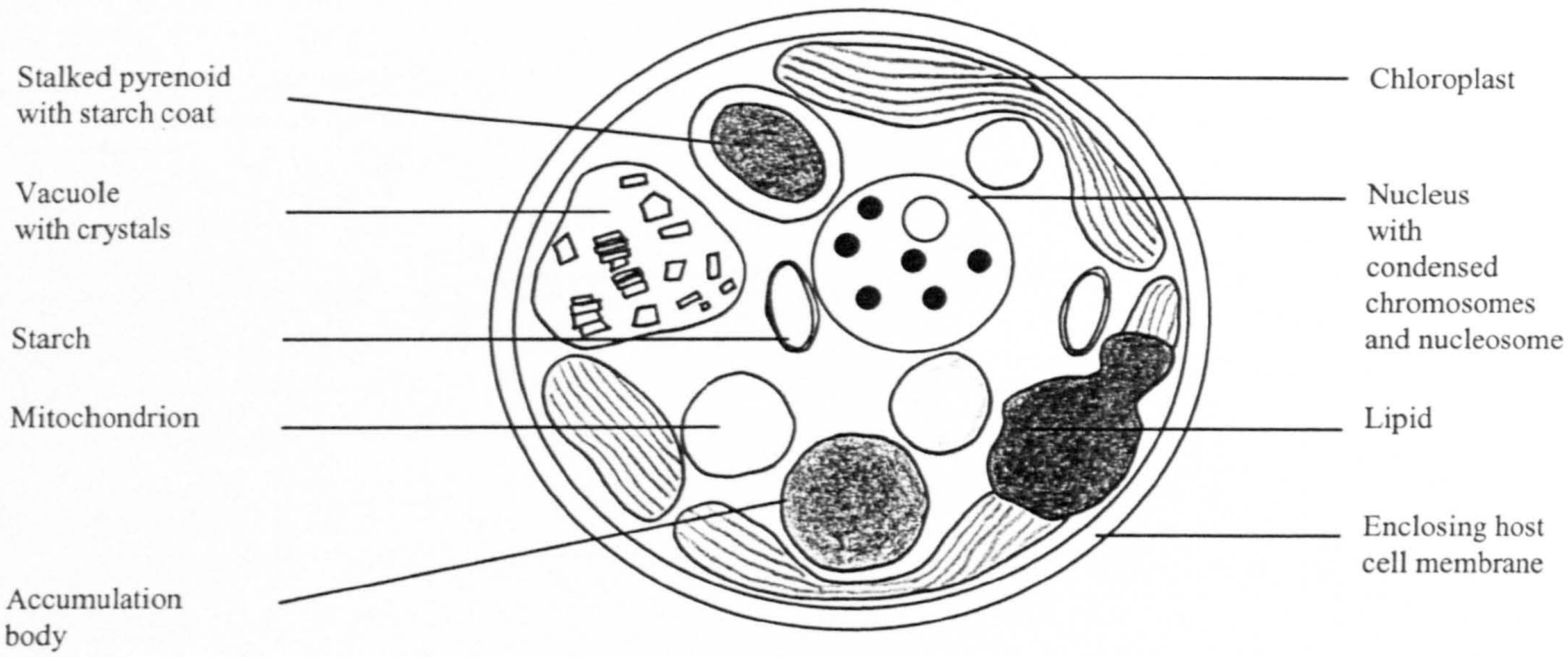
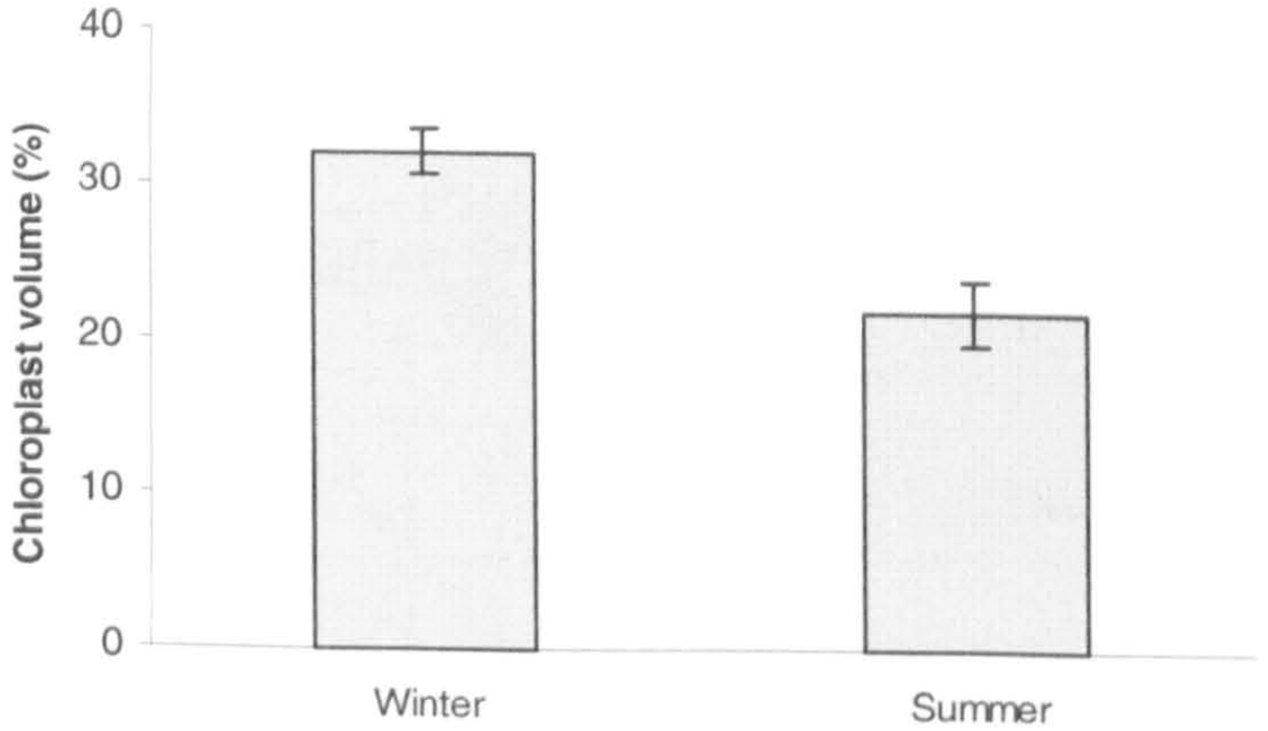


Figure 5.1. The main ultrastructural features of the zooxanthella *Symbiodinium*.

Figure 5.2. Volume fractions (%) of a) chloroplasts and b) starch in zooxanthellae from intertidal *Anemonia viridis* collected during summer (8/98) and winter (2/98) from Castle Island Bay, Lough Hyne, Ireland. Error bars show ± 1 standard error. Chloroplast volume is significantly lower in summer anemones.

a)


Chloroplast volume (%)
40
30
20
10
0
Winter
Summer

b)

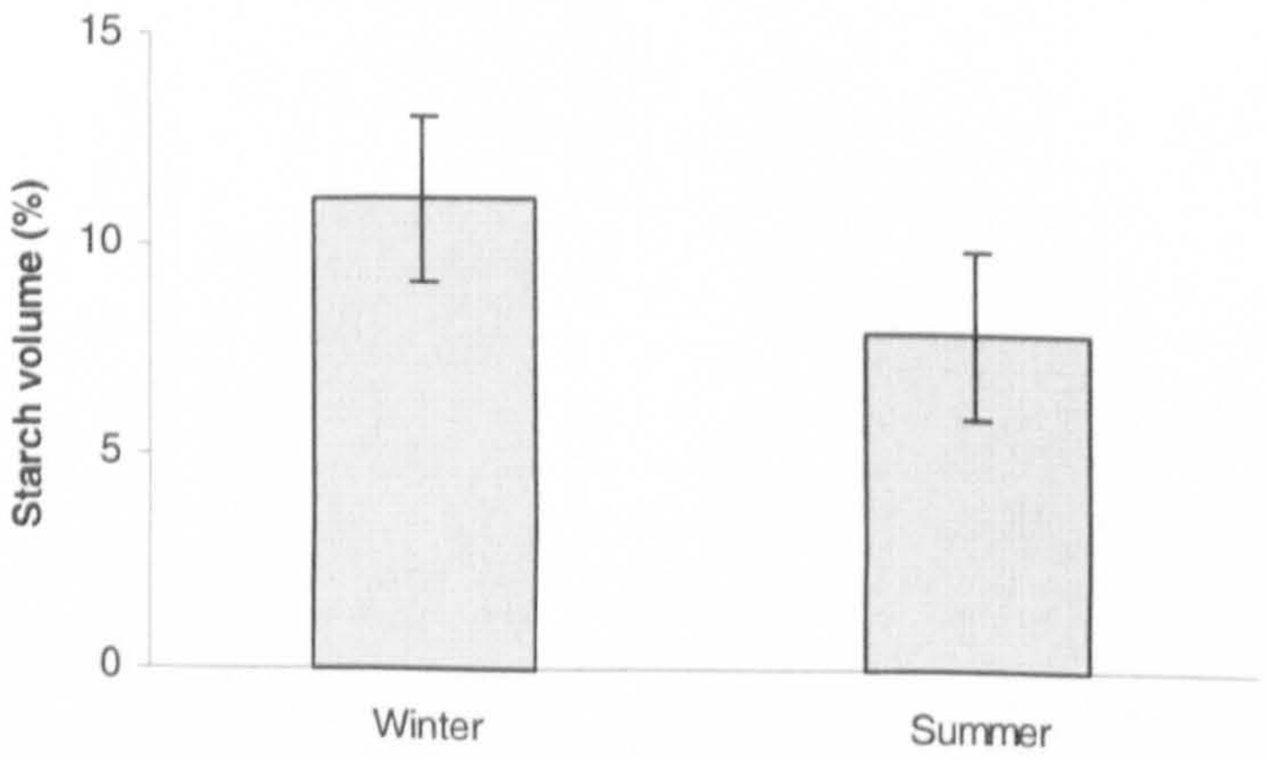
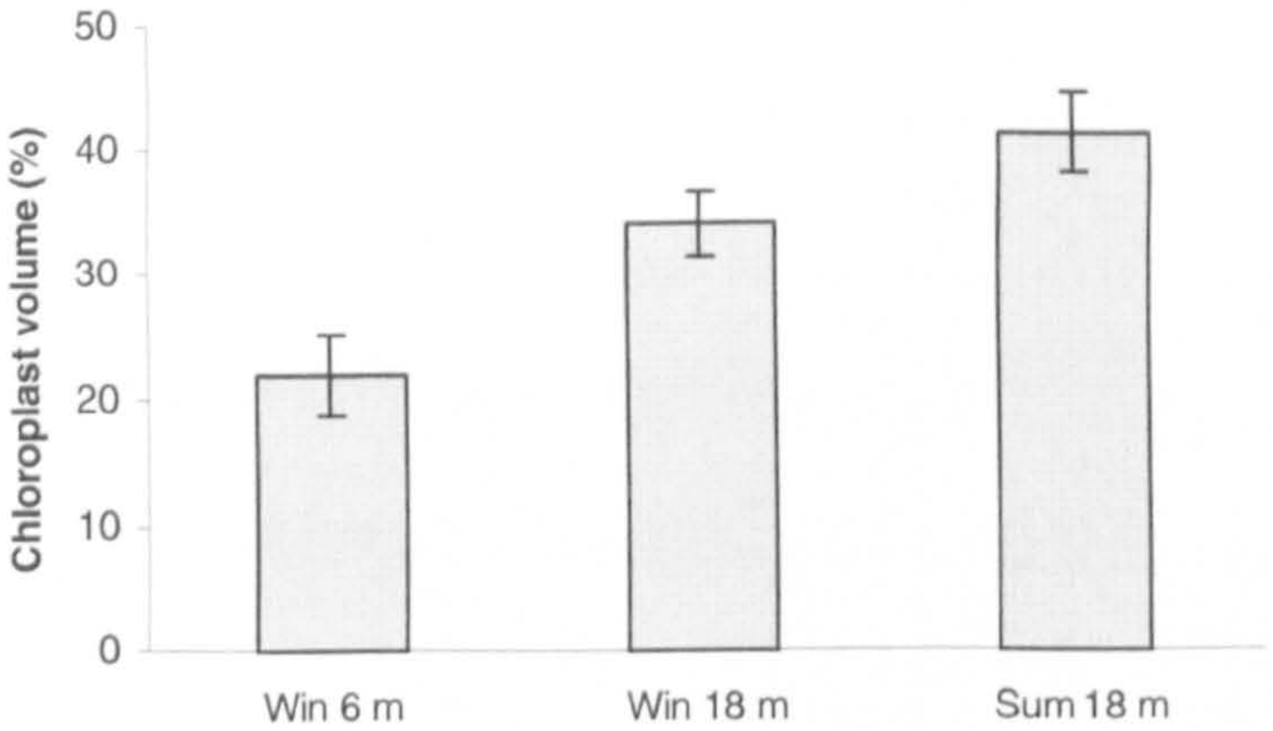

Starch volume (%)
15
10
5
0
Winter
Summer

Figure 5.3. Volume fractions (%) of a) chloroplast and b) starch in zooxanthellae from subtidal *Anthopleura ballii* at 6 m and 18 m collected during summer (8/98) and winter (2/98). Error bars show ± 1 standard error.

a)


Chloroplast volume (%)
50
40
30
20
10
0
Win 6 m
Win 18 m
Sum 18 m

b)

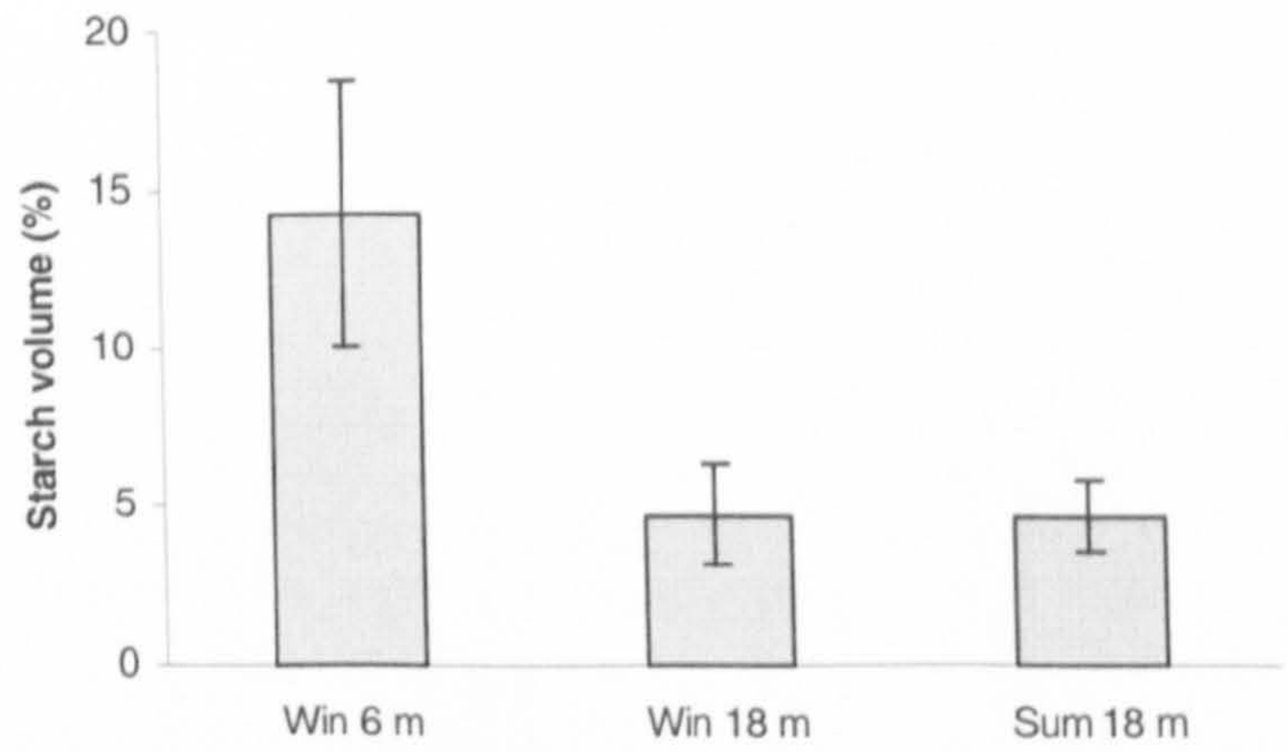
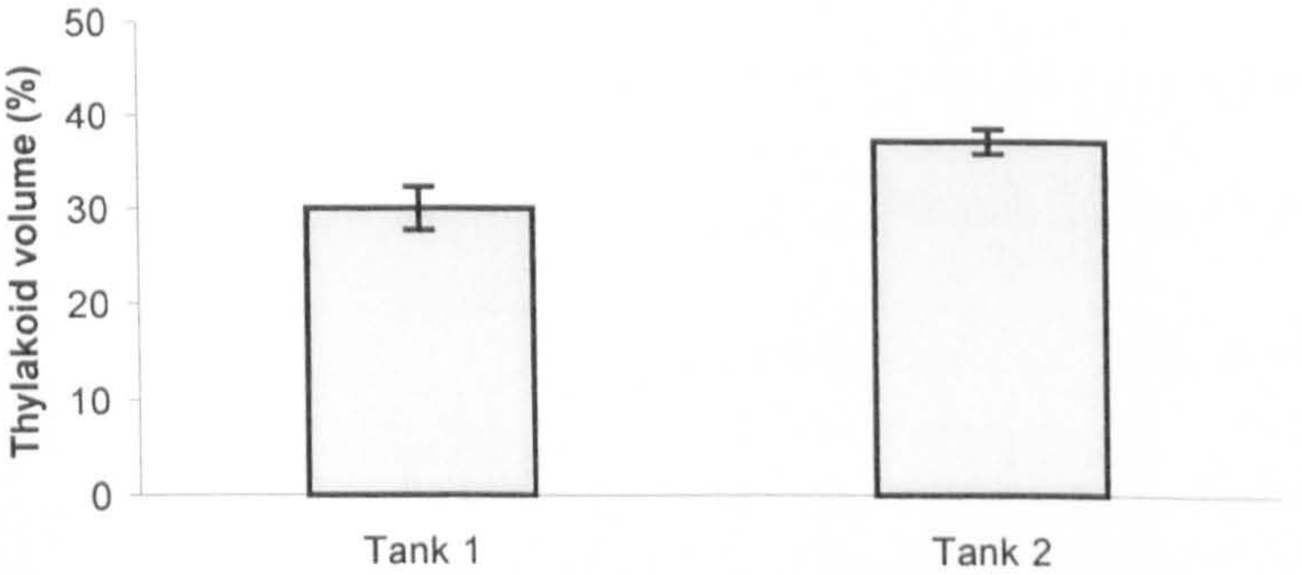

Starch volume (%)
20
15
10
5
0
Win 6 m
Win 18 m
Sum 18 m

Figure 5.4. Thylakoid volume fraction (%) in zooxanthellae chloroplasts from laboratory maintained *Anemonia viridis* in a) tank 1 = 4 μmol m^{-2} s^{-1}; tank 2 = 20 μmol m^{-2} s^{-1} and b) tank 1 = 4 μmol m^{-2} s^{-1} full spectrum irradiance; tank 4 = green irradiance (3 μmol m^{-2} s^{-1}); tank 3 = red irradiance (3 μmol m^{-2} s^{-1}). Error bars show ± 1 standard error.

a)


Thylakoid volume (%)
50
40
30
20
10
0
Tank 1
Tank 2

b)

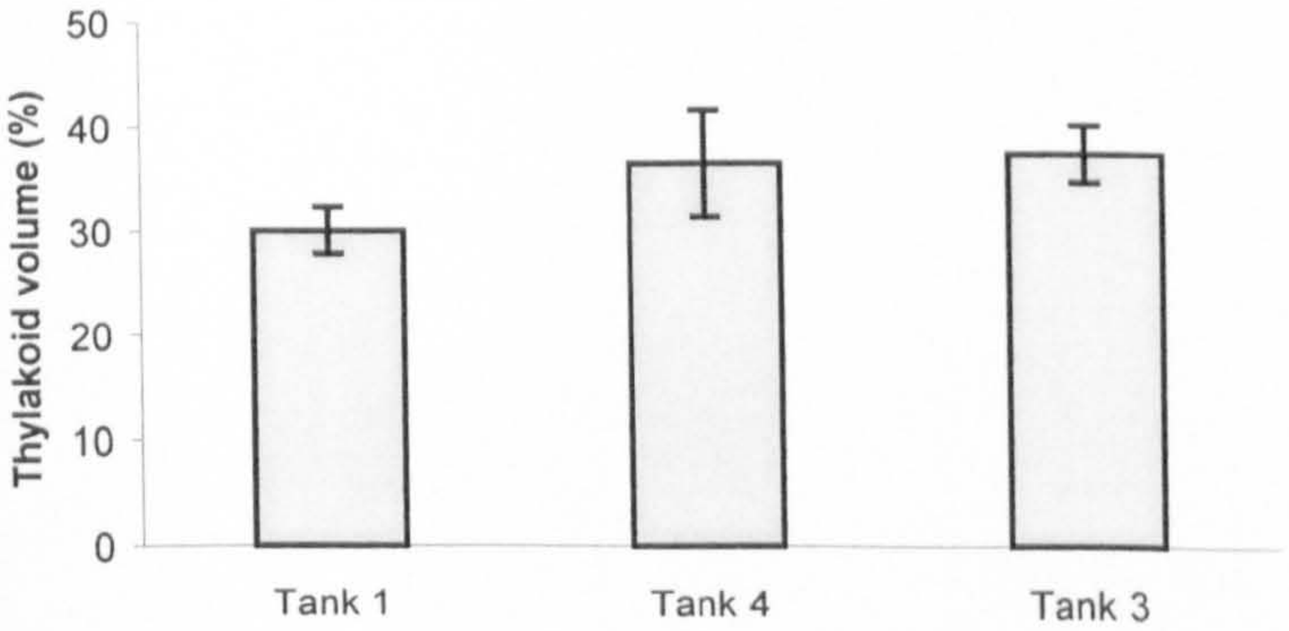
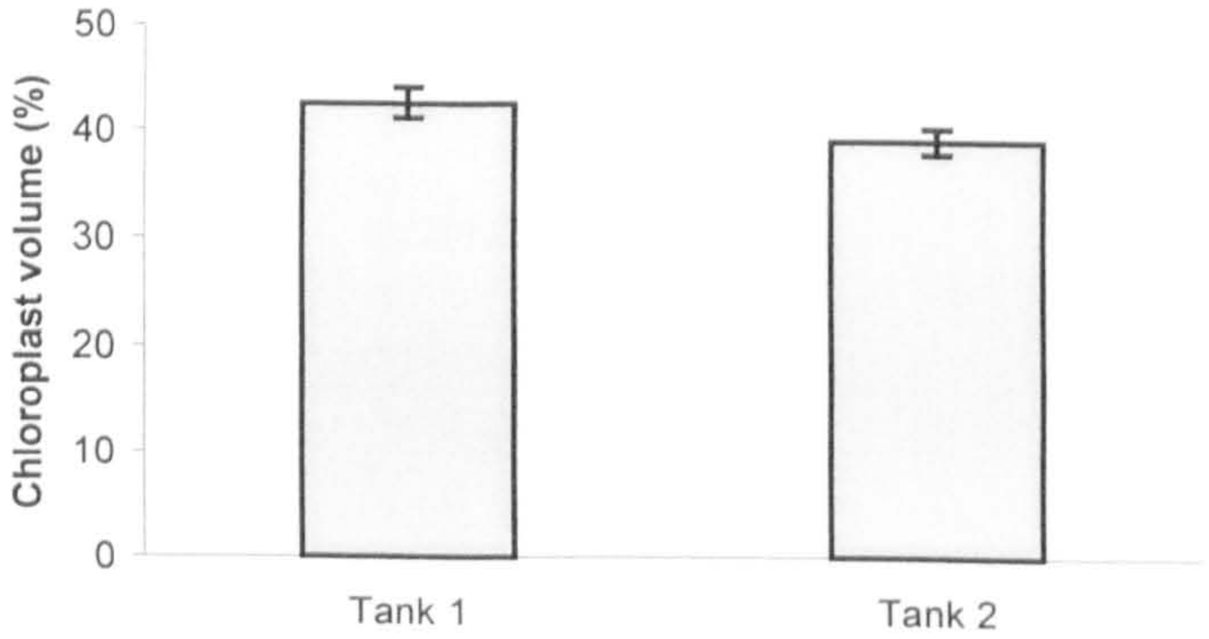

Thylakoid volume (%)
50
40
30
20
10
0
Tank 1
Tank 4
Tank 3

Figure 5.5. Volume fractions (%) of a) chloroplasts and b) starch in zooxanthellae from laboratory maintained *Anemonia viridis* in 4 μmol m^{-2} s^{-1} (tank 1) and 20 μmol m^{-2} s^{-1} (tank 2) artificial irradiance. Error bars show ± 1 standard error.

a)


Chloroplast volume (%)
50
40
30
20
10
0
Tank 1
Tank 2

b)

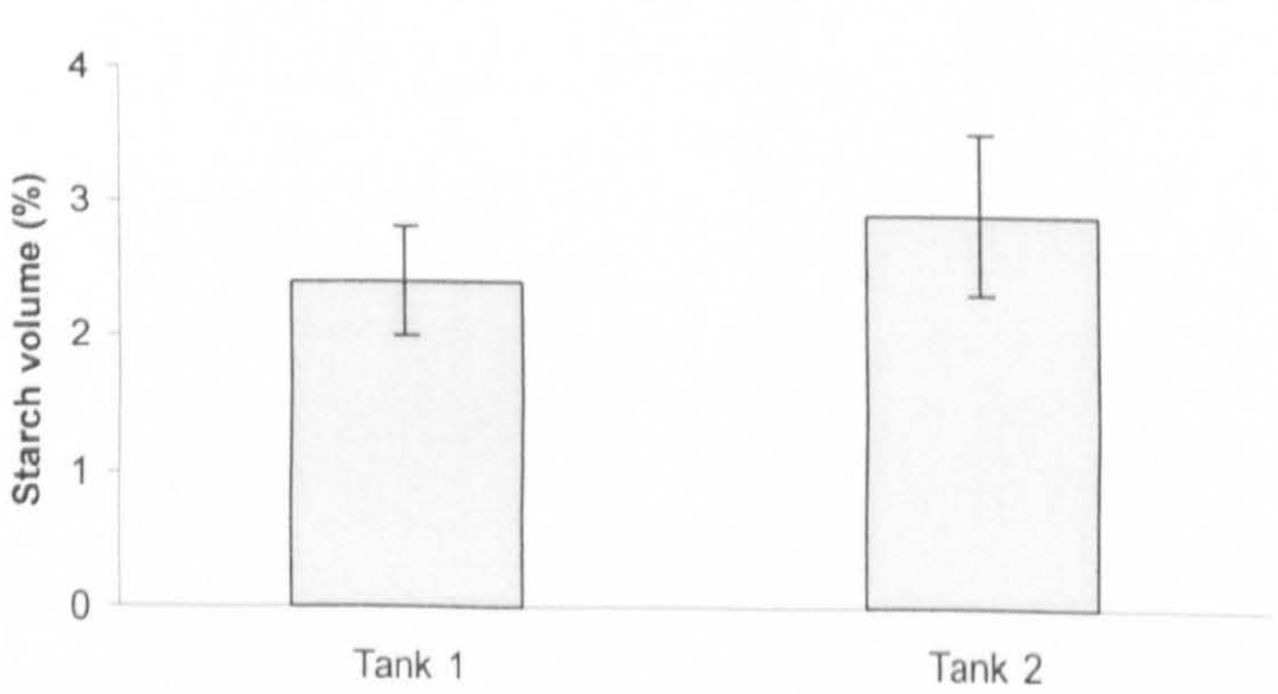
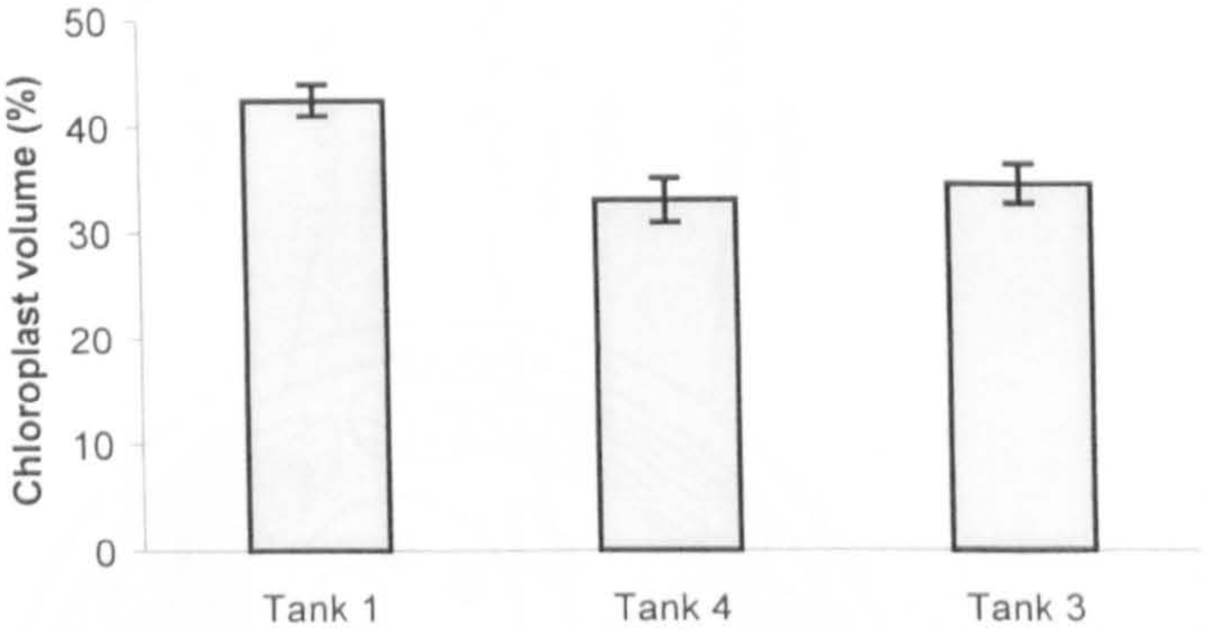
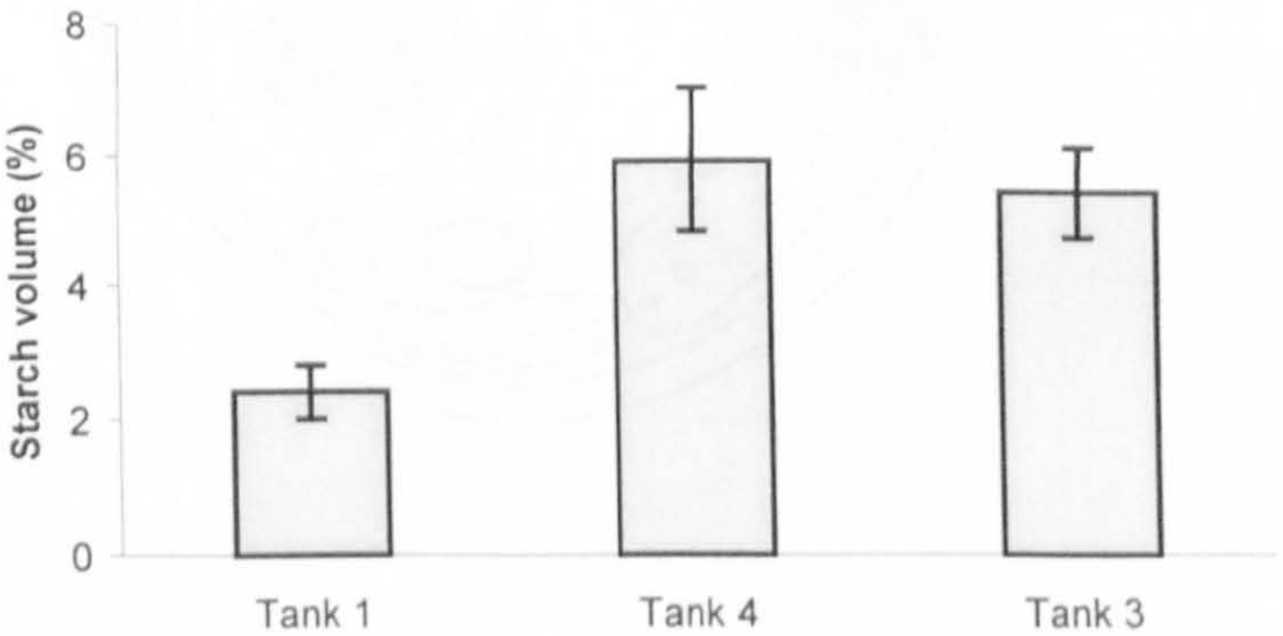

Starch volume (%)
4
3
2
1
0
Tank 1
Tank 2

Figure 5.6. Volume fractions (%) of a) chloroplast volume b) starch and c) accumulation bodies in zooxanthellae from laboratory maintained *A. viridis* in full spectra irradiance (tank 1, 4 μmol m^{-2} s^{-1}) green irradiance (tank 4, 3 μmol m^{-2} s^{-1}) and red irradiance (tank 3, 3 μmol m^{-2} s^{-1}). Error bars show ± 1 standard error.

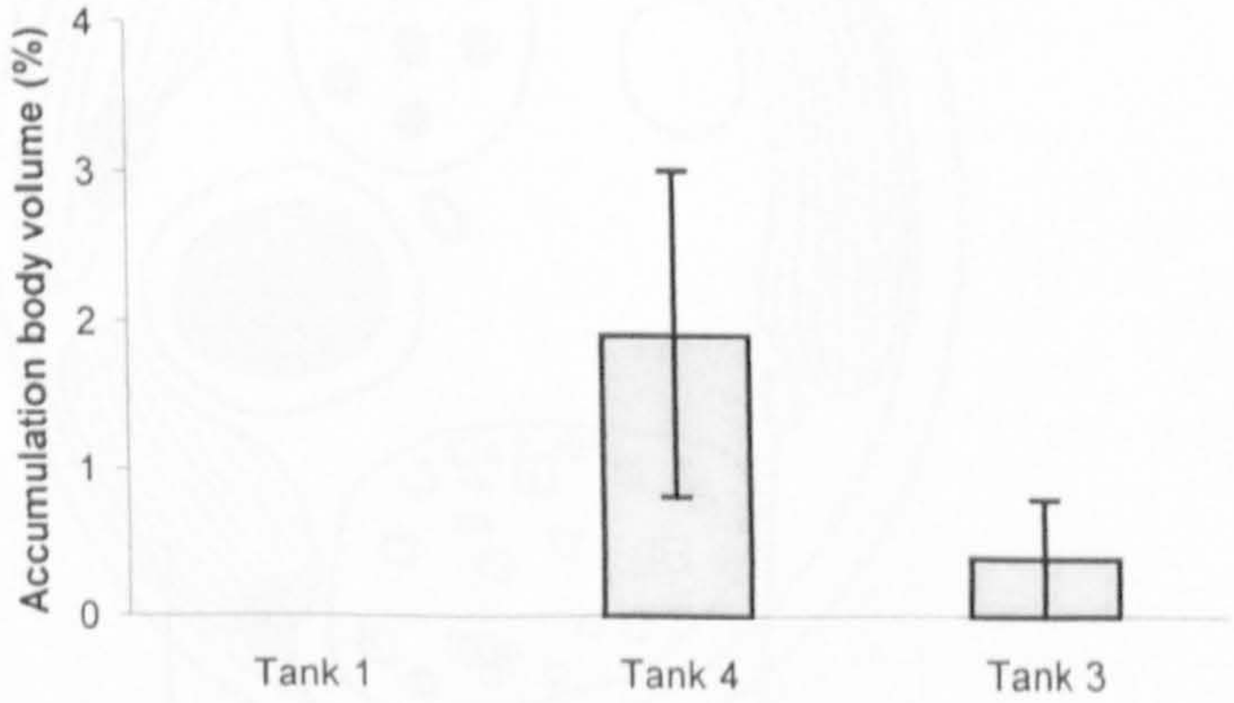
a)


Chloroplast volume (%)
50
40
30
20
10
0
Tank 1
Tank 4
Tank 3

b)


Starch volume (%)
8
6
4
2
0
Tank 1
Tank 4
Tank 3

c)


Accumulation body volume (%)
4
3
2
1
0
Tank 1
Tank 4
Tank 3

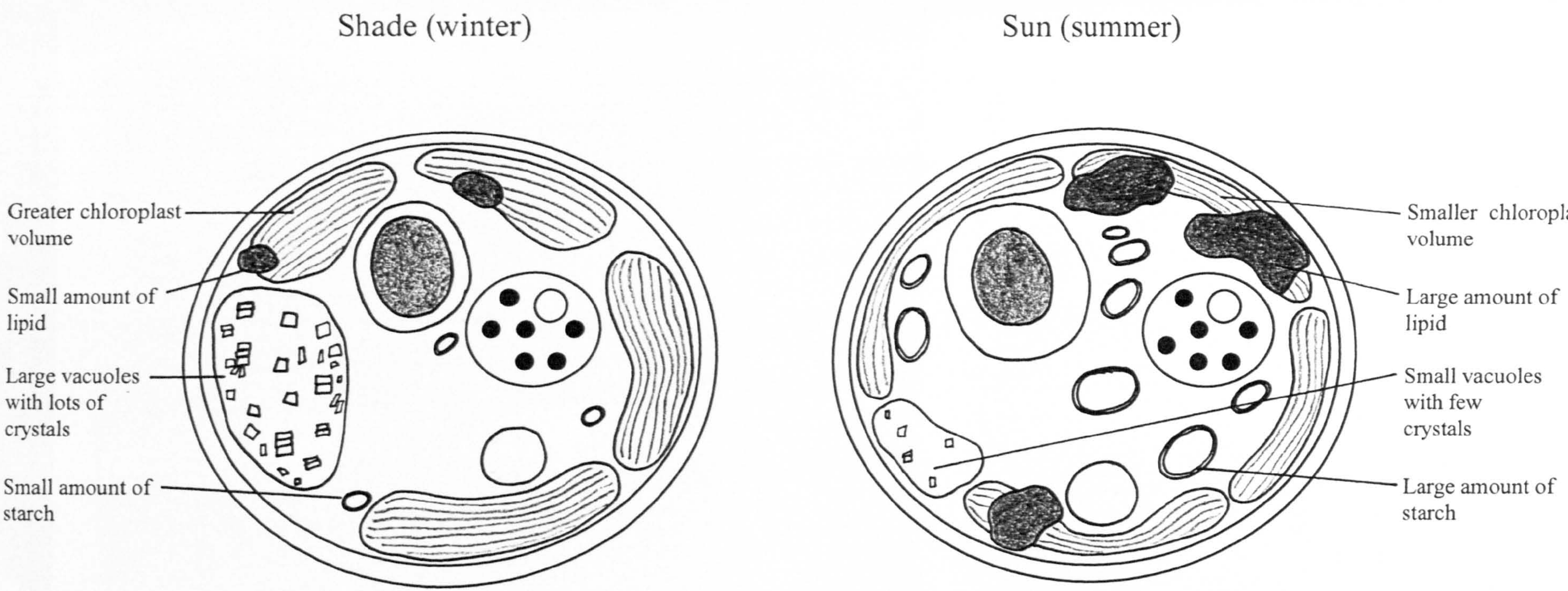
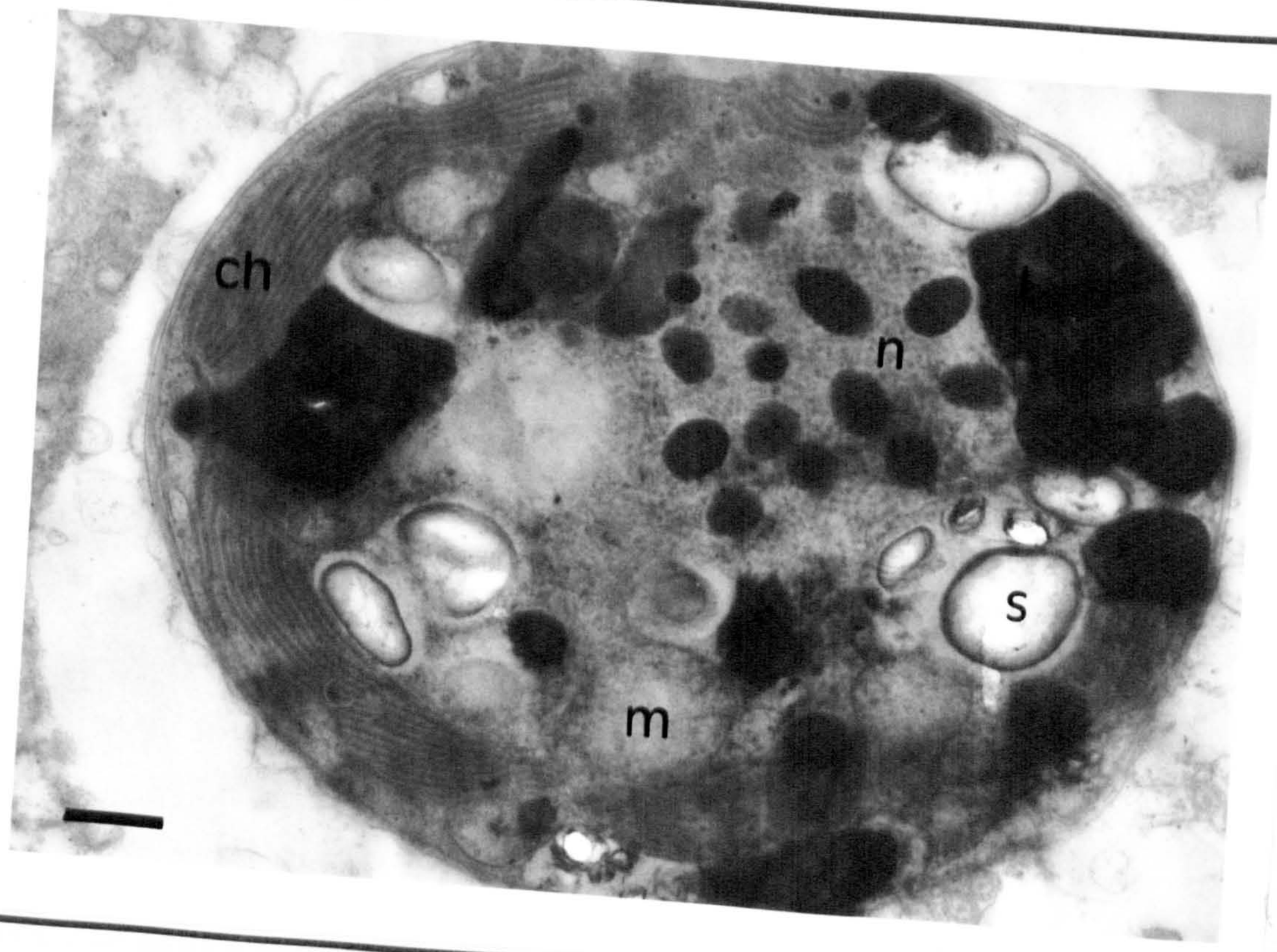


Figure 5.7. Summary of the effects of shade (winter) and sun (summer) on zooxanthellae ultrastructure in temperate Anthozoa.

Plate 5.1a. The ultrastructure of a zooxanthella from the tentacle of intertidal *Anemonia viridis* from Trearddur Bay during summer (7/98). ch= chloroplast, l= lipid, m= mitochondrion, n= nucleus, s= starch. Scale = 2 μm.

Plate 5.1b. The ultrastructure of a zooxanthella from the tentacles of intertidal *Anemonia viridis* from Trearddur Bay during winter (1/98). p=pyrenoid, s= starch. Scale = 2 μm.


ch
n
s
m

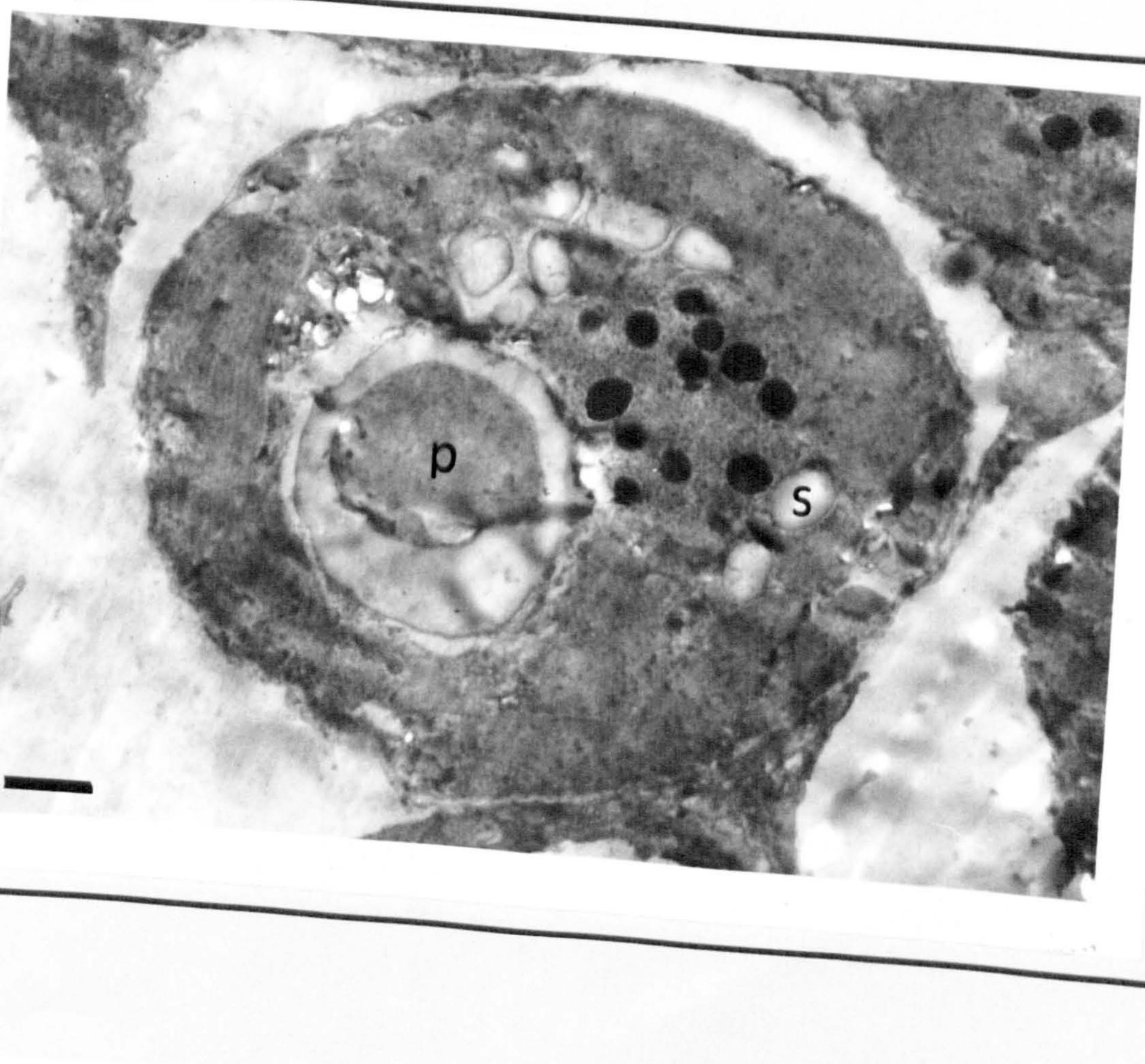
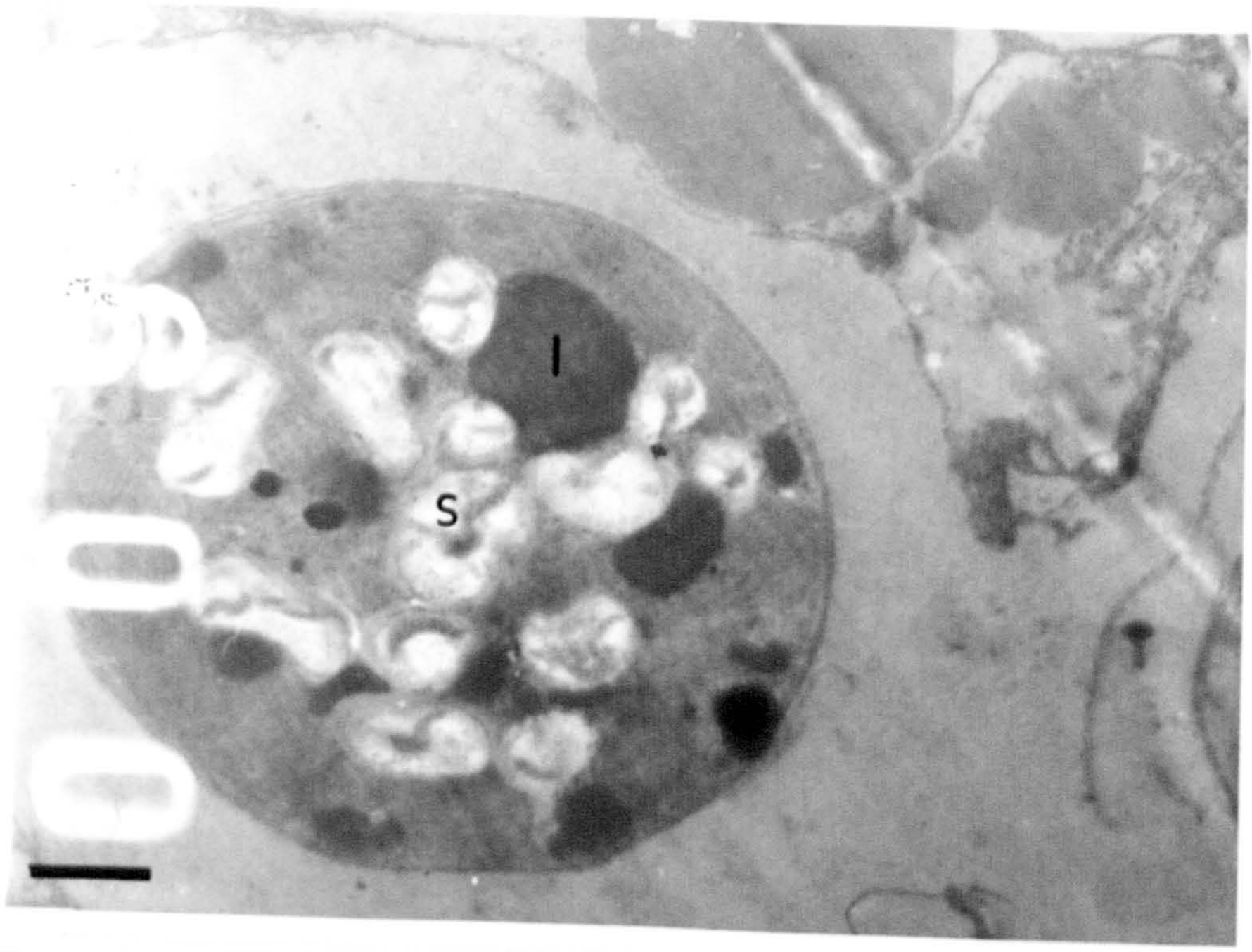

p
s

Plate 5.2. The ultrastructure of a zooxanthella from the tentacles of subtidal *Anemonia viridis* from Castle Island Bay, Lough Hyne during winter (2/98). l = lipid, s= starch. Scale = 1 μm.

Plate 5.3a. The ultrastructure of a zooxanthella from the tentacles of subtidal *Anthopleura ballii* from Glannafeen cliff. Lough Hyne at 6 m during winter (2/98). l= lipid, p=pyrenoid. Scale = 1 μm.


I
S

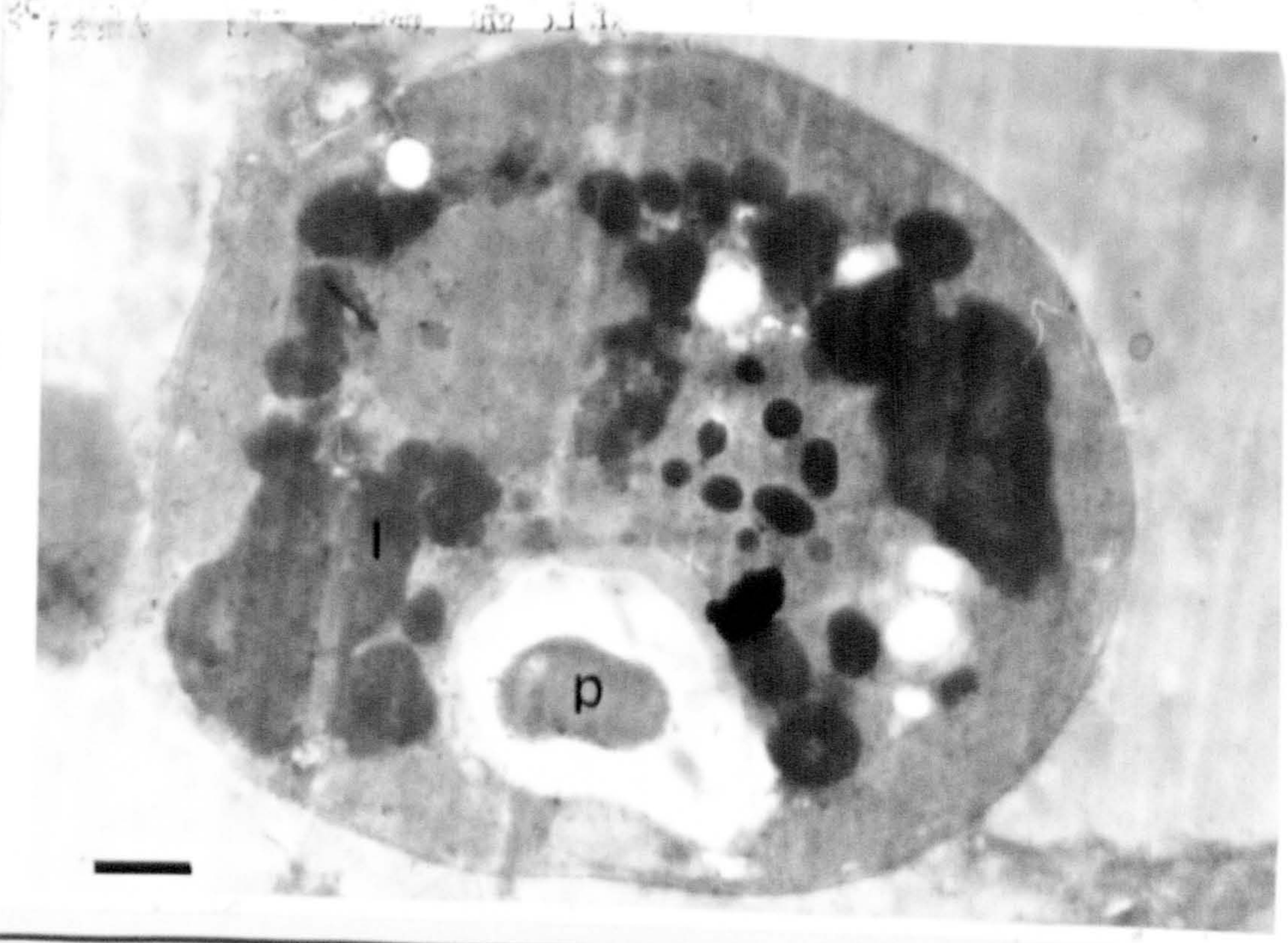

I
p

Plate 5.3b. The ultrastructure of a zooxanthella from the tentacles of subtidal *Anthopleura ballii* from Glannafeen cliff, Lough Hyne at 18 m during winter (2/98). *s*= starch. Scale = 2 μm.

Plate 5.3c. The ultrastructure of a zooxanthella from the tentacles of subtidal *Anthopleura ballii* from Glannafeen cliff, Lough Hyne at 18 m during summer (7/98). s= starch, *va*= vacuole with "calcium oxalate crystals". Scale = 2 μm.


S

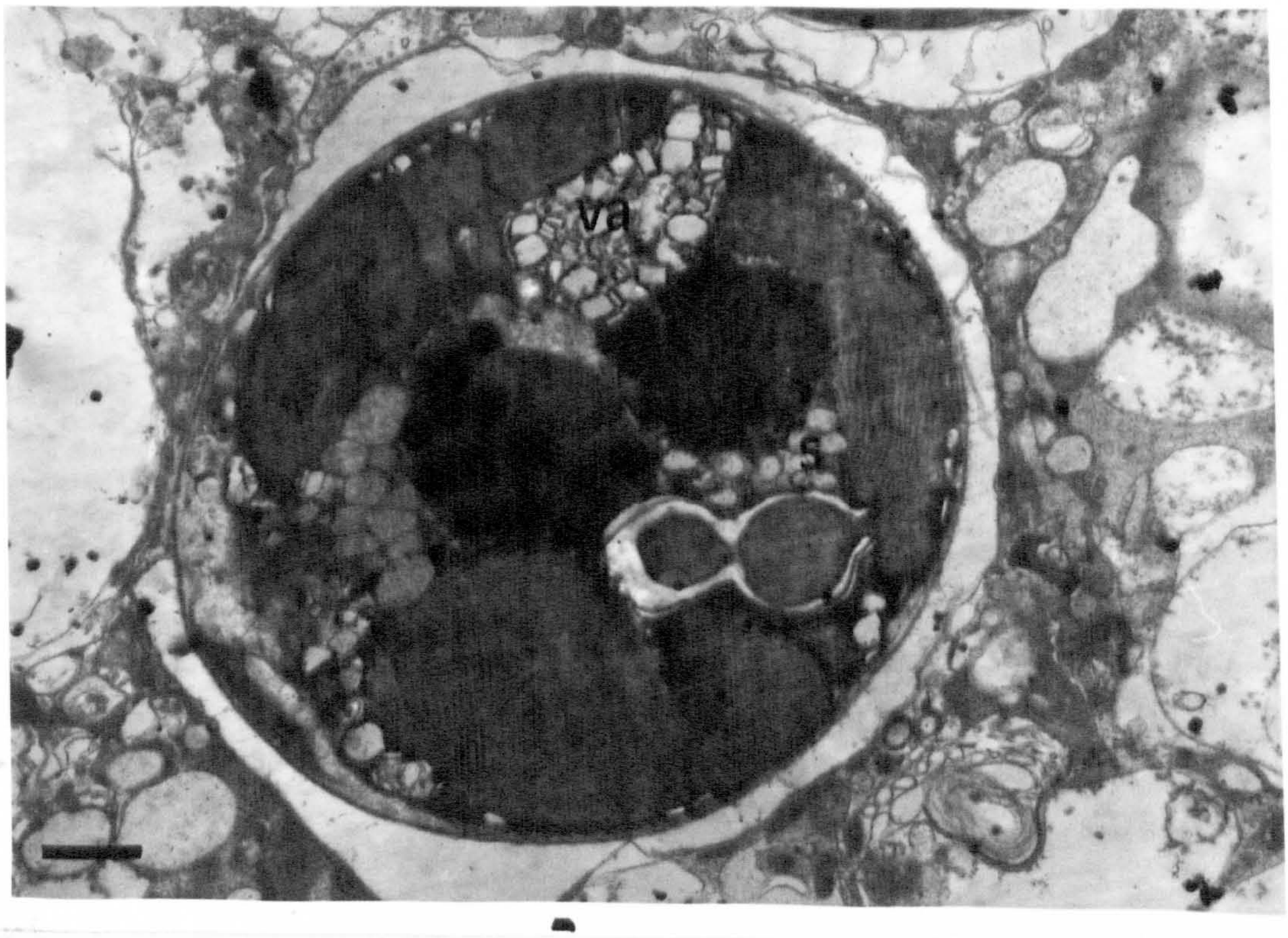
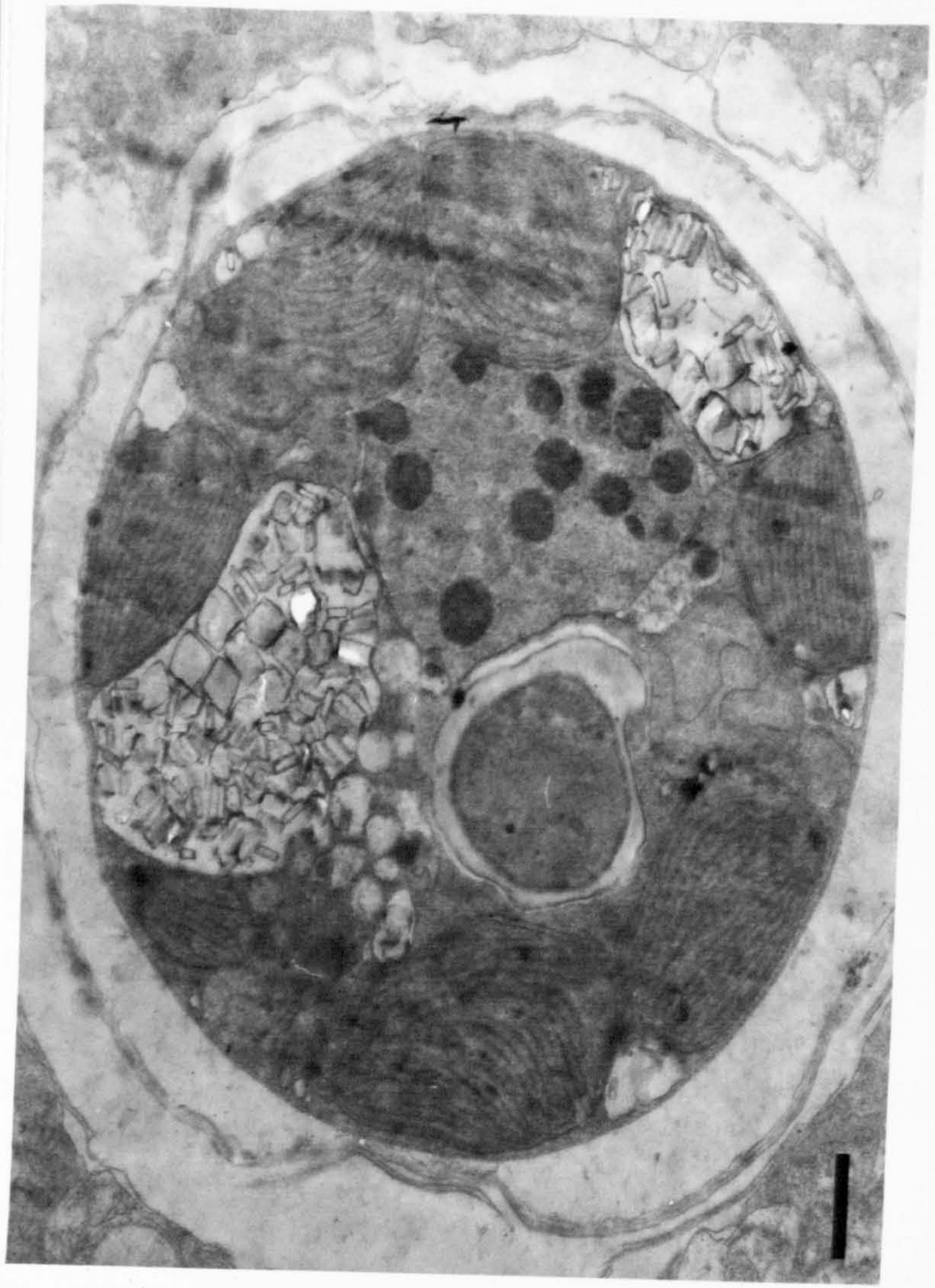

va
S

Plate 5.4a. The ultrastructure of a zooxanthella from the tentacles of *Anemonia viridis* maintained 4 μmol m^{-2} s^{-1} artificial irradiance (tank 1). Scale = 1 μm.

Plate 5.4b. The ultrastructure of a zooxanthella from the tentacles of *Anemonia viridis* maintained 20 μmol m^{-2} s^{-1} artificial irradiance (tank 2). Scale = 1 μm.



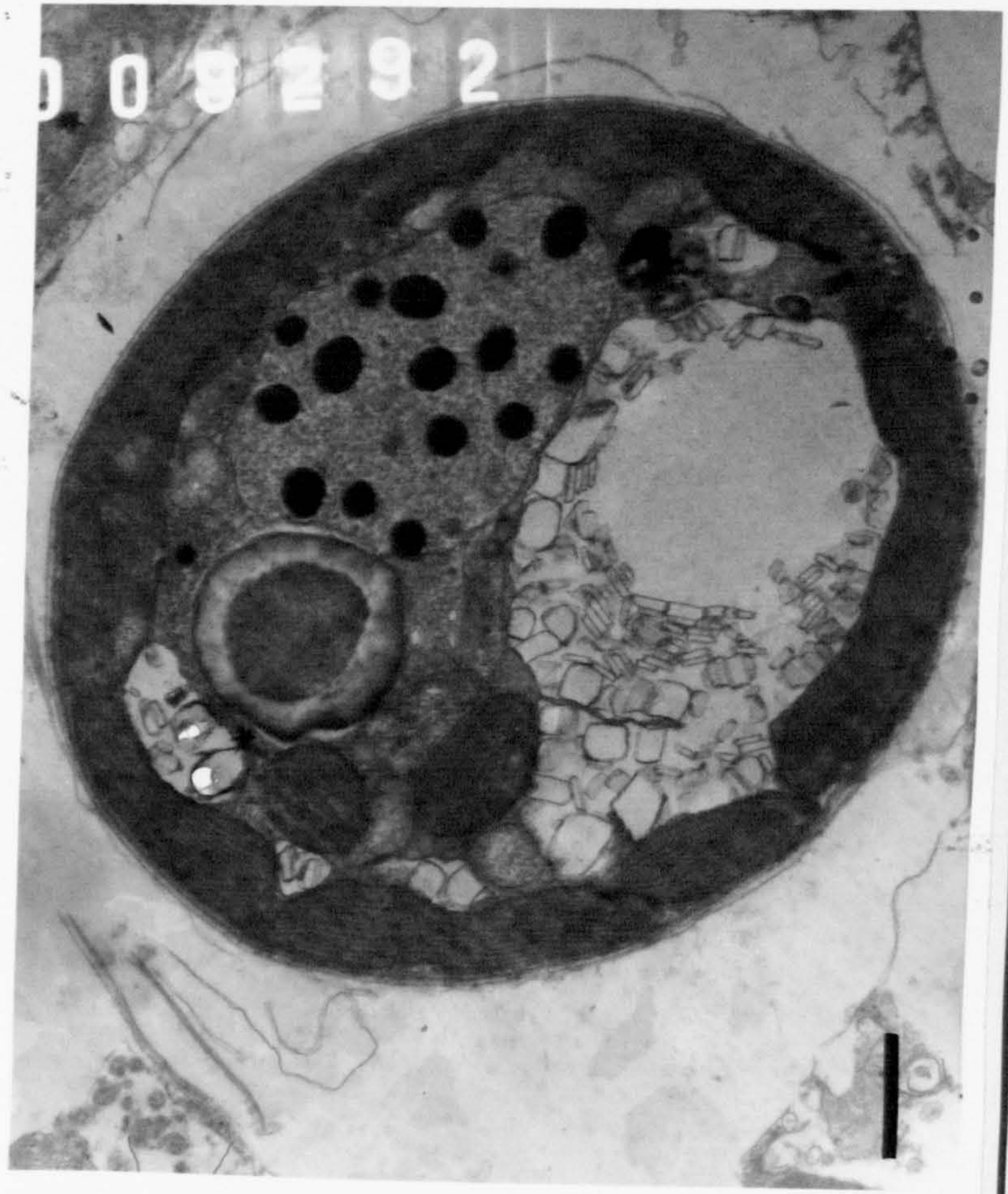
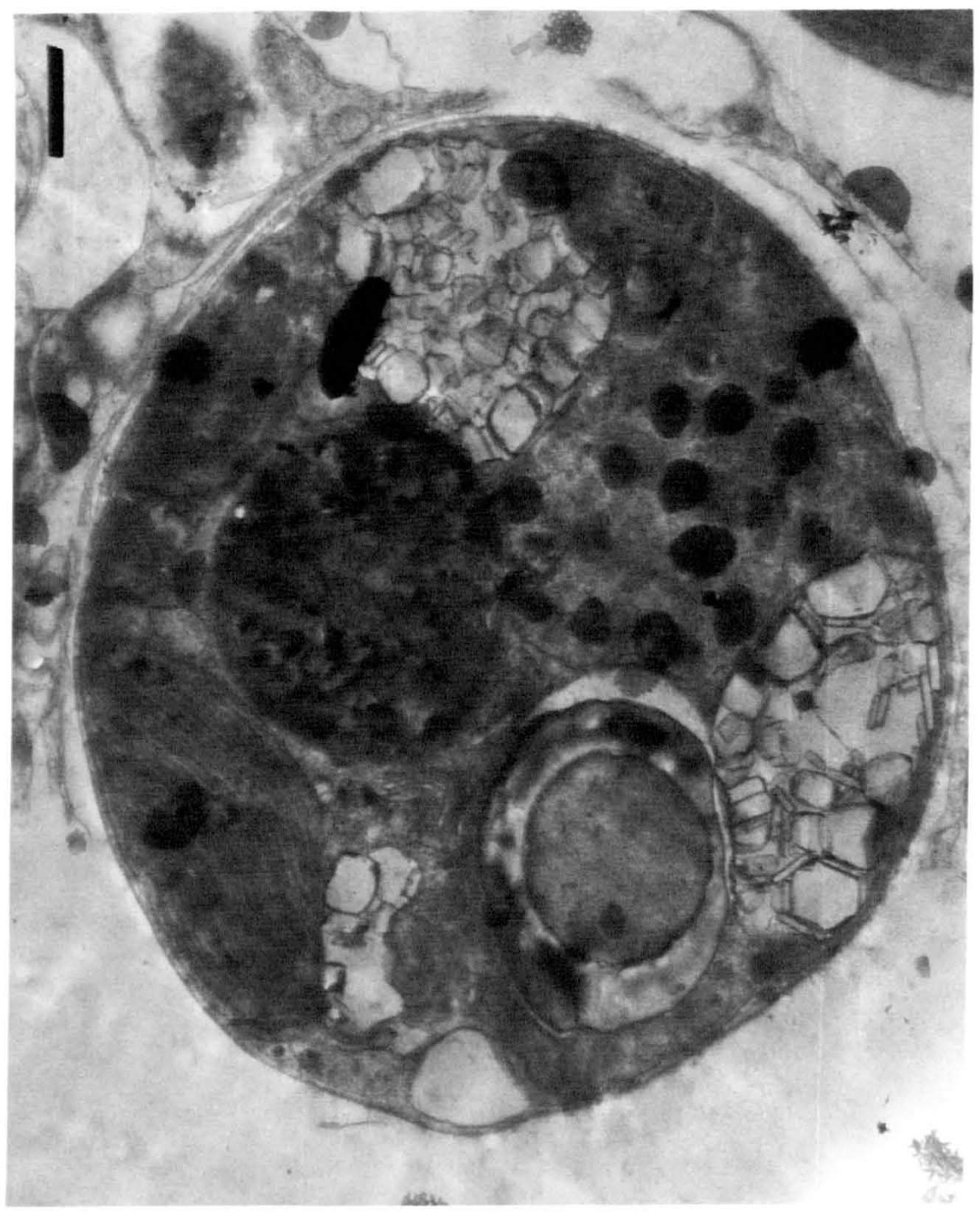
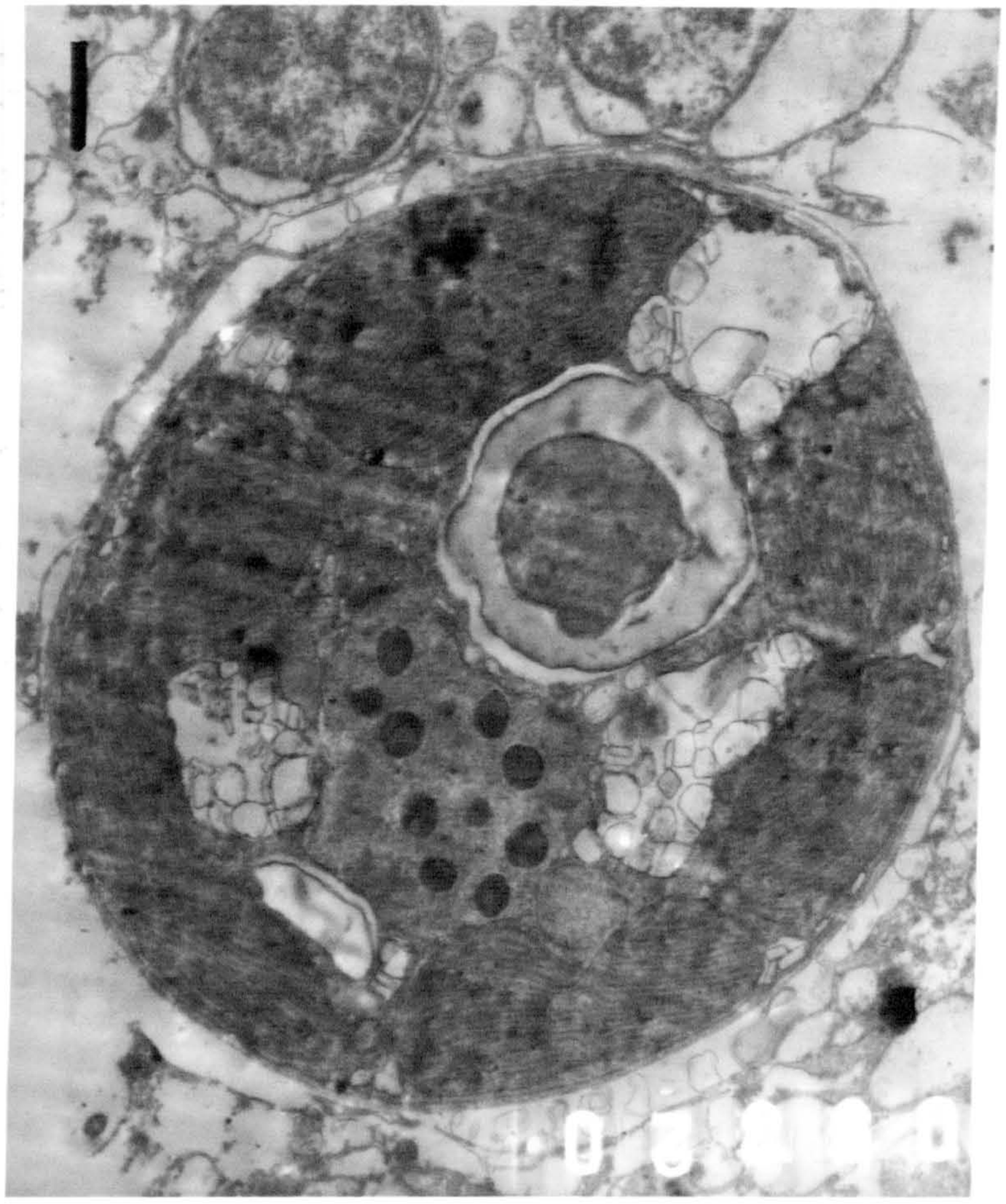

D 0 3 2 9 2

Plate 5.5a. The ultrastucture of a zooxanthella from the tentacles of *A. viridis* maintained under a red filter in artificial irradiance (3 μmol m^{-2} s^{-1}, tank 3) . Scale = 1 μm.

Plate 5.5b. The ultrastucture of a zooxanthella from the tentacles of *A. viridis* maintained under a green filter in artificial irradiance (tank 4, 3 μmol m^{-2} s^{-1}). ab= accumulation body. Scale = 1 μm.





CHAPTER 6

General Discussion

The aim of this chapter is to collate the most important aspects of each chapter and provide a focus for the thesis and future work. The temperate symbiotic anemones *Anemonia viridis* and *Anthopleura ballii* show variations in zooxanthellae characteristics which correspond to variations in day-to-day weather, season and water depth in Lough Hyne and Trearddur Bay (see Table 1, Appendix VII for summary of results). Zooxanthellae from intertidal *A. viridis* in Trearddur Bay showed variations which correlated with seasonal variations in environmental parameters. High zooxanthellae mitotic index (MI) recorded in May in Trearddur Bay corresponded with the peak phytoplankton density in the Menai Strait (Owen, 1998). This phytoplankton bloom occurred after high rainfall and peak nitrate levels in March/April, peak sunshine in April/May and increasing daylength. These factors combined to create optimal conditions for photosynthesis and proliferation of phytoplankton, and appeared to stimulate similar peak division of zooxanthellae. In the succeeding summer months, when nitrate levels were depleted, division rate of zooxanthellae returned to a low background level.

Unlike free-living microalgae, zooxanthellae are able to obtain nutrients from host metabolism (Cook 1972, Cates & McLaughlin 1979, Smith 1979, Steen 1986). Feeding by the host is believed to provide endosymbiotic algae with nitrogen and phosphorus, presumably from the digestion of zooplankton prey (Cook *et al.* 1988). For example, Smith & Douglas (1987) reported that the zooxanthellae of *Anthopleura elegantissima* were able to take up host waste nitrogen, and Shick (1991) reported that

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prolonged starvation of the host may lead to nutrient limitation in its zooxanthellae. In *Aiptasia pallida* anemones not fed for 1.5-2 months consistently had higher rates of inorganic phosphate and ammonium uptake than recently fed specimens (Muller-Parker *et al.* 1988). In anemones starved for 1 month, zooxanthellae division decreased and chlorophyll *a* content per cell decreased. Conversely, Cook *et al.* (1988) demonstrated that the MI of zooxanthellae in *Aiptasia pallida* increased when inorganic nitrogen or phosphorous was added to anemone culture water. They concluded that nutrient sufficiency of the zooxanthellae in *A. pallida* cultured in low nutrient seawater depended on the availability of particulate food to the host. All of these results indicate that zooxanthellae may become nutrient limited when their hosts are unfed, and that under normal conditions nutrients are provided by the host. Furthermore, when water column nutrients are depleted the anemone host is able to provide essential nutrients for photosynthetic carbon fixation via heterotrophic feeding. Consequently, zooxanthellae may not suffer nutrient limitation but still appear to respond to high levels of nutrients in the water column as observed during May in Trearddur Bay *A. viridis*.

In Trearddur Bay during 1997-1998, zooxanthellae density showed an inverse relationship with temperature, daylength and sunshine. McCloskey *et al.* (1996) observed that reduced irradiance resulted in diminished expulsion rates for zooxanthellae from *Anthopleura elegantissima*. Reduced autumn irradiances may have produced a similar diminution of zooxanthellae expulsion in *A. viridis*, resulting in a subsequent increase in zooxanthellae in December and January. Conversely, although zooxanthellae density was not high during or after the March/April peak in nitrate levels, it was highest during the winter when water column nutrients were higher than in summer. However, as previously discussed, nutrients may be obtained

by the zooxanthellae from the host, reducing the need for a nutrient supply from sea water. Thus, it is most likely that seasonal fluctuations in temperature and solar radiation governed variations in zooxanthellae density.

Alternatively, changes in zooxanthella density may have reflected seasonal differences in the size of anemones. Because the number of zooxanthellae was standardised to anemone biomass, it is possible that the increase in mean zooxanthellae density in November, December and January represented somatic shrinkage of the anemones in response to low winter temperature, irradiance and prey availability. Size frequency distributions showed that anemones were significantly smaller in winter than summer at Trearddur Bay. Hence, the actual number of zooxanthellae present may not have increased. However, similar observations of seasonal variation in zooxanthellae density in recent studies of tropical symbioses (Stimson 1997, Brown *et al.* 1999, Fagoonee *et al.* 1999, Fitt *et al.* 2000) would suggest that the data from the present study are not anomalous. For example, Fitt *et al.* (2000) reported significantly higher zooxanthellae densities in 5 species of tropical corals during winter, when solar irradiance and water temperature were lower. The lowest densities occurred in late summer or early fall when water temperatures were warmest, and hence were attributed to annual bleaching of the colonies sampled.

The observed similarities in the response of tropical and temperate zooxanthellae to seasonal variations, suggest that tropical and temperate symbiotic Anthozoa respond in a similar way to variations in environmental parameters despite differences in magnitude of these variations. Although evidence reported by Brown *et al.* (1999) and results of the present study (Chapter 2) have demonstrated that tropical marine environments show more seasonality than previously suggested (Adey, 1998) temperate marine environments are subject to far greater seasonal fluctuations. In the

present study, air and water temperature, sunshine and daylength all showed much greater variation at the temperate sites than in tropical Mauritius. The low zooxanthellae densities observed during summer in intertidal and subtidal *Anemonia viridis* may be attributed to annual bleaching, in response to high levels of solar radiation and high water temperatures, as suggested by Fitt *et al.* (2000) for tropical corals. Alternatively, zooxanthellae density may increase during winter as a photoadaptation to low solar irradiance. Whatever the explanation, the evidence that zooxanthellae characteristics vary seasonally in tropical environments (Fagoonee *et al.* 1999, Brown *et al.* 1999, Fitt *et al.* 2000) where fluctuations are less marked than in temperate environments, lends support to the suggestion that zooxanthellae characteristics show similar predictable variations in temperate symbiotic Anthozoa. Further consideration of seasonal variation in temperate Anthozoa requires larger sample sizes and replication over several years to confirm the observations from Trearddur Bay. Simultaneous measurement of meteorological and local water column parameters is also required during years when conditions are typical, and not affected by unusual climatic events such as El Nino.

When MI and cell size are considered, the results suggest that light intensity may be an important regulatory factor of zooxanthellae density. In the laboratory, where temperature, food and daylength were controlled and light intensity was altered, no variation in zooxanthellae density was observed at the irradiances used. This may simply suggest that light intensity has no effect on zooxanthellae density, consistent with the studies of Harland & Davies (1994) and Bythell *et al.* (1997) on *Anemonia viridis* in high and low light treatments (300 & 10 μE. m^{-2} s^{-1}) and in intertidal (300-1700 μmol m^{-2} s^{-1}) and subtidal (30-90 μmol m^{-2} s^{-1}) habitats. Furthermore, this observation may indicate that variations in daylength, water temperature or feeding

may be important. Similar to field observations in summer, higher MI and corresponding smaller cell size were observed in higher intensity irradiance in laboratory maintained *A. viridis*. Accordingly, no increase in zooxanthellae density was observed, suggesting regulation of zooxanthellae numbers despite variations in light intensity. Perhaps if *A. viridis* had been maintained at the experimental irradiances for a period comparable to a winter of low solar irradiances, a different pattern would have emerged.

It appears that zooxanthellae density is regulated in *A. viridis* from Trearddur Bay throughout the spring and summer despite changes in irradiance and high MI during May. In December and January zooxanthellae abundance is allowed to increase unregulated, suggesting a change in some regulatory factor. This could be light intensity, daylength or temperature, all of which decrease towards December. For example, the intensity of solar radiation may fall below a threshold level for zooxanthellae density regulation in December, and thus trigger proliferation of the zooxanthellae population. Longer periods of low intensity irradiance in the laboratory may have produced a comparable irradiance regime to December, and thus triggered a similar zooxanthellae proliferation. Observation of zooxanthellae ultrastructure however, would suggest otherwise. Large vacuoles with conspicuous crystals were observed in all zooxanthellae from laboratory maintained *A. viridis*, whilst vacuoles were absent from zooxanthellae of the intertidal *A. viridis*, Trearddur Bay. This observation may suggest that lower irradiances were experienced in the laboratory than in the summer or winter (Doyle & Doyle, 1940). Similarly no storage lipids were observed in the zooxanthellae of laboratory *A. viridis*. Further investigation in the laboratory may resolve such speculation.

During the spring and summer months, when firstly nutrients and light and then only light conditions were favourable for photosynthesis, and zooplankton was abundant for anemone feeding zooxanthellae from intertidal *A. viridis* at Trearddur Bay were accumulating lipid reserves. The amount of carbon passed to the host depends upon the rate of photosynthesis. Davy *et al.* (1996) demonstrated that at 1.5 m on sunny days in summer (Lough Hyne), zooxanthellae in *A. viridis* would require 1.80 to 5.89 % of the carbon fixed in photosynthesis for respiration and growth, and translocate the remaining 94.11 to 98.20 % to the host. Carbon produced in excess of metabolic requirements is available for storage as lipids. Comparable results were obtained by Harland *et al.* (1992) for fed *A. viridis*, who observed an increase in zooxanthellae storage lipids after 60 days, which was proportional to irradiance level. The lipid reserves observed in *Anemonia viridis* from Trearddur Bay during summer were used in subsequent months when both light levels for photosynthesis were low, and plankton availability for heterotrophic feeding was reduced. Furthermore, Muller-Parker *et al.* (1996), Berner & Izhaki (1994) and Ambariyanto & Hoegh-Guldberg (1996) observed that feeding or nutrient enrichment produced decreases in lipid and starch stores of zooxanthellae from tropical hosts (p.93). Hence, a possible explanation for the absence of lipid from zooxanthellae in intertidal *A. viridis* during winter.

Feeding by the host may also affect chloroplast volume in high and low light. Muller-Parker *et al.* (1996) observed that starvation of *A. pallida* produced a decrease in zooxanthellae chloroplast volume at high and low irradiance. In the present study lower chloroplast volume was observed in zooxanthellae from intertidal *A. viridis* during summer (when food supplies were abundant) than winter. Hence, it is unlikely

that variation in chloroplast volume was due to nutrient limitation, but was higher in winter due to photoadaptation to low winter irradiances.

An increase in the proportion of chlorophyll *a* per zooxanthellae was observed during December which may also have been due to photoadaptation. Figure 4.15, chapter 4 shows the absorption spectrum for zooxanthellae from *A. viridis*. Chlorophyll *a* has two absorption peaks at 650-680 nm and 400-440 nm. The higher proportion of chl *a* per zooxanthellae during December would suggest adaptation to the prevailing light conditions under water in Trearddur Bay. In contrast, Dykens & Shick (1984) observed a disproportional winter increase in chl c_2 in the zooxanthellae *Anthopleura elegantissima*. This may reflect differences in the attenuance spectra of the water at the two sites. For example, a higher proportion of chl c_2 in zooxanthellae from *A. elegantissima* would suggest a greater availability of 440-460 nm wavelength light under water in winter at Bodega Harbor, California than in Trearddur Bay.

Seasonal variation in zooxanthella density of *A. viridis* from a shallow subtidal habitat was also observed. Although an incomplete picture of zooxanthellae variation through the seasons was provided by sampling anemones during one winter (2/98) and one summer (7/98) month, the effects of extreme seasonal conditions were observed. In February zooxanthellae had higher density, were larger but divided more frequently than in July. These results are comparable with the seasonal variations observed in intertidal *A. viridis* in Trearddur Bay for these months, and suggest a similar response to seasonally varied environmental parameters. By comparison, small amounts of lipid and small vacuoles with crystals were present in the zooxanthellae of subtidal *A. viridis* during winter (2/98), whilst neither were observed in intertidal *A. viridis* during winter (2/98).

These observations may reflect three differences between the intertidal and subtidal habitats. Firstly, prevailing physical conditions in the intertidal rock pool habitat are likely to be much more variable than in the subtidal habitat. Extremes of temperature and salinity cause physiological stresses for the anemone, which would not be present in a subtidal habitat. Secondly, greater water depth in the subtidal habitat would increase attenuation of downwelling irradiance, reducing the level of ultraviolet (UV) and photosynthetically active radiation (PAR) and the spectral composition of irradiance reaching anemones, compared with those in the intertidal, rock pool habitat. High levels of UV and PAR may have a damaging influence on the symbiosis (Lesser & Shick 1989, Lesser *et al.* 1990, Kinzie 1993, Brown *et al.* 1994, Hannack *et al.* 1997) and cause photoinhibition (Lesser & Shick 1989, Jokiel & York 1982). Finally, differences in the optical properties of the water at the two sites may have a similar effect on prevailing light conditions. The resulting presence of lipid in subtidal anemones and absence from intertidal anemones during winter suggest larger reserves were accumulated in the subtidal zooxanthellae during summer. This may be attributed to the more stable conditions of a subtidal existence. In addition, the presence of small vacuoles would suggest lower prevailing irradiance (Doyle & Doyle, 1940) in the subtidal habitat than the intertidal during winter. It is unfortunate that no summer comparison of ultrastructure was available for zooxanthellae in subtidal *A. viridis*. Future work would include sampling through all 4 seasons, to investigate variation over a consecutive annual cycle.

The effects of extreme seasonal conditions and water depth on the zooxanthellae of *A. ballii* were investigated in Lough Hyne. In February zooxanthellae density, MI and diameter showed no difference between 6 m and 18 m. By contrast, zooxanthellae density was lower at 6 m than 18 m in summer on Glannafeen cliff, whilst MI and

diameter showed no depth variation. Thus, in winter depth did not affect zooxanthellae density, whilst in summer it did. In addition, at 18 m season did not affect zooxanthellae density, whereas at 6 m it did. Altering both depth and season also produced significant variation in zooxanthellae density, with lower values observed at 6 m in summer than 18 m in winter.

Attenuation of irradiance with water depth in Lough Hyne provides an explanation for the observed differences (Chapter 2). The irradiance level at which photosynthesis just equals respiration is termed the compensation irradiance (Kirk, 1994). *Anthopleura ballii* from Lough Hyne has a compensation irradiance of 89.5 μmol m^{-2} s^{-1} (Davy, 1994). Downwelling irradiance measured at 6 m on a bright day in winter (50 μmol m^{-2} s^{-1}) and at 18 m during both winter (5 μmol m^{-2} s^{-1}) and summer (21 μmol m^{-2} s^{-1}; Table 1, Appendix V) was below the compensation irradiance of *A. ballii*. Hence, zooxanthellae from anemones at 18 m during both winter and summer and at 6m in winter, may have been experiencing irradiance below the compensation irradiance for photosynthesis. In comparison, irradiance measured at 6 m on a bright day in summer was 350 μmol m^{-2} s^{-1} which exceeds the compensation irradiance of *A. ballii*. Bathymetric differences in prevailing irradiance regimes may also have contributed to the observed seasonal differences in zooxanthellae ultrastructure. For example, higher starch and lipid volumes were observed in zooxanthellae at 6 m in winter than at 18 m in winter or summer. Downwelling irradiance measured at 6 m in summer (350 μmol m^{-2} s^{-1}) was much higher than the irradiance measured at 18 m in summer (21 μmol m^{-2} s^{-1}). The high irradiance at 6 m would have produced a higher rate of photosynthesis than at 18 m for example, Turner (1988) observed that *Anemonia viridis* could live autotrophically above 9 m in the summer and 1.5 m in the winter in Lough Hyne. Carbon fixed in excess of metabolic requirements may have

been available for storage as lipid and starch, and used during the subsequent winter months. The greater reserves accumulated during summer at 6 m than 18 m (due to a higher rate of photosynthesis) would last longer during winter, resulting in the larger starch and lipid reserves observed at 6 m than at 18 m in the winter. Investigation of downwelling irradiance in Lough Hyne (chapter 2) revealed that attenuation was much greater during summer (7/98) than winter (2/98) due to an increased abundance of plankton and yellow substance in the summer. Thus, irradiance underwater was diminished much more rapidly during summer. The result was prevailing irradiances at 18 m in summer and 18 m in winter which were of comparable magnitude, and a deeper euphotic zone in winter. Furthermore, total daily illumination varied according to the weather and seasonal daylength. Overall, in winter total sunshine was lower and daylength shorter than in summer, so that total daily illumination underwater was lower in winter.

The chloroplast volume of zooxanthellae also showed evidence of photoadaptation to lower solar irradiances experienced at 18 m in summer (7/98) than at 6 m in winter (2/98). At 18 m in summer zooxanthellae from *A. ballii* had significantly higher chloroplast volume than those at 6 m in winter. Measurement of downwelling irradiance indicated that irradiance levels were lower at 18 m in summer (21 μmol m^{-2} s^{-1}) than at 6 m in winter (50 μmol m^{-2} s^{-1}; table 1, Appendix V) in Lough Hyne. In comparison, no variation in chloroplast volume was observed between winter and summer at 18 m, possibly due to prevailing irradiance being consistently lower than the compensation irradiance for *A. ballii* as previously discussed. Unfortunately no data were available for zooxanthellae from 6 m in the summer; such a comparison may have revealed depth-related photoadaptation, as the difference in irradiance between these depths was considerable (Table 1, Appendix V).

Alternatively, differences in the level of ultraviolet radiation present at 6m and 18 m may have affected zooxanthellae characteristics. For example, Hannack *et al.* (1997) observed an intensity-dependent decrease in the number of zooxanthellae present in *Cereus pedunculatus* exposed to high levels of artificial UV radiation. However, UV levels are unlikely to have been sufficiently high to cause inhibition of photosynthesis or growth, at 6 m in Lough Hyne.

Accompanying the diminution in irradiance with water depth is variation in the spectral composition. Thus, differences observed between zooxanthellae from 6 m and 18 m anemones may have been a response to variations in spectral composition of the prevailing irradiance. For example, Figure 1, Appendix VI and observations from chapter 2 suggest that as depth increases, irradiance underwater in Lough Hyne becomes progressively richer in wavelengths which correspond to the green waveband. From these observations, zooxanthellae from anemones maintained under the green filter in the laboratory might be expected to show a similar response to zooxanthellae in anemones from 18 m water depth. Furthermore, observation of the transmission spectrum for the green filter (Figure 4.2, section 4.3.1, chapter 4) indicates penetration of a broad spectrum of irradiance. This corresponds well with the spectrum of downwelling PAR measured for Lough Hyne in winter (Figure 1, Appendix VI). For example, at 18 m the prevailing irradiance was rich in mid-spectrum wavelengths, with 665 nm (red irradiance) absent and 412 nm (violet-blue irradiance) impoverished. By comparison, at 6 m all wavebands were present with the most notable addition being red irradiance. Thus, prevailing irradiance at 6 m was compared with red irradiance in the laboratory and 18 m compared with green irradiance. Anemones experienced low level irradiance and low water temperature in

the laboratory, more comparable with natural conditions experienced during the winter than the summer in Lough Hyne.

The spectral composition of irradiance tested in the laboratory produced variation in zooxanthellae characteristics. Similar to 6 m and 18 m zooxanthellae in the winter, no significant variation in zooxanthellae density or MI was observed in green and red light. By contrast, zooxanthellae in green irradiance were smaller (10.6 ± 0.08 μm) than those in red irradiance (11.0 ± 0.11 μm), whilst no variation was observed in zooxanthellae size from 6 m and 18 m. Spectral composition produced little variation in zooxanthellae ultrastucture in laboratory maintained *A. viridis*. Although no variation in chloroplast volume was observed between zooxanthellae in green (33.0 ± 0.21 %) or red (34.5 ± 1.9 %) irradiance, a higher volume was observed in full spectrum irradiance (42.2 ± 1.5 %). Similarly, no variation was observed between 18 m and 6 m zooxanthellae in winter in Lough Hyne. Furthermore, starch volume showed no variation between green and red irradiance, whilst starch volume was lower at 6 m than 18 m in Lough Hyne.

Thus, laboratory and field investigations reveal similar responses in zooxanthellae exposed to irradiance of different spectral composition. Nevertheless, the laboratory observations in the present study contradict the conclusions of Kinzie *et al.* (1984) who reported that overall the coral algal system exhibited "sun" type changes when grown in red waveband irradiance. Zooxanthellae from *A. viridis* maintained in the laboratory, showed relative increases in cellular pigment concentrations (Chapter 4) which were more characteristic of shade adaptation described in the literature, whilst other zooxanthellae characteristics were unaffected. Hence, the results of the present study were not conclusive and require further investigation at different irradiances.

Zooxanthellae number, division rate, size, pigment content and ultrastructure in *A. viridis* and *A. ballii* showed variations which correspond with natural variations in environmental parameters. Photoadaptations to fluctuations in both depth and season were observed which were comparable with sun and shade adaptations, and seasonal variations observed in tropical symbiotic Anthozoa. Overall, it is hoped that the findings described in this thesis will provide valuable preliminary data for further studies that examine variations in zooxanthellae characteristics, in temperate symbiotic Anthozoa.

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Appendix I

Experiment to test the suitability of wet weight of tentacles as a standard for zooxanthellae densities

To be able to compare zooxanthellae densities from different anemones, a standard amount must be sampled from each animal. The preferred method was non-destructive allowing repeated sampling of the same animals in the future, and required isolation of intact zooxanthellae. By comparing wet and dry weights of 60 pairs of tentacles from *Anemonia viridis*, wet weight was demonstrated to be a suitable standard for zooxanthellae density. Tentacles were excised from each animal; pairs of tentacles were used to increase the amount of tissue present. Each pair of tentacles was blotted dry and weighed, then dryed in an oven at 60 °C for 72 hours and weighed again. Analysis of the data showed there was a highly significant correlation between wet and dry weight (Pearson correlation, $p = < 0.001$, $r^2 = 0.91$). Wet weight was therefore deemed suitably rigorous and used to standardise zooxanthellae densities from different animals.

Experiment to investigate proportion of zooxanthellae in *Anemonia viridis* tentacles

The aim was to determine whether zooxanthellae density was dependent on the weight of anemone tissue present. Small and large tentacles of different wet weights were sampled and the zooxanthellae per g of animal tissue determined. The densities were then compared with wet weight and analysis demonstrated a highly non-significant correlation (Pearsons correlation, $p = <0.05$, $r^2 = 0.092$). Hence, density is independent of weight.

Appendix II

Table 1. Abundance and distribution of benthic organisms along a vertical depth profile of **Glannafeen** cliff, Lough Hyne. Numbers show mean percent cover of organisms at different depths. Communities were sampled by replicates of 36-point quadrats. For example, a mean cover of 5.6 % was recorded for *Hildenbrandia* sp. at a depth of 6 m. The list of species is not definitive, but represents those able to be recognised underwater on a limited number of dives with confirmation from samples identified in the laboratory.

Species	18 m	15 m	12 m	9 m	6 m	3 m
MACROALGAE						
Hildenbrandia sp.				1.4	5.6	
Lithothamnion sp.					7	9.7
Foliaceous red algae	5.6	36.2	13.9	4.2	2.8	4.2
Filamentous red algae		5.6	33.3	7	2.8	
Corallina sp.				5.6	5.6	5.6
Jania rubens					1.4	
Mesophyllum lichenoides						4.2
Dictyota dichotoma			25	19.5		
Asperococcus turneri				16.7	5.6	
Stilophora rhizoides				19.5	12.5	100
Ulva sp.					7	
ANTHOZOA						
Isozoanthus sulcatus	4.2	2.8	4.2			
Caryophyllia smithii	16.7	4.2	4.2			
Corynactis viridis						
Anthopleura ballii			2.8	15.3	16.7	8.4
Anemonia viridis			1.4	4.2	2.8	
Parerythropodium sp.					2.8	
HYDROZOA						
Bougainvillea sp.		1.4				
BRYOZOA						
erect bryozoan		1.4	4.2	4.2		
ASCIDIACEA						

Ascidiella aspersa	7	13.9	2.8			
Aplidium punctum			1.4	4.2	2.8	
Sidnyum sp.		2.8			4.2	
Diplosoma listeranium	1.4	1.4		1.4		
PORIFERA						
Raspailia sp.	7		2.8			
Polymastia mammilaris	2.8	2.8	1.4			
Polymastia boletiformis			1.4			
Suberites ficus					2.8	
Suberites carnosus	1.4	1.4				
Encrusting orange sponge	16.7	13.9	4.2	2.8		
Encrusting yellow sponge			1.4			
Encrusting red sponge					1.4	2.8
Dysidea fragilis					1.4	
Axinella dissimilis	1.4	8.3	1.4			
Axinella damicornis			1.4		1.4	

Appendix III

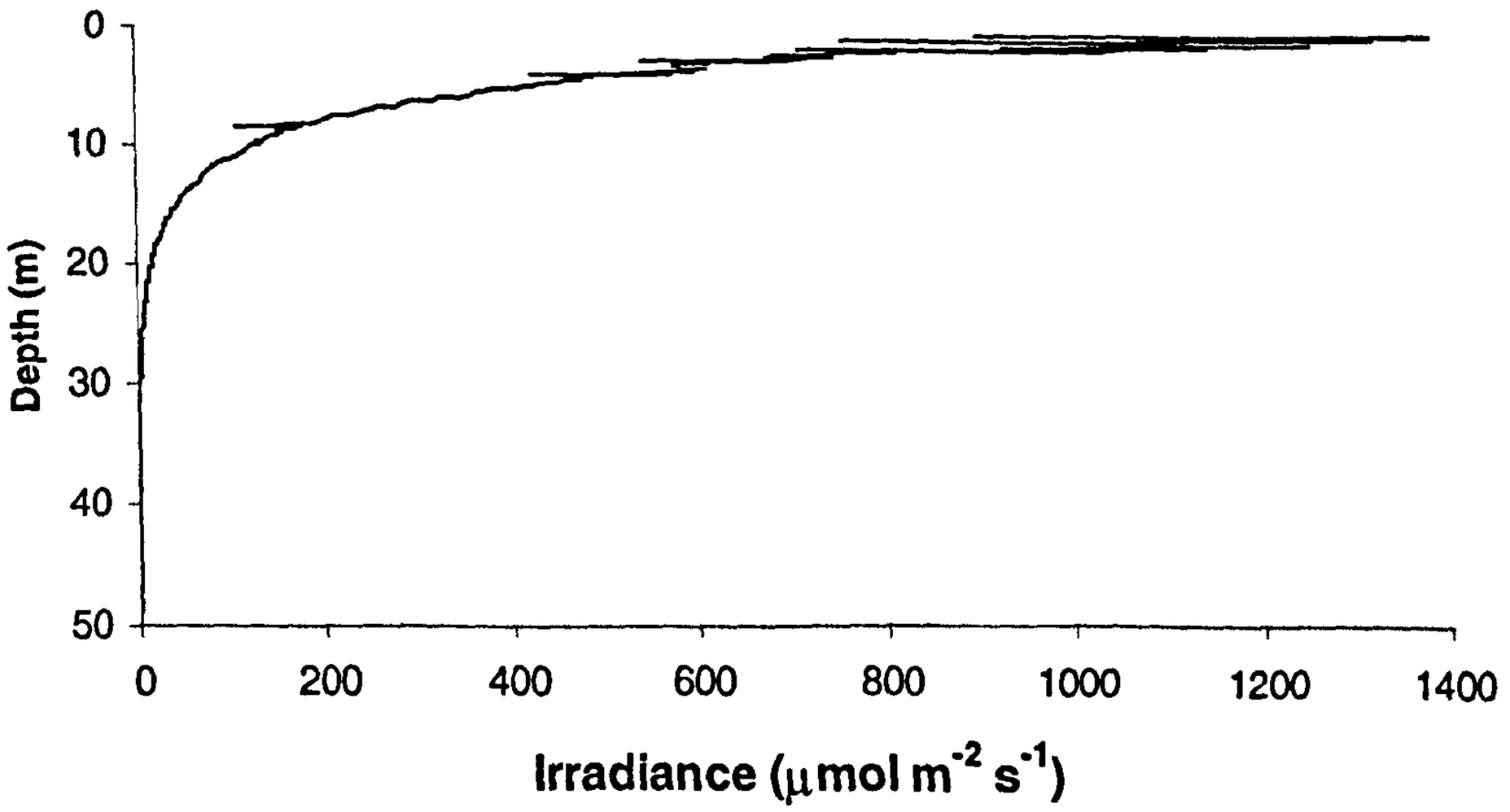
Table 1. Attenuation coefficients (K_d, m^{-1}) for different wavebands in Lough Hyne during summer (7/98) and winter (2/98). The ratio of summer:winter K_d at each wavelength is shown to illustrate the relative proportions of each waveband present during winter and summer. For example, proportionately greater attenuation of the blue waveband (443 nm) occurs during summer.

Wavelength (nm)	Colour of waveband	Summer K_d (m^{-1})	Winter K_d (m^{-1})	ratio of sum K_d:win K_d
412	violet	0.405	0.312	1.30
443	blue	0.311	0.229	1.36
490	blue-green	0.214	0.166	1.29
510	green	0.200	0.161	1.24
555	yellow	0.178	0.164	1.09
665	red	0.565	0.485	1.16
Total PAR		0.239	0.179	

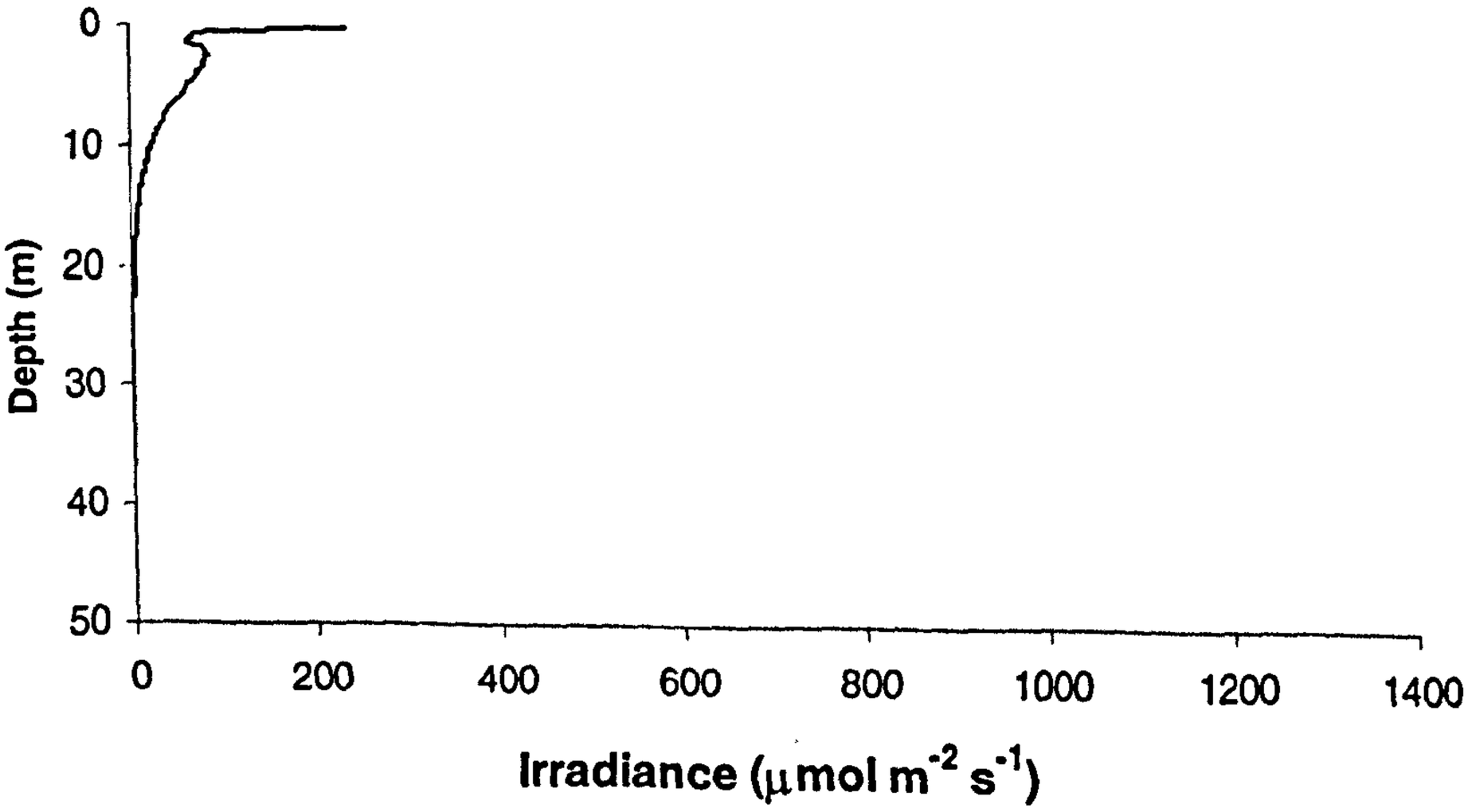
Appendix IV

Figure 1. Attenuation of downwelling irradiance (PAR, µmol m^{-2} s^{-1}) in Lough Hyne, Ireland during a) summer (8/98) and b) winter (2/98).

a)



b)



Appendix V

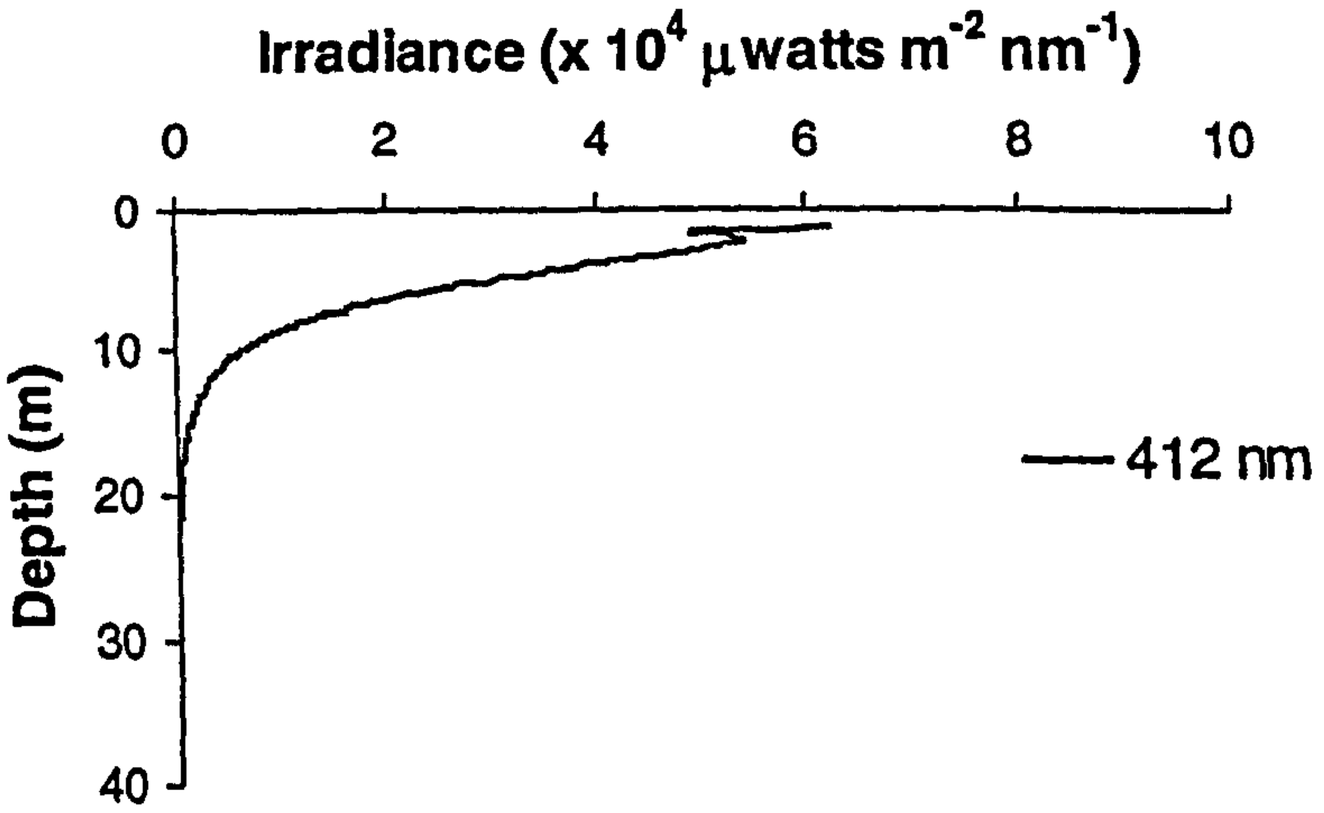
Table 1. Downwelling irradiance ($\mu mol\ m^{-2}\ s^{-1}$) at 6 m and 18 m in Lough Hyne on bright days in summer (7/98) and winter (2/98).

	Depth (m)	Irradiance ($\mu mol\ m^{-2}\ s^{-1}$)
SUMMER	6 m	350
	18 m	21
WINTER	6 m	50
	18 m	5

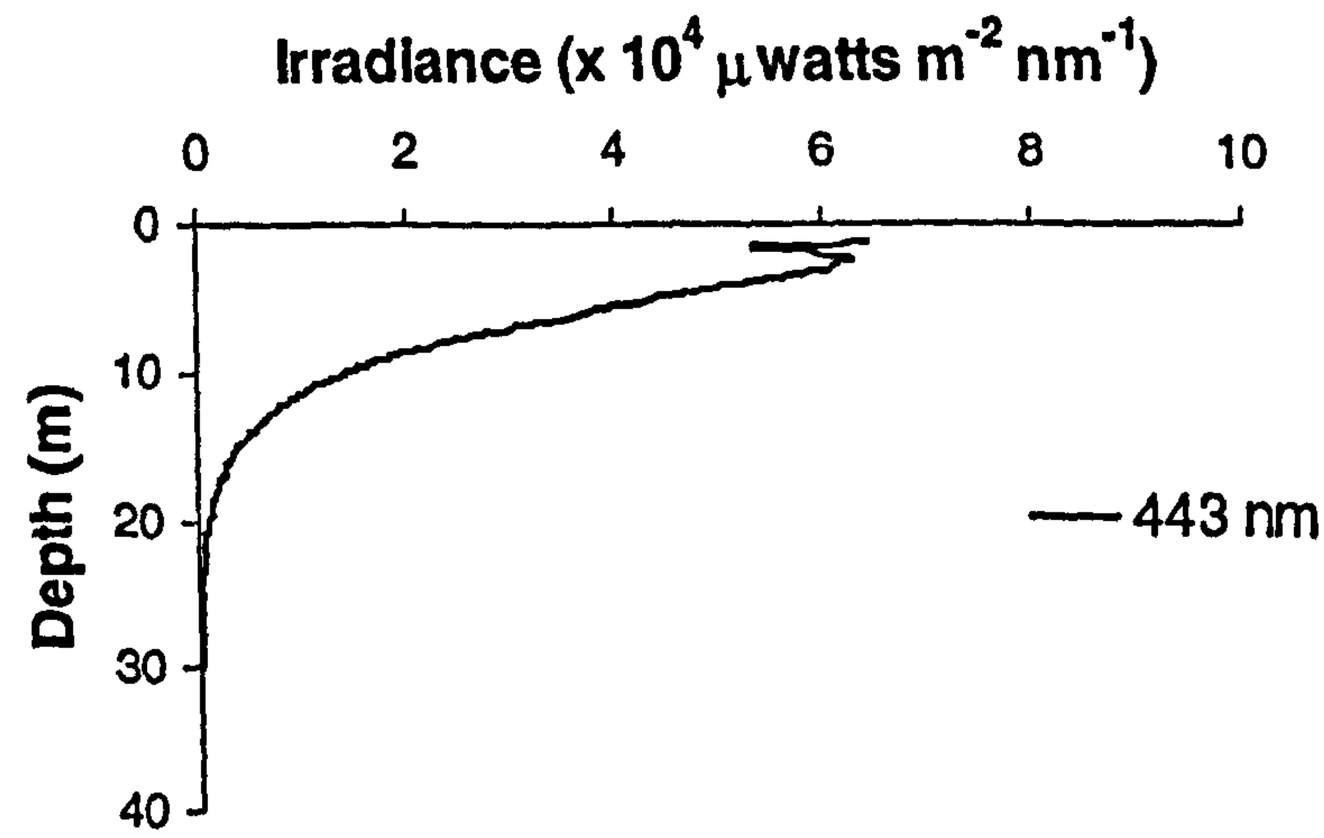
Appendix VI

Figure 1. Attenuation of the different wavebands of downwelling PAR with water depth in Lough Hyne during winter (2/98). Wavebands measured were a) 412, b) 443, c) 490, d) 510, e) 555 and f) 665 nm.

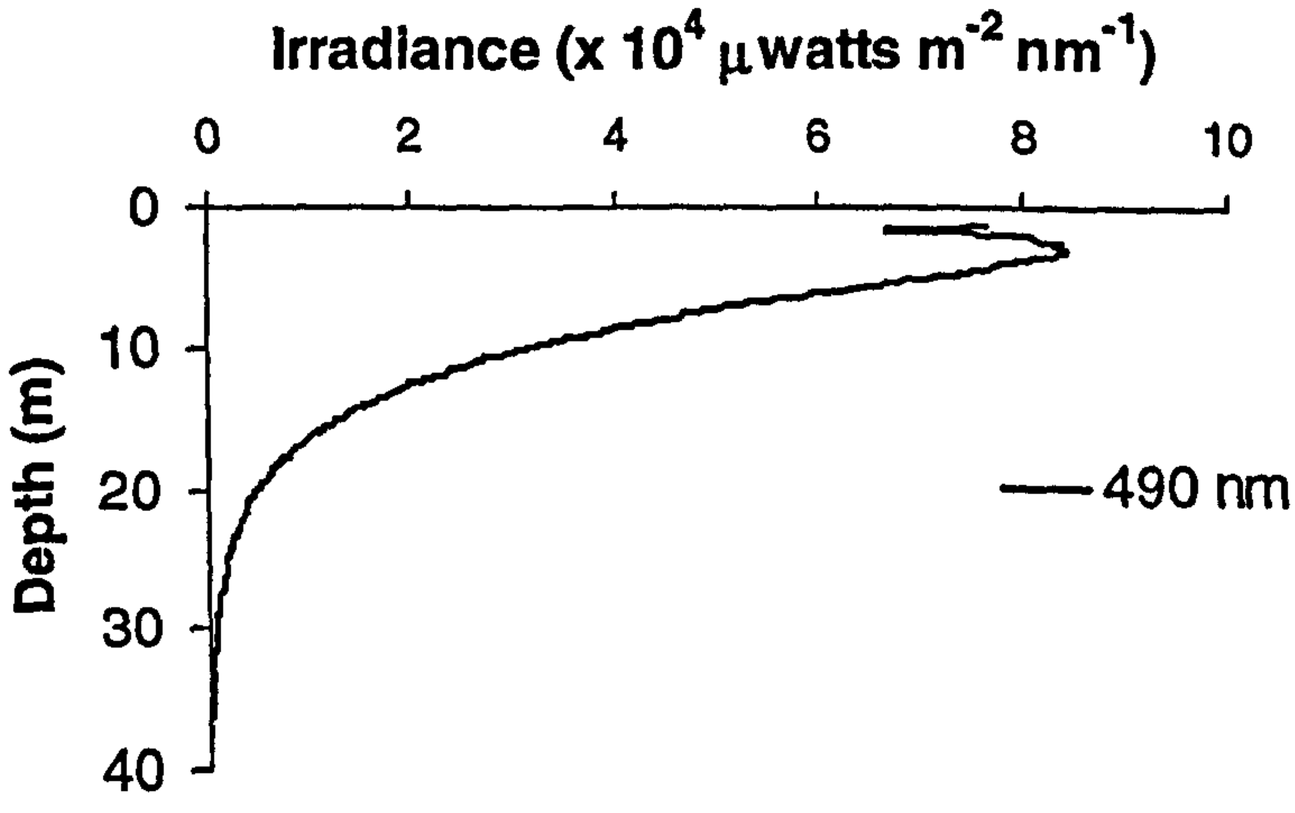
a)



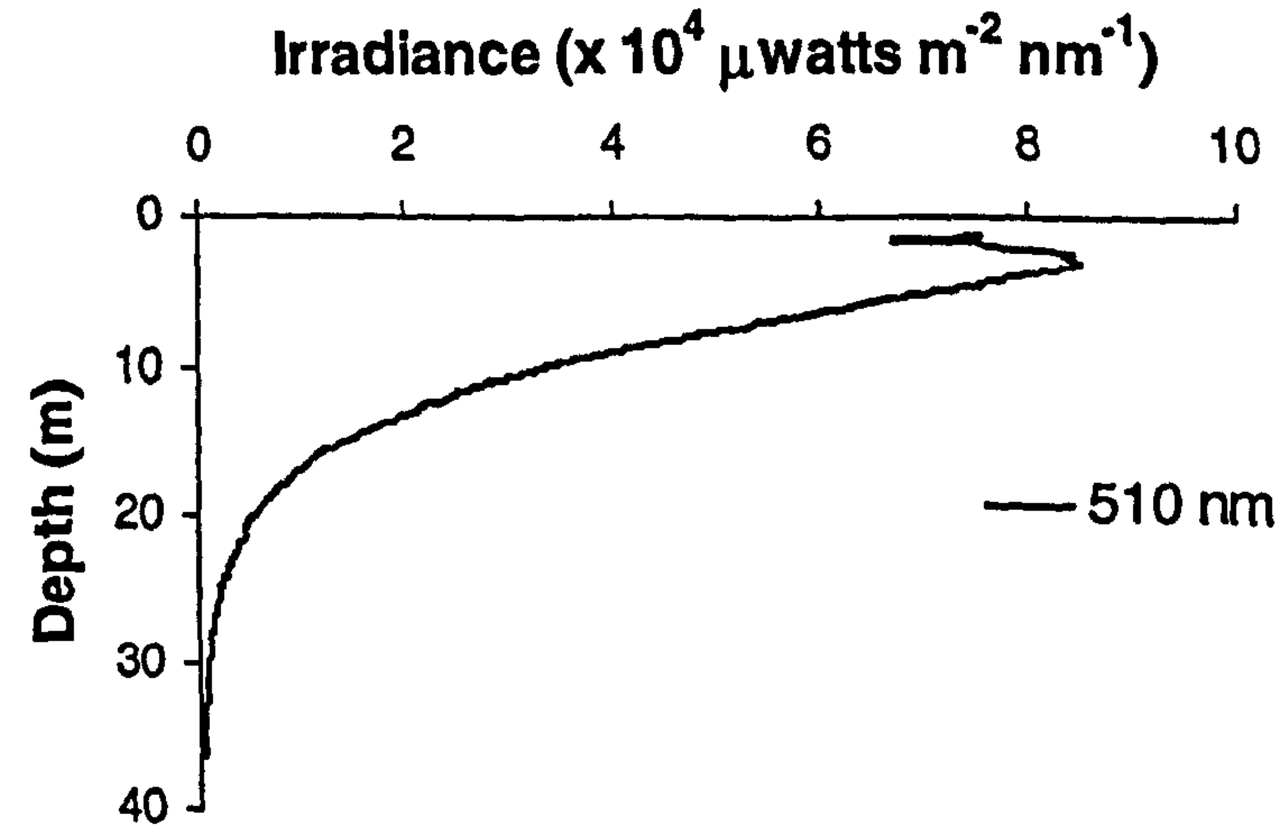
b)



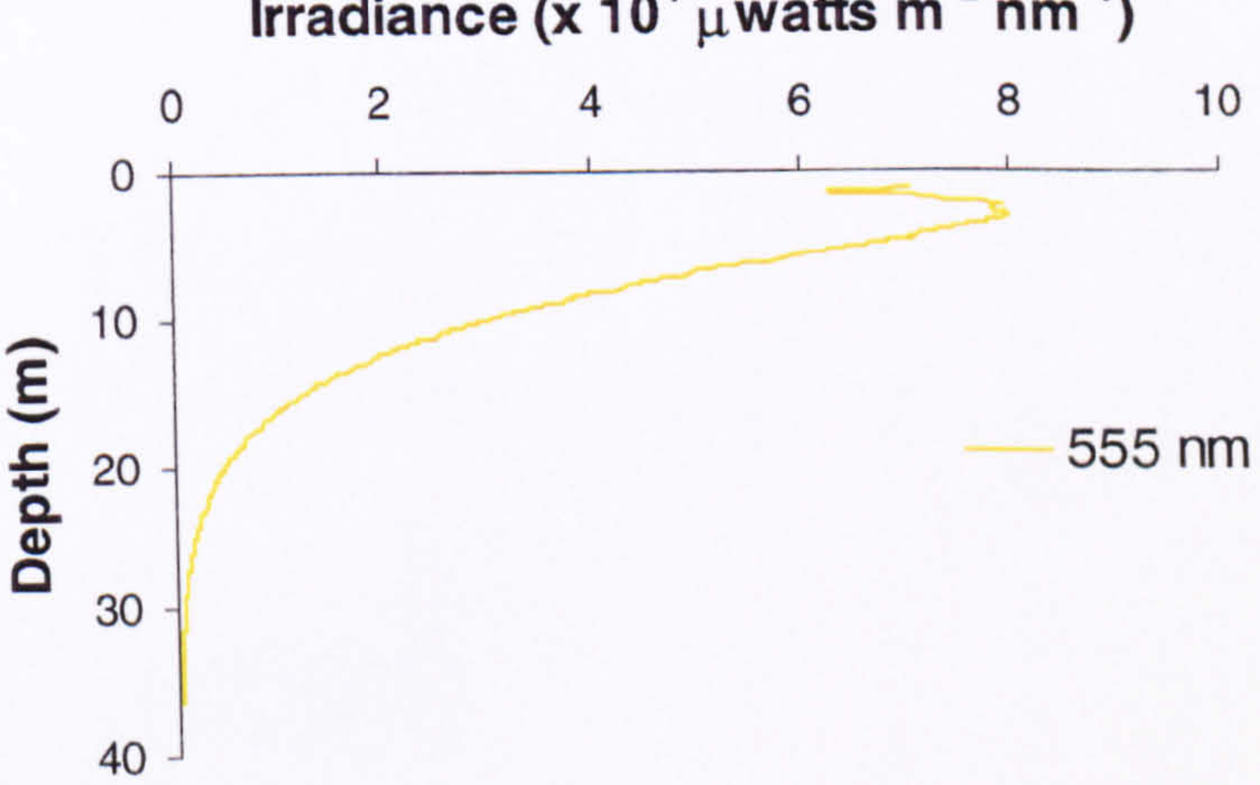
c)


Irradiance (x 10^4 μwatts m^-2 nm^-1)
0
2
4
6
8
10
0
10
20
30
40
Depth (m)
490 nm

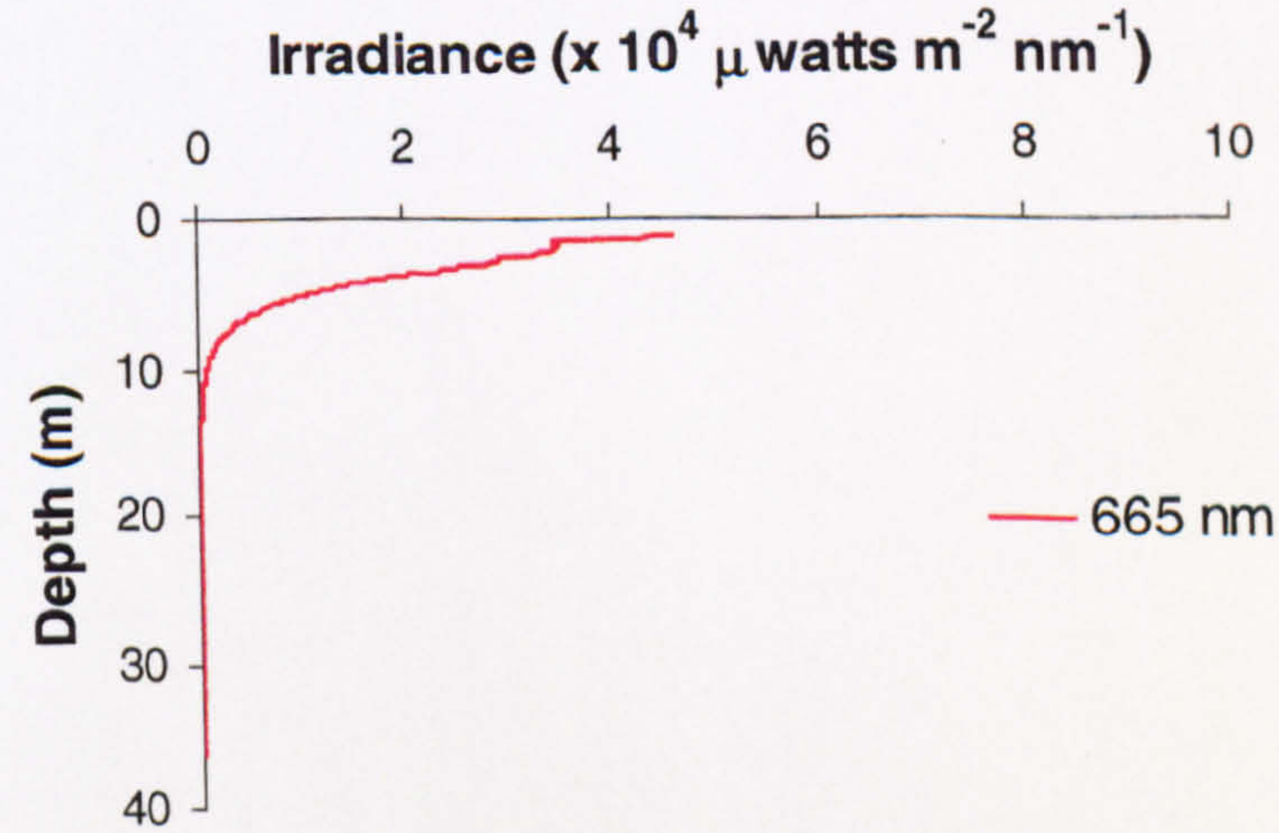
d)


Irradiance (x 10^4 μwatts m^-2 nm^-1)
0
2
4
6
8
10
0
10
20
30
40
Depth (m)
510 nm

e)


Irradiance (x 10^4 μwatts m^-2 nm^-1)
0
2
4
6
8
10
0
10
20
30
40
Depth (m)
555 nm

f)


Irradiance (x 10^4 μwatts m^-2 nm^-1)
0
2
4
6
8
10
0
10
20
30
40
Depth (m)
665 nm

Appendix VII

Table 1. Summary of field and laboratory zooxanthellae characteristic data. Values shown are means (± 1 standard error). Red, green, tank 1 (4 μmol m^{-2} s^{-1}) and tank 2 (20 μmol m^{-2} s^{-1}) refer to artificial irradiances used in the laboratory. Mitotic index data were not available for subtidal *A. ballii*, or green and red irradiance treatments, instead the presence or absence of dividing cells was compared. Summer anemones at 6 m had a greater number of dividing zooxanthellae than winter anemones at 6m. All other pairwise comparisons revealed no significant differences. Tank 1 anemones had significantly more samples with dividing zooxanthellae than both red or green irradiance. treatment anemones.

		Month of year											Light treatment			
		J	F	M	A	M	J	J	A	S	O	N	D	Green	Red	Tank 1	Tank 2
A. viridis																	
Intertidal	**Density** (x 10^8 cells g^{-1})	5.33 (0.20)	1.58 (0.08)	1.58 (0.08)	1.48 (0.06)	1.61 (0.09)	0.68 (0.06)	1.24 (0.09)	1.01 (0.05)	1.70 (0.17)	1.61 (0.12)	1.76 (0.07)	2.73 (0.18)	0.44 (0.09)	0.57 (0.03)	0.69 (0.06)	0.82 (0.07)
	MI (%)	-	0.53 (0.12)	1.02 (0.13)	0.88 (0.13)	3.07 (0.26)	0.29 (0.10)	0.93 (0.10)	0.65 (0.19)	0.74 (0.10)	1.01 (0.25)	0.61 (0.20)	0.77 (0.20)	-	-	0.55 (0.08)	0.33 (0.05)
	Diameter (µm)	9.87 (0.07)	10.00 (0.05)	9.91 (0.09)	9.78 (0.09)	9.07 (0.05)	9.38 (0.15)	8.81 (0.12)	9.94 (0.10)	9.71 (0.06)	9.23 (0.16)	8.50 (0.12)	9.16 (0.10)	10.6 (0.08)	11.0 (0.11)	10.8 (0.10)	10.4 (0.05)
(µg g^{-1})	**Chl *a***	1112 (145)	434 (25)	317 (14)	399 (26)	367 (24)	-	304 (28)	319 (22)	375 (61)	395 (43)	-	810 (39)	212 (24.5)	250 (16.8)	263 (23.6)	424 (33.0)
	Chl c_2	484 (83.6)	261 (35.3)	236 (24.4)	215 (16.1)	193 (12.9)	-	178 (15.7)	194 (23.1)	440 (119)	270 (41.2)	-	309 (28.2)	100 (11.1)	109 (7.1)	125 (8.5)	191 (14.0)
	ratio of Chl *a*: c_2	2.49 (0.24)	1.90 (0.20)	1.50 (0.11)	1.88 (0.07)	1.96 (0.13)	-	1.79 (0.18)	1.82 (0.20)	1.14 (0.23)	1.59 (0.15)	-	2.76 (0.16)	2.11 (0.05)	2.31 (0.03)	2.09 (0.07)	2.22 (0.05)
(pg $cell^{-1}$)	**Chl *a***	2.3 (0.66)	2.8 (0.25)	2.1 (0.10)	2.7 (0.22)	2.4 (0.16)	-	2.7 (0.38)	3.2 (0.27)	-	2.6 (0.35)	-	3.0 (0.28)	3.7 (0.31)	6.0 (0.63)	4.0 (0.52)	5.4 (0.67)
	Chl c_2	1.1 (0.35)	1.7 (0.27)	1.5 (0.15)	1.5 (0.12)	1.3 (0.11)	-	1.6 (0.24)	2.0 (0.32)	-	1.7 (0.22)	-	1.3 (0.16)	1.74 (0.14)	2.61 (0.28)	1.92 (0.22)	2.50 (0.29)
	ratio of Chl *a*: c_2	2.01 (0.09)	1.90 (0.20)	1.39 (0.16)	1.89 (0.06)	1.96 (0.13)	-	1.86 (0.18)	1.82 (0.20)	-	1.63 (0.17)	-	2.76 (0.19)	2.13 (0.04)	2.31 (0.03)	2.09 (0.07)	2.20 (0.14)

A. viridis		Summer	Winter
Subtidal	**Density** (x 10^8 cells g^{-1})	1.01 (0.09)	2.06 (0.11)
	MI (%)	0.39 (0.07)	0.68 (0.10)
	Diameter (µm)	8.08 (0.10)	9.02 (0.05)

A. ballii		Summer		Winter	
		6 m	18 m	6 m	18 m
Subtidal	**Density** (x 10^8 cells g^{-1})	0.45 (0.04)	0.98 (0.09)	0.97 (0.10)	0.82 (0.10)
	MI (%)	-	-	-	-
	Diameter (µm)	11.3 (0.20)	11.1 (0.21)	11.5 (0.15)	11.7 (0.18)